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EFFECT OF FEEDING 17α– METHYLTESTOSTERONE AND WITHDRAWAL ON FEED UTILIZATION AND GROWTH OF NILE TILAPIA *(OREOCHROMIS NILOTICUS* L.)FINGERLINGS

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Abstract

Fingerlings Nile tilapia (Oreochromis niloticus) were orally administered 17α -methyltestosterone (MT.). Fish received 0, 2, 5, or 8 mg MT/Kg diet for 20 weeks (phase-1) followed by 8 weeks withdrawal period (Phase II). At the end of Phasel -1, fish received 2mg MT/Kg diet (best treatment) exhibited significantly higher (P <0.05) weight gain over the control by 44.5%. Likewise, food conversion ratio (FCR),protein efficeincy ratio (PER), and productive protein value (PPV) among group receiving 2mg MT/Kg diet were significantly better than the control (2.82 versus 3.40, 1.14 versus 0.94 and 18.15 versus 14.98 respectively).

The anabolic property of dietary steroids became more visible after 10 weeks. Increasing the dietary concentration of MT more than 2mg / Kg diet reduced fish growth and feed utilization efficiency, but not below the level of control.

The results of the present study showed increase in moisture, protein, fat and ash deposition in fish body with the decrease of MT levels in the diet. In other words, the nutrients were more retentive in fish body with lower doses than with higher doses of dietary MT.Fish group received 2mg MT / Kg diet had greater retention values than of those the control group by 47.7%, 44.9%, 20.8% and 35.1% for moisture, protein, fat and ash respectively.

Viscro-somatic indices (VSI) of the MT-treated fish were slightly but not significantly (P>0.05) higher than those of the control. The gonado-somatic indices were significantly (P<0.05) higher in the MT-treated fish than the control.

Hormone withdrawal reduced fish growth and feed efficiency values to below the level of control in all the treatments, and greater declines ($P<0.005$) occurred for MT treated fish fed the diet containing 2mg MT/Kg. Hormone withdrawal negated MT stimulated increases in moisture protein, fat, and ash retention in fish body that occurred in Phase-I.

No differences were observed between final mean daily weight gain, nutrient gains (Protein, fat, ash and mositure) or feed efficiency (FCR, PER and PPV) values of fish initially fed MT and control fish calculated for the entire 28 weeks of the study.

The results demonstrated that, incorporation of MT into diets of Nile tilapia fingerling following hormone withdrawal offers no potential for improving either growth or food utilization efficiency if treatment and withdrawal period similar to those used in this study are employed.

INTRODUCTION

Steroids have been used to increase growth, protein synthesis, and efficient utilization of feeds in a number of terrestrial animals (Matty and Cheema 1978). Research on the effects of anabolic steroids in fish has been mainly given to sex reversal (Shelton et al. 1982, Shelton 1986) or to the inducement of sterility (Stanley 1982, Mazor Ali and Satyanarayana Roa 1989).

The increased growth rate obtained by supplementing diets with anabolic steroids has an important implication for the fish culturist. It is known that the aquacultural industries are handicapped by the steadily increasing cost of feedstuffs. Growth promoter hormones are means to increase the efficiency of feeds so that fish can be raised to desired size in shorter time at less cost. In this context, using hormone supplemented diets may improve the economics of operation and utilization of facilities.

17 α-methyltestosterone (MT), one of the synthetic steroids hormones, has been produced by modification of the molecular structure and it possess anabolic activity, while the androgenic effect is suppressed.

Not all fish responses to this compound have been positive, however, Higgs et al. (1982) in a review, indicated that, species, size of fish, dietary dose, environment and nutritional status can influence the response to feeding steroids. Increase

in weights has been reported for salmonids 17 α-methyltestosterone (Higgs et al. 1982, Schreck and Folwer 1982, Ostrowski and Garling 1988). Also, Common carp (*Cyprinus carpio*), American eel (*Anguilla rostrata*) and Blue tilapia (*Oreochromis* aureaus) have responded positively to MT feeding (Lone and Matty 1980, Degani and Gallagher 1985 and Guerro 1975), respectively. Reduced growth rates have been reported for European eel (*Anguilla anguilla*) (Degani and Gallagher 1985) and Channel catfish (*Ictalurus punctaturs*) (Simone 1990, Gannam and Lovell 1991). These results suggest that fish species may vary in their metabolism of MT, or in their response to various dietary levels of hormone.

To ensure safe drug use, a withdrawal period may be necessary before treated fish are marketed (Fagerlund and McBirde 1978). Consequently, it is important to determine whether initial improvements in growth and feed efficiency obtained with hormone treatments are maintained through a period of hormone withdrawal to justify the incorpation of these agents into fish feeds.

Tilapia are indigenous to Africa, and early in the twentieth century were already estabished as one of the world most nutritious fish. To day, no other fish with the probable exception of the common carp (*Cyprinus carpio*), is more widely cultured than Tilapia (Balarin, 1979).

Nile tilapia is the most common cichlid species in the inland fisheries of Egypt (Ishak et al. 1979). Although Nile tilapia have rapid growth and efficiency able to use artificial food, further impovements in diet efficency may be important to increase the fish production.

The purposes of the present study were to evaluate the anabolic effect of 17 α–methyltestosterone and its potential for enhancing growth rate when fed to Nile tilapia (*Oreochromis niloticus*), and to examine the effects of the hormone withdrawal from diet on fish growth and feed efficiency values.

MATERIALS AND METHODS

The experiment was conducted over 28 weeks period divided into an initial 20 weeks hormone treatment (Phase-I) and a subsequent 8 weeks hormone withdrawal (Phase-II). Fish receiving the hormone supplemented diets were immediately

switched to their respective control diets upon completion of the 20 weeks hormone treatment (Phase-I). Control fish were fed hormone-free diet for the entire 28 weeks.

Fish and Culture Techniques :

Nile tilapia (*O.niloticus*) were obtained from one of the experimental ponds of the field station of the Central Laboratory of Aquaculture Research "CLAR". Prior to the start of the experiment, all fish were kept indoor in the laboratory and placed in a fiberglass tank and fed a normal diet for two weeks acclimatization period for the laboratory conditions. The fish were then divided into 12 groups, each contained 30 fish of average weight 3.60 ± 0.5 Kg per fish. Each group of fish was transferred at random into galss aquatium 40 L capacity. Dechlorinated tap water was used throughout the study. In order to avoid accumulation of the metabolites, water of the aquarium was changed daily. Each aquarium was also suuplies with air produced by a central compressor placed in the "CLAR". Water temperature and dissolved oxygen were recorded daily. The mean values for the entire period of the study were 26.8oC and 6.4 mg/L, respectively. The photoperiod was set on a 12 light-dark cycles using fluorescent tubes as the light source.

Diet Preparation and Feeding Regime :

A basic diet calculated to contain 300 g protein / Kg was prepared. The composition of the basic diet and the results of the proximate analysis according to the Standard methods of AOAC (1980) are shown in Table 1.

The ingredients were blended in Hobart mixer and divided into four equal portions. Four 200 ml 95% ethanolic solutions of MT were prepared to provide diffferent hormonal concentrations of 8,5,2 and 0 mg MT/Kg of diet. Each of the MT solutions was added and extruded through a meat mincer. The diets were air-dried for 24 h, broken into pellets and stored at -20 ^{o}C in sealed polyethyethylene bags. Each of the four diets was offered to triplicate groups of fish. The fish in each aquaria daily received restricted rations at 5% of their body weight. The fish were fed twice a day and six days a week. The rations were adjusted every two weeks so that feed intake as percentage of toal fish weight was identical for the groups receiving these rations. Feeding of the hormone supplemented diets commenced on 7 June and continued for 20 weeks. After this period, all fish groups were immediately placed on control diet (0 mg MT / Kg) for a further 8 weeks. During Phase-II, the number of fish was reduced to 21 in each aquarium to accommodate the increasing

This reduction process was conducted at random. All other conditions remained unchanged.

Somatic Indices :

Table 1. Composition and proximate analysis of the basal diet.

Ingredients	%	Analysed Components	%
Fish meal * ·	32.75	Protein	34.50
Soybean meal dehulled	32.75	Lipids	7.10
Rice bran	22.50	Carbohydrates + Ash	44.10
Corn oil	5.00	Gross Energy (Kcal/ g diet) ++	14.30
Starch	3.00		4.43
Vitamins Mixture **	2.00		
Minerals Premix ***	2.00		

* Commercial preparation

** Vitamins mixture contained (as g / kg of premix) : Thiamine 2.5; Riboflavin 2.5; Pyridoxine 2.0; Inositol 100.0; Biotin 0.3; Pantothenic acid 100.0; Folic acid 0.75; Para-aminobenzoic 2.5; Choline 200.0 Nicotinic acid 10.0; Cyanocobalamine 0.005; -tocopherol acetate 20.1; Ascorbic acid 50.0; Menadione 2.0; Retinol palmitate 100.000 IU; Cholecalciferol 500.000 IU.

*** Minerals premix (as g / Kg of premix) : CaHpo4.2H20 727.7775; MgSo4. H20 127.5; Kcal 50.0; Nacl 60.0; FeSo4. 7H20 25.0; ZnSo4. 7H20 5.5; MnSo4.4H20 2.53; CuSo4. 5H200.785; CoSo4. 7 H20 0.4775; Calo3. 6H2 0.295; CrCl3.6H20 0.1275.

The supplements of vitamins and minerals were originally formulated fo trout (Tacon *et al.* 1982), but has proven successful with tilapias (Jauncey adn Ross 1982).

\+ Calculated by difference.

\+ + Gross energy were calculated, the energy value (Kcal / g) for protein 5.65; for lipids 9.45 and for carbohydrate 4.1 (Brett 1973, Jobling 1983).

biomass.

The Viscero Somatic Index (VSI): Fifteen fish from each treatment (3 fish / replicate) were individually weighed to the nearest 0.01 g and the entire mass in the abdominal cavity of each fish was removed and weighed. The equation used for the calculation was, VSI = viscera weight X 100 / fish weight.

The Gonadosomatic Index (GSI) : The gonads of the fifteen fish which have been taken for the VSI's determination were also individually removed and weighed. The equation used for caculation was, GSI = weight of gonads X 100 / weight of fish.

Chemical Analysis of Fish and Diet:

At the beginning of the experiment, 10 fish of average sizes similar to those of tested fish were taken for chemical analysis. At the end of Phase-I and Phase=II, samples of 9 fish from each treatment (3 fish / replicate) were also taken for analysis. They were kept in labelled plastic bags and held frozen at 20°C until prior to proximate analysis. The fish samples were analysed for tissue composition using the standard methods of AOAC (1980). Thawed fish in each replicate were dried at 65°C for 48 hours. The fish were coarsely ground in an electric blender and were processed further in a wiley mill from which standard sub-samples were taken for the chemical analysis of protein, lipids, ash and moisture contents.

Statistical Analysis :

A one-way analysis of variance was used to determine if there were differences in growth and feed utilization efficiency data among treatments. The data were subjected to Duncan's Multiple Range Test to compare mean values and to evaluate the significance ($P<0.05$) of the differences among obtained means.

Results and Discussion

The effect of the anabolic property of the dietary steroid on growth improvement of treated fish became visible after 10 weeks and became more pronounced as the study progressed (Fig.1). The present study demonstrated that the steroid MT was effective as a growth accelerator for Nile tilapia during th ehormone treatment period of 20 weeks. The growth was more pronounced with lower than with higher

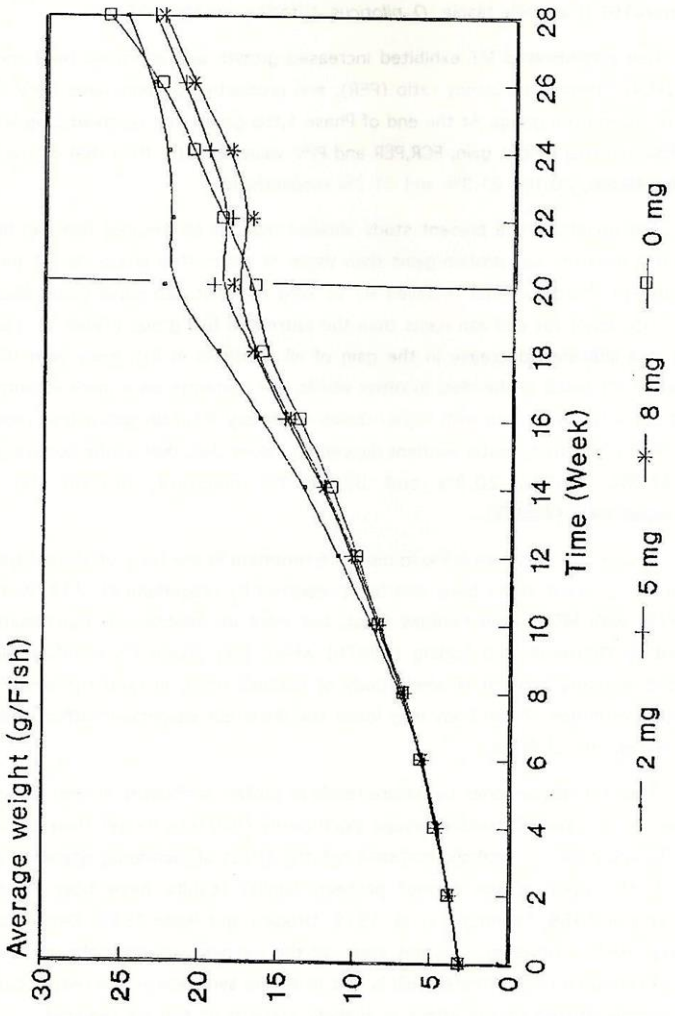

Fig. 1. Effect of feeding different levels of 17 α-methyltestosterone on the growth of Nile tilapia (Oreochromis niloticus) for 20 week and withdrawal for 8 week.

ceived 2mg MT/Kg feed (best treatment) (Table 2). This result generally agrees with studies with cannal catfish where fish fed high level of MT had less gain than those fed low level of MT (Gannam and Lovell 1991). Previous studies showed that, MT induced growth accelaration in fry stages of blue tilapia *O. aureus* (Guerrero1975) and Nile tilapia *O. niloticus* (Ufodike and Madu 1986).

Fish administered MT exhibited increased growth and improved feed conversion (FCR), protein efficiency ratio (PER), and productive protein value (PPV) over that of the control group. At the end of Phase 1,the group that received 2mg MT/Kg diet had terminal weight gain, FCR,PER and PPV values greater than that of the control by 44.5%, 20.1%, 21.3% and 21.2% respectively.

The results of the present study showed that, all MT-treated fish had higher ($P<0.05$) moisture and protein gains than those of the control group. All MT-treated fish, except the group that received MT at 2mg /Kg diet had equal or significantly ($P<0.005$) lower fat and ash gains than the untreated fish group (Table 3). The results also indicated decrease in the gain of all nutrients in fish body with the increase of MT levels in the diet. In other words, the nutrients were more retentive in fish body with lower than with higher doses of dietary MT. Fish group that received 2mg MT/Kg diet had greater nutrient deposition values than that of the control group by 47.7%, 44.9%, 20.8% and 35.1% for moisture, protein, fat and ash,respectively (Table3).

The results of the increase in moisture retention in the body of steriod treated fish in the present study have also been reported by (Fagerlund *et al.*1979, YU *et al.*1979) with MT-treated rainbow trout, but were on contrary to the results reported by Ostrowski and Garling (1987b) where they found MT significantly decreased moisture content in whole body of rainbow trout. In fact, the increase in moisture retention of fish body may lower the dress-out weightsand affect the texture and quality of fillets.

The steroid hormones by nature result in protein anabolism. In this study, the protein gain of the MT-treated groups significantly ($P<0.05$) greater than untreated fish showing that the anabolic hormone has the effect of increasing nitrogen retention in the body in the formof protein. Similar results have been reported (Kruskenper 1968, Fagerlund et al. 1979, Ufodike and Madu 1986, Ostrowsk and Garling 1987b). However, it is not clear, at the moment, whether this increase in protein retention of MT-treated fish is due to active synthesis or decreased catabolism. Further studies on the effect of anabolic steroids on fish are required.

Table 2. Effects of Feeding Various Levels of 17 α– methyltestosterone (MT) and Withdrawal Growth and Food Utilization Efficiency of Nile Tilapia.

Treatments MT mg / Kg diet	Weight gain g / fish	Growth rate g / day	FCR	PER	PPV
	Hormone Treatment (Phase - I)				
2 mg	19.41 A	0.1386 A	2.82 B	1.14 A	18.15 A
5 mg	16.23 B	0.1159 AB	3.14 AB	1.02 AB	17.56 AB
8 mg	14.95 BC	0.1068 B	3.37 A	0.96 B	15.78 B
0 mg (Control diet)	13.43 C	0.0959 B	3.40 A	0.94 B	14.98 B
	Hormone Withdrawal (Phase - II)				
2 mg	2.55 C	0.046 C	12.66 A	0.23 C	5.20 C
5 mg	3.91 B	0.070 B	7.21 B	0.40 B	5.48 C
8 mg	4.51 B	0.081 B	5.88 C	0.49 B	9.06 B
0 mg (Control diet)	6.36 A	0.167 A	3.02 D	0.96 B	16.79 A
	The Entire Experiment (Phase - II)				
2 mg	21.93 AB	0.112 AB	3.96 A	0.777 A	12.76 B
5 mg	20.05 AB	0.102 AB	3.95 A	0.782 A	12.94 B
8 mg	19.49 B	0.099 B	3.93 A	0.786 A	13.30 B
0 mg (Control diet)	23.00 A	0.117 A	3.21 A	0.957 A	15.73 A

Means with same superscripts in the same columns are not significantly different at ($P>0.05$)

Table 3. Effects of Feeding Various Levels of 17 α– methyltestosterone (MT) and Withdrawal on Nutrient Gains in Body of Nile Tilapia and Effect of the Hormone on the Somatic Indices.

MT mg per Kg diet	VIS	GSI	Moisture	Protein	Lipid	Ash
Hormone Treatment (Phase - I)						
2 mg	9.97 A	1.45 A	14.02 A	3.10 A	1.45 A	0.77 A
5 mg	10.74 A	1.87 A	11.52 B	2.79 B	1.23 B	0.56 B
8 mg	11.17 A	1.89 A	10.93 B	2.47 B	1.06 C	0.45 C
0 mg	9.96 A	0.55 B	9.49 C	2.14 D	1.20 B	0.57 B
Hormone Withdrawal (Phase - II)						
2 mg			1.70 B	0.58 B	-0.12 C	0.40 A
5 mg			2.97 B	0.53 B	0.18 B	0.32 A
8 mg			2.91 B	0.83 B	0.30 B	0.47 A
0 mg (Control)			6.75 A	1.64 A	0.50 B	0.51 A
The Entire Experiment (Phase - II)						
2 mg			15.72 A	3.60 A	1.33 A	1.17 A
5 mg			14.49 A	3.32 A	1.41 A	0.98 A
8 mg			13.84 A	3.30 A	1.36 A	0.92 A
0 mg (Control)			16.24 A	3.78 A	1.70 A	1.08 A

Mean with same superscripts in the same columns are not significantly different at ($P>0.05$).

The steroid hormones by nature result in fat catabolism. This study showed significant ($P<0.05$) decrease in fat gain in fish body with increasing dietary MT concentration (Table 3). Moderate level of MT (5mg/Kg ddiet) had neutral effect on fat gain of fish body comparedd with the control group but high level of MT (8mg/Kg diet) had negative effect, and low level of MT (2 mg/kg diet) had positive effect (Table 3). These results suggest that, fish may vary in their metabolism of fat to various dietary levels of MT. However, Killian and Kohler (1991) reported that, not all researches to-date on the effect of MT on body fat content in fish are in complete agreement.

Among the values of ash retention in the body of MT-treated fish, the group that received MT at 2mg/Kg diet was the only significantly greater than that of the control group. This increase in ash retention can be attributed to increased bone mineralization. The other groups of treated fish had equal or even lower ash gain than that of control group (Table 3). Increase in ash deposition in MT-treated fish body has been reported by Fagerlund *et al.* (1979), Ostrowski and Garling (1987).

In the present study, Viscero-somatic indices (VSI) of the MT -treated fish were slightly, but not significantly ($P>0.05$) higher than that of the control. The values of VSI increased with the increase of the dietrary MT levels (Table 3). The gonado-somatic indices (GSI) of the MT-treated fish were significantly ($P<0.05$) with the increase of the dietary MT levels. Killian and Kohler (1991) reported increase in VSI of MT-treated Red tilapia more than that of untreated fish. On the other hand, Lone and Matty (1980) reported decrease in VSI of MT-treatedcommon carp more than that of untreated fish. However, the increases in the VSI and GSI, as wellas the previously mentioned increase in retained moisture of MT-treated Nile tilapia may significantly lower the dress-out weight percentages.

After withdrawal of the MT-hormone from the diet, drop in weight gain (g/fish), FCR, PER, PPV and growth rate (g/day) of fish compared with the control were observed in all experimental groups (Table 2). The greater increases in feed efficiency parameters and growth of fish fed 2mg MT/Kg diet (best treatment in Phase1)have been reversed to greater declines after the hormone withdrawal from the diet (Table 2). These results agree with finding reported by Ostrowski and Garling (1987b), that rainbow trout lost all previous gain growth after MT was removed from the diet with reduction in relative daily gain being apparent within two to for weeks. Lone and Matty (1980) reported opposite results with common carp, that the adventageous gained through the use of MT were not lost. Therfore with-

drawal effect could be species specific with the results being acceptable for the culture of common carp, but are not so for Nile tilipia. The advantageous of better gains of protein, fat and ash in the fish group that received 2mg/Kg diet over the control during Phase2 (Table 3). Reasons for the negative effects at the withdrawal period are not well known. These reversal effects mayhave been resulted due to hormone withdrawal itself, or to carry-over effects of a prolonged hormone treatment period. However, long periods of anabolic steroid treatments have promoted hormone insensitivity and reduced growth in mammals (Kochakian, 1976).

When growthand nutritional parameters were calculated and averaged for tha entire 28 weeks experimental period, there were no observed adventageous in MT-treated fish over control group in the values of the final fish weight gains (g/fish), FCR, PER, PPV and nutrient's gains (of protein, fat, ash or moisture) in fish body (Tables 2 and 3). Indeed, the values of these growths and nutritional parameters of the control group were better than those of MT-treated fish. Therefore, 17α-methyltestosterone does not appear promising for use with Nile tilapia if treatment and withdrawal period similar to those used in this study are employed. Also, MT is relatively expensive compound, thus, its use in practical Nile tilapia feeding is not encouraging at the present time.

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دراسة النمو ومعدلات الأستفادة من الغذاء فى أسماك البلطى النيلى عند التغذية بعلائق تحتوى على معدلات مختلفه من هرمون ١٧ - ألفا ميثيل - تيستوستيرون ثم أعادة التغذية بعليقة خالية من الهرمون

على عز الدين عبد الغنى

المعمل المركزى لبحوث الثروة السمكية - العباسة - مركز البحوث الزراعية - مصر.

تم تغذية أسماك البلطى النيلى بعلائق متماثلة تماما فى جميع محتوياتها ولكنها تختلف فقط فى تركيزات هرمون ١٧ الف ميثيل تيستوستيرون بها ، حيث كانت التركيزات كالاتى : صفر - ٢- ٥ - ٨ ملليجرام هرمون / كجم علف وأستمرت فترة التغذية مدة ٢٠ أسبوعا (وهى المرحلة الأولى) تم بعدها تغذية جميع الأسماك فى المعاملات المختلفة على العليقة الضابطة التى لا تحتوى على هرمون (صفر هرمون / كجم علف) مدة ٨ أسابيع أخرى (وهى المرحلة الثانية). وجد فى المرحلة الأولى أن تأثير الهرمون على الأسماك كان إيجابيا حيث زادت معدلات النمو والأستفادة من الغذاء فى الاسماك التى تناولت الهرمون بالمقارنة بالأسماك التى تناولت عليقة خالية من الهرمون ، كذلك أيضا اكتسب جسم الأسمالك التى تناولت الهرمون فى غذائها زيادة فى أوزان البروتين - الدهون - الرماد - الرطوبة بالمقارنه بالاسماك التى تناولت عليقة خالية من الهرمون ، وبالتالي كان الهرمون سببا في انتاج أسماك ذات قيمة غذائية عالية بالمقارنة بالأسماك التى لم تتناول الهرمون في غذائها. تلاحظ أيضا إن الاسماك التى تناولت الهرمون فى غذائها تتصف بزيادة فى وزن الأحشاء والغدد التناسلية بالمقارنة بالأسماك التى تتناول الهرمون.

فى المرحلة الثانية التى تم تغذية جميع الأسماك على عليقة خالية من الهرمون : أدى ذلك الى أن الأسماك فقدت المميزات التى أكتسبتها فى المرحلة الأولى سواء من حيث النمو فى الوزن أو الزيادة المكتسبة فى أجسامها من البروتين - الدهون - الرماد - الرطوبة أو من حيث زيادة معدلات الاستفادة من الغذاء ، ليس هذا فقط بل تفوقت الاسماك التى لم تتناول الهرمون فى الغذاء منذ البداية على نظائرها من الأسماك التى تناولت الهرمون ثم توقف تناولها للهرمون فيما بعد، وبالتالى يتضح أن أستخدام الهرمون فى علائق أسماك البلطى النيلى لتشجيعها على النمو لم يؤدى الغرض المستهدف منه.