جامعة الأزهر
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جامعة الأزهر
مركز الدراسات
والاستشارات الزراعية

المؤتمر الدولي
الثروة السمكية والأمن الغذائى في الدول العربية والإسلامية

في الفترة من ٢٢-٢٤ أكتوبر ٢٠٠٣م

دراسة النمو ومعدلات الاستفادة من الغذاء فى أسماك البلطى النيلى عند التغذية لفترة بعلائق تحتوى على معدلات مختلفة من هرمون ١٧ الفا ميثيل تيستوستيرون ثم إعادة التغذية بعلائق خالية من الهرمون

إعـــــــــــداد

على عز الدين عبدالغنى

المعمل المركزى لبحوث الثروة السمكية

العباسة- أبو حماد - الشرقية

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دراسة النمو ومعدلات الاستفادة من الغذاء فى أسماك البلطى النيلى عند التغذية لفترة بعلائق تحتوى على معدلات مختلفة من هرمون ١٧ الفا ميثيل تيستوستيرون ثم إعادة التغذية بعلائق خالية من الهرمون

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ملخص

تم تنفيذ هذه التجربة على مرحلتان مدتهما ثمانية وعشرون أسبوعا. فى المرحلة الاولى تم تغذية أسماك البلطى النيلى بعلائق متماثلة تماما فى جميع محتوياتها ولكنها تختلف فقط فى تركيزات هرمون ١٧ الفا ميثيل تيستوستيرون المضاف اليها، حيث كانت التركيزات كالاتى: صفر – ٢ – ٥– ٨ ملليجرام هرمون/كجم علف، واستمرت فترة التغذية مدة ٢٠ إسبوعا فى نهايتها بدات المرحلة الثانية حيث تغذت فيها الاسماك مدة ثمانية أسابيع على عليقة الكنترول الغير مضاف لها الهرمون، وكانت النتائج كالآتى:

كان تاثير التغذية بالاعلاف المضاف لها الهرمون خلال المرحلة الاولى إيجابيا حيث تفوقت الاسماك فى معدلات النمو والاستفادة من الغذاء والوزن المكتسب من البروتين والدهن والرماد بالمقارنة بالاسماك التى تغذت على العليقة الضابطة (الكنترول) الخالية من الهرمون.

أدى التوقف عن التغذية بالاعلاف المضاف لها الهرمون خلال المرحلة الثانية ان فقدت الاسماك المميزات التى سبق لها إكتسابها فى المرحلة الاولى وتفوقت بها على الاسماك التى تغذت على العليقة الضابطة الخالية من الهرمون مثل معدلات النمو والاستفادة من الغذاء والوزن المكتسب من البروتين والدهن والرماد.

ليس هذا فقط بل حدث ان تفوقت الاسماك التى لم تتناول الهرمون فى غذائها من بداية التجربة (أسماك معاملة الكنترول) على الاسماك التى غذيت بالهرمون لفترة ثم منع عنها بعد ذلك.

وبالتالى يتضح ان إستخدام الهرمون فى علائق أسماك البلطى النيلى لم يؤدى الى الوصل الى النتائج المستهدفة منه.

Effects of Feeding 17 α– Methyltestosterone and Withdrawal on Feed Utilization and Growth of Nile Tilapia Oreochromis niloticus L., Fingerlings

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ABSTRACT

Fingerling Nile tilapia, Oreochromis niloticus, were fed diets containing 17 α-methyltestosterone (MT) at a doses of 0 (control), 2, 5, or 8 mg MT / kg diet for 20 weeks (Phase I), followed by an 8- week withdrawal period (Phase-II). At the end of Phase I, fish receiving 2 mg MT /kg diet exhibited significantly higher ($p < 0.05$) weight gain than fish fed the control diet. Likewise, food conversion ratio (FCR), protein efficiency ratio (PER), and productive protein value (PPV) of fish fed 2 mg MT / kg diet were significantly better than those of control fish (2.82 versus 3.40, 1.14 versus 0.94, and 18.15 versus 14.98, respectively). The anabolic property of the dietary steroids became more visible after 10 weeks. Increasing the dietary concentration of MT above 2 mg / kg diet reduced fish growth and feed utilization efficiency, but not below that of control fed fish.

The results of the present study indicated an increase in moisture, protein, fat and ash deposition in fish body, with the decrease of MT levels in the diet. Fish receiving 2 mg MT / kg diet had greater retention values (by 47.7%, 44.9%, 20.8% and 35.1%)than did the control group for moisture, protein, fat, and ash, respectively. Hormone withdrawal reduced fish growth and feed efficiency values to below the level of control in all the treatments, and greater declines ($p < 0.05$) occurred for fish fed the diet containing 2 mg MT / kg. Hormone withdrawal negated MT stimulated increases in moisture protein, fat, and ash retention in fish body that occurred in Phase I. Results demonstrated that incorporation of MT into diets of Nile tilapia fingerling offers little potential for improving either growth or food utilization efficiency after hormone withdrawal 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 directed towards 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 important implications for the fish culturist. It is known that the aquacultural industries are handicapped by the steadily increasing cost of feedstuffs. Growth promoting hormones are a means to increase the efficiency of feeds so that fish can be raised to desired size in shorter time. In this context, using hormone supplemented diets may improve the economics of operation and the utilization of facilities.

The synthetic steroid hormone, 17 α-methyltestosterone (MT) possess enhanced anabolic activity, while the androgenic effect is suppressed. It was postulated that the presence of the 17 α-methyl group enhanced efficacy of the synthetic steroid over endrogenous steroids (Donaldson et al. 1979). 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. Increases in weights have been reported for salmonids fed MT (Higgs et al. 1982; Schreck and Folwer 1982; Ostrowski and Garling 1988), as well as for blue tilapia, *O. aureaus*; common carp, *C. carpio*, and American eel, *Anguilla rostrata* (Guerrero 1975; Lone and Matty 1980; Degani and Gallagher 1985 respectively). Reduced growth rates have been reported for European eel, *Anguilla anguilla* (Degani and Gallagher 1985) and channel catfish, *Ictalurus punctatus* , (Simone 1990; Gannam and Lovell 1991b). These results suggest that fish species may vary in their metabolism of MT or in their response to various dietary levels of hormone.

To insure 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 incorporation of these agents into fish feeds.

Nile tilapia, *Oreochromis niloticus*, is the most common cichlid species in the inland fisheries of Egypt (Ishak et al 1979). Though Nile tilapia have rapid growth and are able to efficiently use artificial foods, further improvements in diet efficiency may be important to increase the

economy of 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, 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 for 28 weeks divided into an initial 20 weeks of hormone treatment (Phase-I) and a subsequent 8 weeks of hormone withdrawal (Phase-II). Fish receiving the hormone supplemented diets were immediately switched to their respective control diets upon completion of the 20 week hormone treatment (Phase-I). Control fish were fed hormone free diet for the entire 28 weeks.

Fish and Culture Techniques

Nile tilapia 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 indoors and placed in a fiberglass tank where they were fed a standard diet for two weeks as an acclimation period for the laboratory conditions. The fish were then divided into 12 groups, each containing 30 fish (average weight 3.06 ± 0.5 g). Each group of fish was transferred at random into a 40 L glass aquarium. Dechlorinated tap water was used throughout the study. In order to avoid accumulation of the metabolites, the water of the aquarium was changed daily. Each aquarium was also supplied with air produced by a central compressor. Water temperature and dissolved oxygen were recorded daily using Model PENN-PLAX thermometer and Model YSI-55 oxygen meter, respectively. The mean values for the entire period of the study were 26.8 ° C and 6.4 mg/L respectively. The photoperiod was set on a 12 hour light-dark cycles using fluorescent tubes as the light source.

Diet Preparation and Feeding Regime

The diet contained 30% protein and its manufacturer composition determined according to the Standard methods of AOAC (1980) is shown in Table I. The ingredients were blended in a mixer and divided into four equal portions. Four 200 mL 95% ethanolic solutions of MT were prepared to provide different hormonal concentration of 0, 2, 5, and 8 mg MT / kg of diet. Each of the MT solutions was added to one of the four equal portions of the basal diet. Each portion was separately remixed and extruded through a meat mincer. The diets were air-dried for 24 hour, broken into pellets and stored at - 20 C in sealed polyethylene bags.

Each of the four diets was offered to triplicate groups of fish. Fish in each aquarium were fed at 5% of their body weight. The fish were fed twice a day, six days a week. The rations were adjusted every two weeks according to the new weights of fish so that feed intake as percentage of fish weights was identical for the groups receiving these rations. Feeding of the hormone supplemented diets commenced on 7 June and was continued for 20 weeks. After this period all fish groups were immediately placed on control diet (0 mg MT / kg) for an additional 8 weeks (Phase II). During Phase-II, the number of fish was reduced to 21 in each aquarium to accommodate the increasing biomass. This reduction process was conducted at random. All other conditions remained unchanged.

Chemical Analysis of Fish and Diet

At the beginning of the experiment, 10 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. All samples were kept in labeled plastic bags and frozen at - 20 ° C until proximate analysis. The fish samples were analyzed 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 sub samples were taken for the chemical analysis of protein, lipids, ash and moisture contents.

To process the data, the food conversion ratio (FCR), protein efficiency ratio (PER), and productive protein value (PPV) were calculated according to the equations: FCR = food intake [dry weight (g)] / body weight gain [wet weight (g)]; PER = [gain in weight (g) / protein intake in food (g)]; and PPV = [protein gain in fish (g) / protein intake in food (g)].

Statistical Analysis

A one-way analysis of variance was used to determine if there were differences in growth and feed utilization efficiency 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 the obtained means.

RESULTS AND DISCUSSION

The present study demonstrated that the steroid MT was effective as a growth accelerator for Nile tilapia during the hormone treatment period of 20 weeks. The growth was more pronounced with lower doses than with higher doses of the steroid and the maximum growth effect was seen

in fish receiving 2 mg MT / kg feed (Table 2). This result generally agrees with studies with channel catfish where fish fed a high level of MT had less weight gain than those fed low level of MT (Gannam and Lovell 1991a, 1991b). Previous studies showed that MT induced growth acceleration in fry stages of blue tilapia (Guerrero 1975) and Nile tilapia (Ufodike and Madu 1986).

At the end of Phase-I 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. The group receiving 2 mg 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 indicate that all MT-treated fish had higher ($P < 0.05$) moisture and protein gains than that of the control group (Table 3). In this study, protein gain of the MT-treated groups was significantly ($P < 0.05$) greater than untreated fish showing that the anabolic hormone has the effect of increasing nitrogen (protein) retention in the body. Similar results have been reported in other fish (Kruskenper 1968; Fagerlund et al, 1979 Ufodike and Madu 1986; Ostrowski and Garling 1987). It is not clear at the moment, however, if this increase in protein retention by MT-treated fish is due to active synthesis or decreased catabolism. Further studies on the effect of anabolic steroids on fish are required.

Among the values of ash retention in the body of MT-treated fish, the group receiving MT at 2 mg / kg diet was the only one significantly greater than 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 has been reported previously (Fagerlund et al. 1979 ; Ostrowski and Garling 1987).

After withdrawal of the hormone from the diet, decreases 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 2 mg MT / kg diet (best treatment in Phase-I) were reversed to greater declines when the hormone was withdrawn from the diet (Table 2). These results agree with Ostrowski and Garling (1987) study that states that rainbow trout lost all previous gain in growth with reduction in relative daily gain being apparent within two to four weeks after MT was removed from the diet. Lone and Matty (1980) reported the opposite results with common carp, i.e., that the advantages gained through the use of MT were not lost. Therefore, the withdrawal effect could be species

specific with the results being acceptable for the culture of common carp but not for Nile tilapia.

The advantages of better gains of protein, fat and ash in the fish group receiving 2 mg / kg diet over the control during Phase-I were reversed in MT withdrawal from the diet during Phase-II. Reasons for the negative effects at the withdrawal period are not well known. These reversal effects may have resulted from hormone withdrawal itself or be due to carry over effects of a prolonged hormone treatment period. Long periods of anabolic steroid treatments, however, have promoted hormone insensitivity and reduced growth in mammals (Kochakian 1976).

When growth and nutritional parameters were calculated and averaged for the entire 28 weeks experimental period, there were no differences at all in MT-treatments over the control group in the values of the final fish weight gains (g/fish), FCR, PER, PPV and nutrient gains (protein, fat, ash or moisture) in fish carcass. Indeed, the values of these growth and nutritional parameters of the control group were better than that of MT-treated fish. Therefore, 17α-methyltestosterone does not appear promising for use with Nile tilapia if treatment and withdrawal periods similar to those used in this study are employed. Also, MT is a relatively expensive compound and thus, its use in practical Nile tilapia feeding is not encouraged at the present time.

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Table 1. Composition (%) and proximate analy. f the basal diet

Ingredients	%
Fish meal	32.75
Soybean meal dehulled	32.75
Rice bran	22.50
Corn oil	5.00
Starch	3.00
Vitamin mix[1]	2.00
Mineral mix[2]	2.00
Chemical analysis	
Protein (%)[3]	34.50
Lipid (%)[3]	7.10
Ash (%)[3]	14.30
Gross energy (kcal/g diet)	4.43

1- Vitamin mixture contained (as g / kg of premix): thiamine, 2.5; riboflvin, 2.5; pyridoxine, 2.0; inositol, 100.0; biotin, 0.3; pantothenic acid, 100.0; folic acid, 0.75; para-aminobenzoic acid, 2.5; choline, 200.0; nicotinic acid, 10.0; cyanocobalamine, 0.005; α-tocopherol acetate, 20.1; ascorbic acid, 100.0; menadione, 2.0; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU. It was prepared according to (Jauncey and Ross, 1982).

2- Mineral mix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7$ $3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03.

3- As dry matter basis.

Table 2. Effects of feeding various levels of 17 α- methyltestosterone (MT) and withdrawal on growth and food utilization efficiency of Nile tilapia

MT mg/kg diet	Weight gain (g / fish)	Growth rate (g / day)	FCR	FER	PPV
Hormone treatment (Phase-I)					
0 (Control)	13.43 c	0.0959 b	3.40 a	0.94 b	14.98 b
2	19.41 a	0.1386 a	2.82 b	1.14 a	18.15 a
5	16.23 b	0.1159 ab	3.14 ab	1.02 ab	17.56 ab
8	14.95 bc	0.1068 b	3.37 a	0.96 b	15.78 b
Hormone treatment (phase-II)					
0 (Control)	9.36 a	0.167 a	3.02 d	0.96 a	16.79 a
2	2.55 c	0.046 c	12.66 a	0.23 c	5.20 c
5	3.91 b	0.070 b	7.21 b	0.40 b	5.48 c
8	4.51 b	0.081 b	5.88 c	0.49 b	9.06 b
Phase-I & II (Combined)					
0 (Control)	23.00 a	0.117 a	3.21 a	0.957 a	15.73 a
2	21.93 ab	0.112 ab	3.96 a	0.777 a	12.76 b
5	20.05 ab	0.102 ab	3.95 a	0.782 a	12.94 b
8	19.49 b	0.099 b	3.93 a	0.786 a	13.30 b

Means with same superscripts in the same columns are not significantly different at ($P > 0.05$). FCR = Food conversion ratio; PER = Protein efficiency ratio; PPV= Productive protein utilization.

Table 3. Effects of feeding various levels of 17 α– ethyltestosterone (MT) and withdrawal
of the hormone on nutrient gains (g/fish) in body of Nile tilapia.

MT mg/kg diet	Moisture	Protein	Lipid	Ash
Hormone treatment (Phase-I)				
0 (Control)	9.49 c	2.14 d	1.20 b	0.57 b
2	14.02 a	3.10 a	1.45 a	0.77 a
5	11.52 b	2.79 b	1.23 b	0.56 b
8	10.93 b	2.47 c	1.06 c	0.45 c
Hormone treatment (phase-II)				
0 (Control)	6.75 a	1.64 a	0.50 a	0.51 a
2	1.70 b	0.58 b	- 0.1 c	0.40 a
5	2.97 b	0.53 b	0.18 b	0.32 a
8	2.91 b	0.83 b	0.30 b	0.47 a
Phase-I & II (Combined)				
0 (Control)	16.24 a	3.78 a	1.70 a	1.08 a
2	15.72 a	3.60 a	1.33 a	1.17 a
5	14.49 a	3.32 a	1.41 a	0.98 a
8	13.83 a	3.30 a	1.36 a	0.92 a

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