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The Effects of Combined Phytoestrogen Administration on Growth Performance, Sex Differentiation and Body Composition of Sharptooth Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract

The present study was conducted to investigate the effects of Genesis (G, a commercial mixture of phytoestrogens) on growth, body composition and sex differentiation of Sharptooth catfish *Clarias gariepinus*. Genesis is a commercially available product on USA and European markets used for menopausal complaints as an alternative medicine. Different concentrations (0 mg G 30 L^{-1}, 210 mg G 30 L^{-1}, 420 mg G 30 L^{-1}, 630 mg G 30 L^{-1}, 750 mg G 30 L^{-1} and 1500 mg G 30 L^{-1}) of Genesis were administered by immersion of newly hatched larvae (averaging 2.27±0.12 mg) every 3 days for 30 days then immersion treatments were ceased and all groups reared in the same condition for the later 90 days, a total 120 days experimental period. At the end of the trial, specific growth rate of the females increased with the increasing concentration of Genesis up to 420 mg 30 L^{-1} ($P<0.05$), but different concentrations of Genesis did not affect the growth performance of males ($P>0.05$). The highest value of protein content (21.60%) was observed from the 420 mg G 30 L^{-1} group and found significantly different from the control and other groups ($P<0.05$). Also, lipid contents were significantly affected by the Genesis concentrations and the highest lipid contents were detected in the 210 mg G 30 L^{-1} (5.98%) and the 630 mg G 30 L^{-1} (6.66%) groups. Although among the dosages used in the present study, 1500 mg G 30 L^{-1} was the most effective dosage that ensured maximum female ratio (69.77%), the sex ratios observed for 0, 210, and 420 mg G 30 L^{-1} treatment groups were nearly the expected ratio of 1:1 (male: female). Therefore, it was concluded that usage of higher doses and treatment durations of Genesis could be more effective for all-female production of the Sharptooth catfish population.

Keywords: *Clarias gariepinus*, genesis, immersion, sex determination, growth.

Introduction

Generally, the synthetic hormones are administered for only 30-45 days period, from the fish larvae hatched or take their first feed and hence hormonal residues will have disappeared from the flesh of the fish long before they are harvested. In fact, the levels of two hormones, estradiol-17β and 17α-methyltestosterone, which are the most commonly used for sex differentiation or/and reversal, are unmeasurable quantities (Stepherd and Bromage, 1995). Although the administered synthetic steroids are eliminated from the fish in a short time period and have no harmful effect to fish, it sometimes generates some problems in marketing and health public concern (Tave, 1992). Feminization of catfish can be produced by direct synthetic hormonal treatment that is efficient and straightforward (Liu *et al*., 1996). However, synthetic hormones are more expensive than plant extracts and their administration in fish is time-consuming, labor-intensive and requires specialist expertise. Furthermore, the synthetic hormones have been reported to have the potential to accumulate in the sediment water and aquatic biota (Contreras-Sanchez *et al.*, 2001; Çek *et al.*, 2004). These factors have forced the investigators to find new alternative applications, which are being shown to be both effective and safe (Çek *et al.*, 2007a; Çek *et al.*, 2007b). Phyto-pharmacology has therefore been encouraged to support the production of new and safe phytochemicals with minimal undesired toxic effects (Adimoelja, 2000). Nowadays, in response to modern sophisticated artificial chemical medicine, there has been growing interest on medicinal plants to apply in fish culture as well as human therapy (Lotke, 1998). Phytoestrogens have also been used to promote growth in poultry (Jurani *et al.*, 1987) and fish (Turan and Akyurt, 2005). The positive effects of the phytoestrogens on growth in poultry and fish attracted to research into possible benefits in fish culture.

Genesis is a commercially available product on USA and European markets used for menopausal complaints as an alternative medicine (hormone replacement therapy in women). It contains naturally occurring mixture of plant substances (soy concentrate, wild yam, vitex, dong quai, black cohosh, licorice root, gentian root) that are recognized to have varying degrees of estrogenic activity. There have been growing bodies of literature about these compounds (Gambacciani *et al.*, 1994; Lampe *et al.*, 1994). Sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) is widely distributed in the southern part of Turkey where it has high commercial importance. To our knowledge, there has been no previous work conducted to evaluate the effects of the different levels of Genesis (a commercial mixture of

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the phytoestrogens) on growth, sex differentiation and body composition of Sharptooth catfish, *Clarias gariepinus*. Therefore, the objective of the present study was to investigate the possible effects of Genesis on growth, sex differentiation and body composition in Sharptooth catfish as an alternative technique.

Materials and Methods

Immersion Treatments

Immersion technique was used as an alternative to the more common, oral/diet application method because Genesis is a soluble product in water. Immersion method also ensures synergic induction, cheaper than dietary treatment and requires almost no skill (Pandian and Sheela, 1995). In diet supplementation technique, hormone suffers degradation in digestive tract. Its purity and also its solubility varies with the solvent used: uniformity of its distribution in feed may vary. Size hierarchy may lead to differential feed uptake and hence hormone intake (Pandian and Sheela, 1995).

The ingredients of Genesis (a phytoestrogen mixture, Life Time Nutritional Specialist Inc. USA), obtained from Çagdas Medical Ltd. Izmir-Turkey, are given in Table 1. The aqueous extracts of Genesis was prepared by boiling fine extract of Genesis in 1500 ml distilled water for 30 minutes, filtered with a whatman filter paper twice (Kavumpurath and Pandian, 1993; Gauthaman and Adaikan, 2005) and added to the aquaria medium. The fish remained in the solution for three days, and then the water of the aquaria was changed entirely every 3 days for 30 days. This solution was not a stock but was freshly prepared 10 times every 3 days during the immersion period. Then immersion treatments were ceased and larvae in all groups reared in the same condition for the later 90 days, with a total 120 days experimental period.

Fish Larvae, Laboratory Conditions and Experimental Design

The newly hatched Sharptooth catfish larvae (averaging 2.27±0.12 mg) were randomly removed from the hatching tank, counted, measured and placed in 18 glass aquaria, each containing 30 L of water, which was continuously aerated with a 4 cm length air stone. The aquarium system was static bath with changing water manually. The photoperiod was maintained on a 12-h light: 12-h dark schedule and controlled temperature (25±1ºC). Each aquarium comprised 100 larvae and a total 1800 larvae were used for the experiment. Larvae were randomly assigned to six different treatment groups that each received one of the six immersion doses of Genesis tested: 0 (control), 210, 420, 630, 750 and 1500 mg G 30 L^{-1}. The six treatments, each consisted of three replicates, were done simultaneously (full randomized experimental design). The larvae were fed five times a day with freshly hatched *Artemia salina* (Subreme Bay Brand INC., San Francisco, USA) and live tubifex for the first five days after yolk absorption then weaned to commercial trout starter (58% crude protein, CP) and pelleted diets (43%, CP) (Skretting Trouw, France). Diet diameters were increased with the increasing mouth opening of fish.

Sampling Procedure Chemical and Statistical Analysis

At the end of the experiment, all fish (120 days old) were anaesthetized with 5 mg L^{-1} quinaldine sulphate (Sigma Chemical Company, Germany) (Yanar and Genç, 2004) and stored at -20ºC for determination of mean final weight, specific growth rate by Clark *et al*. (1990), sex ratio, gonadosomatic and hepatosomatic index calculations described by Çek *et al.* (2001).

Standard methods were used to determine the final body proximate composition (AOAC, 1990) as follows: moisture was determined by oven-drying at 105ºC for 24 h, crude protein (N x 6.25) by the Kjeldahl method and crude ash by combustion in a muffle furnace at 550ºC for 16 h. Total lipid concentration was determined by extract with chloroform-methanol method described by Bligh and Dyer (1959). Secondary sexual characteristics (especially genital papilla) were used to distinguish males from females. Also, the structure of the testis and ovaries were observed by naked eye and examined under light microscopy (Olympus BX 50).

Final fish weight, specific growth rates, carcass

Table1. Components of the phytoestrogens (Genesis) used (mg) per 30 liters water by immersion technique during 30 days

	Concentration (mg 30 L^{-1})				
Ingredient	210	420	630	750	1500
Soy total isoflavones	11.60	23.20	34.80	41.41	82.82
Wild yam	51.53	103.06	154.59	184.05	368.10
Vitex	38.65	77.30	115.95	138.04	276.08
Dong quai	38.65	77.30	115.95	138.04	276.08
Black cohosh	30.92	61.84	92.76	110.43	220.86
Licorice root	25.77	51.54	77.31	92.02	184.04
Gentian root	12.88	25.76	38.64	46.01	92.02

compositions (moisture, crude protein, crude lipid and crude ash), gonadosomatic and hepatosomatic index values were all subjected to one-way analysis of variance to detect if significant differences occurred among experimental groups. Data were statistically analyzed with one-way ANOVA and Duncan's multiple range tests and expressed as mean values ± SEM. In addition, Chi-Square (χ^2) test was performed to determine the differences between sex ratios of the experimental groups (Zar, 1984). Effects with a probability of $P<0.05$ were considered significant. Statistical analyses were performed using SPSS for Windows (Standard Version 9.0 SPSS Inc. Chicago, Illinois).

Results

Effects of Genesis on the Growth, Sex Ratio and Some Body Indices of *C. gariepinus*

The effects of different immersion concentrations of Genesis on growth, sex ratio and some body indices of Sharptooth catfish for 120 days are shown in Table 2. At the end of the experiment, specific growth rate of the females increased with increasing rates of Genesis up to 420 mg G 30 L^{-1} concentration level ($P<0.05$), but different concentrations of Genesis did not affect the growth performance of males ($P>0.05$). The highest value of specific growth rates (SGR) for male and females were 8.36 and 8.17 at 210 mg G 30 L^{-1} and 420 mg G 30 L^{-1}, respectively. The sex ratios observed for 0, 210, and 420 mg G 30 L^{-1} treatment groups were nearly expected ratio of 1:1 (male: female). The sex ratio determined in fish taken from the 630, 750 and 1500 mg G 30 L^{-1} groups were 43.97:56.03, 38.46:61.54, 30.23:69.77 (male: female) respectively, but these ratios were not significant ($P>0.001$). In the present study, no inter-sex fish were recorded.

There were highly significant overall differences between groups at gonadosomatic index values for males and females ($P<0.05$). The highest gonadosomatic index values for males and females were detected from 210 mg G 30 L^{-1} and 630 mg G 30 L^{-1} groups as 0.049% and 0.350%, respectively. Gonadosomatic index values of females treated with different levels of Genesis were higher than that of the control group. Moreover, hepatosomatic index values increased with the increasing concentrations of Genesis and affected by the immersion technique applied ($P<0.05$).

Effect of Genesis on the Body Composition

Moisture contents of the fish treated with 420 and 630 mg G 30 L^{-1} decreased significantly and all the other groups were found similar except from these groups (420 and 630 mg G 30 L^{-1}). The highest value of protein content (21.60%) was detected from the 420 mg G 30 L^{-1} group and found significantly different from the control and other groups ($P<0.05$), while there were no significant differences between protein contents of four dosage (210, 630, 750, and 1500 mg G 30 L^{-1}) and control groups ($P>0.05$). Also, lipid contents were significantly affected by the Genesis concentrations and the highest lipid contents were detected in the 210 mg G 30 L^{-1} (5.98%) and the 630 mg G 30 L^{-1} (6.66%) groups. On the contrary, ash contents of all groups did not change significantly (Table 3).

Table 2. The effects of different concentrations of Genesis (mg 30 L^{-1}) on final body weight, specific growth rate, sex ratio and some body indices of the African catfish, *Clarias gariepinus* for 120 days*

Body weight (g $fish^{-1}$) and sex ratio (%)				Body indices (%)	
G (mg 30 L^{-1})	FW ♂	SGR ♂	SR ♂ : ♀	GSI ♂	HIS ♂
0	57.85±3.75 ^{a}	8.33±0.05^{a}	47.46: 52.54	0.036±0.003^{ab}	0.69±0.02^{a}
210	61.98±4.83 ^{a}	8.36±0.06 ^{a}	48.51: 51.49	0.049±0.008^{b}	0.87±0.02^{b}
420	48.82±3.64 ^{a}	8.19±0.05 ^{a}	47.49: 52.51	0.035±0.004^{ab}	0.95±0.02^{c}
630	50.19±4.23 ^{a}	8.18±0.07 ^{a}	43.97: 56.03	0.024±0.002^{a}	0.91±0.03^{bc}
750	55.55±4.28 ^{a}	8.30±0.06 ^{a}	38.46: 61.54	0.026±0.002^{a}	0.96±0.03^{c}
1500	52.92±6.89 ^{a}	8.23±0.15 ^{a}	30.23: 69.77	0.040±0.017^{ab}	0.97±0.05^{c}
G (mg 30 L^{-1}) ♀					
0	42.87±2.44^{abc}	8.09±0.04^{a}		0.280±0.009^{a}	0.69±0.02^{a}
210	45.29±3.87^{bc}	8.13±0.06^{a}		0.330±0.013^{c}	0.81±0.03^{b}
420	48.03±3.41^{c}	8.17±0.05^{a}		0.310±0.009^{abc}	0.90±0.02^{c}
630	38.11±2.99^{abc}	7.91±0.06^{b}		0.350±0.011^{c}	0.87±0.02^{bc}
750	33.88±2.42^{a}	7.84±0.05^{b}		0.290±0.012^{ab}	0.91±0.03^{c}
1500	35.32±3.69^{ab}	7.89±0.09^{b}		0.320±0.014^{bc}	0.92±0.04^{c}

* Values (mean ± standard error of triplicate) superscripted by different alphabets within the same column are significantly different ($P< 0.05$)
Initial live mean length and weight of the larvae were 6.14±0.11 mm and 2.27±0.12 mg, respectively
SGR (specific growth rate; %)= [(lnW2-lnW1)/(T2-T1)]x100, where W1 and W2 are mean body weight at times when the first and second samples were taken (T2-T1)
GSI (gonadosomatic index, %) = (gonad weight/body weight) x 100
HSI (hepatosomatic index, %) = (liver weight/body weight) x 100
G, Genesis; FW, final weight; SR, sex ratio

Table 3. The effects of different concentrations of Genesis (mg 30 L^{-1}) on the muscle tissue composition of the African catfish, *Clarias gariepinus* for 120 days* (wet basis)

G (mg 30L^{-1})	Moisture	Crude protein	Crude lipid	Crude ash
0	77.03±0.36^{a}	16.57±0.36^{a}	4.93±0.27^{a}	1.47±0.06^{a}
210	75.61±0.54^{ab}	17.15±0.54^{a}	5.98±0.08^{d}	1.26±0.27^{a}
420	73.15±1.86^{c}	21.60±1.86^{b}	3.95±0.03^{bc}	1.30±0.04^{a}
630	74.45±0.67^{bc}	17.42±0.67^{a}	6.66±0.19^{d}	1.47±0.09^{a}
750	75.21±0.48^{abc}	18.77±0.48^{a}	4.54±0.37^{ab}	1.48±0.03^{a}
1500	76.93±0.47^{a}	18.13±0.47^{a}	3.35±0.28^{c}	1.59±0.06^{a}

* Values (mean ± standard error of triplicate) superscripted by different alphabets within the same column are significantly different ($P< 0.05$)
Body composition data were presented on a wet basis
G, Genesis

Discussion

The goal of the present study was to find environmentally friendly, easily obtainable and more effective feminization method for fish culture by using the Sharptooth catfish, *Clarias gariepinus*, as a model species. Genesis treatment is a better method than synthetic hormonal treatment and is environmentally friendly. Since synthetic hormones and hormone metabolites persistence and their fate in fish, water and sediment will provide information on the potential risks of using hormonal sex control technology (Contreras-Sanchez *et al.*, 2001). Fish offered to the consumer will not be treated with synthetic hormones and producers may have an alternative method for producing of monosex populations based on natural products. The use of Genesis (a commercial combined phytoestrogen mixture) as an alternative method to produce all-female populations of Sharptooth catfish may address environmental safety issues. It can be applied with ease to a large number of individuals simultaneously.

The potential of improving fish growth by natural and synthetic steroid treatments were examined in several fish species such as *Oncorhynchus mykiss*, *Cyprinus carpio*, *Oreochromis niloticus, O. aureus* and *Perca flavescens* (Pandian and Sheela, 1995; Malison *et al.*, 1988). Park *et al.* (2003) reported that tamoxifen-incorporated feed to access the relative growth promoting efficiency on bagrid catfish (*Pseudobagrus fulvidraco*) through pelleted diets, and a promoted growth rate to a significant level compared to control and the 50 ppm. concentration recorded a maximum growth rate. Furthermore, Turan and Akyurt (2005) determined significantly improved growth for Sharptooth catfish fingerlings receiving the 75 mg red clover (a phytoestrogen) kg^{-1} diet compared to control group. Moreover growth-promoting effects of *Tribulus terrestris* extract (a phytoandrogen) on Convict Cichlid (*Cichlisoma nigrofasciatum*) (Çek *et al.*, 2007a) and guppy (*Poecilia reticulate*) (Çek *et al.*, 2007b) were recorded. Our results indicated the increase in the Genesis concentrations enhanced weight gain up to a definite level (420 mg G 30 L^{-1}) for females, but after this level specific growth rates decreased significantly.

From a chemical composition point of view, Genesis application in lower and moderate concentrations (420 mg G 30 L^{-1} and, 210 and 630 mg G 30 L^{-1}, respectively) increased the level of protein and lipid in Sharptooth catfish. It was reported that oral administration of 75 mg red clover kg^{-1} diet increased both protein and lipid contents of the Sharptooth catfish fingerlings significantly (Turan and Akyurt, 2005). Similar findings were also reported on bagrid catfish (*Pseudobagrus fulvidraco*) in which tamoxifen increased the level of lipid and ash contents (Park *et al.*, 2003).

We found that genesis was effective, only at one dose 1500 mg G 30 L^{-1}, in producing female populations. However the treatment of newly-hatched progenies using higher doses of Genesis increased the percentage of females, Genesis application for tested concentrations by immersion technique was ineffective in producing 100% female population. Therefore, usage of higher doses and/or treatment durations of genesis may lead to the production of all-female Sharptooth catfish population. To our knowledge so far, our study documents the first reported investigation to evaluate genesis as a potent feminizing agent in Sharptooth catfish. Although the present research provides evidence that genesis treatment by immersion technique resulted in a high rate of feminization (~70%), whether this potency is caused by estrogen increase can not be deduced from the present study, as we did not measure plasma estrogen levels during the experiment.

We concluded that further studies to measure the amount of estrogen levels after Genesis application in Sharptooth catfish could provide more accurate evidence as regards to the effects of Genesis on sex differentiation. Also, further investigations on higher doses and/or treatment durations of Genesis should be conducted to determine the possible effects and to find the optimum concentration for producing all-female population of Sharptooth catfish and other cultivable fish species.

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