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Effect of dietary estradiol-17β on growth performance, body composition and blood indices in Stellate sturgeon, *Acipenser stellatus*

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ABSTRACT

This study was investigated the effects of dietary estradiol-17β (E2) on growth, body composition and blood indices in *Acipenser stellatus*. Fish (40.9 ± 1.1 g average initial weight; n = 60 per group) were fed with three different diets containing 0 (control), 25 and 50 mg kg^{-1} dietary estradiol contents to apparent satiation for seven months. The results suggested that growth rate were decreased as the E_2 level was increased. No significant difference was observed in condition factor among dietary treatments. The highest survival rate was observed in fish fed control diet, but was not significantly different among the treatments (P>0.05). Body composition did not show significant changes among dietary treatments. Number of white blood cells and red blood cells, hemoglobin and hematocrit values were significantly decreased as the E2 levels were raised (P<0.05). Plasma biochemical parameters including glucose, total protein, cholesterol, triglyceride, calcium and phosphorus levels were dose dependent with the lowest levels in control with the highest levels in fish treated with 50 mg E2. The results of the present study revealed growth suppression of dietary E2 and changes of blood indices with providing some basic information on the effect of estrogen hormone on physiology of sturgeon.

Keywords: *Estradiol, Blood indices, body composition, Growth, Stellate sturgeon, Acipenser stellatus*

INTRODUCTION

In general, the principle that dictates which type of hormone to be used is the need to supplement or replace the particular hormone type that is deficient in the animals to be treated. The estradiol-17β (E2) is one of the most important compounds which commonly used for anabolic purposes in food animals. Gender and maturity of an animal influence its growth rate and body composition. E2, on the other hand, may act by stimulation of the somatotropic axis to increase growth hormone (Degani, 1986). In theory, growth enhancement can be able to occur through improvement in appetite, digestion, absorption of metabolic re-arrangements leading to overall anabolism, and the contribution of each of these factors varies somewhat among different species and type of steroid used (Woo *et al.*, 1993).

E2 promoted growth in yellow perch *Perca flavescens* by stimulating appetite (Malison *et al.*, 1988). On the other hand, E2 has been proven to depress growth in many fish species (Johnstone *et al.*, 1978; Blázquez *et al.*, 1998). Akhundov and Fedorov (1994) examined the effect of estradiol dipropionate on ovarian development in Sterlet, *Acipenser ruthenus*. Juvenile paddlefish, *Polyodon spathula*, implanted with 17-methyltestosterone showed changes in sex ratio (Shelton and Mims 1998). Also, dietary E2 feminized all shortnose sturgeon, *Acipenser brevirostrum*, after a 9-month trial (Flynn & Benfey 2007).
Sturgeons are valuable species, which are currently highly endangered. The Caspian populations are under massive pressure from overfishing (including poaching) and loss of spawning sites and the stocks are declining very fast. The rearing of these

species has seen considerable progress during past two decades. Stellate sturgeon (*Acipense*r *stellatus*) is one of the Caspian sturgeon species with relatively suitable growth rates and appears to be very suitable for aquaculture (Falahatkar *et al*., 2014). Despite the economic importance of Stellate sturgeon, there is little information on using of E2 supplementation in fish diet and its effect on physiological functions. The present study, for the first time, assesses the effectiveness of E2 supplementation to growth performance, body composition and blood indices in Stellate sturgeon.

MATERIALS AND METHODS

Experimental fish

One hundred and eighty, five-month old, juvenile Stellate sturgeon reared at Dr. Yousefpour Fish Hatchery Center in Siahkal, Guilan, Iran, were used for this study. Twenty fish within average weight of 40.9 ± 1.1 g were randomly distributed into each of nine circular concrete tanks containing a water volume of 900 L and supplied with flow-through freshwater at 17 ± 0.5 L/min. During the experimental period, water was aerated continuously with compressed air delivered through an air stone in each tank. The fish were kept and reared under this condition for approximately seven months. Water parameters were recorded daily; average water temperature and dissolved oxygen concentrations were 18 ± 2 ^{o}C and 8.7 ± 1.5 mg/L.

Diet preparation

E2 (Sigma- Aldrich St. Louis, MO, USA) was dissolved in alcohol, sprayed on the feed, and then stored at 4 ^{o}C in a refrigerator. The experiments were divided into three group treatments with different concentrations of dietary E2: 0 (control treatment), 25, and 50 mg/kg (Lord et al., 2009). The fish was kept under natural photoperiod and fed 2-6 times a day depending on water temperature according to their satiation with a formulated diet (Biomar, France). Every day, each tank was partially cleaned of the fish feces and the water was partially changed (about 50%). Fish were weighed every three weeks intervals during the experimental period to monitor the growth performance.

Growth performance

Body weight (g) and total body length (cm) of individual fish were measured to the nearest of 1 g and 0.1 cm, respectively, every three weeks. The individual fish from each tank was anesthetized by 300 mg l^{-1} of clove powder and then weighted. Fish were deprived of food for 24 h before each weighing. At the end of the trial, specific growth rate (SGR), body weight increase (BWI), weight gain (WG), feed conversion ratio (FCR) and condition factor (CF) were calculated using the following formulas to compare growth performance among experimental groups (Biswas *et al.,* 2008):

SGR (% day^{-1}) = 100 × (ln final body weight - ln initial body weight) / time (days)
BWI (%) = 100 × [final body weight (g) - initial body weight (g) / initial body weight (g)]
WG (g) = final body weight (g) - initial body weight (g)
FCR= feed intake (g) / wet weight gain (g)
CF = 100 × [final body weight (g) / total body length $(cm)^3$]

Chemical analysis

At the end of the experiment, five fish were randomly sampled from each replicate and then pooled in plastic bags and stored at -20 ^{o}C for whole body composition analysis. The proximate body composition (moisture, crude protein, crude lipid, and ash) was determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1995). Protein was determined by estimating the Kjeldahl method (N × 6.25), moisture by heating at 105 ^{o}C to constant weight, ash by incinerating in a crucible at 600 ^{o}C and crude lipid was determined by using Soxhlet apparatus.

Blood sampling

At the end of the trial, five fish from each tank were quickly captured and sampled after being anesthetized. Then, approximately 2 mL of blood samples were collected from the caudal vasculature using a 5 mL heparinized syringe equipped with a 25 gauge needle. Blood samples were divided into two portions. Half of the blood was used for separating plasma and the remaining blood was used for hematological analysis. Plasma was

separated by centrifugation (1500 × g for 10 min) and stored at -70 °C until subsequent analysis. Hematocrit (Hct) value was determined by the standard microhematocrit method and expressed in percentage. Hemoglobin (Hb) concentration was determined using the cyanmethemoglobin method. The numbers of white blood cells (WBC), red blood cells (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Ranzani-Paiva *et al.* (2004). Plasma glucose was analyzed based on the colorimetric glucose oxidase-peroxidase reaction (Bayunova *et al.,* 2002). Plasma cholesterol and triglyceride levels were determined by the CHOD-PAP and GPO-PAP methods, respectively (Chatzifotis *et al.,* 2004), using commercial available kits (Pars Azmun, Karaj, Iran). Total protein levels were measured by the Biuret method (Ziest Chem Diagnostics, Tehran, Iran). Total plasma calcium was determined by the colorimetric method (Sigma-Aldrich procedure no. 587). Plasma phosphorus was measured using an endpoint colorimetric assay (Sigma-Aldrich procedure no. 360).

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) after confirmation of normality of data and homogeneity of variance based on Kolmogorov–Smirnov and Levene's tests, respectively. Significance of the differences between means was tested using Duncan's multiple range test ($P<0.05$). All assays were performed in triplicates and data are shown as mean ± standard deviation (SD) for each dietary group.

RESULTS

The final body weight of the groups fed 25 and 50 mg E2 kg^{-1} diet were significantly lower than those of control group ($P>0.05$). The FCR and SGR of the control fish were significantly higher ($P<0.05$) than those of all E2 treated groups (Table 1). There was no significant difference in survival rate and CF among the dietary treatments. Different concentrations of E2 in the diets significantly affected blood indices. Highest Hb and Hct were observed in control group compared to groups treated with E2 (Table 2). Levels of calcium, cholesterol, triglyceride, protein and phosphorus were significantly increased as the E2 levels were raised (Table 3). In all experimental groups, body composition including crude protein, crude lipid, moisture and ash content did not show significant differences (Table 4).

Table 1. Growth performance of Stellate sturgeon fed with different levels of E_2 in diet.

E2 levels (mg kg^{-1})	Initial body weight (g)	Final body weight (g)	FCR	SGR (%/day)	CF	Survival rate (%)
Control	40.9 ± 1.1	171.7 ± 6.5 ^{a}	1.15 ± 0.04 ^{b}	1.38 ± 0.02 ^{a}	0.24 ± 0.001	100
25	40.9 ± 1.1	119.4 ± 3 ^{b}	1.46 ± 0.07 ^{b}	1.2 ± 0.02 ^{b}	0.26 ± 0.002	95 ± 5
50	40.9 ± 1.1	80.6 ± 1.4 ^{c}	2.01 ± 0.18 ^{a}	1.03 ± 0.04 ^{c}	0.25 ± 0.007	81.7 ± 7.3

Different superscripts in columns show the significant differences between data ($P<0.05$).

Table 2. Blood indices in Stellate sturgeon fed with different levels of E_2 in diet.

Parameters	Control	25 mg kg^{-1}	50 mg kg^{-1}
RBC (×10^3 iL^{-1})	525.3 ± 43.7 ^{a}	435.8 ± 23.2 ^{a}	304.4 ± 22.6 ^{b}
WBC (×10^3 iL^{-1})	13.6 ± 0.86 ^{a}	12.8 ± 0.68 ^{a}	7.9 ± 0.78 ^{b}
Hemoglobin (mg dL^{-1})	6.55 ± 0.46 ^{a}	5.54 ± 0.28 ^{a}	3.8 ± 0.3 ^{b}
Hematocrit (%)	26.6 ± 1.7 ^{a}	21.1 ± 1 ^{a}	15.4 ± 1.2 ^{b}
MCV (fL)	517 ± 11	511 ± 8	538 ± 7
MCH (pg)	12.6 ± 0.22	12.7 ± 0.11	12.4 ± 0.21
MCHC (g dL^{-1})	24.5 ± 0.34	25 ± 0.34	24.8 ± 0.40
Lymphocyte (%)	77.7 ± 2.1	78.7 ± 2.2	79.1 ± 1.9
Monocyte (%)	1.9 ± 0.3	2.1 ± 0.3	1.9 ± 0.3
Neutrophil (%)	17.8 ± 1.3	17.5 ± 1.7	18.7 ± 1.7
Eosonophil (%)	2.6 ± 0.9	1.7 ± 0.5	1 ± 0.2

Different superscripts in columns show the significant differences between data ($P<0.05$).

Table 3. Blood biochemistry parameters in Stellate sturgeon fed with different levels of E_2 in diet.

E2 levels (mg kg^{-1})	Calcium (mg dL^{-1})	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})	Protein (g dL^{-1})	Phosphorus (mg dL^{-1})
Control	6.8 ± 0.7 [c]	75.6 ± 3.7 [b]	59.2 ± 3.1[b]	526.7 ± 28.2 [c]	2.21 ± 0.04 [c]	15.6 ± 0.78 [c]
20	113.9 ± 5.5 [b]	97.3 ± 7.3 [a]	255.9 ± 12.4 [a]	1427 ± 111 [b]	12.64 ± 1.5 [b]	61.7 ± 4.6 [b]
50	136.9 ± 9.1 [a]	88.7 ± 8.5 [b]	298.1 ± 23.4 [a]	2070 ± 217 [a]	21.11 ± 2.6[a]	135.2 ±16.9 [a]

Different superscripts in columns show the significant differences between data (P<0.05).

Table 4. Whole body composition (%) of Stellate sturgeon with different levels of E_2 in diet.

E2 levels (mg kg^{-1})	Moisture	Crude protein	Crude lipid	Ash
Control	75.6 ± 0.4	14.62 ± 0.19	9.2 ± 0.4	2.91 ± 0.1
25	74.6 ± 0.2	15.05 ± 0.12	8.7 ± 0.5	2.57 ± 0.1
50	74.7± 0.4	14.45 ± 0.17	8.4 ± 0.4	2.54 ± 0.1

DISCUSSION

The results of the present study showed that E2 significantly increased the growth of Stellate sturgeon. Fish in E2 treated groups grew significantly slower than control fish during the period of treatment, indicating significant growth depression of E2, especially in the high dose of 50 mg E2. This result is consistent with most of previous reports. Funk *et al.,* (1973) in pink salmon *Onchorhynchus gorbuscha*, Johnstone *et al.* (1978) in rainbow trout *Onchorhynchus mykiss*, Blázquez *et al.* (1998) in European sea bass *Dicentrarchus labrax*, Woo *et al.* (1993) in red sea bream *Chrysophrys major*, Hendry et al. (2003) in Atlantic halibut, *Hippoglossus hippoglossus* and Flynn & Benfey, (2007) in shortness Sturgeon, *Acipenser brevirostrum* have reported E_2-related growth depression. Goetz *et al.,* (1979) also found a dose dependent decrease in the size of Coho salmon treated with high E_2 concentrations. However, estrogen enhanced growth in *Pleuronectes platessa* (Cowey *et al.* 1973), Coho salmon, *Oncorhynchus kisutch* (Yu *et al.,* 1979), yellow perch and Japanese eel *Anguilla japonica* (Satoh & Nimura, 1991). In European eels, *Anguilla anguilla* an increase of the concentration of E2 from 1 mg kg^{-1} to 15 mg kg^{-1} increased the growth of fish (Degani, 1986). These discrepancies in the growth effects caused by E2 may indicate difficulties in the method of hormone treatment in hormone dosage, duration of hormone treatment and age of the fish receiving the hormone.

The results of present study showed that diet supplemented with E2 resulted in significant changes in blood indices of Stellate sturgeon. These changes in WBC, RBC, Hb and Hct values were clearly observed in E2 treated groups. With increasing doses of hormone in the diet, WBC, RBC, Hb and Hct values were decreased. However, other blood indices were not significantly influenced by E2 treatment. The results of this study are in line with other studies which reported Hb and Hct values were significantly lower in lake trout *Salvelinus fontinalis* (Schafhauser-Smith and Benfey, 2003), chum salmon *Oncorhynchus keta* (Haney *et al.,* 1992), *Carassius auratus* (Wang & Belosevic, 1994) and *Cyprinus carpio* (Watanuki *et al.,* 2002) when E2 administrated in the diet.

In the present study, we found that values of blood biochemical parameters except glucose in E2 treated groups were considerably higher than control treatment. Levels of these parameters significantly increased as the E2 levels were raised. The results of this study are inconsistent with Mei-Ping *et al.* (2009) in Siberian sturgeon, *Acipenser baerii* fed with doses of 1 and 10 mg kg^{-1} E2 for 90 days. Chiba *et al.* (1993) stated that E_2 may play a role in food consumption and conversion in fish. Other roles of E_2 in growth may involve protein and fat synthesis. Japanese eel (Satoh & Nimura, 1991) and slow growing eel *Anguilla anguilla* (Degani,

1986) showed alternations in amount of protein and fat in the muscle following hormone treatment. In contrast, we did not observe any alteration in body composition of *A. stellatus*.

In summary, considering the effects of E2 on survival, growth, blood indices and body composition, we conclude that the E2 in even low dose can suppress the growth and changes in blood and biochemical parameters. Further study is also required to understand the growth improvement mechanism in the Stellate Sturgeon.

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اثر ۱۷-بتا استرادیول جیره بر عملکرد رشد، ترکیب بدن و شاخص های خونی ازون برون، *Acipenser stellatus*

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چکیده

این مطالعه روی اثرات ۱۷-بتا استرادیول جیره بر عملکرد رشد، ترکیب بدن و شاخص های خونی ماهی ازون برون انجام شد. ماهیان (میانگین وزن اولیه ۱/۱ ± ۴۰/۹ گرم، تعداد= ۶۰ عدد در هر گروه) با مقادیر مختلف ۱۷-بتا استرادیول در جیره شامل صفر (کنترل)، ۲۵ و ۵۰ میلی گرم در کیلوگرم غذا به مدت ۷ ماه تغذیه شدند. نتایج نشان دادند که رشد با افزایش سطح استرادیول کاهش می یابد. بالاترین نرخ بقا در ماهیان گروه کنترل مشاهده شد، ولی اختلاف در میان تیمارها معنی دار نبود ($P>0.05$). ترکیب بدن ماهیان در تیمارهای مختلف جیره تفاوت معنی دار نشان نداد. تعداد گلبول های سفید و قرمز، هموگلوبین و هماتوکریت با افزایش سطح ۱۷-بتا استرادیول تفاوت معنی دار کاهشی داشت ($P<0.05$). سطوح پارامترهای بیوشیمیایی پلاسما شامل گلوکز، پروتئین تام، کلسترول، تری گلیسرید، کلسیم و فسفر به سطوح مختلف ۱۷-بتا استرادیول بستگی داشت، بطوریکه در گروه کنترل کمترین مقدار و در گروه با دوز ۵۰ میلی گرم در کیلوگرم غذا بیشترین مقدار را به خود اختصاص داده بودند. نتایج مطالعه حاضر سرکوب رشد با جیره حاوی سطوح بالای ۱۷-بتا استرادیول و تغییرات شاخص های خونی را به همراه برخی اطلاعات پایه بر روی اثر هورمون استروژن در فیزیولوژی ماهیان خاویاری را نشان داد.

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