Effects of Aflatoxin B1-contaminated feeds on growth performance, blood parameters and liver enzymes of farmed *Acipenser stellatus* fingerlings

J Jalilpour[1], A Sepahdari[2*], S Kakoolaki[2], H Vahabzadeh Roodsari[3], Z Pajand[1], M. Masoumzadeh[1], M. Alizadeh[1], S. Bazari Moghaddam[1]

[*1] International Sturgeon Research Institute, Agricultural Research Education and Extension Organization (AREEO) Rasht, Iran, P. O. Box: 41635-3464

[2] Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran, P. O. Box: 14155-6453

[3] Department of Fishery and Aquaculture, Faculty of Natural Resource, Lahijan Branch, Islamic Azad University, Lahijan, Iran, P.O. Box: 1616

Received: February 2018 **Accepted:** April 2018

Abstract

Aflatoxins are toxic chemicals that are produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi. Aflatoxicosis caused by consumption of aflatoxin-contaminated feeds represents one of the serious diseases in most fish species. Due to the lack of information regarding the effects of aflatoxin B_1 (AFB_1) on farmed *Acipenser stellatus*, providing practical information for rearing of *A.stellatus* is imperative. *A. stellatus* fingerlings with the mean initial weight of 7.90 ± 0.12 g were kept in 50 Liter tanks with 10 fish per tank in a one way through water system with well water. Fish were adapted to the rearing conditions. The experimental diets were formulated to contain 1500, 1850, 2300, 2850 and 3500 ppb AFB_1 kg^{-1} diet. Two control diets were also considered. Fish were fed each of the experimental diets in triplicate groups three times a day for 52 days. At the end of the experimental period, there were significant effects ($p<0.05$) on WG%, final biomass, condition factor (CF), specific growth rate (SGR) and survival rates with the increase in aflatoxin levels in diets. There were significant differences ($p<0.05$) in blood parameters including WBC, RBC, HB and PCV % and also liver enzymes such as AST, ALT and ALP with the increase in aflatoxin levels in diets. 50% of fingerlings perished after 52 days of feeding at the concentration of 3500 ppb AFB_1 kg^{-1}. The results of this study revealed that *Acipenser stellatus* was relatively resistant to aflatoxin B_1.

Correspondence A Sepahdari, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO) Tehran, Iran. (e-mail: asepahdari@yahoo.com).

Keywords: Farmed *Acipenser stellatus*, Aflatoxin B_1, Growth, Survival, Liver enzymes, blood parameters.

[DOR: 20.1001.1.2345315.2018.4.1.9.3]

[DOI: 10.29252/ijaah.4.1.95]

Introduction

Aflatoxicosis caused by consumption of aflatoxin - contaminated feeds represents one of the serious diseases in most fish species. Aflatoxins are toxic chemicals that are produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi. Aflatoxins are generally known as fungus. Oil seeds such as cottonseed, ground nut, corn, wheat, sunflower seeds, fish food and generally all foods can be contaminated with aflatoxins. The four main aflatoxins are B_1, B_2, G_1 and G_2 and aflatoxin B_1 is one of the most powerful cancer-causing agents in animals (Smith 1997, Mortazavi & Tabatabaei 1998, Royes 2002). Biological effects of aflatoxin B_1 on fish is directly influenced by factors such as age and sex of fish as well as the concentration of toxin in food (Eaton & Groopman 1994). Fry are more susceptible to aflatoxicosis than adults. The reported symptoms of toxicity in fish include weight loss, changes in blood parameters and necrosis of liver cells (Raghavan, Zhu, Lei, Han, Yang & Xie 2011; Abdelhamid, Salem, Mehrim & EL-Sharavi 2007). Inappropriate storage of food is one of the most common predisposing factors for fungal growth and mold production which can be controlled by fish culturists. Synthesis of aflatoxins in feeds is increased at temperatures above 27°C, humidity levels greater than 62% and moisture levels in the feed above 14% (Santacroce, Conversano, Casalino, Lai, Zizzadoro, Centoducati & Crescenzo 2008). *Acipenser stellatus* is one of the most important economic sturgeon species in the Caspian Sea. Due to the severe reduction of brood stocks of this species in recent years (Ghaninejad 1997) the maximum exploitation of farmed sturgeon fingerlings must be carried out. On the other hand, fingerlings are more susceptible to pathogens than adults, and hence high mortality is recorded during their adaptation to formulated diets. Moreover this stage is physiologically the most important stage in fish growth. Therefore, producing a healthy diet with the high nutritional value in order to improve the quality of produced fish and increase survival rates in this species were the reasons for choosing this study. The research regarding the effects of AFB_1 on sturgeon fish in Iran was carried out by Farabi, Yousefian & Hajimoradloo (2006) and Sepahdari, Ebrahimzadeh Mosavi, Sharifpour, Khosravi, Motallebi, Mohseni, Kakoolaki, Pourali & Hallajian (2010) on juvenile farmed beluga (*Huso huso*). The effect of aflatoxin B_1 was widely studied in fishes such as rainbow trout (Halver 1969 Hendricks, Putnam & Sinnhuber 1980) common carp (Svobodova, Piskac, Havlikova & groch 1982) channel catfish (Jantrarotai & Lovell 1990) Rohu (Sahoo & Mukherjee 2001, Sahoo, Mukherjee & Jain 2003) tilapia (Abdelhamid *et al*. 2007, Zaki, Sharaf, Roshad, Mostafa & Fawzi 2008) sea bass (El-Sayed, Khali 2009) *Acipenser ruthenus* ♂ × *A. baeri* ♀ (Raghavan *et al*. 2011) Crustaceans like *P. vanami* and *P. stylirostris* (Lightner, Redman, Price & Wiseman 1982) and *P.monodon* (Boonyaratpalin, Supamattaya, Verakunpiria

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

& Supra sert 2001). Due to the lack of information regarding the effect of aflatoxin B_1 on farmed *Acipenser stellatus*, providing practical information for rearing of *A.stellatus* is imperative.

Materials and Methods

The experimental system and fish selection

A total of 210 *A. stellatus* fingerlings with a mean initial weight of 7.90 ± 0.12 g (were obtained from International Sturgeon Research Institute, Rasht, Iran) were kept in 50-L tanks in a one way through water system with aerated well water. Fish were adapted to the rearing conditions and all the experimental treatments were kept under the same rearing conditions. Seven treatments (2 control groups and 5 experimental groups with different concentrations of aflatoxin) with three replicates were considered for the experiment.

Preparation of experimental diets

A stock solution was prepared by dissolving 1mg of aflatoxin B_1 (Sigma Chemicals, St. Louis, MO, USA) in 10 ml of methanol (Jantrarotai & Lovell 1990, Jantrarotai *et al*. 1990). The concentrations, 0.75, 0.925, 1.15, 1.425 and 1.75 mL from this stock solution, were determined based on the mean body weight of fish and added to the experimental diets. The concentrations of 1500, 1850, 2300, 2850 and 3500 ppb AFB_1 kg^{-1} were obtained by logarithmic calculations.

The determined concentrations were added to each treatment based on 2% of the biomass in each treatment. A diet with no aflatoxin and a diet including 1 cc of methanol were considered as the negative control and positive control diets, respectively. Some parts of the experimental and control diets were transferred to the laboratory to determine the toxin concentration by HPLC (Waters E 2695. USA). The amount of AFB_1 in experimental diets was estimated based on ISO 16050:2003 and Iranian National Standards (ISIRI No. 6872 2003). The fish were fed at 08:00 and 16:00 h (Raghavan *et al*. 2011). Temperature, dissolved oxygen concentration and pH were measured by a portable oxygen meter and pH meter (WTW-Multi 340I).

Determination of growth parameters

According to the information obtained from length and weigh determinations in fish, the total amount of consumed food as well as rearing period and growth performance analysis were calculated as follows:

TW = Total weight (g) and TL = Total length (cm). Biomass (g) = Total weight × number of fishs.

WG (Weight gain) (%) = 100 × (final weight – initial weight) / initial weight (Hung, Aikins, Lutes, Xu 1989).

CF (Condition Factor) = (weight (g) / $lenght^3$) × 100 (Shapawi ustafa & Ng 2011).

SGR (Specific growth rate) = [(Ln final weight - Ln initial weight) / experiment days] × 100 (Ronyai & Ruttkay 1990).

Hematology studies

At the end of the rearing period (52 days), twelve 12 fish from each experimental group (four fish from each replicate) were randomly sampled and blood samples were collected

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

from caudal vein by 1 ml syringes. Blood samples were transferred into the vial containing heparin to determine blood parameters included white blood cells (WBC), red blood cells (RBC), Hemoglobin (Hb) and Haematocrit (PCV) to vials without heparin (Caspian Tamin Pharmaceutical. Co. Iran) to investigate the blood serum parameters. The blood was centrifuged (Heraeus Labofuge 200. Germany) at 7000 *Rpm* for 7 min (Houston 1990). PCV% (D-78532 Tuttilingen Hettich Co., Germany) values were determined using microhaematocrit heparinized capillary tubes. Hb was determined using commercial kits (Pars Azmon Co. Iran) by spectrophotometer (6505 UV/Vis, England). The WBC and RBC blood cells counts were determined by Hemocytometer (Neubauer imroved, Precicolor HBG, Germany) per cubic millimeter of blood for each sample (Gao, Wang, Abbas, Zhou, Yang, Diana, Wang, Wang, Li & Sun 2007, Klontz 1994). Liver enzymes such as Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) were determined using Pars Azmon kits by IFCC (International federation of Clinical chemistery) method.

Statistical analysis

In order to determine the normal distribution of data in experimental groups and replicates, both general and descriptive statistics were used. Kolmogorov-Smirnov test and histogram plot were also used. After confirming the homogeneity of variance, data were analyzed by One-way ANOVA (SPSS version 17). When a significant treatment effect was observed, the Duncan's multiple range test was performed. Treatment effects were considered at $p< 0.05$ level of significance.

Results

Growth

According to Kolmogorov-Smirnov test, data in replicates groups showed a normal distribution. According to one-way ANOVA, there were no significant differences ($p>0.05$) in mean weight, mean length and biomass of fingerlings among experimental groups in the first biometry (Table1). There were no significant differences ($p>0.05$) in mean weight and length among fish fed diets containing aflatoxin and control diets at the end of the rearing period. But final weight and length of fish fed diets containing aflatoxin were lower than those of fish fed control diets. Diets containing the highest concentrations of aflatoxin (2500 and 3500 ppb AFB_1 kg^{-1}) resulted in the lowest mean weights among treatments. Final biomass, body weight increase (BWI %), condition factor (K) and specific growth rate (SGR) of fish fed diets containing different concentrations of aflatoxin were significantly lower than those of fish fed control diets ($p<0.05$) and this reduction was more severe in fish fed diets containing 2500 and 3500 ppb AFB_1 kg^{-1} than in other treatments (Table1).

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

Table 1. Growth performance of *Acipenser stellatus* in experimental treatments throughout 52 days exposure period. all values are mean ± SE, n=3 (each replicate is the combination of ten fish)

Items	Treatment						
	Control +	Control -	T_1 (1500 ppb)	T_2 (1850 ppb)	T_3 (2300 ppb)	T_4 (2850 ppb)	T_5 (3500 ppb)
Initial							
TW (g)	7.51 ± 0.13	7.73 ± 0. 2	7.78 ± 0.17	8.07 ± 0.05	8.52 ± 0.12	8.05 ± 0.12	7.65 ± 0.05
TL (cm)	14.85 ± 0.2	14.80 ± 0.1	15.51 ± 0.52	15.765 ± 0.14	15.83 ± 0.2	14.85 ± 0.2	14.85 ± 0.2
Biomass (g)	75.15 ± 1.4	77.35 ± 1.5	77.78 ± 4.78	80.70 ± 0.50	85.29 ± 1.23	80.59 ± 1.64	76.50 ± 0.50
FINAL							
TW (g)	12.04 ± 0.06	12.10 ± 0.3	11.4 ± 0.3	10.10 ± 0.2	11.30 ± 0.06	10.60 ± 0.05	10.30 ± 0.60
TL (cm)	17.60 ± 0.1	17.82 ± 0.2	17.51 ± 0.5a	17.83 ± 0.2	17.83 ± 0.04	17.86 ± 0.09	17.87 ± 0.02
Biomass (g)	120.4 ± 0.6 ^{a}	121.4 ± 3.1 ^{a}	114.45 ± 10.2 ^{ab}	109.8 ± 1.8 ^{ab}	101.88 ± 0.5 ^{bc}	85.24 ± 1.5 ^{cd}	76.91 ± 0.7 ^{d}
WG (%)	60.25 ± 2.1 ^{a}	56.92 ± 0.9 ^{a}	46.80 ± 1.4 ^{ab}	36.07 ± 3.1 ^{b}	32.76 ± 2.6 ^{b}	32.21 ± 0.4 ^{b}	34.63 ± 2.7 ^{b}
CF	0.22 ± 0.004 ^{a}	0.22 ± 0.001 ^{a}	0.21 ± 0.01 ^{ab}	0.19 ± 0.01 ^{bc}	0.19 ± 0.01 ^{bc}	0.18 ± 0.001 ^{c}	0.18 ± 0.01 ^{c}
SGR (%bw day $^{-1}$)	0.90 ± 0.024 ^{a}	0.86 ± 0.011 ^{a}	0.73 ± 0.018 ^{b}	0.59 ± 0.043 ^{c}	0.34 ± 0.037 ^{d}	0.10 ± 0.006 ^{e}	0.01 ± 0.005 ^{f}

a,b,c... Means in the same row bearing different letter significantly ($p<0.05$).

TW: Total weight (g), TL= Total length (cm), Biomass (gr): Total weight × number of fishs.

WG: weight gain rate (%) = 100 × (final total weight – initial total weight) / initial total weight (Hung *et al.* 1989).

CF: (weight (g) / lenght3) × 100 (Shapawi *et al*. 2011).

S.G.R: Specific growth rate = [(Ln final weight- Ln initial weight) ÷ experiment days] × 100 (Ronyai & Ruttkay 1990).

Hematology

Blood parameters

Blood parameters including RBC, Hb and PCV % significantly decreased with the increase in toxin in diets, (Table 2). While a significant increase was observed in WBC when toxin levels increased in diets compared to that in the control groups ($p<0.05$)

Table 2. Hematogram of *Acipenser stellatus* in experimental treatments with AFB_1 throughout 52 days exposure period, all values are mean ± SE

Items	Treatment						
	Control +	Control -	T_1 (1500 ppb)	T_2 (1850 ppb)	T_3 (2300 ppb)	T_4 (2850 ppb)	T_5 (3500 ppb)
WBCs (10^3 mm^{-3})	15.75 ± 1.75^{c}	21.75 ± 3.25^{bc}	16.50 ± 2.5^{c}	28 ± 7^{b}	27 ± 0.5^{b}	32.75 ± 4.25^{a}	33 ± 2.5^{a}
RBCs (10^6 mm^{-3})	1.17 ± 0.2^{a}	1.05 ± 0.05^{a}	1.01 ± 0.52^{a}	0.95 ± 0.14^{ab}	1.005 ± 0.2^{ab}	0.83 ± 0.2^{b}	0.85 ± 0.2^{b}
Hb (g dl^{-1})	4.26 ± 0.68^{a}	5.26 ± 0.32^{a}	2.96 ± 0.02^{b}	2.76 ± 0.04^{b}	2.52 ± 0.32^{b}	2.85 ± 0.13^{b}	2.23 ± 0.38^{b}
P.C.V%	23.5 ± 1. 5^{a}	22. 5 ± 0.54^{a}	20.50 ± 1.5^{ab}	18.5 ± 0.5^{b}	12 ± 0.5^{b}	14. 5 ± 0.5^{b}	16 ± 1^{b}

a,b,c... Means in the same row bearing different letter significantly ($p<0.05$).

Plasma biochemical parameters

Based on the results obtained from this study, significant differences in ALP, ALT and AST values were observed among fingerlings fed different concentrations of aflatoxin and control diets at the end of the feeding trial ($p<0.05$). The values of ALP, ALT and AST in

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

the blood of fingerlings significantly increased with increase in toxin levels in diets and showed significant differences compared to that in control groups ($p<0.05$) (Table 3).

Table 3. Serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities of control and AFB1 exposed *Acipenser stellatus* throughout 52 days exposure period

Items	Treatment						
	Control +	Control -	T_1 (1500 ppb)	T_2 (1850 ppb)	T_3 (2300 ppb)	T_4 (2850 ppb)	T_5 (3500 ppb)
AST ($U\,L^{-1}$)	293 ± 5.5^{b}	325.5 ± 3^{b}	275 ± 40.5^{a}	441.5 ± 1.5^{ab}	501.5 ± 48.5^{ab}	613.5 ± 33.5^{a}	501 ± 32^{a}
ALT ($U\,L^{-1}$)	3 ± 0.5^{b}	3.5 ± 0.5^{b}	5 ± 0.7^{a}	4 ± 0.14^{ab}	3.5 ± 0.5^{ab}	4.5 ± 1^{a}	6 ± 1^{a}
ALP ($U\,L^{-1}$)	247.5 ± 2.5^{b}	304 ± 6^{b}	473 ± 28^{a}	409 ± 1^{ab}	387.5 ± 2.5^{ab}	346 ± 19^{a}	424 ± 19^{a}

a,b,c... Means in the same row bearing different letter significantly ($p<0.05$).

Survival

The survival rates in fingerlings fed control diets were higher than those of fish fed diets containing aflatoxin. The results indicated that the survival rates decreased with toxin increase in diets. The lowest survival rate was recorded in fish fed the diet containing 3500 ppb AFB_1 kg^{-1} (treatment 5) which had significant differences compared to that in the control groups and other treatments ($p<0.05$) (Table 4).

Table 4. Survival of *Acipenser stellatus* fed various levels of AFB_1 supplemented diets for throughout 52 days exposure period n = 3 (each replicate is the combination of ten fish)

Items	Treatment						
	Control +	Control -	T_1 (1500 ppb)	T_2 (1850 ppb)	T_3 (2300 ppb)	T_4 (2850 ppb)	T_5 (3500 ppb)
Survival (%)	100^{a}	100^{a}	93.75 ± 1.8^{a}	80 ± 2.7^{ab}	65 ± 1.9^{ab}	57.5 ± 2.5^{b}	47.5 ± 2.5^{b}

a,b,c... Means in the same row bearing different letter significantly ($p<0.05$).

At the end of 52 days (8-week) feeding trial (Fig. 1.), there was no mortality in control groups. A total of 10% mortality was recorded in fish fed the diet containing 1500 ppb AFB_1 kg^{-1} (treatment 1) from the 7^{th} week of rearing. 20% of mortality was recorded in fish fed the experimental diet containing 1850 ppb AFB_1 kg^{-1} (treatment 2) from the 4^{th} up to 8^{th} week of rearing. 40% of mortality was recorded in fish fed the diet containing 2300 ppb AFB_1 kg^{-1} (treatment 3) from the 3^{rd} up to 8^{th} week of rearing. 40% of mortality was recorded in the treatment fed the diet containing 2850 ppb AFB_1 kg^{-1} (treatment 4) from the 2^{nd} up to 8^{th} week of rearing and 50% of mortality was recorded in fish fed the diet containing 3500 ppb AFB_1 kg^{-1} (treatment 5) from the 1^{st} up to 8^{th} week of rearing.

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

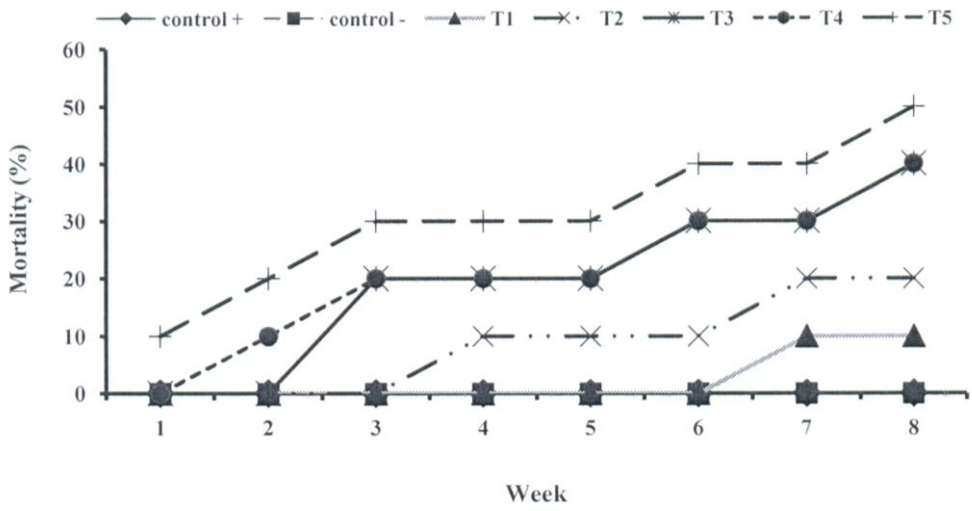

Figure 1. Progression of mortality (%) *Acipenser stellatus* fed various levels of AFB_1 supplemented diets throughout 52 days (8-week) exposure period**.**

Discussion

Growth

There were no significant differences in mean weight among experimental treatments and control diets in the last biometry ($p>0.05$), but final weight of fish fed diets containing aflatoxin decreased compared to those of fish fed control diets.

According to Cagauan, Tayaban, Somga and Bartolome (2004), the different levels of aflatoxin contamination (0, 10, 50 and 100 %) did not significantly affect the mean final weight in Nile tilapia ($p>0.05$), while fish fed diets including 10, 50 and 100 % aflatoxin had lower mean weight than those of fish fed control diet. Sepahdari *et al*. (2010) showed that there were no significant differences in average weight of *Huso huso* fed diets containing 0, 25, 50, 75 and 100 ppb AFB_1 kg^{-1} after two months of rearing, while a considerable reduction in weight was found in fish fed aflatoxin-contaminated diets compared to those fed control diets which confirms our results.

In this study, final biomass of fish fed aflatoxin-contaminated diets was lower than those of fish fed control diets. In the current study, fish fed diets with 2850 and 3500 ppb AFB_1 kg^{-1} had a significant decrease in condition factor than those of fish fed control diets. There were no significant differences in mean final biomass of Nile tilapia fed different levels of aflatoxin (0, 25, 50, 75 and 100 ppb) in contaminated diets (Cagauan *et al*. 2004). According to Cagauan *et al*. (2004) the highest mean final biomass (233.83 g) was obtained in the control group and the lowest mean final biomass (93.77 g) was recorded in the treatment containing 100 % moldy feed.

Chavez-Sanchez, Martinez Palacios and Osorio Moreno (1994) also revealed that the growth and biomass of Nile tilapia decreased when fish were fed a diet containing 1.88 mg AFB_1 during the 25 days of rearing. Abdelhamid *et al.* (2007) in a study on tilapia

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

reported that fish fed diets containing 100 ppb AFB_1 kg^{-1} showed lower weight than those in fish fed control and anti-toxin diets. Experimental studies conducted in channel catfish exposed to 2150 ppb AFB_1 kg^{-1} revealed that there were no significant differences in weight gain during ten weeks of rearing. Only 24 % of fish showed a reduction in weight gain with 10000 ppb concentration of AFB_1 (Jantrarotai & Lovell, 1990). These findings were in agreement with this study. The results of this study indicated that specific growth rate (SGR) decreased as dietary AFB_1 concentration increased. Abdelhamid *et al.* (2007) showed that tilapia fed diets containing 100 ppb AFB_1 kg^{-1} had lower SGR than fish fed control and anti-toxin diets. A study on reducing effects of vitamin C on Aflatoxin-contaminated feeds by Shehata, El-Melegy and Ebrahim (2009) indicated that Nile tilapia fed diets containing 3000 ppb AFB_1 kg^{-1} had lower SGR than fish fed control diets or aflatoxin-contaminated diets supplemented with vitamin C. Chavez-Sanches *et al*. (1994) revealed that the growth of Nile tilapia decreased when fish were fed diets containing 1880 ppb AFB_1 kg^{-1} during the 25 days of rearing. The specific growth rate of tilapia was significantly influenced by diet containing 2.5 mg AFB_1 kg^{-1}, while diet containing 250 ppb AFB_1 kg^{-1} did not significantly affect the SGR of tilapia (Tuan, Grizzle, Lovell, Manning & Rottinghaus 2002). It is generally observed that Nile tilapia is more sensitive to aflatoxin contamination than channel catfish (Tuan *et al.* 2002).

Hematology

Blood parameters including RBC, Hb and PCV % significantly decreased with toxin increase in diets, while a significant increase was observed in WBC with toxin increase in diets compared to control groups ($p<0.05$). Salem, Shehab El-Din1, Khalafallah, Sayed, Amal (2010) showed that the white blood cells of Nile tilapia was higher in fish fed diets containing 150 ppb AFB_1 kg^{-1} than in those fed control or anti-toxin diets. Long-term impairment of the immune system through a substantial reduction in B lymphocytes was observed in Rainbow trout embryos exposed to AFB_1 (Arkoosh & Kaattari 1987; Ottinger & Kaattari 1998).

A reduction in immunoglobulin production as well as increase in lymphocytes was reported by aflatoxin contamination (Ottinger and Kattari 1998). The deleterious effects of aflatoxin B_1 on hematopoietic tissues lead to decreased production of lymphocytes and immunoglobulin (Sahoo & Mukherjee 2001, 2003). In a study on minimum lethal concentration of aflatoxin in *Acipenser ruthenus* ♂ × *A. baeri* ♀ fingerlings, a significant reduction in hematocrit was reported in fish fed diets containing 80 ppb AFB_1 kg^{-1} (80 μg kg^{-1}) after 35 days of rearing (Raghavan *et al*. 2011). Investigations conducted by Tuan *et al*. (2002) showed that increase in aflatoxin B_1 concentrations led to significant decrease in hematocrit values. These findings were in agreement with Stewart and Larson in 2002 on rainbow trout and Jantrarotai and Lovell (1990) on channel catfish. Sepahdari *et al*. (2010) reported that *Huso huso* fed diets with 100 ppb AFB_1 kg^{-1} had significantly lower red blood cells and hemoglobin in comparison with fish

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

fed other treatments and control diet. Salem *et al.* (2010) showed that the red blood cells and hemoglobin concentrations of Nile tilapia were higher in fish fed diets containing 150 ppb AFB_1 kg^{-1} than in those fed control or anti-toxin diets. Increase in dietary aflatoxin B_1 led to a significant increase in ALP, ALT and AST values at the end of the rearing period ($p<0.05$). The changes in levels of ALT and AST in serum are related to the activity of the liver and can be considered as a means to assess health and changes in permeability of cell membranes. Increase in AST and ALT of blood serum in aflatoxin-contaminated diets can not only be caused by the impaired metabolism of tissue proteins by chemical stress but is also in response to the contamination in the kidneys (Sahoo and Mukherjee 2001). Abdelhamid *et al.* (2007) reported that the increase in phosphatase and transaminase enzyme activities in blood of Nile tilapia by the increase in aflatoxin can be caused by liver, kidney and heart necrosis. Due to the destructive effects of aflatoxin B_1 on hepatocytes, the values of ALT, AST and ALP will increase in blood serum. These findings are similar to results obtained from *Acipenser stellatus* in the present study. Santacroce *et al.* (2008) reported suspicious mortalities without any clinical signs in aquatics fed aflatoxin- contaminated diets.

Survival

A significant reduction in survival rates was observed as dietary aflatoxin increased. According to Raghavan *et al*. (2011), mortality of *Acipenser ruthenus* ♂ × *A. baeri* ♀ fingerlings fed diets containing 80 ppb AFB_1 kg^{-1} reached to more than 50% after 25 days of rearing. The first mortality was recorded at the concentration of 80 ppb AFB_1 kg^{-1} on days 12 and 13 of rearing. Mortality was observed on day 18 of rearing at the concentration of 20 ppb AFB_1 kg^{-1}. A total of 8.6% mortality in juvenile *Huso huso* was reported by Farabi *et al*. (2006) after 15 days of feeding at the concentration of 10 ppb AFB_1 kg^{-1}, although the exact time of the first mortality was not specified in their study. However in other species such as Nile tilapia, 17% mortality was reported after 10 weeks of feeding at the concentration of 200 ppb AFB_1 kg^{-1} (El-Banna, Teleb, Hadi & Fakhry 1992). Chavez-Sanchez *et al.* (1994) reported that a dose of 30000 ppb AFB_1 kg^{-1} diet was not lethal to Nile tilapia. An increase in mortality was recorded in Nile tilapia fed diets containing 100000 ppb AFB_1 kg^{-1} for 8 weeks, although it was not significantly different from fish fed diets containing 10000 ppb AFB_1 kg^{-1}.

In this study, more than 50% of fingerlings perished after 52 days of feeding at the concentration of 3500 ppb AFB_1 kg^{-1}. A total of 7% mortality was recorded in fish fed diet containing 1500 ppb AFB_1 kg^{-1}. The magnitude of toxicity is influenced by species susceptibility, toxin concentration, toxin exposure time, age and rearing conditions (Santacroce *et al*. 2008). The results of this study revealed that *Acipenser stellatus* was relatively resistant to aflatoxin B_1 and none of the concentrations used in this study were in the tolerance range of *Acipenser stellatus* fingerlings. It is highly recommended that the effects of aflatoxin B_1 in other weight classes

[DOR: 20.1001.1.2345315.2018.4.1.9.3]
[DOI: 10.29252/ijaah.4.1.95]

and rearing environments (brackish water or sea water) are studied in *Acipenser stellatus*.

Acknowledgment

This study was funded by the Iranian Fisheries Research Organizations. The authors would like to thank Abas Ali Motalebi, Mohammad Pourkazemi, Mostafa sharifrouhani and Mahmoud Bahmani their support and constractive comments, to saeideh soheil naghshi and alireza shenavar masouleh for her comments on the manuscript. We are grateful to the staff of the sturgeon farm for their help in the samples.

Conflict of interests

The authors declare that there is no conflict of interest.

References

Abdelhamid A.M, Salem M.F.I, Mehrim M.A.M, EL-Sharavi M. (2007) Nutritions attempts to detoxify aflatoxic diets of tilapia fish. In: proceedings of the first Annual Scientific Conference on animal and fish production, Mansura university, Egypt, 24-25 septamber, pp. 207-230.

Arkoosh MR, Kaattari SL. (1987) Effect of early aflatoxin B_1 exposure on in vivo and in vitro antibody responses in rainbow trout, Salmo gairdneri. *Journal of Fish Biol*ogy 31, 19–22.

Boonyaratpalin M, Supamattaya K, Verakunpiria V, Supra sert D. (2001) Effects of aflatoxin B_1 on growth performance blood componenents, immune function and histopathological changes in black tiger shrimp (*Penaeus monodon Fabricius*). *Journal of Aquaculture Research* 32, 388-398.

Cagauan AG, Tayaban RH, Somga J. & Bartolome RM. (2004) Effect of aflatoxin-contaminated feeds in Nile tilapia (*Oreochromis niloticus L.*). In: Abstract of the 6th International Symposium on Tilapia in Aquaculture (ISTA6) Section: Health Management and Diseases Manila, Philippines 12, p. 16.

Chavez-Sanchez M.C, Martinez Palacios C.A, Osorio Moreno, I. (1994) Pathological effects of feeding young Oreochromis niloticus diets supplemented with different levels of aflatoxin B_1. *Aquaculture* 127(1), 49-60.

Eaton DL. & Groopman JD. (1994) The toxicology of aflatoxins: human health, veterinary and agricultural significance. Academic Press, San Diego, pp. 6–8.

El-Banna R, Teleb H. H, Hadi N.M, Fakhry F.M. (1992) Performance and tissue residue of tilapia fed dietary aflatoxin. *Veterinary Medical Journal Giza* 40, 17-23.

EL-Sayed Y.S, Khalil R.H. (2009) Toxicity biochemical effects and residue of aflatoxin B_1 In marine water- reared sea bass (*Dicentrarchus labrax L.*). *Journal of food and chemical toxicology* 47(7), 1606-1609.

Farabi S.M.V, Yousefian M. & Hajimoradloo A. (2006) Afla-toxicosis in juvenile Huso huso fed a contaminated diet. *Journal of Applied Ichthyologhy* 22, 234-237.

[DOR: 20.1001.1.2345315.2018.4.1.9.3]

[DOI: 10.29252/ijaah.4.1.95]

Ghaninejad D. (1997) on some biological aspect of *Acipenser stellatus* in the south west of the Caspian sea. *Iranian Journal of Fisheries Sciences*. 6(1) 141–150. [In Persian]

Gao Z, Wang W, Abbas K, Zhou X, Yang Y, Diana J.S, Wang H, Wang H, Li Y. & Sun Y. (2007) Haematological characterization of loach Misgurnus anguillicaudatus: Comparison among diploid, triploid and tetraploid specimens. *Comparative Biochemistry and Physiology* 147 1001-1008.

Halver J.E. (1969) Aflatoxicosis and trout hepatoma. In: Aflatoxin: Scientific Background, Control and Implications (Goldblatt, L.A. ed.) pp. 265-306. Academic Press, New York.

Hendricks J.D, Putnam T.P. & Sinnhuber R.O. (1980) Null effect of dietary Aroclor 1254 on hepatocellular carcinoma incidence in rainbow trout Salmo gairdneri exposed to aflatoxin B_1 as embryos. *Journal Environ Pathol Toxicol*. 4(5-6), 9-16.

Houston A. H. (1990) Blood and circulation. In Methods for Fish Biology (Schreck, C. B. & Moyle, P. B., eds), pp. 273–334. Bethesda, MD: American Fisheries Society.

Hung S. S. O, Aikins K. F, Lutes P. B, Xu, R. (1989) Ability of Juvenile white sturgeon (*Acipenser transmontanus*) to utilize different Carbohydrate source. *Journal Nutrition* 119(5), 272-733.

IFCC. International federation of clinical chemistry and laboratory medicin. http://www.ifcc.org/.

ISO 16050:2003: Foodstuffs. Determination of aflatoxin B1, and the total content of aflatoxins B_1, B_2, G_1 and G_2 in cereals, nuts and derived products. High - performance liquid chromatographic method.

ISIRI 6872:2003: Food and feed stuffs. Determination of aflatoxins B&G by HPLC method using immunoaffinity column clean up-Test method. Institute of Standards and Industrial Research of Iran. ICS: 67.040.

Jantrarotai W, Lovell R.T. (1990) Subchronic toxicity of dietary aflatoxin B_1 to channel catfish. *Journal of Aquatic Animal Health* 2, 248-254.

Jantrarotai, W, Lovell, R.T, Grizzle, J.M. (1990) Acute toxicity of dietary aflatoxin B_1 to channel catfish. *Journal of Aquatic Animal Health* 2, 237-247.

Klontz G. W. (1994) Fish hematology. In Techniquesin fish immunology, FITC 3, Stolen J. S. Fletcher T. C, Rowley A. F, Anderson D. P, Kaattari S.L, Zelikoff J. T, Smith S. A. (eds.). SOS Publications, Fair Haven, New Jersey, pp.121–132.

Lightner D.V, Redman R.M, Price R.L. & Wiseman M.O. (1982) Histopathology of aflatoxin in the marine shrimp *Penaeus stylirostris* and *p. vannamei*. *Journal of Invertebrate Pathology* 40, 279-271.

[DOR: 20.1001.1.2345315.2018.4.1.9.3]

[DOI: 10.29252/ijaah.4.1.95]

Mortazavi A. & Tabatabaei F. (1998) Mycotoxins. Ferdowsi University Press. PP.206. [In Persian]

Ottinger CA, Kaattari SL. (1998) Sensitivity of rainbow, trout leucocytes to aflatoxin B_1. *journal of the International Society of Fish and Shellfish Immunology* 7, 515–530.

Pars Azmon Co., Iran.www.parsazmun.ir

Rajeev Raghavan P, Zhu X, Lei W, Han D, Yang Y, Xie S. (2011): Low level of Aflatoxin B_1 could cause mortalities in juvenile hybrid sturgeon, *Acipenser ruthenus* ♂ × *A. baeri* ♀. *Aquaculture Nutrition* 17, 39-47.

Ronyai A. & Ruttkay A. (1990) Growth and food utilization of wels fry (*Silurus glanis*) fed with tubifex. Aquaculture. Hung. (Szarvas) VI, 193– 202.

Royes JB, Yanong RPE. (2002) Molds in fish and aflatoxicosis, (http://edis.ifas.ufl. Edu/FAO95).

Sahoo p.k, Mukherjee S.C. (2001) Immunosuppressive effect of aflatoxin B_1 in idian major carp (*Labeo rohita*). *Comparative Immunology, Microbiology and Infectious Diseases* 24, 142-149.

Sahoo p.k, Mukherjee S.C, Jain A.K. & Mukherjee A. (2003) Histopathological and electron Microscopic studies of gills and opisthonephros of Rohu, Labeo rohita to acute and subchronic of aflatoxin B_1 toxicity. *Journal of Asian Fisheries Science* 16, 257-268.

Salem M. F. I, Shehab El-Din1 M. T, Khalafallah M.M.M.A, Sayed S. H, Amal S. H. (2010) Nutritional Attempts to Detoxify Aflatoxic Effects in Diets of Tilapia Fish (*Oreochromis niloticus*). *Journal of the Arabian aquaculture socyiet* 5(2), 195-205.

Santacroce M. P, Conversano. M.C Casalino E, Lai O, Zizzadoro C, Centoducati G, Crescenzo G. (2008) Aflatoxins in aquatic species: metabolism, toxicityand perspectives. *Reviews in Fish Biology and Fisheries* 18, 99–130.

Sepahdari A, Ebrahimzadeh Mosavi H.A , Sharifpour I, Khosravi A, Motallebi A.A , Mohseni M, Kakoolaki S, Pourali H.R, Hallajian A. (2010) Effects of different levels of AFB_1 on survival rate and growth factors of Beluga (*Huso huso*).*Iranian Scintific Fisheries Journal*. 9(1):141–150. [In Persian]

Shapawi R, M ustafa S. & W.K. Ng. (2011) A Comparison of the Growth Performance and Body Composition of the Humpback Grouper, Cromileptes altivelis Fed on Farm-made Feeds, Commercial Feeds or Trash Fish. *Journal of Fisheries and Aquatic Science* 6, 522-534.

Shehata S.A, El-Melegy Kh.M. & Ebrahim M.S. (2009) Toxicity Reduction of Aflatoxin B_1 by Vitamin C in Fish. *Journal of the thearabian Aquaculture society* 4 (2), 73-85.

Smith, J.E. (1997) Aflatoxins. In: Felix D'Mello, J.P. (Ed.), Plant and Fungal

[DOR: 20.1001.1.2345315.2018.4.1.9.3]

[DOI: 10.29252/ijaah.4.1.95]

Toxicants. Florida, USA: CRC Press, Boca Raton, pp. 269–285.

SPSS (2007). Statistical package for the social sciences, Revisions 17, spss Inc, Chicago, IL 60606-6412, USA.

Stewart D, Larson E. (2002) Aflatoxicosis in wildlife. Information Sheet 1582. Mississippi State University. Extension Service, Cooperating with U.S. Dept. of Agriculture.

Svobodova Z, Piskac A, Havlikova J. & groch L. (1982) The influence of feed With different contents of B_1 aflatoxin on the carp health condition. Zivocisna Vyroba – UVTIZ, 25, 811-820.

Svobodava Z, Piskac A, Havlikova J, Groch, L. (1999) The influence of feed with different contents of Aflatoxin B_1 on the carp health condition, Zivocisna Vyroba – UVTIZ, 27(11), 811.

Tuan N.A, Grizzle J.M, Lovell R.T, Manning B.B, Rottinghaus G.E. (2002) Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B_1.*Aquaculture* 212, 311-319.

Wieser W., Krumschnalbel G. & Ojwang-Okwor J. P. (1992) The energetics of starvation and growth after refeeding in juveniles of three cyprinid species. *Environmental Biology of Fishes* 33, 63–71.

Xie S., Zhu X., Cui Y., Wootton R. J., Lei W. & Yang Y. (2001) Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. *Journal of Fish Biology* 58(4), 999–1009.

Zaki S, Sharaf E, Roshad H, Mostafa O. & Fawzi M. (2008) Diminution Dietary Supplementation With fix in toxin and Nigella Sativa Oil. *Journal of Agriculture and Environmental Sciences* 3(2), 211-215.

[DOR: 20.1001.1.2345315.2018.4.1.9.3]

[DOI: 10.29252/ijaah.4.1.95]

اثر سمیت غذای آلوده به آفلاتوکسین B_1 بر رشد، فاکتورهای خونی و آنزیم‌های کبدی بچه ماهی ازون برون پرورشی (*Acipenser stellatus*)

جلیل جلیل پور[1]، ابوالفضل سپهداری[2]*، شاپور کاکولکی[2]، حبیب وهاب زاده رودسری[3]، ذبیح الله پژند [1]، مهدی معصوم زاده[2]، مهدی علیزاده[2]، سهیل بازاری مقدم[2]

[1] موسسه تحقیقات بین المللی تاسماهیان دریای خزر، سازمان تحقیقات، آموزش و ترویج کشاورزی، رشت، ایران، صندوق پستی: ۳۴۶۴- ۴۱۶۳۵
[2] موسسه تحقیقات علوم شیلاتی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران، صندوق پستی: ۶۴۵۳- ۱۴۱۵۵
[3] دانشگاه آزاد اسلامی، واحد لاهیجان، دانشکده منابع طبیعی، گروه شیلات، لاهیجان، ایران صندوق پستی: ۱۶۱۶

چکیده

آفلاتوکسین‌ها مواد شیمیایی سمی هستند که بوسیله انواع قارچ‌ها (*Aspergillus flavus* & *Aspergillus parasiticus*) تولید می‌شوند که بطور کلی بعنوان کپک شناخته می‌شوند و آفلاتوکسیکوزیس بیماری است که در اکثر انواع ماهیان بر اثر تغذیه از غذای آلوده به آفلاتوکسین‌ها ایجاد می‌شود. عدم وجود اطلاعات در ارتباط با تأثیرات آفلاتوکسین B_1 در مورد گونه ازون برون پرورشی *Acipenser stellatus*) ایجاب می‌نماید که اطلاعات کاربردی در جهت موفقیت کامل در زمینه پرورش این گونه فراهم گردد. بچه ماهیان ازون برون با میانگین وزن اولیه ۰/۱۲ ± ۷/۹۰ گرم پس از عادت دهی، با تراکم ۱۰ عدد در مخازنی با حجم ۵۰ لیتر در یک سیستم جریان آب یکطرفه و در شرایط یکسان و تحت کنترل برای همه تیمارها در غلظت‌های پیش بینی شده با مقادیر ppb ۱۵۰۰، ۱۸۵۰، ۲۳۰۰، ۲۸۵۰ و ۳۵۰۰ آفلاتوکسین B_1 و دو شاهد در ۳ تکرار برای هر تیمار بررسی شدند. تغذیه بچه ماهیان در تیمارهای آزمایشی و شاهد تا پایان آزمایش روزانه در ۳ نوبت و به مدت ۵۶ روز انجام شد. نتایج نشان داد که افزایش میزان غلظت سم تأثیر معنی‌داری بر درصد افزایش وزن (WG%)، بیومس نهایی، ضریب چاقی (CF)، نرخ رشد ویژه (SGR)، و بازماندگی داشت ($p<0.05$). در مطالعه فاکتورهای خونی و آنزیمی با افزایش مقدار سم در غذا تغییرات و اختلاف های معنی‌دار آماری در فاکتورهای خونی شامل WBC، RBC، HB و PCV% و آنزیم‌های کبدیALP، ALT، AST مشاهده شد ($p<0.05$). پس از ۵۲ روز در غلظت ppb ۳۵۰۰ بیش از ۵۰٪ بچه ماهیان تلف شدند. نتایج این بررسی نشان داد که ازون برون گونه‌ای نسبتاً مقاوم نسبت به سم آفلاتوکسین B_1 می‌باشد.

کلمات کلیدی: ازون برون پرورشی (*Acipenser stellatus*)، آفلاتوکسیکوزیس، رشد، بقا، آنزیم‌های کبدی، پارامترهای خونی.

***نویسنده مسئول:** asepahdari@yahoo.com