Effects of different dietary levels of AFB$_1$ on survival rate and growth factors of Beluga (*Huso huso*)

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Abstract
In the present study, the impacts of various concentrations of Aflatoxin B$_1$ (AFB$_1$) on Beluga, *Huso huso*, under controlled conditions were investigated. Belugas (120±10g) were fed on diets containing 0, 25, 50, 75, and 100 ppb AFB$_1$/kg of diet for 3 months. Results showed various levels of AFB$_1$ do not significantly affect the specific growth ratio (SGR) (P<0.05) of fish in different treatments. However, weight gain and food conversion ratio (FCR) were varied significantly (P<0.05 between control and treatments with diets contaminated with 75 and 100ppb AFB$_1$/kg after 90 days). The increase AFB$_1$ level of did not affect on the percent of survival rate(SR) and no mortality was observed in treatments (SR=100%) suggesting that various AFB$_1$ levels under experimental conditions of the present study affect some growth factors, such as, weight gain and FCR but have no significant impact on SR and SGR. Histopathological studies showed that different level of AFB$_1$ can cause broad range of change in liver tissue, including progressive fat deposition, hepatocyte degeneration and necrosis, particularly at concentration of 75 and 100ppb AFB$_1$/kg of diets after 60 days.

Keywords: *Huso huso*, AFB$_1$, FCR, SGR, SR, Pathology, Liver

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Introduction

The primary objective in fish nutrition is to provide a nutritionally balanced mixture of ingredients to support the maintenance, growth, reproductive performance, flesh quality and health of the animals at an acceptable cost. The diet also has an important effect on water quality and culture systems. In order to achieve these goals the diet must provide all required nutrients in the correct balance and must be formulated to keep any anti-nutritional components below concentrations that would impede the performance and health of the fish (NRC, 1993). Over the last 10 years plant-based ingredients have been increasingly used in fish diets. This change is the product of increased economic/market pressures on the fish meal and oil manufacturing industries and animal feed compounders, and the drive to produce lower cost, sustainable alternatives by the aqua feed manufacturing sector (Tacon, 2004). The extent of the damage produced by aflatoxins depends on the toxin concentration present in foods or feeds and also on the time period of exposure, as well as animal species susceptibility (Stewart & Larson, 2002). Within a given species, the magnitude of toxicity is influenced by age, sex, weight, diet, and exposure to infectious agents. Fry are more susceptible to aflatoxicosis than adults, and certain fish species are more sensitive than others (Royes & Yanong, 2002). Since the discovery of the nature of aflatoxins, aflatoxicosis has been investigated predominantly in freshwater aquatic species, especially in rainbow trout, (*Oncorhynchus mykiss*) (Halver, 1969; Lovell, 1989; Hendricks, 1994; Gallagher & Eaton, 1995) and, also, in American channel catfish (*Ictalurus punctatus*) (Lovell, 1984; Jantrarotai & Lovell, 1990; Jantrarotai et al., 1990; Plakas et al., 1991; Hendricks 1994; Gallagher & Eaton, 1995), Nile tilapia (*Oreochromis niloticus*) (Chavez-Sanchez et al., 1994; Tuan et al., 2002), Indian major carp (*Labeo rohita*) (Sahoo et al., 2001; Sahoo et al., 2003; Murjani 2003), mosquitofish (*Gambusia affinis*) (McKean et al., 2006), guppy (*Lebistes reticulatus*) (Sato et al., 1973), and in some invertebrate species such as *Penaeus monodon*, *Penaeus stylirostris*, *Penaeus vannamei* (Wiseman et al., 1982; Lightner et al., 1982; Boonyaratpalin et al., 2001), brine shrimp (*Artemia salina*) (Reiss 1972a) and a copepod (*Cyclops fuscus*) (Reiss, 1972b).

The only research regarding the effects of AFB$_1$ on sturgeon fish was carried out by Farabi et al. (2006) on juvenile beluga (*Huso huso*). Therefore, the purpose of the present study was to asses the effect of different levels of AFB$_1$ on the growth and survival rate of *Huso huso* as economical and native Iranian species to provide additional information to the current knowledge.

Materials and methods

Five hundred fish of approximately the same size (100±10g) were obtained from the Shahid Beheshti Sturgeon Rearing Centre in north of Iran. The fish were acclimated to 500 liter tank and fed on a control diet for 30 days before the AFB$_1$ experiment began. Twelve fish of approximately the same size (120g average)
were stocked into each of the 15 tanks which contained 200 liter of water. Water from a reservoir was passed continuously into tanks and water exchange was about 200% during 24 hours. Water temperature was maintained at 18±2°C. Aeration was provided by an air blower and distributed continuously to each tank through an air-stone to maintain dissolved oxygen at a level of at least 6 mg/l. Three tanks were randomly assigned to each of the five dietary treatments. The fish were fed 3% body weight three times a day at 08:00, 12:00, and 16:00h for 3 month. The remained feed and feces siphoned daily at 16:00 and tanks were cleaned weekly.

The experimental diets were formulated to contain 0, 25, 50, 75, and 100ppb AFB/kg diet from pure SIGMA product. Ingredients were mixed and then AFB₁ which was dissolved in Methanol was sprayed during mixings process, for five minutes on each experimental diet. Then diets extruded to produce semi moist pellets and moisture content decreased to about 10%, through drying. Dried diets were stored in paper bags in cold room until fed. The amount of AFB₁ in experimental diets, confirmed by HPLC prior to feeding.

Feed consumption was recorded daily. Fish were anesthetized in clove flower powder solution (250-300ppb in water) in two weeks intervals, then length and weight of each individual determined and recorded. Growth rate was determined as weight gain (g) at 2-weeks intervals. WG, FCR, SGR and SR were calculated as follows:

W.G. =W₁ – W₀

W₀ =Initial weight
W₁ = Final weight
F.C.R. = Dry feed consumption/W₁-W₀
S.G.R. =LnW₁-Ln W₀ / T (days of culture)
S.R. = (Number of live fish/ Initial number of fish) ×100

For histopathology, at the end of each month the liver were removed from three fish from each tank and fixed in Bouin’s solution, dehydrated in Isopropanol, cleared in Histosol, infiltrated in Paraffin, and sectioned at a thickness of 5µ. Sections were stained with hematoxylin and eosin (H & E), and examined with a light microscope.

Mean weight gain, feed conversion ratio, and specific growth rate were analyzed by Analysis of Variances. Duncan's multiple range tests were used to compare different treatment groups (P level = 0.05).

**Results**

Table 1 summarized average body weights of experimental fish after 90 days feeding with different levels of dietary AFB₁.

There was significant difference in average body weight (ABW) of fish between T₃ and T₄ with T₁ and control in third month biometry (P<0.05) (Table 1).
Table 1: Monthly comparison of the ABW ±SD in different treatments

<table>
<thead>
<tr>
<th>treatment</th>
<th>Control (25ppb)</th>
<th>T1 (25ppb)</th>
<th>T2 (50ppb)</th>
<th>T3 (75ppb)</th>
<th>T4 (100ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First month</td>
<td>362.2±42.9c</td>
<td>339.2±46.8abc</td>
<td>329.1±51.2abc</td>
<td>356.2±58.2abc</td>
<td>323.6±60.3f</td>
</tr>
<tr>
<td>Second month</td>
<td>484.3±54.7e</td>
<td>454.8±43.1def</td>
<td>429.2±58.2d</td>
<td>418.4±75.4d</td>
<td>429.2±65.8ed</td>
</tr>
<tr>
<td>Third month</td>
<td>502.4±51.3f</td>
<td>470.0±69.5g</td>
<td>443.4±69.7f</td>
<td>421.9±86.9f</td>
<td>431.5±76.9f</td>
</tr>
</tbody>
</table>

Common scripts show no significant differences at the 0.05 level.

Similar results were acquired for FCR and SGR (Table 2). There was significant difference between FCR in treatment two (diet contained 75ppb AFB1) and control diet (P<0.05). No significant difference was observed between other treatments. Also, there was no significant difference between treatments in relation with SGR. No mortality observed in experimental fish and survival rate was 100% in all treatments.

Tissue liver of the experimental fish in different treatments was subject to various changes (Figs. 1 & 2). The range of changes varied widely and these were chronic manifestation (Table 3).

Table 2: Comparing of FCR and SGR in experimental treatments after 90 days (±SD)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AFB1 levels (ppb)</th>
<th>SGR ±SD</th>
<th>FCR ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1</td>
<td>100</td>
<td>0.83±0.03</td>
<td>2.22±0.16ab</td>
</tr>
<tr>
<td>Diet 2</td>
<td>75</td>
<td>0.80±0.04</td>
<td>2.56±0.30a</td>
</tr>
<tr>
<td>Diet 3</td>
<td>50</td>
<td>0.89±0.04</td>
<td>2.02±0.20ab</td>
</tr>
<tr>
<td>Diet 4</td>
<td>25</td>
<td>0.91±0.04</td>
<td>1.88±0.13ab</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.94±0.02</td>
<td>1.70±0.07b</td>
</tr>
<tr>
<td>P -Value</td>
<td></td>
<td>0.034</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Common scripts show no significant differences at the 0.05 level.
Figure 1: Hemorrhage, fat deposition, degeneration of hepatocytes, mild necrosis, presence of MMC in liver parenchyma in T₁ at 2nd sampling (H&E ×40).

Figure 2: Degeneration of hepatocytes, necrosis, increase MMC and fibrocytes, granuloma like structure, T₄ at 2nd sampling (H&E ×20).

Table 3: Histopathological changes of the liver tissue in the present study

<table>
<thead>
<tr>
<th>Time/Head</th>
<th>First month</th>
<th>Second month</th>
<th>Third month</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Congestion, hepatocyte swelling, fat deposition</td>
<td>Hemorrhage, fat deposition, hepatocyte degeneration, mild necrosis, presence of MMC,</td>
<td>Hepatocyte degeneration, mild necrosis, increase MMC</td>
</tr>
<tr>
<td>T2</td>
<td>Congestion, fat deposition, hepatocyte degeneration, nuclear pyknosis and mild necrosis</td>
<td>Hemorrhage, fat deposition, hepatocyte degeneration, nuclear pyknosis and mild necrosis</td>
<td>Fat deposition, hepatocyte degeneration, focal necrosis, increase inflammatory cells and MMC</td>
</tr>
<tr>
<td>T3</td>
<td>Severe congestion, fat deposition, hepatocyte degeneration, nuclear pyknosis and mild necrosis</td>
<td>Fat deposition, hepatocyte degeneration, focal necrosis, increase MMC</td>
<td>Fat deposition, hepatocyte degeneration, confluent necrosis, increase MMC</td>
</tr>
<tr>
<td>T4</td>
<td>Severe congestion, fat deposition, hepatocyte degeneration, nuclear pyknosis and mild necrosis</td>
<td>Hepatocyte degeneration, confluent necrosis, increase MMC and fibrocytes, granuloma</td>
<td>Hepatocyte degeneration, confluent necrosis, increase MMC, granuloma, fibrosis</td>
</tr>
</tbody>
</table>
Discussion

Farabi et al. (2006) in a study on juvenile Huso huso reported 8.6% mortality during 15 days feeding contaminated diet (amount of contamination in this study was not defined). Similarly, there was a significant difference in the survival rate between Nile tilapia fed with the diet containing 100mg AFB/kg and the other diets (Tuan et al. 2002). In this study, survival of fish fed with 100mg AFB/kg was 55% after 6 weeks of feeding, and at the end of the feeding period (8 weeks) it was 40%. In contrast, in the present study, however, Beluga was fed with various levels of AFB$_1$ (25, 50, 75 and 100ppb/kg of diet) for three months at 18±2°C, no mortality occurred. This may be due to the sensitivity of sturgeon fish to aflatoxins. Further research on sensitivity of sturgeons is necessary to clarify this matter. Since early 1990s, channel catfish have been shown to be refractory to AFB$_1$ toxicity. Also, Coho salmon and Zebra fish have been reported to be resistant to AFB$_1$ hepatocarcinogenesis (Jantrarotai & Lovell, 1990; Plakas et al. 1991; Hendricks, 1994; Gallagher & Eaton, 1995; Tsai, 1996). Experimental studies conducted in catfish exposed to a wide range of AFB$_1$ concentrations in diets (ranged from 100 to 2,154ppb AFB$_1$), revealed that there was no significant reduction in weight gains or histopathological findings (Jantrarotai & Lovell, 1990). Our study showed that there was significant reduction in weight gain in 75 and 100ppb concentrations of AFB$_1$ with control after two months and liver pathological changes from sever congestion to confluent necrosis is noticeable in all treatments after three month. Early clinical signs of acute aflatoxicosis, e.g., decreased growth performance, anaemia, liver and gastric necrosis, were observed in catfish only when levels of 10,000ppb AFB$_1$ were fed (Jantrarotai & Lovell, 1990). The 10-day median LD50 of AFB$_1$ for channel catfish by i.p. administration is 11.5mg/kg body weight (Jantrarotai et al., 1990), which is about 14- and 20-fold higher than the LD50 of rainbow trout by i.p. administration or oral administration, respectively.

Another fish species may be moderately resistant to AFB$_1$ is Nile tilapia, however, it is more susceptible than channel catfish to the acute effects of AFB$_1$ (Tuan et al. 2002). For instance, Chavez-Sanchez et al. (1994) reported that a dose of 30mg AFB/kg diet was not lethal to this species. When aflatoxin-contaminated feed (115.34ppb) was administered to Nile tilapia for 120 days, only chronic granulomatous inflammation was observed in the liver, with no tumor formation (Cagauan et al., 2004). These results were close to our finding in relation to lethal dose. However, we did not observe tumor formation but liver damages were more progressive in Beluga. Furthermore, Nile tilapia that were fed diets with 10mg AFB/kg for 8 weeks had 90% reduction in growth rates compared to 24% growth reduction in channel catfish fed the same amount (Jantrarotai & Lovell, 1990; Tuan et al., 2002).
According to Tuan et al. (2002) the intensity of adverse effects induced in Nile tilapia, by AFB$_1$ dietary administration for 8 weeks, increased proportionally along with the aflatoxin concentration ingested: fish fed diets containing 2.25mg AFB/kg had changes in growth rate, whereas 10mg AFB/kg produced hepatic lesions, and 100mg AFB/kg caused severe hepatic necrosis and 60% mortality, as reviewed by Gallagher and Eaton (1995).

Average body weight and FCR of Beluga were significantly affected by 75 and 100ppb AFB$_1$ in diet but not by 50ppb or less. Although, there was significant differences between FCR in treatment two (Diet with 75ppb AFB$_1$) with control diet and no statistical different observed between other treatments, but functional differences could have economical effects on large farm production. There was no significant difference in SGR between experimental treatments and control after three months. SGR has a close relation with weight gain, but it was not affected. Cha´vez-Sa{n}chez et al. (1994) reported similar findings for Nile tilapia; growth was not affected by the 0.94mg/kg diet, but was reduced by diets containing 1.88mg AFB/kg or higher. However, these authors reported that FCR was not affected by AFB levels as high as 30mg/kg. An inverse relation between AFB concentration and growth rate of Nile tilapia was reported by El-Banna et al. (1992).

In conclusion, diets containing 100mg AFB/kg were not lethal to Beluga, 25, 50, 75 and 100mg/kg caused different degree of hepatic lesions. The severity of hepatic lesions in 75 and 100ppb after two months suppressed appetite and food digestion which could affected weight gain and FCR significantly.

Aquatic species have shown dissimilar susceptibility to the hepatotoxic and carcinogenic effects of AFB$_1$ that depends on the particular species. The differential susceptibility seems to be correlated with interspecies variation in the biotransformation efficiency of AFB$_1$. Even though the problem of aflatoxosis in fish was discovered about 40 years ago, sudden outbreaks of fish mortality continue to be reported, suggesting that the problem is still misunderstood and that scant preventive measures have been adopted (Maria Pia Santacroce et al. 2008).

This was one of the first studies in regard to Huso huso and further studies are required for more details.

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