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ORIGINAL RESEARCH ARTICLE

# Effects of aflatoxin contaminated feed on the fingerlings of tilapia (*Oreochromis niloticus* Linnaeus, 1758)

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**ABSTRACT**

Aflatoxin contamination, particularly common in cultured fishes in Asian countries, are considered unsafe both for fish and human health. However, the presence of aflatoxin in cultured fish feed and their effect are still under estimated in Bangladesh. The present study aimed to assess the effects of aflatoxin on growth performance and residues in tilapia (*Oreochromis niloticus*) fingerlings. Fish feed were treated with several concentration of aflatoxin as 0 ppb ($T_0$, control), 25 ppb ($T_1$), 50 ppb ($T_2$) and 100 ppb ($T_3$) and fed the tilapia fingerlings (n=10) in individual glass aquaria (24×12×12 inch, 105-litre capacity) conditions for 12 weeks. Comparatively higher body length (cm) and weight gain (g) were observed in treatment $T_0$(1.68 and 4.98) and $T_1$(1.60 and 5.48) than those of treatment $T_2$ (1.31 and 4.06) and $T_3$(1.20 and 3.10), respectively. The specific growth rate (SGR) were almost similar in treatment $T_0$(52%), $T_1$ (51%) and $T_2$ (52%) whereas declined significantly ($p$<0.05) in $T_3$ (39%). Higher survival rate was also demonstrated in treatment $T_0$ (90%) and $T_1$ (90%) whereas significantly decreased in treatment $T_2$ (60%) and $T_3$ (40%). The residue of aflatoxin was not detected in $T_0$ and $T_1$. On the contrary, the residual effect in tilapia fingerling was evident in $T_2$ and $T_3$ treatment. The findings of the present study revealed that aflatoxin contaminated feed is harmful for the growth performance and survival of *O. niloticus* fingerlings. Further study is necessary to safeguard the aquaculture production as well as to produce healthy food for human consumption.



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## INTRODUCTION

Aflatoxins are toxic secondary metabolites produced from certain strains of fungi *Aspergillus flavus* and *A. parasiticus* under suitable temperature and humid conditions and mainly grows in improperly stored feeds having lower quality ingredients (Rao *et al*., 2020; Huang *et al*., 2014; Deng *et al*., 2010). Several studies have been reported the aflatoxin contamination in foodstuffs like nuts, cereals, and spices in many countries, mostly in Africa and Asia (Bankole *et al*., 2010; Soubra *et al*., 2009). According to Chen and Rawlings (2008), aflatoxins found in 96.1% of the 334 tested commercial feeds and raw materials collected from Asia. The major aflatoxin is $B_1$, $B_2$, $G_1$, and $G_2$ usually found together in foods and livestock feeds in various proportions (Benkerroum, 2020). However, $B_1$ ($AFB_1$) is the most prevalent and toxic for humans, land animals and aquatic organisms

(Santacroce *et al*., 2008; Han *et al*., 2008). The aflatoxin $AFB_1$ affects the growth, reproduction, immuno suppression, behavior of animals (Binder *et al*., 2007; Bintvihok *et al*., 2003). The aflatoxins can be taken up by human customs through the food chain which can impair the health of humans (Boonyaratpalin *et al*., 2001; El-Sayed and Khalil, 2009).

Aflatoxin $B_1$ ($AFB_1$) is the major mycotoxin that contaminates aquafeeds globally and mostly common in tropical countries and regarded as a causative agent in illnesses and the mortality of aquacultural species (Wu *et al*., 2019; Murjani, 2003). Aflatoxin might still be a serious concern in aquaculture because of the vast use of plant feedstuffs in diet formulations, and the spread of $AFB_1$ by lethal deposits in the fish may be a threat to humans as well (El-Sayed and Khalil, 2009; Manning *et al*., 2005; Raghavan *et al*., 2011). Many feed ingredients used in aquacultures, such as cottonseed, peanuts, corn, soybean, maize, rice, dried fish, shrimp, and fish meals, are frequently contaminated (Cagauan *et al*., 2004; Fegan, 2005; Spring and Fegan, 2005). The carcinogenic effect of aflatoxin $B_1$ has been studied in fishes such as salmonid, rainbow trout, channel catfish, guppy and Indian major carps (Wu, 1998; Murjani, 2003) and *Penaeus monodon* (Bautista *et al*., 1994). The effects of aflatoxin in fishes are directly linked to their level of feed intake and the age and species (Eaton and Groopman, 1994). Marine and freshwater cultured rainbow trout are extremely sensitive to single-dose $AFB_1$ and caused a substantial outbreak of hepatocellular carcinoma (Williams *et al*., 2009). On the other hand, channel catfish is much less responsive and affected only at high doses and resulted in reduced body weight gains, haematological abnormalities, and necrotic hepatocytes (Manning *et al*., 2005).

Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) commonly known as aquatic chicken (Jhingran and Pullin, 1985). Tilapia is one of the most important species for the 21st-century aquaculture and is produced in more than 100 countries (Diana *et al*., 2004). Interesting high yield tilapia production in Bangladesh was about 298062 metric tonnes in the 2013-2014 financial year (DoF, 2016). Besides this huge production sometime report rise to decrease tilapia production due to the outbreak of disease, nutritional deficiency, and other unknown causes. In Bangladesh, we provide pelleted feeds for feeding tilapia fishes which may produce inappropriate procedures for bagging, transport and storage. In addition to high temperature and humidity may help for the growing fungus. The potentiality of fungal growth may produce aflatoxicosis which is a serious health hazard for fishes like tilapia fingerling as well as humans. Very few reports are available on the toxicity of $AFB_1$ to cultured aquatic fish species in Bangladesh. Considering the above facts, the study aimed to asses the effects of aflatoxin on the growth and survival of tilapia fingerlings, and trace out the amount of aflatoxin residue presence in tilapia fingerlings (muscles, kidney and liver tissue).

## MATERIALS AND METHODS

As a part of the research, this experiment was done twelve (12) weeks from April 2016 to June 2016. The methodology followed and the materials used are described below.

### Preparation of aflatoxin-contaminated feed

The commercially available floating feed was collected from the fish feed market. The composition of the selected feed was crude protein (23%), fat (2.5%), fibre (4.5%) and moisture (10%). The aflatoxin (*Aspergillus flavus*) for this study was collected from the Bangladesh Council of Scientific and Industrial Research (BCSIR). Then different doses of aflatoxin such as, 25ppb, 50ppb and 100ppb were mixed with the selected feed for the experimental purposes. For this, the "spray gun" method was used to add aflatoxin on feed, where different doses were sprinkled over the feed and dried it overnight by a dryer. Afterwards, the contaminated feed was kept in airtight bottle favourable for the growth of moulds such as moist conditions and high temperatures. Some feed was also treated without aflatoxin (0) as a control. After 24 hours the feed sample was collected and the feed mixture was covered with a plastic sack. The final feed was labelled as $T_0$(Control feed), $T_1$(feed mixed with 25 ppb aflatoxin), $T_2$(feed mixed with 50 ppb aflatoxin) and $T_3$ (feed mixed with 100 ppb aflatoxin).

### Experimental design

A total of 12 leak proof glass aquaria (24×12×12 inch) of 105 litre capacity of water each were prepared at the laboratory of Fish Biology and Genetics, Sylhet Agricultural University (SAU). Then two filters and two air-stones were set in each aquarium to provide filtration and sufficient aeration during the experimental period. Four treatments including control were designed ($T_0$, $T_1$, $T_2$, and $T_3$) each with 3 replications ($R_1$, $R_2$ and $R_3$) according to completely randomized design (CRD) (Table 1).

### Stocking of tilapia fingerling and feeding with an aflatoxin-contaminated diet

The fingerlings of tilapia (*Oreochromis niloticus*) were collected from local fish hatchery named "Khidirpur Bohumukhi Khamar" near Khadimnagar, Sylhet. The average body length and weight of fingerling were 6.44-6.7 cm and 6.02-6.87g, respectively. The collected fishes were acclimatized in an aquarium for overnight. Then the equal number of tilapia fingerling was stocked in each of the aquariums. The fingerlings were fed by a previously prepared diet ($T_0$, $T_1$, $T_2$ and $T_3$) in accordance with the treatments and replications (Table 1). The feeding was performed three times in a day at an apparent satiation level of fishes. The water quality parameter was monitored and recorded during the study period as temperature (23.61 - 27.09° C), dissolved oxygen (5.16 - 6.07 ppm), and pH (7.77 - 7.87). All of the water quality parameters were found satisfactory in all aquaria.

### Sample collection and preparation

The tilapia fingerlings were collected from both control and experimental aquarium at day 7, 14 and 21 after the onset of the experiment. Three fingerlings were collected randomly from

each aquarium and subjected for measuring the different growth parameters. For analysis of aflatoxin residue in the fish body, the fish was kept into the refrigeration immediate after harvesting. Thereafter, they were transported to BSCIR laboratory using icebox for detection and quantification of aflatoxin residue in the fish body at different time intervals.

### Measurement of growth parameter

To calculate and monitor the various growth parameters, the fish were weighed individually, and zoometric measurements were taken at 7$^{th}$, 14$^{th}$, and 21$^{st}$-day intervals during the experiment. The following equations were used for the calculation of growth and survival rate of tilapia fingerlings.

$$\text{Mean weight gain (g)} = \text{Mean final weight} - \text{Mean initial weight}$$

$$\text{Mean length gain (cm)} = \text{Mean final length} - \text{mean initial length}$$

$$\text{Percent weight gain (\%)} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \times 100$$

$$\text{Percent length gain (\%)} = \frac{\text{Mean final length} - \text{Mean initial length}}{\text{Mean initial length}} \times 100$$

$$\text{Specific growth rate (SGR \%/day)} = \frac{\ln FWt - \ln IWt}{T} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Present number of fishes}}{\text{Total number of fishes}} \times 100$$

### Detection of aflatoxin in fish body

High-Performance Liquid Chromatography (HPLC) with fluorescence detector was used to detection and quantification of four main types of Aflatoxin: $B_1$, $B_2$, $G_1$, and $G_2$ in tilapia fingerling fish samples. The analysis was done in the laboratory of the Institute of Food Science and Technology (IFST) at BCSIR, Dhanmondi, Dhaka-1205. In brief, the samples from the tilapia fingerling were collected in accordance with the experimental regimes as day 7, 14, and 21 after the onset of the experiment. Muscle, liver, and kidney were taken and mixed homogenously to form a paste. Then 10 grams of paste was taken into the conical flask and added with 2 times of distilled water into the conical flask and weighed. Then 80 ml of acetone was added into the conical flask and mixed homogeneously for 30 minutes using a vortex. The sample passed through a filter paper (Whatman No.1) and taken into another conical flask. Then 10 ml filtered samples were taken into measuring cylinder. Thereafter, 2 ml of 10% lead acetate, 10 ml of methanol and distilled water were added to prepare a 150 ml solution. This solution was transferred to the vacuum manifold glass through "Bond Elut Reservoir" and "Bond Elut pH". Bond Elut pH is used to trace the aflatoxin. A pump was added to the SPE vacuum manifold (Supelco Visiprep) to dry the Bond Elut pH. When all the solution passed out, then 5 ml methanol and 5 ml distilled water were added to clean the Bond Elut Reservoir. Thereafter the vial tube is placed into the SPE vacuum manifold (SupelcoVisiprep). $SaSO_4$ and fluorescent were added to pass through the Bond Elut reservoir because $SaSO_4$ limits the water and fluorescent prevent colour compound. After that, it was placed in the dryer at 60°C for complete drying. Then aflatoxin was taken in vial tube from the mobile phase (Acetonitrile: Methanol: Water = 22.5:22.5:55) by using a micropipette and placed on the vortex machine for homogenous mixing. Then filtered the sample by using a syringe filter and transferred it to another vial. Thus the vial was prepared and injected 20 µL samples in HPLC column: C18 250mm (L) × 4.6 mm (ID) 10µ/5µ ( Alltech/Graces or equivalent). The fluorescent detector (Agilent, G1321A) was used to detect the aflatoxin from the injected vial and it was visualized in computer software, Agilent chem station for 3D system Rev.A.02.

### Statistical analysis

The data were expressed as the mean ± standard deviation of the means and analyzed by a one-way analysis of variance (ANOVA) using SPSS (Statistical package for social science, version, 20) software. Differences were considered statistically different at *P*-values< 0.05. The amount of aflatoxin in the fish body was determined through HPLC machine-reading and summarized the results in tabulated form.

**Table 1.** Layout of the experimental design with stocking densities and dietary doses of aflatoxin.

| Treatments | Replication | Stocking density of *O. niloticus* | Dose of aflatoxin (ppb) | Fed with assigned diet | Assigned name |
|---|---|---|---|---|---|
| $T_0$ (Control) | $R_1$ | 10 | 0 | Diet 1 (Control) | $T_0R_1$ |
| | $R_2$ | 10 | 0 | Diet 1 (Control) | $T_0R_2$ |
| | $R_3$ | 10 | 0 | Diet 1 (Control) | $T_0R_3$ |
| $T_1$ (Treatment 1) | $R_1$ | 10 | 25 | Diet 2 | $T_1R_1$ |
| | $R_2$ | 10 | 25 | Diet 2 | $T_1R_2$ |
| | $R_3$ | 10 | 25 | Diet 2 | $T_1R_3$ |
| $T_2$ (Treatment 2) | $R_1$ | 10 | 50 | Diet 3 | $T_2R_1$ |
| | $R_2$ | 10 | 50 | Diet 3 | $T_2R_2$ |
| | $R_3$ | 10 | 50 | Diet 3 | $T_2R_3$ |
| $T_3$ (Treatment 3) | $R_1$ | 10 | 100 | Diet 4 | $T_3R_1$ |
| | $R_2$ | 10 | 100 | Diet 4 | $T_3R_2$ |
| | $R_3$ | 10 | 100 | Diet 4 | $T_3R_3$ |

## RESULTS AND DISCUSSION

### Effect of aflatoxin on growth performance and survival of tilapia fingerling

In terms of economic standpoint, Aflatoxins contamination is one of the most severe problems for the livestock and feed industries (de Freitas Souza *et al*., 2020). Aflatoxin has known to hamper the growth performance of several fishes (Tuan *et al*., 2002; Abdelhamid, 2008; Selim *et al*., 2014; Mahfouz and Sherif, 2015). In the present study, it has also been observed that aflatoxin has an negative impact on the growth and survival of the studied fish species. It was found that the weight gain significantly decreased ($p$<0.05) in aflatoxin treated fishes as compared to the fish kept in control ($T_0$) condition. The lowest average body weight gain (3.10 gm) was observed in treatment $T_3$. On the contrary, the highest average body weight gain (4.98 gm) was recorded in fish under the $T_0$ treatment. The growth rate, specific growth rate, and percent body weight gain was also high in treatment $T_0$ and decreased gradually in treatment $T_2$ and $T_3$ (Table 2). A similar trend was also demonstrated in body length gain. It has been shown that the average body length gain and percent body length gain was also significantly decreased ($p$<0.05) in $T_1$, $T_2$, and $T_3$ in compare to the fish reared under $T_0$ (Table 1). The survival rate of different treatments was significantly different. The lowest survival rate was found in treatment $T_3$ (40%) and $T_2$ (60%). On the other hand, treatment, $T_0$ and $T_1$ were exhibited about 90% of the survival rate. The mortality rate was increased as the aflatoxin level increased in the dietary feed. Available data showed that the ingestion of low to moderate doses of $AFB_1$ over a long period caused significant growth decrease in Nile tilapia (Abdelhamid, 2008; Selim *et al*., 2014). According to Mahfouz and Sherif (2015), the exposure of AFB1 at 100 ppb for 6 or 12 weeks has significantly reduced growth indices (total weight gain, average daily gain and relative growth rate) but not the survivability, in comparison with the exposure of 20 ppb. Cagauan *et al*. (2004) found different levels of aflatoxin contamination did not significantly ($p$>0.05) affect the final average length, weight and weight gain of fish but percent survival of fingerlings was significantly ($p$<0.001) influenced by aflatoxin level. The aflatoxin ($AFB_1$) had a negative impact on tilapia weight gain and feed efficiency over a relatively short period of 10 weeks (Zychowski *et al*., 2013). The present study found similar to the previous study (Ruby *et al*., 2013) where aflatoxin-contaminated feed significantly declined the growth and survival rate of *Labeo rohita*. The study reveals that aflatoxin contaminated feed decreases the growth performance of tilapia fingerling.

**Table 2.** Effect of aflatoxin treatment on the growth parameters of tilapia fingerlings.

| Growth parameters | Treatments | | | |
|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ |
| Body length (Initial) | 6.81±0.15 | 6.7±0.04 | 6.44±0.22 | 6.7±0.04 |
| Body length (Final) | 8.49±0.32 | 8.3±0.13 | 8.01±0.16 | 7.9±0.27 |
| Average body length gain | 1.68 | 1.60 | 1.31* | 1.2* |
| % Body length gain | 24% | 24% | 20%* | 17%* |
| Initial body weight (g) | 6.70±0.12 | 6.02±0.56 | 6.87±0.29 | 6.75±0.17 |
| Final body weight (g) | 11.68±0.69 | 11.50±0.51 | 10.93±0.06 | 9.85±1.14 |
| Mean body weight gain | 4.98 | 5.48 | 4.06 | 3.10* |
| % Body weight gain | 74.32 | 91.02 | 59.09* | 45.92* |
| Specific growth rate (%) | 52 | 51 | 52 | 39* |
| Survival rate (%) | 90 | 90 | 60* | 40* |

Values are mean ± Std. of fishes from each treatment and asterisks indicate significant change*($p$<0.05).

**Table 3.** Variation in HPLC detection of aflatoxin ($B_1$, $B_2$, $G_1$, and $G_2$) in tilapia fingerlings due to feeding of different dietary level of aflatoxin contaminated feed.

| Name of the sample | (Paste of kidney, liver and muscle) | Feeding diets | Test interval (7/14/21 days) | Detection of aflatoxin (ppb) | | | | Total aflatoxin (ppb) |
|---|---|---|---|---|---|---|---|---|
| | | | | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ | |
| Tilapia (*Oreochromis niloticus*) | 10 gm | $T_0$ | 7 | - | - | - | - | ND |
| | 10 gm | $T_0$ | 14 | - | - | - | - | ND |
| | 10 gm | $T_0$ | 21 | - | - | - | - | ND |
| | 10 gm | $T_1$ | 7 | - | - | - | - | ND |
| | 10 gm | $T_1$ | 14 | - | - | - | - | ND |
| | 10 gm | $T_1$ | 21 | - | - | - | - | ND |
| | 10 gm | $T_2$ | 7 | - | - | - | - | ND |
| | 10 gm | $T_2$ | 14 | 20.859 | 0.124 | - | - | 20.983 |
| | 10 gm | $T_2$ | 21 | 8.947 | - | - | 1.223 | 10.172 |
| | 10 gm | $T_3$ | 7 | - | - | - | - | ND |
| | 10 gm | $T_3$ | 14 | 22.007 | 0.206 | - | 1.656 | 23.869 |
| | 10 gm | $T_3$ | 21 | 13.077 | 0.213 | - | 0.702 | 13.992 |

ND: Not detected.

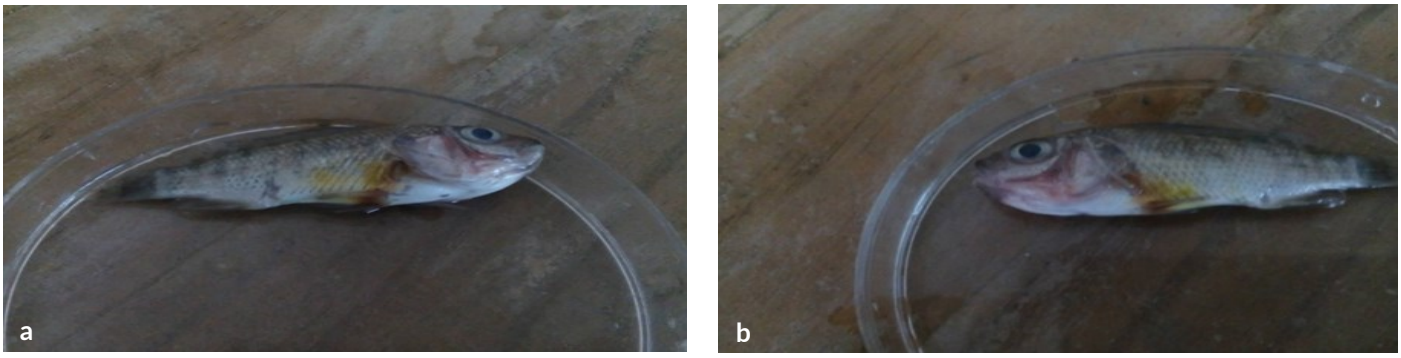


**Figure 1 (a, b).** *Morphological change (yellow color) on the body surface of the tilapia fingerling due to the feeding of aflatoxin contaminated feed during the study period.*

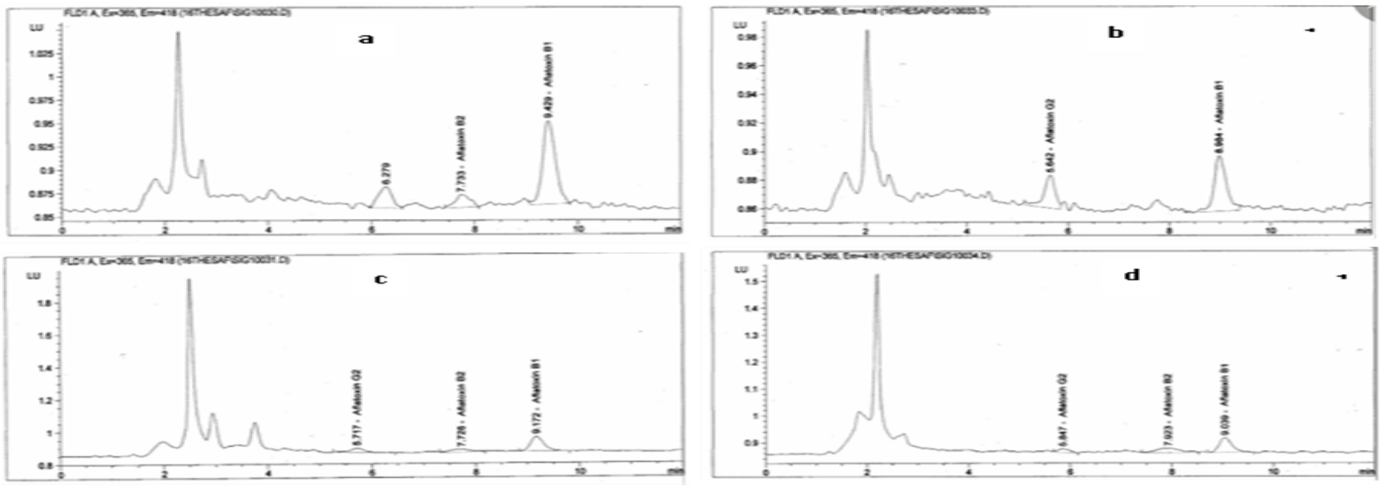


**Figure 2.** *Chromatograms showing the aflatoxin residue in O. niloticus fingerlings in 14 and 21 days intervals at the dose of 50 ppb aflatoxin (a and b) and 100 ppb (c and d), respectively.*

## Morphological changes of tilapia fingerling due to aflatoxin treatment

Several morphological changes were notified in the tilapia fingerlings during the aflatoxin treatment period. The key observed external manifestations in tilapia fingerling were abnormality in feeding, eye opacity leading to cataract and blindness, fin and tail rot, yellowing of the body surface of the fish (Figure 1), irregular swimming, weak and less movement. These abnormalities were more intense in the higher dietary level of aflatoxin treated fish ($T_3$) whereas these symptoms were not shown in treatment $T_0$ as the fish were not consumed any aflatoxin contained feed in this treatment. The low dose of aflatoxin did not show any immediate effect in tilapia fingerlings while high dose demonstrated both external and internal abnormalities in tilapia fingerling similar to the findings of another study (Chavez *et al.*, 1994). The previous studies found behavioural changes in tilapia fingerling (Cagauan *et al.*, 2004) and silver catfish (Anater *et al.*, 2020). The high doses of aflatoxin contamination in feed samples were assumed to be responsible for those kinds of external and internal manifestation in fishes (Wu, 1998; Royes and Yanong, 2002). The present study also in agreement with the study of El-Boshy *et al.* (2008) and Ruby *et al.* (2013).

## Detection and quantification of aflatoxin in tilapia fingerling

The presence of aflatoxin residues in fish muscle is considered a very dangerous problem for food safety as well as human health (Wild and Gong, 2010). The present study quantified the residue of aflatoxin ($AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$) in tilapia fingerling by using High-Performance Liquid Chromatography (HPLC) method. The results indicated that the treatment $T_1$(feeding with 25 ppb aflatoxin) has no aflatoxin residue in fish samples within different sampling periods at day 7, 14 and 21 after treatment of the tilapia fingerlings. In treatment $T_2$(feeding with 50 ppb aflatoxin), aflatoxin residue was observed at days 14 (20.983 ppb) and 21 (10.172 ppb) while there was no aflatoxin residue in the tilapia fingerling at day 7 reared with the same diet containing aflatoxin. Similarly, when the fingerlings were reared with 100 ppb aflatoxin contaminated feed, it has been demonstrated that the fish did not show any residue of aflatoxin at day 7 whereas it was detected within the days of 14 and 21 at the concentration of 23.86 ppb and 13.99 ppb, respectively (Table 3 and Figure 2). This means that the residue of aflatoxin increased in tilapia fingerlings with increasing the dose of aflatoxin contaminated feed. The main target organ for aflatoxin toxicity is the liver, at first aflatoxin absorbed from the diet and passed to different organs (Abdel-Wahhab *et al.*, 2007). The majority of the studies demonstrated higher $AFB_1$ residue in liver tissue in comparison to the muscles of the fishes (Bintvihok *et al.*, 2003; Tuan *et al.*, 2002). The $AFB_1$ residues were detected in the liver of Nile tilapia at 20, 100 ppb aflatoxin level for 6 to 12 weeks (Mahfouz and Sherif, 2015). The aflatoxin $AFB_1$ accumulation in Nile tilapia (*O.niloticus*) and Gibel carp (*Carassius auratus gibelio*) muscles were only detected in fish exposed to the highest inclusion level of $AFB_1$ (Huang *et al.*, 2014; Hussain *et al.*, 2018).

## Conclusion

The findings of the present study revealed that the tilapia fingerlings might able to tolerate the immediate effect of aflatoxin whereas in a later stage the fishes showed external and internal abnormalities and the residue of aflatoxin was mainly observed in fish muscles, liver and kidney. High dose and long-time exposure are mostly responsible for aflatoxin toxicity in tilapia fingerling. However, it can be concluded that the aflatoxin contaminated feed has a negative impact on the growth and survival rate of tilapia fingerling which may accelerate the loss of productivity in the aquaculture system. Moreover, the aflatoxin metabolites found in edible fish muscle and liver, which might be toxic to the human body by biological accumulation through the food chain. It is assumed that the improper feed milling, storage procedure, and unhygienic practice are responsible for the fungal contamination in a tropical country like Bangladesh. Use of well-dried ingredients in producing fish and animal feed, and stored fish feed properly for preventing the growth of fungus. Thus the government authority needs to monitor to safeguard healthy aquaculture feed production. Further study is needed for mass detection of aflatoxin contamination in the commercially available fish feeds in Bangladesh.

## Conflicts of interest

The authors declare that they have no conflict of interest.

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