AN OUTBREAK OF AFLATOXICOsis IN CLARIAS GARIepinus, A MYCOLOGICAL, BIOCHEMICAL AND HistoPATHOLOGICAL STUDIES

DOAA A.H.¹; RAWIA S.M. ADAWY²; KHEDER Z.A.³ and K.A.DEEB⁴

¹Department of Mycology, Animal Health Research Institute, Dokki, Giza, Egypt, El Mansoura Provincial lab.
²Department of Fish Diseases, Animal Health Research Institute, Dokki, Giza, Egypt, El Mansoura Provincial lab.
³Department of Biochemistry, Animal Health Research Institute, Dokki, Giza, Egypt, El Mansoura Provincial lab.
⁴Department of Clinical Pathology, Animal Health Research Institute, Dokki, Giza, Egypt, El Mansoura Provincial lab.

Received: 31 December 2016; Accepted: 30 January 2017

ABSTRACT
A total of one hundred and twenty cat fish (Clarias gariepinus) were collected from two private fish farms in Dakahlia governorate during summer, 2016. We collected fifty diseased fish from first farm which suffered from cumulative mortality and seventy healthy fish from second farm. All collected fish were subjected to clinical, postmortem, histopathological and mycological examination. Aflatoxins were extracted and quantitated from feed and musculature of diseased and healthy fish (5 samples for each) using HPLC. Blood samples were collected for biochemical and hematological parameters. The clinical observation revealed that the diseased fish showed opacity of the eyes, paleness of the gills with presence yellowish greenish infiltration, hemorrhagic, yellowish skin and fins, and the fish were slow in motion. The postmortem examination showed enlargement of the gall bladder, distention of the stomach, yellow/ brownish enlarged liver, swollen, dark red and friable kidneys. Whereas the histopathological examination revealed a severe degenerative and necrotic changes in most internal organs of diseased fish. On the other hand mycological examination of diseased fish yielded isolation of Aspergillus flavus (42%), A. parasiticus (34.8%), A. niger (12%) and A. fumigatus (10.9%). While no fungi was isolated from any organs of healthy fish. The mean levels of aflatoxins in feed of diseased fish was 108.9±0.77 ppb (46.5±0.75 ppb AFB₁, 1.4±0.11 ppb AFB₂, 58.9±0.38 ppb AFG₁, 2.1±0.24 ppb AFG₂), while the mean levels of AFs residues detected in musculature of diseased fish was 20.9±0.39 ppb (11.3±0.25 AFB₁, 0.15±0.007 AFB₂, 8.9±0.20 AFG₁, 0.5±0.44 AFG₂). Whereas Aflatoxins not detectable in feed or muscles of healthy fish.

Keywords: Aflatoxins, Clarias gariepinus mycological examination, biochemical parameters, histopathological changes.

INTRODUCTION
Fish is the most important source of protein, calcium and phosphorus for human consumption especially in African country (Kumolu-Johnson and Ndimels, 2011). Intensive aquaculture conditions can promote the transmission of fungal diseases led to off flavor, offensive odor and unpalatable taste as well as severe economic losses. Moreover mycotoxin producing fungi species is transmitted in aquacultures (Ali et al., 2011). Penicilli and Aspergilli were recorded as the most common fungi present in high number in water bodies associated with sediment, and biofilms (Goncalves et al., 2006). Toxigenic Aspergillus flavus strains that possess all necessary genes foraflatoxins biosynthesis produce either AFB₁ and / or AFB₂. Aspergillus parasiticus strains produce AFG₁ and AFG₂, in addition to AFsB (Eaton and Groopman, 1994, Var and Kabak, 2004, Milita et al., 2010 and Huang et al., 2011). The four major naturally known aflatoxins include AFB₁, AFB₂, AFG₁, AFG₂ (Trasher and Crawley, 2009, Reddy and Waliyar 2012). The increase in the incorporation vegetal ingredients into fish feed formula enlarges the risk of contamination coming from that origin namely fungi and their mycotoxins. Aflatoxins are polycyclic aromatic hydrocarbons toxin that known as a hepatocarcinogen in various animal species including fish, birds, rodents and non-human primates (Abbott, 2002, Abdel – Wahhab et al., 2002, Bintvinhok, 2002 and Allameh et al., 2005). AflatoxinB₁ is the most frequent of all aflatoxins in contaminated food (Kennedy et al., 1998, Hussein and Brasel, 2001, Keller 2007, Martin et al., 2008, Cavaglieri et al., 2011).
Aflatoxins are heat resistance thus the presence of the toxin residues in fish tissues is very dangerous for human even after processing the fish (Galvano et al., 2005 and Jay et al., 2005). All animal species is not resistant to the acute toxic effects of aflatoxins. Animal species, environmental factors, exposure level and duration of exposure beside age, health and nutrition status of diet can influence the toxicity (Cong et al., 2003, Turner et al., 2003 and Thrasher, 2012). Young fish are more vulnerable, warm water species are generally less sensitive to AFB1 than cold water species. The sensitive benchmark species is the rainbow trout, which has an LD50 of 0.5 – 1 ppm of AFB1 in feed (Lovell, 1989). While other species including the warm water channel cat fish can tolerate increased levels up to around 10 ppm (LD50 11.5 ppm). The clinical aflatoxin cases in fish caused severe external lesions as cloudy eye, yellow greenish infiltration near the gills, erosion of caudal fins and abdomen, slow motion and greater opercular movement due to increase oxygen demand, thicken mucus (Cagauan et al., 2004). Aflatoxins cause significant increase in liver enzymes as Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline Phosphatase with significant decrease in total protein, potassium, sodium and chloride (Fernandez et al., 1995, Sahoo and Mukherjee, 2001, Rauber et al., 2007, El Sayed and Khalil, 2009 and Oluwafemi and Dahunsi, 2009). Joner et al. (2000) reported that aflatoxin reacts negatively with different cell protein which lead to inhibition of carbohydrates and lipid metabolism and protein synthesis. So, the decrease in growth rate may be due to disturbance in metabolic process (Abdelhamed, 2008 and Selim et al., 2013).

Therefore the aim of this study to isolate pathogenic fungi, detect the levels of aflatoxins in fish feed and muscle and evaluate the biochemical and pathological changes in diseased fish.

MATERIALS AND METHODS

Fish samples:
A total of one hundred and twenty cat fish of 750 gm – 1 kg obtained from two private extensive farm in Dakahlia governorate during summer, 2016. Fifty fish were diseased and collected from the first farm which suffered from cumulative mortality while seventy healthy fish were obtained from the second farm. All fish were transported alive (with acid electric oxygen operators and the save parameters of water to minimize all stress effect) or freshly dead in plastic bags to fish laboratory of animal health research institute, El Mansoura for clinical examination, mycotic isolation and identification.

Feed and muscles samples: five samples of feed and five samples of muscles were collected from each diseased and healthy fish for detection of aflatoxins.

Mycological examination:
Fish were washed in running water to remove of sediment, samples were taken from kidney, liver, muscle and inoculated onto duplicate plates of Sabouraud's dextrose agar incubated at 25°C,37°C for at least 2 weeks during which the growing fungal species were examined and identified. Macroscopic examination was carried out using the morphological characters of colonies including gross appearance of culture, rate of growth, texture and color of the colonies (above and reverse side) following the identification keys according to (Raper and Fennel, 1965; Dovorak and Actenasek, 1969 and Samason et al., 2000).

Detection of aflatoxinsin feeds and muscles of fish:
The aflatoxins from musculature of fish were extracted and measured by HPLC (Jemmali and Murthy, 1976). It consists of extraction of AFs samples with methanol, treatment of the residue with a mixture of dimethoxy methane– methanol evaporation of dimethoxymethylene followed by a liquid –liquid defatting with hexane before the transfer of AFs into chloroform. The chloriform extracts are further purified by silica gel- acidic alumina-anhydrous sodium sulfate column chromatography. While Feed samples were done according to Akyama et al. (1996). Quantification of AFs was done by using high performance liquid chromatography (HPLC). Agilent Series 1050 quaternary gradient pump, Series 1050 auto sampler, Series 1050 FLD detector, and HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France). The chromatographic separation was performed with a reversed-phase column (Extend-C18, Zorbax column, 4.6 mm i.d., 250 mm, 5um, Agilent Co.). Sample extraction were analyzed isocratically using 60:20:20 water/methanol/ acetonitrile mixture as the mobile phase. The column temperature adjusted at 30 °C at a flow rate of 1.0 mL/min to achieve the optimum resolution of the aflatoxins. The injection volume was maintained at 20 µL for both the sample and standard.

Biochemical parameters:
The blood samples were collected from 40 fishes (20 diseased and 20 apparently healthy) the collected samples were divided into 2 portions, the first was added to anticoagulant (EDTA) for measurement of
The results in table (3) showed that the liver was the site of predilection for infection with the fungi \(A.\text{flavus} \) 40.6\%, \(A.\text{parasiticus} \) 52.6\%, \(A.\text{niger} \) 50\%, \(A.\text{fumigatus} \) 44.4\% followed by muscles (33.3\%, 38.6\%, 40\%, 27.8\%) respectively and latter on the kidney (26\%, 8.8\%, 10\%, 27.8\%) respectively.

On the other hand the results in table (4) revealed isolation of 164 isolates of Aspergillus species from diseased fish which identified as \(A.\text{parasiticus} \) (42\%), \(A.\text{parasiticus} \) (34.8\%), \(A.\text{niger} \) (12\%) and \(A.\text{fumigatus} \) (10.9\%). \(A.\text{flavus} \) is the most common species isolated from diseased fish followed by \(A.\text{parasiticus} \), \(A.\text{niger} \) finally \(A.\text{fumigatus} \). While the mycological examination of healthy fish recorded absence of fungal isolates from all organs.

Results in table (5) showed that the mean levels of AFs in feed of diseased fish were 108.9±0.77ppb (46.5±0.75 ppb \(A\text{FB}_1 \), 1.4±0.11ppb\(A\text{FB}_2 \), 58.9±0.38ppbAFG\(_1 \) and 2.1±0.24 ppbAFG\(_2 \)).

The results in table (6) shown that, AFs residues in musculature of diseased fish were at level of 20.9 PPb (11.3ppb, 0.15ppb, 8.9ppb and 0.5ppb) respectively. While aflatoxins not detectable in feed or muscles of healthy fish.

Regarding the histopathological findings in diseased fish, in skin samples the epidermal cells showed proliferation of mucus and alarm cells with subepithelial leukocytic infiltration, melanomachrophage proliferation and dermal edema (Fig.7) while in skullle muscle, Inter and intramuscular edema with degeneration and mild leukocytic infiltration were encountered (Fig.8). Whereas, all the hepatic cells of the liver suffered from necrotic or degenerative changes which characterized by vaculation of cytoplasm and pyknotic or karyolitic nuclei (Fig 9). On the other hand, the renal tubular epithelia of kidney suffered from degenerative changes mainly hydropic or hyaline degeneration beside depletion of hemopoietic centers (Fig10) and mild leukocytic infiltration (Fig11). While regenerative attempts in the renal parenchyma adjacent to necrotic and degenerated renal tissues were prevalent (Fig12). The mucus of the stomach and intestine showed hyperplasia of goblet cells or desquamation of their lining epithelium with presence of excess mucus and intense submucosal leukocytic infiltration mainly lymphocytes and the latter usually invade the muscular coat (Fig13). While some muscle fibers in the tunica muscularis may showed partial degeneration or necrosis. Considering the pathological finding in gills, the Gills arch showed edema, leukocytic infiltration mainly lymphocytic type (Fig14).
Table 1: Mean values of some serum biochemical parameters of Clarias gariepinus N=20 for each group.

<table>
<thead>
<tr>
<th>Item</th>
<th>Parameters</th>
<th>Healthy fish</th>
<th>Diseased fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.total protein (g/dl)</td>
<td>3.7±0.24</td>
<td>2.1±0.09 *</td>
<td></td>
</tr>
<tr>
<td>S.Albumin (g/dl)</td>
<td>2.9±0.19</td>
<td>2.2±0.11 *</td>
<td></td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>0.71±0.10</td>
<td>1.22±0.11 **</td>
<td></td>
</tr>
<tr>
<td>S. AST (Iu/I)</td>
<td>161±3.60</td>
<td>183±5.38 **</td>
<td></td>
</tr>
<tr>
<td>S. ALT (Iu/I)</td>
<td>25.00±1.14</td>
<td>36.3±1.32 ***</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>82.5±3.11</td>
<td>68.4±2.64 **</td>
<td></td>
</tr>
</tbody>
</table>

*=significant at P < 0.05 **=high significant at P < 0.01 ***=very high significant P < 0.001

Table 2: Mean values of some hematological parameters of Calarias gariepinus (N=20 for each group).

<table>
<thead>
<tr>
<th>Item</th>
<th>Parameters</th>
<th>Healthy fish</th>
<th>Diseased fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB (g/dl)</td>
<td>8.90±0.44</td>
<td>7.10±0.38 **</td>
<td></td>
</tr>
<tr>
<td>RBCs (x10^6/mm3)</td>
<td>2.14±0.16</td>
<td>1.44±0.23 *</td>
<td></td>
</tr>
<tr>
<td>WBCs (x10^3/mm3)</td>
<td>30.67±0.92</td>
<td>38.06±1.07 ***</td>
<td></td>
</tr>
</tbody>
</table>

#=significant at P < 0.05 **=high significant at P <0.01 ***=very high significant at P < 0.001

Table 3: Prevalence of isolated mould from different organs of diseased catfish (n=50).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Organ</th>
<th>Liver</th>
<th>No</th>
<th>%</th>
<th>Kidney</th>
<th>No</th>
<th>%</th>
<th>Muscle</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Flavus</td>
<td></td>
<td></td>
<td>28</td>
<td>40.6</td>
<td>18</td>
<td>26</td>
<td>33.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. parasiticus</td>
<td></td>
<td></td>
<td>30</td>
<td>52.6</td>
<td>5</td>
<td>8.8</td>
<td>22</td>
<td>38.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td></td>
<td></td>
<td>10</td>
<td>50</td>
<td>2</td>
<td>10</td>
<td>8</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td></td>
<td></td>
<td>8</td>
<td>44.4</td>
<td>5</td>
<td>27.8</td>
<td>5</td>
<td>27.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Incidence of isolated mould genera from the diseased cat fish (n=50).

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>A. flavus</th>
<th>A.parasiticus</th>
<th>A.niger</th>
<th>A.fumigatus</th>
<th>Total No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>(42%)</td>
<td>(34.8 %)</td>
<td>(12%)</td>
<td>(10.9%)</td>
<td>164</td>
</tr>
</tbody>
</table>

Fig. (1): Chromatography of total aflatoxins standards, AFB1 eluted at Retention time (tR)=5.122 min., AFB2 tR=9.269, AFG1 tR=6.379, AFG2 tR=12.604

Table 5: The mean level of AFs (ppb) in feed of diseased cultured Calarias gariepinus (N=5).

<table>
<thead>
<tr>
<th>Total AFs</th>
<th>AFB1</th>
<th>AFB2</th>
<th>AFG1</th>
<th>AFG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>107.5</td>
<td>44.4</td>
<td>1.3</td>
<td>59.5</td>
</tr>
<tr>
<td>2</td>
<td>110.6</td>
<td>47</td>
<td>1.1</td>
<td>60.1</td>
</tr>
<tr>
<td>3</td>
<td>110.9</td>
<td>49</td>
<td>1.5</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>108.2</td>
<td>46</td>
<td>1.5</td>
<td>58.6</td>
</tr>
<tr>
<td>5</td>
<td>107.3</td>
<td>46</td>
<td>1.8</td>
<td>58.4</td>
</tr>
<tr>
<td>Mean</td>
<td>108.9±0.77</td>
<td>46.5±0.75</td>
<td>1.4±0.11</td>
<td>58.9±0.38</td>
</tr>
</tbody>
</table>

The permissible limit of AFs must not more than 15 ppb according to (WHO, 1979) and not more than 20 ppb according to (FAO,1995)
Table 6: The mean level of AFs (PPb) residues in musculature of diseased cultured Clarias gariepinus (N=5).

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>AFs</th>
<th>AFB1</th>
<th>AFB2</th>
<th>AFG1</th>
<th>AFG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.05</td>
<td>11.9</td>
<td>0.15</td>
<td>9.6</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>20.03</td>
<td>10.9</td>
<td>0.13</td>
<td>8.4</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>20.7</td>
<td>11.4</td>
<td>0.14</td>
<td>8.7</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>21.5</td>
<td>11.7</td>
<td>0.16</td>
<td>9.2</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>20.07</td>
<td>10.5</td>
<td>0.17</td>
<td>8.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Mean</td>
<td>20.9±0.39</td>
<td>11.3±0.25</td>
<td>0.15±0.007</td>
<td>8.9±0.20</td>
<td>0.5±0.44</td>
</tr>
</tbody>
</table>

Figure (2): Skin and muscles of Clarias gariepinus showing yellowish colour and hemorrhage on skin and fins, gall bladder was enlarged and congested.

Figure (3): Female Clarias gariepinus showing congested and yellowish ovary.

Figure (4): Kidney of Clarias gariepinus showing enlargement, friable and congested.

Figure (5): Female of Clarias gariepinus showing enlarged, friable and yellowish liver and gills pale in color.

Figure (6): Male of Clarias gariepinus showing spleen was enlarged and congested. Tests were congested.
Figure (7): Skin of Clarias gariepinus fed on contaminated ration with AFB1 showing proliferation of alarm and mucus cells in epidermis and dermal edema. H&E X120

Figure (8): Skeletal muscle of Clarias gariepinus fed on contaminated ration with AFB1 showing mild leucocytic infiltration, hyaline degeneration or necrosis of some muscle fibers. H&E X300

Figure (9): Liver of Clarias gariepinus fed on contaminated ration with AFB1 showing vacuolar degeneration or necrotic changes of the hepatic cell. H&E X300

Figure (10): Kidney of Clarias gariepinus fed on contaminated ration with AFB1 showing necrosis of tubular epithelium and depleted hemopoietic tissues. H&E X300

Figure (11): Kidney of Clarias gariepinus fed on contaminated ration with AFB1 showing mild leucocytic cells infiltration of renal tubular epithelium. H&E X300

Figure (12): Kidney of Clarias gariepinus fed on contaminated ration showing regenerative attempts in some tubular epithelium. H&E X300

Figure (13): Stomach of Clarias gariepinus fed on contaminated ration with AFB1 showing submucosal leukocytic aggregations, excess mucus and goblet cells. H&E X120

Figure (14): Gills of Clarias gariepinus fed on contaminated ration with AFB1 showing edema and leukocytic infiltration in gill racker. H&E X120
DISCUSSION

Diseased fish suffered from signs of aflatoxicosis aspaleseness of the gills, with presence of yellowish greenish infiltration, hemorrhage and yellowish color of the skin and fins these signs were in agreement with those obtained by (Hussein et al., 2000, Soliman et al., 2000, Abdelhamid et al., 2002b and Xinxia et al., 2016). The post mortem lesions showed enlarged stomach, gall bladder and liver, the liver become yellowish and friable. Kidney become darken swollen and friable while viscera covered with thick layer of mucus. These findings were also reported by (Cagauan et al., 2004 and Mehrim et al., 2006). The mortality rate in diseased fish farm is about 45% (Abdelhamid et al., 2002a and Durre et al., 2013). The clinical signs, post mortem lesions of liver and kidney damage indicated aflatoxicosis which confirmed by biochemical, mycological, histopathological examination, as well as extraction of aflatoxins from feed and muscles of diseased fish.

The results in table (1): Showed a significant decrease in total protein of diseased fish compared to healthy ones, decrease total proteins are common in gills, liver and kidney damage due to ingestion of AFB1. Also there was a significant decrease in serum albumin. The liver is responsible for clearing the blood from bilirubin, so liver damage can result in pale yellow color of fish body which described as possible symptoms of mycotoxicosis in fish (Oluwafemi and Taiwo, 2004, Rauber et al., 2007 and Xinxia et al., 2016). There is a high significant increase in serum creatinine of diseased fish which indicated kidney damage. AFs have the ability to inhibit several metabolic systems such as protein synthesis thus leading to liver, kidney and heart damage (Sharma et al., 2011, Soliman et al., 2012). The liver enzymes of infected fish showed a high significant increase in aspartate aminotransferase (AST) and very high significant increase in alanine aminotransferase (ALT) in comparison with normal fish. As in animals, in fish some enzymes can be used as indicators of hepatotoxic damage, one of such enzyme is (AST) which is found in high concentration in liver parenchymal cells, tissue damage cause release of AST also ALT is an enzyme which is present in hepatocytes (liver cells) when a cell is damaged, it leaks this enzyme into the blood and rises dramatically in acute liver damage or hepatic necrosis (Oluwafemi and Taiwo, 2004, Oluwafemi and Daushi, 2009, Varior and Philip, 2012 and Magdy and Ahmed, 2015). The decrease in serum glucosehas been also described as valuable parameters of hepatic injury and function (Mathur et al., 2000, Kasmani et al., 2012).

The decrease in Hb concentration and RBCs count of diseased fish were confirmed with the results obtained by (Wimol et al., 1990, Islam, 2004, Reddy and Waliyar, 2012, Magdy and Ahmed 2015 and Xinxia et al., 2016). The decrease in Hb and RBCs indicated anemia, possibly due to erythropoiesis, hemesynthesis and osmoregulatory dysfunction that led to inhibit the activities of several enzymes involved in heme biosynthesis (ATSDR, 2005). As well as aflatoxins cause abnormal decrease level of protein, these protein have been associated with hemolysis and increased break down of red blood cells (Jenkins and Smith, 2003). The increase in WBCs which is mainly neutrophils suggested that the toxin is eliciting an inflammatory response, and in turn causes alteration in bone marrow and the function of the immune system (Sharma et al., 2011).

The current results in table (3) showed that liver is the site of predilection for infection of fungi followed by muscles while kidney is the lowest infected organ. These results were in agreement with those obtained by (Ali et al., 2004).

On the other hand the results in table (4) revealed isolation of 164 isolates of Aspergillus species from infected fish A.flavus is the most common species followed by A. parasiticus, A.niger finally A. fumigatus, nearly similar isolates were reported by (Allinezhad et al., 2011, Ismail et al., 2013, Etelevina et al., 2015 and Phammidh et al., 2015). Aspergillus flavus produce either AFB1 and / or AFB2, Aspergillus parasiticus strains produce AFG1 and AFG2 in addition to AFsB (Milita et al., 2010 and Huang et al., 2011). From our results it clear that Aflatoxins make the fish become more susceptible to fungal infection due to its immunosuppressive effect (Tuan et al., 2002).

Results in table (5) showed that the mean levels of AFs in feed of diseased fish were 108.9±0.77 ppb (46.5±0.75 ppb AFB1, 1.4±0.11ppb AFB2, 58.9±0.38 ppb AFG1 and 2.1±0.24 ppb AFG3). The detected levels of AFs were over the permissible limits in Food reported by (WHO,1979) who stated that mycotoxins must be not more than 15ppb, also FAO, (1995) and FDA, (2000) who mentioned that the levels of AFs must be not more than 20 ppb in food and (EU, 2010) who stated that the levels of aflatoxins in food must be not more than 50 ppb, also our results were above the permissible limit, in comparison with Egyptian standards quality control (10 ug/kg AFB1 and 20μg/kg AFB1,B2,G1,G2). According to the annual BIOMIN Mycotoxin survey in 2014, a look at aqua feed for fish and shrimp showed that, out of 35 feed samples analyzed 63% of the aqua feed samples were contaminated with AFTs at a high average concentration 49 parts per billion (ppb), with some samples reaching to 221ppb. The results in table (6) showed that, AFs residues in musculature of diseased fish were at mean levels of 20.9±0.39 PPb (11.3±0.25ppb AFB1, 0.15±0.007ppb AFB2, 8.9±0.20ppb AFG1 and 0.5±0.44ppb AFG3). These results were similar to those reported by Hussain et al. (1993) who mentioned that aflatoxins B1,G1 and G2 were detected in the musculature of walleye fish (Sander vitreus) at a concentration of 5 to 20 ppb after received feed treated with 50 and 100 ppb
aflatoxin for 30 days which were, however, no longer detectable after two weeks of mycotoxins with withdrawal. Dimitri et al. (1997) detected aflatoxin B₁ and G₁ at levels of 11 and 20 ppb respectively from muscles of rabbit which received ration contained 2 ppm aflatoxin for 6 months. Also Bintvihok and Danis (2003) detected Aflatoxins B₁, B₂, G₁, M₁ from 20.3% of liver and 10.2% of muscles samples of swine in Bankok and –by, Thailand.

The histopathological examination confirm the aflatoxicosis of diseased fish, the epidermal cells showed proliferation of mucus and alarm cells with subepithelial leukocytic infiltration. Similar results were obtained by Amany et al. (2009). While Inter and intramuscular edema with degeneration and mild leukocytic infiltration were encountered in skeletal muscles. All the hepatic cells suffered from necrotic or degenerative changes which characterized by vacuolation of cytoplasm and pyknotic or karyolitic nuclei. Similar results were reported by (Hussein et al., 2000, Soliman et al., 2000, Abdelhamid et al., 2002a and Amany et al., 2009). Abdelhamid et al. (2004) who reported that 100-200 ppm AFB₁ in the O.niloticus fish diet led to severe histopathological alteration in the liver. The renal tubular epithelia of kidney suffered from degenerative changes mainlyhydropic or hyaline degeneration beside depletion of hemopoietic centers and mild leukocytic infiltration. These results nearly similar to those obtained by (Abdelhamid et al., 2002b, Abdelhamid et al., 2004 and Merhim et al., 2006). The mucus of the stomach and intestine showed hyperplasia of goblet cells or desquamation of their lining epithelium with presence of excess mucus and intense submucosal leukocytic infiltration mainly lymphocytes. These results were in accordance with those obtained by (Kandil et al., 1991, Amany et al., 2009). The Gill’s arch showed edema, leukocytic infiltration mainly lymphocytic type. These results were similar to those obtained by (El-Bouhy et al., 1993, Anjum 1994, Mohamed and Mokhbatly, 1997) who mentioned that these lesions developed as a result of immunosuppressive effect of aflatoxins.

CONCLUSION

We can concluded that the high levels of aflatoxins in fish feed could be considered as a high risk for aquaculture as well as for the human health. The detected residues of aflatoxins in fish increased the awareness of use these fish for human consumption. Therefore, constant monitoring of fish feed for AF-producing fungi is an urgent need which enable us to safe the public health of fish consumers.

REFERENCES


Amany, M.K.; Hala, M.E.; Mohammed, M.N.; Authman and Abdel-Wahhab, M.A. (2009): Pathological studies on effects of aflatoxin on Oreochromis niloticus with application of


