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Effect of Aflatoxins in Aquaculture: Use of Bentonite Clays as Promising Remedy

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Abstract

Aflatoxins are among all known mycotoxins, the most widespread source of contamination of food and feed throughout the world. In aquaculture and aqua-feed formulations the increased use of plant based materials has intensified the risks of aflatoxicosis in farmed fish because of the high load of aflatoxin by the vegetable sources. The aflatoxins production by toxic fungal strains can take place in the field directly, during different processing stages like insiling, aqua feed formulation, preparation and due to improper feed storage. However, the thermal treatments can only destroy the fungus but have no effect on the heat-resistant mycotoxins present in mycelium and spores of the mold. So, the aflatoxin accumulation in fish meal and fish feed represents a great threat for the aqua-cultured species and ultimately for the consumer's health and safety. Consequently, the issue of aflatoxin contamination in fish and aquaculture industry has intensified. This review focused on the deleterious effects of aflatoxins in fish and aquaculture and the possible remedy by the use of adsorbents.

Key words: Aflatoxins, Fisheries, Aquaculture, Adsorbents, Calcium bentonite

Introduction

The most serious problem facing the aquaculture sector today, is removing food borne toxin exposure, such as aflatoxin B_1 (AFB_1). Aflatoxins (AFs) are small molecular weight compounds produced mainly by two fungal species, *Aspergillus flavus* and *A. parasiticus*, which are normally occur in hot and humid regions of the world. Recently, a large number of studies focusing on aflatoxin contamination in foodstuffs, mainly in nuts, cereals and spices, have been reported in many countries, especially those in Africa and Asia (Bankole, Adenusi, Lawal, & Adesanya, 2010; Ding, Li, Bai, & Zhou, 2012; Ok et al., 2007; Soubra, Sarkis, Hilan, & Verger, 2009). From the class of aflatoxins, aflatoxin B_1 is the most lethal naturally occurring carcinogen (Ayyat, Abd-Rhman, El-Marakby, Mahmoud, & Hessan, 2013; Binder, Tan, Chin, Handle, & Richard, 2007). The toxicity of AFB_1 resulted to poor growth, immunity inhibition, reproduction damage, behavioral abnormalities, necrotic hepatocytes and the traces of aflatoxins in the liver and other edible tissues (Binder et al., 2007; Bintvihok, Thiengnin, Doi, & Kumagai, 2002; Deng et al., 2010; Farabi, Yousefian, & Hajimoradloo, 2006; Groopman, Wang, & Scholl, 1996; Sahoo and Mukherjee, 2001). However, there have been few studies reported on the contamination and the effects of AFB_1 in farmed aqua feeds (Barbosa et al., 2013). On the other hand, AFB_1 might still be a serious concern in aquaculture

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since the vast use of plant sources in aqua feeds, and spread of AFB_1 by lethal deposits in the fish may be a danger to humans as well (El-Sayed & Khalil, 2009; Manning, Li, & Robinson, 2005; Raghavan et al., 2011).

Animal feeding studies have demonstrated that clay additives in the animal feed such as bentonites have the ability to sorb and mitigate the toxic effects of aflatoxins. Bentonites are mostly consists as smectite minerals (montmorillonite) and are found almost everywhere around the globe. In the commercially available animal feeds clays has been added to improve the quality of the food. The use of clays as protective measures against aflatoxicosis has been proven beneficial in many animal studies and this result in the advancement in the clay additive research. The animal feeding studies with some commonly available clays like Novasil, Na-bentonite, Astra Ben 20A, sepiolite, and many others has actually been proven beneficial against the toxic effects of mycotoxins such as AFB_1 (Jayens & Zartman, 2011).

Impact of AFB_1 On Fish Health

A reduction in growth is one of the main adverse effects reported due to contamination aflatoxin B_1. Several studies reported the decrease of growth rates in channel catfish (10 mg AFB_1/kg; Jantrarotai & Lovell, 1990) and Nile Tilapia, Nile tilapia (L.), (0.1 mg/kg AFB_1 - Encarnacao, Srikhum, Rodrigues, & Hofstetter, 2009; 1.88 mg/kg AFB_1 - Chavez–Sanches, Palacios, & Moreno, 1994; 0.1 mg/kg AFB_1 - El-Banna, Taleb, Hadi, & Fakhry, 1992). Further effects of high aflatoxin levels include low hematocrit levels above 0.25 mg/kg, severe liver necrosis in the Nile tilapia with levels of 100 mg/kg AFB_1 (Tuan, Manning, Lovell, & Rottinghaus, 2002) and immunosuppression in common carp, *Cyprinus carpio* (L.) (Sahoo & Mukherjee, 2001).

The results of many studies revealed the negative effect of AFB_1 on the health performance of Nile tilapia as Zychowski et al. (2013b) reported the deleterious effects of AFB_1 in tilapia at lower concentrations such as 1.5 mg/kg. Mehrim and Salem (2013) have revealed serious toxic effects of AFB_1 in the Nile tilapia with levels of 0.15 mg/kg AFB_1 addition to the hepatotoxic effects on the liver than the control group. El-Banna et al. (1992) have reported that the growth of Nile tilapia decreases significantly when it was exposed to a diet with 0.1 mg/kg AFB_1 for a period of 10 weeks, and was observed 16.70% mortality when exposed to a dose of 0.2 mg/kg.

In the majority of aquatic organisms exposed to AFB_1, the toxic signs as anorexia, yellowing of the body surface, weight loss, feed efficiency reduction, liver dysfunction and histological damage are commonly observed. The results of the study of Cagauan, Tayaban, Somga, and Bartolome (2004) revealed that when Nile tilapia offered a diet containing 0.005–0.039 mg/kg $AFB_{1,}$ survival rate was lowered 67% as compared to the fish having no aflatoxin B_1. When concentration of AFB_1 was exceeded more than 0.029 mg AFB_1/kg feed, the surface of tilapia becomes more yellowish. On the other hand, Tuan et al. (2002) concluded that tilapia has no deleterious effects when exposed to 0.25 mg AFB_1/kg diet. They also concluded that growth performance and hematocrit of tilapia affected only at high dose as 2.5 mg AFB_1/kg feed. Moreover, Chavez-Sanchez et al. (1994) concluded that tilapia can tolerate as high as 30 mg/kg AFB_1 of feed without mortality.

Aflatoxins exert a substantial impact on the fish farming production, causing disease with high mortality and a gradual decline of reared fish stock quality, thus representing a significant problem in aquaculture systems. Han, Xie, Zhu, Yang, and Guo (2010) investigated the toxic effects of AFB_1 in terms of growth performance, histological

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changes and physiological responses in juvenile gibel carp (*Carassius auratus gibelio*). At the end of 90 days trial % weight gain (WG) by the fish fed 0.018 mg/kg of AFB_1 was 112.6% as compared to the control (without AFB_1), no prominent difference in % WG in fish fed 0.028 mg/kg and the control. The results of the study of Salem, Shehab, Khalafallah, Sayed, and Amal (2010) reported that the aflatoxin contaminated diets resulted in poor growth performance, protein and feed utilization, survival, carcass composition, somatic indices, some blood parameters, activities of plasma enzymes, protein profile and the residues of AFB_1 in the whole body of fish.

Under laboratory conditions, Sepahdari et al. (2010) studied the effects of different dietary levels of AFB_1 on *Huso Huso*. At the end of 3 months trial, the specific growth rate was not differing significantly among the treatments when exposed to different levels of AFB_1. While there observed a significant difference in % weight gain and feed conversion ratio (FCR) among the treatments (diets contaminated with 0.075 and 0.1 mg AFB_1/kg) and also with the control). Mortality and survival was not affected by the increasing concentration of AFB_1. In line of these results Ruby, Masood, and Fatmi (2013) also concluded the same trend as observed in terms of weight gain and survival when *Labeo rohita* was exposed to increasing levels of AFB_1.

Aflatoxins and their animal biotransformation products, known as food-borne carcinogens, have been associated with serious harmful effects on the health of humans and animals. Zychowski et al. (2013a) concluded that aflatoxin B_1 affected negatively increasing weight of red drum, feed efficiency, survival, hepatosomatic index (HSI), concentration in serum lysozyme, liver histopathology score, the levels of whole body lipid, as well as the inhibition of trypsin as low as 0.1 mg/kg of AFB_1. The interaction between aflatoxin (0.025 mg/kg) and various treatments (Ozone (0.5 mg/L/minute; Coumarin 5000 mg/kg diet; 2000 mg of clay/kg) irrelevant influenced the final body weight, daily gain and the feed conversion ratio (FCR). The results showed higher final body weight, increased daily gain and FCR in the groups treated with ozone fish, followed by clay treated groups (Ayyat et al., 2013). Huang et al. (2010) conducted a 24-week feeding trial to investigate the toxic effects of dietary AFB_1 on the growth performance, histological changes, physiological responses, fertility and accumulation of AFB_1 in Gibel Carp (*Carassius auratus gibelio*) during the stage of development of the gonads. The results showed that, there was no significant difference after 24 weeks of AFB_1 exposure, in the final body weight in fish groups treated with AFB_1 and the control.

Different mycotoxins reveal a number of lethal effects in different animals, largely in the hepatic and nephritic regions. Chronic exposure of AFB_1 resulted in specific variations and injury to the liver of Nile tilapia (Chavez-Sanchez et al., 1994; El-Banna et al., 1992; Tuan et al., 2002). Kenawy, El-Genaidy, Authman, & Abdel-Wahhab (2009) observed that the liver of Nile tilapia enlarged and faded with grayish patches with the spleen also weighed in the available-treated fish. Kidneys were also swollen and friable, while the gills were dark and filled with mucus in color. Such changes were increased in mild (AFS + EM) treated group but treated in (AFS + EB) group in severity. It was found that EM could effectively alleviate lesions of AFS and prevent the presence of AFS residues in fish tissue to be safe for human consumption.

Raghavan (2011) conducted a study to evaluate the effect of AFB_1 on hybrid sturgeon. The results showed non-significant difference in hepatosomatic index between the groups. Zychowski et al. (2013a) found that aflatoxin B_1

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has negative effect on red drum's hepatosomatic Index (HSI) at different concentrations of AFB_1 (0.1, 0.25, 0.5, 1.0, 2.0, 3.0 and 5.0 mg/kg).

Impact of AFB_1 on Fish Immune Response

Aflatoxin B_1 significantly alters the immune system. Controlled laboratory studies on animals have shown that AFB_1 immunosuppression increases the risk of secondary infection (Pier, 1986). Immunosuppressive effects arising as a result of AFB_1 exposure are cited studies in aquaculture. If trout embryos are exposed to AFB_1, they suffer long term immunodeficiency (Ottinger & Kaattari, 2000). In this study, rainbow trout were exposed to 0.5 mg/kg AFB_1 for 30 minutes. Anterior kidney immunoglobulin production was about 2/3 of the levels of control in AFB_1-exposed embryos. Lysozyme is a bacteriolytic enzyme that is one of several humoral parameters and is present in serum, secretions, and mucous membranes. The kidney and intestine have the highest concentrations of lysozyme (Hoover et al., 1998).

A wide variety of biological effects such as toxicity, carcinogenicity, teratogenicity, genotoxicity, impairment of immune and reproductive system have been recorded in most animal species. Zychowski et al. (2013a) found that aflatoxin B_1 negatively affected red drum immune system by changing serum lysozyme concentration and trypsin inhibition. When fish exposed to 0.1 mg/kg AFB_1, it showed maximum plasma lysozyme values (246 units ml^{-1}), while at 5 mg/kg AFB_1 levels fish exhibit the lowest values (45 units ml^{-1}). Nile tilapia decreased macrophage phagocytosis after exposure to 0.2 mg/kg AFB_1 (El-Boshy, El-Ashram, & El-Ghany, 2008). Other species such as *Labeo rohita* experience suppressed serum bactericidal activity, neutrophil and globulin levels after AFB_1 exposure. Aflatoxin injection in this species at 1.25 mg/kg body weight led to lymphocytolysis (Sahoo and Mukherjee, 2001).

AFB_1 may induce irregularities in blood biochemical and hematological factors. RBC's, WBC's, hematocrit and hemoglobin content of the fish are affected by AFB_1 (Tuan et al., 2002). Lesser amounts of total serum protein, albumin and globulin were observed in rohu and tilapia when subjected to different levels of AFB_1 (Deng et al., 2010).

The results of similar studies shows that AFB_1 have significant influence on the immune system of fish as described by El-Enbaawy, Adel, Marzouk, and Salem (1994), Sahoo and Mukherjee, (2001). El-Sayed and Khalil (2009) reported epizootic hepatoma in fish due to the contamination of food by Aspergillus species in sea bass (*Dicentrarchus labrax L*). An abnormal behavior with signs of toxicity of aflatoxin B_1 were observed with significant elevations of alkaline phosphatase and serum transaminases activities and a significant decrease in plasma proteins. Varior and Philip (2012) studied the effect of dietary crude aflatoxin B_1 on the lysosomal membrane stability in *Oreochromis mossambicus* at biochemical and cellular level. The results showed significant difference in lysosomal membrane stability in different diets contaminated with AFB_1. Hassan, Kenawy, Abbas, and Abdel-Wahhab (2010) conducted a study to examine the effects of two bentonite clays against the AFB_1 contaminated diets in Nile tilapia. The results showed an overall decreasing trend in some of the blood proteins like albumin, globulin and total proteins.

AFB_1 Residues in Fish

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Many studies revealed that animals have AFB_1 residues in their tissues after exposure to AFB_1 that causes poor health effects after ingestion (Han et al., 2010; Messonnier et al., 2007; Puschner, 2002; Santacroce et al., 2011; Tacon and Metian 2008). El-Sayed and Khalil (2009) concluded that delayed exposure of sea bass with small amounts of AFB_1 (0.0018 mg/kg) resulted in poor health performance and the accumulation of the AFB_1 residues in the edible tissues of the fish which ultimately have threat for the consumers. In agreement with these results Han et al. (2009) reported that when gibel carp exposed to more than 0.01 mg/kg of AFB_1 in the diet, the accumulation of AFB_1 residues was detected in the muscles and ovaries above the safety levels of 0.002 mg/kg as set by European Union. In Nile tilapia, AFB_1 residues in the liver regions were noted exposed to as low as 2 mg/kg (Deng et al., 2010). Abdel Hamid, Abdelkhalek, Mehrm, and Khalil (2004) concluded that AFB_1 residues in fish muscle represented a combined effect, which was based on the duration and the exposure of AFB_1.

In another study, high concentrations of AFB_1 residue (0.005 mg/kg) were detected in sea bass muscles. So, seabass was a highly susceptible species to aflatoxin B_1 and it was found that consumption of contaminated fish lead to negative health effects in humans (El-Sayed and Khalil, 2009). Nomura et al. (2011) observed the absorption and excretion of aflatoxins by rainbow trout (*Oncorhynchus mykiss*) on exposure of 21 days.

AFB_1 accumulation in fish ovaries and muscles significantly increased as AFB_1 concentration in the diets increased. AFB_1 in muscles in all groups were all below the safety limit of 0.005 mg/kg wet weight. These results show that AFB_1 exposure to about 2 mg/kg diet have no apparent effect on the growth and liver function of gibel carp, but it would harm fertility of fish (Huang et al., 2011). Raghavan (2011) studied the toxicity of aflatoxin B_1 in juvenile hybrid sturgeon *Acipenserruthenus baeri*. Increased levels of AFB_1 in the diet led to an accumulation of AFB_1 in fish muscle and liver. The maximum level of AFB_1 that could be allowed in feed for sturgeon was found to be 0.01 mg/kg of diet. Seabass exposed to 1.8 mg/kg of AFB_1 for 42 days and resulted 5 ppb AFB_1 accumulation in the muscle (El-Sayed & Khalil, 2009).

So it is concluded that the toxicity caused by AFB_1 have detrimental effects on humans and animals health (Agag, 2004; Messonnier et al., 2007), which is largely motivated by the increasing use of plant substances in feed of animals. Therefore, we can link the exposure AFB_1 exert a bio-accumulative effect throughout the food chain and humans, the farm-raised fish are consuming at risk for AFB_1 exposure.

Efficacy of Bentonite Clays Against Aflatoxicosis

The addition of binders into aflatoxin contaminated feed to adsorb the toxic molecules had caused an increasing attention from scientists over the years. The adsorbents are added to the diet of exposed animals to prevent uptake and metabolism of the toxins (Phillips, Lemke, & Grant, 2002). Bentonites, zeolites and activated carbon are the most studied aflatoxin adsorbents. Hussain et al. (2017) conducted an experiment to evaluate the efficacy of 4TX bentonite clay at 0.5 and 10% levels against 2 and 4 mg/kg AFB_1 induced toxicity in Nile tilapia along with control. The inclusion method of the clay in the remaining diet ingredients was also compared. Clay was added in the diet as powder mix (PM) and water dispersal (WD) form. The results of the study showed an improved growth performance in clay supplemented fish groups when compared to control. Additionally, clay added as PM form showed better results as compared to WD inclusion type. The results by the study of Zychowski et al. (2013b) also revealed the

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protective effect of bentonite clay (NovaSil) against the AFB_1 induced toxicity in Nile tilapia. Hauptman et al. (2015) tested the efficacy of two mycotoxin adsorbents; grain distillers dried yeast (GDDY) and mycofix plus in Rain bow trout. The results showed an increased feed intake and FCR fed 30% (GDDY) diet while minor increase in fish growth, protein and energy retention were noticed with mycofix plus supplemented diets. In line of these findings Zychowski et al. (2013a) concluded that overall health of AFB_1 exposed red drum is improved by NovaSil supplementation in the diets.

Several reports indicated that the phyllosilicate clay, hydrated sodium calcium aluminosilicate (HSCAS), which is currently available as an anticaking agent for animal feeds, may prevent disease associated with aflatoxicosis in animals, including chickens, turkey poults, pigs and minks (Phillips, Kubena, Harvey, Taylor, & Heidelbaugh, 1988; Phillips, 1999). Huwig, Freimund, Kappeli, and Dutler (2001) reviewed the efficiency of different materials, such as activated charcoal, zeolites, HSCAS, other clays, polymers, yeast and yeast products, as adsorbents for different mycotoxins. They found that HSCAS showed almost total protection against the adverse effects of aflatoxins but were very limited in counteracting the mycotoxin zearalenone. In most studies where both zeolites and smectites were studied, smectites seemed to be more effective, with an exception of the study by Tomasevic-Canovic (2001) in which it was concluded that clinoptilolite had a higher chemisorption index, less desorption after adsorption of AFB_1, than montmorillonite. Over all, over the past two decades previous studies have indicated that literature smectite clays are effective adsorbents of aflatoxin and successfully protected many farm animals from aflatoxicosis. Calcium montmorillonite clay, which is both inexpensive and abundant, has a dioctahedral structure that is known to sequester AFB_1 in its negatively-charged interlayer, thereby reducing systemic bioavailability. In another study of Velazquez, Bailey, Deng, and Dixon (2010), the clays were introduced into the feed by the dry bentonite powder was mixed with the feed for 12 minutes in a mechanical mixer. Body weight was increased by 21% with 4TX and 14% clay 1TX in aflatoxin-diet. The concentration of total iv aflatoxins in liver was reduced by 36% with the addition of clays. Liver optics has also been improved from pale red to a reddish color similar to the healthy red liver. Chickens fed with clean feed showed significantly higher body weights than those with highly contaminated feed.

Dosage of clay-based additives in animal feeds to prevent aflatoxin toxicity has varied substantially. Two-percent bentonite clay inclusion has been used in rainbow trout feed to reduce toxicity resulting from 0.02 mg/kg of AFB_1 exposure and significantly decreased liver cancer rates (Ellis, Clements, Tibbetts, & Winfree, 2000). Hassan et al. (2010) concluded that Egyptian bentonite (EB) and montmorillonite (EM), which have the ability to bind tightly with AFB_1 in the gastrointestinal tract of the fish decreases its bioavailability. Moreover, the two tested clays were safe and use as potential aflatoxin binder in animal feed.

Structural information is contained on various adsorbent minerals and noted the specificity of HSCAS for AFB_1 adsorption. Mechanisms of adsorption on the surface HSCAS discussed and their indications that aflatoxins react close to more sites on the clay surface, especially in the intermediate layer. They postulated conceivable risk from dietary intake of smectites on the animal's health and explained the need to pay special attention to mineral interactions in susceptible animals (Phillips et al., 2002). Selim, Elhofy, & Khalil (2013) conducted a trial to investigate the protective effect of three mycotoxin adsorbents, a hydrated sodium calcium aluminum silicates

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(HSCAS), Saccharomyces cerevisiae (SC) and an esterified glucomannan (EGM), to AFB_1 contaminated feed (0.2 mg/kg) in Nile tilapia. The results of the study revealed that treatments having adsorbents along with AFB_1 showed decreased AFB_1 residues in fish muscle tissues when compared to alone AFB_1 treated fish. HSCAS reduced AFB_1 more efficiently as compared to S. C. and EGM.

Montmorillonite can adsorb high concentrations of aflatoxins from aqueous solutions. Kannewischer (2006) described that smectite clay has been used in animal studies over the past 20 years to sorb and mitigate the toxic effects of aflatoxin B_1 and thereby decrease its toxic effect on animals. In the similar study, Jaynes & Zartman (2011) concluded that aflatoxin toxicity can be reduced by the addition of clays such as bentonites in the animal feeding trials. The results of the study showed that montmorillonite treated with choline and creatinine absorbed and reduced more AFB_1 from the aqueous solution of corn flour as compared to untreated groups.

The bioaccumulation of aflatoxin B_1 in the whole body of the fish was expressively increased in fish groups fed contaminated diets with aflatoxin B_1. Fish group treated with clay, ozone and coumarin reduces the residual aflatoxin, when compared with the control group. It could be recommended to use natural clay or ozone treatments tominimize the toxic effects of aflatoxin B_1 contaminated diets (Ayyat et al., 2013).

In some other studies it is reported that phyllosilicate clay, hydrated sodium calcium aluminosilicate (HSCAS), might be use to prevent against toxic effects of aflatoxins in several animals like chickens, poults, pigs, turkey and minks (Phillips et al., 1988; Phillips, 1999). It is now proved by the results of several studies that the incorporation of HSCAS, montmorillonite or bentonite to the diets having aflatoxins can significantly decrease the bio-availability of toxins in the digestive tract of animals due to the great adsorptive properties of these clays (Abdel-Wahhab & Aly, 2005; Mayura et al., 1998). In line of these conclusions, Lindemann, Blodgett, Kornegay, and Schuryg (1993) proposed that 85% growth performance improved by the addition of 5mg/kg clay against AFB_1 induced toxicity. The efficacies of another montmorillonite (Cu-MMT) have been proved beneficial for the growth of fish by the results of Hu, Xu, Xia, Xiong, and Xu (2008).

Chronic aflatoxin (AF) exposure has been reported to have adverse effects on nutritional status of many animal species. In poultry, aflatoxins have been noticed to expressively decrease nutrients like vitamin A (VA) in liver (Pimpukdee et al., 2004) and vitamin D in plasma (Glahn et al., 1991). Vitamin A deficiencies have been shown to compromised immune responses and ultimately increased disease risk in poultry (Aye et al., 2000; Dalloul, Lillehoj, Shellem, & Doerr 2002).

Conclusion

Aquaculture is very important natural sources both strategic and vital for all in the world. Aquaculture will continue to play an important role in the global supply of fish in the future. Negative effects of waste from aflatoxins to aquatic environment are increasingly recognized in Aquaculture. To minimize the risk of aflatoxin exposure, close tripartite cooperation among the trade, the public and the government is essential. Therefore, monitoring of environmental effects of aflatoxins in aquaculture is very important for aquatic ecosystems conservation.

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