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The Investigation of Total Aflatoxin and Total Fumonisin Residues in Fish Feed Collected In Some Provinces of Turkey

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Abstract

In this study 87 fish feed samples were randomly collected from feed mills and fish farming establishments from 14 provinces in Turkey to determine the occurrence of total aflatoxin (AF) and total fumonisin contamination. The quantitative analysis of total AF and total fumonisin of the samples was carried out using commercial kits. The samples were analysed by microplate reader. Total AF residues were found in 86 (98.85%) of 87 analyzed samples. The avarage value for total AF (AFB_1, AFB_2, AFG_1, AFG_2) in positive samples was found as 8.776±4.178 µg/kg ranged from 1.023-17.566 µg/kg, and these values were found to be less than the recommended level 20 ppb (Food Drug Administration). Total fumonisin (B_1, B_2, B_3) residues were found in 49 (56.98%) of 86 samples analysed. The avarage value for total fumonisin in positive samples was found as 0.3028±0.2584 mg/kg ranged from 0.0107-0.9278 mg/kg. These values were below the tolerable limit of 10 ppm for fumonisin (B_1, B_2) announced by Ministry of Agriculture and Forestry in Turkey. It was suggested that although mycotoxin residues were detected in the collected feeds, these values remained below the tolerable values.

Keywords: Aflatoxin, aquaculture, fish feed, fumonisin, mycotoxin residue.

INTRODUCTION

Aquaculture is a growing and developing industry, therefore fish welfare has become an important issue today. To eliminate the factors that reduce productivity such as stress, disease and for adequate growth and resistance, fish should be well fed and dietary needs should be met (Oliva-Teles, 2012). Fish feed is the most important cost item in the aquaculture industry (Enyidi et al., 2017; Chavanne et al., 2016). Cost effective feeds can be formulated for large commercial aquaculture species (Lovell, 1991). Aquaculture started with carp production and later trout production in Turkey in 1965-1970. Later sea bream and sea bass production was put into practice and it became a sector that gradually develops itself (Emiroğlu İşgören et al., 2019). As this sector develops, the need for qualified, clean and economical feed production required to meet the needs of the sector. Infact qualified feed is needed for good yield and qualified product in aquaculture. This can be achieved by optimizing all conditions including selection of raw material in feed, transportation and storage (Kop and Korkut, 2002).

Mycotoxins are toxic and carcinogenic substances produced by many of fungi and can multiply on agricultural products. There are many types of mycotoxins such as aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, patulin and zearelenone (Whitaker et al., 2005). Mycotoxins were first considered as a serious and important problem with poisoning cases in turkeys in England in the 1960' s (Kaya, 2014). Today they are known as the most important causes of cancer. Ingestion of mycotoxins with feed and feedstuffs may cause liver and kidney disease and chronic disorders that cause suppressions of the immune system (Anater et al., 2016). The importance of mycotoxins in aquaculture first emerged with outbreaks of rainbow trout (*Onchorynchus mykiss*) raised in hatcheries in the United States. The source of mycotoxicosis has been identified as aflatoxin contaminated cotton seed meal. Recent years mycotoxins such as ochratoxin A, deoxynivalenol, fumonosin were also taken into consideration (Manning, 2005).

Consumption of feed and feed ingredients contaminated with mycotoxins may result in various health problems and even death in animals. This situation affects the livestock industry globally, thus mycotoxins have become a major threat to animal production systems (Santos Pereira et al., 2019). Mycotoxins are transmitted to the feed source directly or indiectly. Direct contamination occurs as a result of the growth of mold in feed material. Mold growth can ocur in almost all feedstuffs during production, storage and transportation (Abdel-Wahhab and Kholif, 2008). The most common mycotoxins in animal feeds are aflatoxins. *Aspergillus flavus* or *A. parasiticus* strains grow under favorable conditions for aflatoxin formation. The aflatoxins are AFB_1, AFB_2, AFG_1, AFG_2 (Nibbelink Stuart, 1986). Aflatoxins mainly cause liver damage in animals. The poisoning status varies according to the animal species, age, sex, nutritional status. Aflatoxins cause dysfunction of liver, decrease in animal productivity, suppression of immunity. The consumption of feeds containing low concentrations of aflatoxin for a long time causes toxicity

in the embryo. Young animals are more sensitive to aflatoxin. The clinical symptoms of aflatoxicosis are digestive disorders, decreased feed yield and anemia (Dhanasekaran et al., 2011). Fumonisins are toxins produced by *Fusarium* fungi (especially *Fusarium verticilloides and Fusarium proliferatum*). They were discovered in South Africa with the occurrence of esophageal cancer in humans. It was determined that the cause of cancer occured as a result of ingestion of *Fusarium* infected corn (Altınok and Dikilitaş, 2011; Kaya, 2014). Fumonisin B_1 causes leukoencephalomalacia in horses and pulmonary edema in pigs. The most serious toxic effect is cancer. Therefore toxins produced from Fusarium moniliforme have been classified as Group 2B carcinogens for humans (Shier, 2000)

The results of mycotoxins contaminations are not different from other animal species avaible for human consumption. Therefore, fish feed and its production and control are extremely important (Anater et al., 2016). Mycotoxins may cause a decrease in growth rates, liver damage, decrease in immune response, increase in mortality of the fish. The continuous and gradual decreases in quality of the fish performance will cause serious problems in fish industry (Marijani et al., 2017). Therefore the aim of this study is to determine the levels total aflatoxin and fumonisin in fish feed collected from Antalya, Elazığ, Erzincan, İzmir, Ankara, Samsun, Giresun, Trabzon, Sakarya, Tunceli, Gaziantep, Erzurum, Manisa, Muğla provinces. The results will be an auxiliary parameter to protect human and animal health.

MATERIALS AND METHODS

Feed Sampling

Totally 87 fish feed samples (500 g each) were randomly collected from feed mills and fish farming establishments from 14 provinces in Turkey to determine the occurrence of total AF and total fumonisin contamination (Table 1). The samples were collected in 2019-2021. The collected feed material is consists of 50 feed for rainbow trout, 14 feed of sea bream - bass, 21 feed for sea bass and 2 feed for carp. Among these feed 34 of them were juvenile fish feed and 53 of them were adult feed (Table 2). The feed samples were transported and stored at -20°C until analysis.

Table 2. The number of juvenile and other fish feed

Feed Type	Number of samples
Fish feed (Adult) (Growth and finishing feed	53
Juvenile Fish feed	34
Total	87

Total aflatoxin and Total fumonisin analysis

The quantitative analysis of total AF and total fumonisin of the samples were carried out using commercial kit (HELICA Biosystems, Inc., HELICA for total aflatoxin-981AFL01LM-96, and HELICA for fumonisin 951FUM01C-96). The extraction of feed and the procedure of analysis were performed according to the instructions of the manufacturer.

Data analysis

The samples were read on an optical density scale at 450 nanometers in the microplate reader (Biotek 800 Ts, USA). Quantitavie guantification was done by comparing with standarts according to the standart curve obtained from the software provided by the relavant company. The obtained data were interpreted according to the avaible tolerable limits given in the announcement on undesirable substances in animal feed issued by the Ministry of Agriculture and Forestry of the Republic of Turkey (2014) for total fumonisin (10 ppm in fish) and; FDA (2019) for total AF (20 ppb for all animal feed) because there is no determined limit for total AF. The data were presented as arithmetic mean ± standard deviation (SD), and the minimum and maximum values were also recorded.

RESULTS

The results of the study showing the concentrations of total AF and total fumonisin in collected fish feed were given in Table 3, 4 respectively. Total AF (AFB_1, AFB_2, AFG_1, AFG_2) residues were found in 86 (98.85%) of 87 analyzed samples, and these values were found to be less than the recommended level 20 ppb. Total fumonisin (B_1, B_2, B_3) residues were found in 49 (56.98%) of 86 samples analysed. These values were below the tolerable limit of 10 ppm for total fumonisin (B_1, B_2).

Table 1. The number of feed samples collected from the provinces in Turkey

Provinces	Number of samples
ANKARA	2
ANTALYA	19
ELAZIĞ	3
ERZURUM	3
GAZİANTEP	2
GİRESUN	15
İZMİR	4
KONYA	5
MANİSA	9
MUĞLA	2
SAKARYA	4
SAMSUN	10
TRABZON	3
TUNCELİ	6
Totally: 14 provinces	87 samples

Table 3. The level of total AF (µg/kg) in fish feed samples

		Positive samples (µg/kg)			
Feed sample	**Tested** ***n***	**Positive** ***n (%)***	**min-max**	**Mean ±SD**	**Exceed regulation** ***n* (%)**
Juvenile fish feed	34	34 (100%)	2.388-16,709	9.697±4,276	-
Adult fish feed	53	52 (98.11%)	1.023-17.566	8.174±4.040	-
Total	87	86 (98.85%)	1.023-17.566	8.776±4.178	-

n: number of samples, min: minimum level, max: maximum level
The data were given as mean ±SD: Standart deviation. the maximum tolerable limit for total AF is 20 µg/kg (FDA)

Table 4. The level of total fumonisin (mg/kg) in fish feed samples

		Positive samples (mg/kg)			
Feed sample	Tested *n*	Positive *n (%)*	min-max	Mean ±SD	Exceed regulation *n* (%)
Juvenile fish feed	33	16 (48.48%)	0.0139-0.9278	0.3002±0.2638	-
Adult fish feed	53	33 (62.26%)	0.0107-0.9134	0.3040±0.2597	-
Total	**86**	**49 (56.98%)**	0.0107-0.9278	0.3028±0.2584	-

n: number of samples, min: minimum level, max: maximum level.
The data were given as mean ±SD. The maximum tolerable limit for total fumonosin for fish is 10 mg/kg (The Republic of Turkey's Ministry of Agriculture and Forestry Announcement No: 2014/11 named undesirable substances in animal feed)

DISCUSSION AND CONCLUSION

Aflatoxicosis in fish emerged in the 1960s due to the use of dry pelleted foods containing cottonseed oil containing high levels of AF in Rainbow trout. AFs are described a potent carcinogen responsible for widespread hepatocarcinoma formation in cultured trout. Aflatoxin B_1 is the strongest naturally occuring animal carcinogen (Royes, 2001). In a study conducted to evaluate the effect of AF experimentally, *Nil tilapia Oreochromis niloticus* were fed on 20 or 100 ppb AFB_1 contaminated feed for 6 or 12 weeks; it has been demonstrate that 100 ppb AFB_1 negatively affects *O. niloticus* weight gain, feed efficinecy, hematological profile and liver histopatholohgy. A decrease in the resistance of fish to deseases has also been shown (Mahfouz and Sherif, 2015). In another experimental study, Dicentrarchus labrax L (sea bass fish) was administered 0,18 mg/kg, which is the oral LD_{50} (96 hours, LD_{50}) of AFB_1, and abnormal behaviour and signs of intoxication were observed. When given 0,018 mg/kg AFB_1 for 42 days, an increase in serum transaminases and alkaline phosphate activities and a decrease in plasma proteins were seen. Researchers detected high levels of (5ppb) AFB_1 residue in fish muscles, which was thought to adversely affect human health (El Sayed and Khalid, 2009).

Yaroğlu and Gül (2007) investigated aflatoxins in juvenile trout feeds collected in Erzurum region and they stated that the AFB_1 residue in 24 samples was acceptable. Altuğ and Beklevik (2003) collected 85 fish feed samples between 1998-2000, and found the total aflatoxin level ranged 2-42.4 ppb in 20 fish feeds; and in samples with a total aflatoxin above 20 ppb, AFB_1 was detected ranged 18.4-36.3 ppb.

Kaymak (2000) analyzed the AFB_1, B_2, G_1, G_2 levels of trout feeds obtained from fish feed producers in Turkey by immunoaffinity column method and high performance liquid chromotograhy method. It was found that the total aflatoxin levels were ranged between 0.48-3.46 ppb in 29 of 59 feed samples analysed. These levels did not exceed the legal limit.

Aktüre (2005) investigated AFB_1 and total AF levels in 33 fish feed samples collected from Adana and its districts. Although AFB_1 was found in 8 samples none of their levels exceeded the legal limit. Bintvihok et al (2003) detected the highest level of AFB_1 contamination as 0.651 ppb in their study conducted on 150 shrimp feed in Eastren and southern regions of Thailand. Gonçalves Nunes et al. (2015) studied to determine the contamination of *Aspergillus, Penicillium* and *Fusarium* type fungi and AFB_1 in raw feed ingredients and feed samples collected from fish farms in Piaui Brazil; and isolated *Aspegillus flavus* and *Penicillium citrinum* from all samples. AFB_1 was determined in all samples, on the other hand the levels were below the recomended limit 20 µg/kg.

Barbosa et al (2013) found AFB_1 residues in 55% of 60 fish feed samples they collected from Rio de Janeiro. However these residue remained below the limit that can be measured with ELISA. Marijani et al (2017) detected AF residues in 64,3% of fish feed samples collected in East Africa. Researchers declared AFB_1 level between 2-806 µg/kg, and AFB_2 level between 2-74.5 µg/kg. Alinezhad et al (2011) studied the level of AFB_1 levels in trout feed and its feed ingredients by HPLC. Except pellet feed and gluten all components tested were contaminated with different levels of AFB1 in the range of 1,83-67.35 µg/kg. AFB1 levels were found above the legal limit in soybean, fish meal and wheat. In the sea bass feeds collected in Portugal, no AFB_1 residues were found which were measured by HPLC (Almeida et al.,, 2011). In our study although 98.85 % of the collected feed were contaminated these residues were below the recommended level 20 µg/kg. The maximum value for total AF was 17.566 µg/kg.

Fumonisin B_1 is the most toxic fumonisin. It is synthesized by *Fusarium verticillioides*, *Fusarium proleferatum ve Fusarium nygamai*. Contamination with fumonisin mostly occurs in corn and its by products. Fumonisin level in feeds for fish should not exceed 10 mg/kg (Oliveira and Vasconcelos, 2020). Yıldırım et al (2000) applied 20 and 40 mg/kg FB_1 to canal catfish for 10 weeks, and decreases in weight gain of fish received 20 mg/kg FB_1 were detected. 40 mg/kg FB_1 decreased the hematocrit value in fish. Fumonisin B_1 caused an increase in the ratio of free sphinganine to free sphingosine in the liver. Barbosa et al (2013) found FB1 residue in 90% of 60 fish feed in Rio de Janerio. These residues were range between 0.3-4.94 µg/g, with a medium level of 2.6 µg/g.

Marijani et al (2017) detected fumonisin residues between 33.2 and 3970 µg/kg in 57.1% of fish feeds in their study. Compatable with these results in our study the maximum value for fumonisin residue was 0.9278 mg/kg which was well below the tolarable limit 10 mg/kg.

Within the light of this research, it was concluded that although mycotoxin residues were detected in the collected feeds, these values remained below the tolerable values. However the measure and strategies for avoiding mycotoxin contamination should be taken into consideration seriously and awareness should be raised from the producer to the consumer.

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Conflict of Interest
The authors declare that there is no conflict of interest in the content of the article

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