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Effect of Temperature on the Produced Aflatoxins in the Rainbow Trout Feed in West Azerbaijan Province

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Abstract: In the executed research on aquaculture diet in West Azerbaijan Province coldwater fish propagation and culture farms, which was accomplished by the kind cooperation and coordination of West Azerbaijan Province aquaculture department, samples of feed were evaluated during 2 phases, one phase between spring and summer and another phase between fall and winter, based on aflatoxin amount by HPLC technique. The feed samples used in this research were from different factories and of various kinds (SFT-FFT-GFT-BFT) and sizes. After the fulfilling of high performance liquid chromatography (HPLC) and evaluation of the test results in accordance with laboratory standards (the concentration was between 2-4 ppb). Result of samples of the second stage, fall and winter were negative, but of the samples of the first stage, spring and summer there were 5 positive samples. The total concentration of toxin (B₁, B₂, G₁, G₂) was between 1.21 to 6.62 ppb. The sample has been concentration of 6.62 ppb highly exceeded the allowed level. During these examinations, it was revealed that, the farms which had executed the hygienic principals of stocking, showed lower levels of toxin in the diet and vice versa. The toxin levels detected between spring and summer are higher than those of fall and winter due to the high heat and humidity of the warehouse.

Key words: Aflatoxin, fish diet, temperature, Azerbaijan Province, trout

INTRODUCTION

Aflatoxin is a toxic compound produced by *Aspergillus flavus* and *A. parasiticus*. The molds can grow in improperly stored feeds and feeds with inferior quality of ingredients.

Aflatoxins represent a serious source of contamination in foods and feed in many parts of the world (Murjani, 2003). Aflatoxin B₁ is known to be the most significant form that causes serious risk to animals and human health. The carcinogenic effect of aflatoxin B₁ has been studied in fishes such as salmonid, rainbow trout, channel catfish, tilapia, guppy and Indian major carps (Jantrarotai and Lovell, 1990; Lovell, 2001; Tacon, 1992; Wu, 1998; Chavez *et al.*, 1994; Murjani, 2003) and *Penaeus monodon* (Bautista *et al.*, 1994).

Aflatoxicosis is a disease that can affect many species of fish and shellfish and results when feed contaminated with aflatoxins is eaten by the fish (Ashley, 1970; Hernández *et al.*, 2005; Bautista *et al.*, 1994).

The first documented incidences of aflatoxicosis affecting fish health occurred in the 1960s in trout hatcheries. Domesticated rainbow trout (*Oncorhynchus mykiss*) that were fed a pelleted feed prepared with cottonseed meal contaminated with aflatoxins, developed liver tumors (Ashley, 1970). As many as 85% of the fish died in these hatcheries (Taniwaki, 2001).

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In tropical and subtropical conditions, this potential is further increased due to storage under humid and hot conditions. International trade in affected commodities and exposure to aflatoxins are worldwide concerns and the economic impact due to animal losses can be enormous (Golan and Paster, 2008).

Four major aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) are direct contaminants of grains and finished feeds. Factors that increase the production of aflatoxins in feeds include environmental temperatures above 27°C (80°F), humidity levels greater than 62% and moisture levels in the feed above 14%. The extent of contamination will vary with geographic location, feed storage practices and processing methods. Improper storage is one of the most important factors favoring the growth of aflatoxin-producing molds and it is a major element that can be controlled by the fish producer (Payne *et al.*, 1988).

Rainbow trout and Nile tilapia are extremely sensitive to AFB₁, while channel catfish are much less responsive (Jantrarotai and Lovell, 1990; Tuan, 2001).

The Rainbow trout was widespread in the Province of West Azarbaijan. This condition was observed in several farms which administered moldy feeds to their fish. Interview with farmers indicated that moldy feed was caused by high moisture content and improper storage of their feeds.

The purpose of this study was to assess the production of aflatoxin-contaminated feeds in the cold (autumn and winter) and warm (spring and summer) seasons and effect on fish growth. Results from this research will answer some of the questions of trout farmers on their experience on the rainbow trout in the West Azarbaijan.

MATERIALS AND METHODS

Twenty four samples of food (from April 2006 to April 2007) were evaluated during 2 phases warm seasons : spring and summer; cold seasons: autumn and winter in completely random design were used in this study. The compositions of basal diet were from different factories and of various kinds (SFT, FFT, GFT, BFT) and sizes.

These consisted of:

- SFT (Starter Food Trout): containing protein > 45-50% for fry > 5 g
- FFT (Fingerling Food Trout): containing protein ≈ 45-50% for fingerling = 5-30 g
- GFT (Grower Food Trout): containing protein = 35-40% for fish = 30-350 g
- BFT (Brood Food Trout): containing protein = 45-45% for adult fish > 350 g
- The concentration of aflatoxin in diet was adjusted according to aflatoxin B₁, B₂, G₁ and G₂ levels as representative mycotoxin

Determination of Aflatoxin Production by HPLC

The AFB₁, AFB₂, AFG₁ and AFG₂ concentration was determined by HPLC. All samples were threefold extracted with chloroform and this was followed by evaporation at 36°C under nitrogen gas; then the samples were finally dissolved in methanol. The samples were filtered through a Teflon filter (pore size, 0.2 µm; Chromafil; Macherey+Nagel, Düren, Germany) before they were used in HPLC analysis. Forty-microliter aliquots of these filtered extracts were injected for the quantitative determination of the AFB₁ concentration.

The HPLC system consisted of a model L-7100 HPLC-pump (Merck/Hitachi, Darmstadt, Germany), a model L-7200 autosampler combined with a Peltier sample cooler (Merck/Hitachi) and a model HP 1050 diode-array detector (Hewlett-Packard, Böblingen, Germany). The chromatograms were digitally processed by the ChemStation software system (Hewlett-Packard).

For analysis of AFB1, a reversed-phase C₁₈ column (LiChroCART 250-4 RP-18 [5.0 μm]; Merck) protected by a guard column (LiChroCART 4-4 RP-18 [5.0 μm]; Merck) was used with an isocratic mobile phase of acetonitrile-methanol-H₂O (25:25:50, vol/vol/vol) at a flow rate of 1.0 mL min⁻¹. The presence of AFB1 was monitored by the diode array detector at a wavelength of 365 nm (Móricz *et al.*, 2007).

RESULTS AND DISCUSSION

In the Philippines, the limit of aflatoxin in the feed prescribed by the Bureau of Animal Industry is less than 20 ppb. According to national feed legislation in the USA, maize (corn) and peanut (groundnut) products that are to be used for feeding dairy and immature animals (including fish cannot contain more than 20 ppb of aflatoxin (Lovell, 2001).

Table 1 and 2 summarizes the final growth *Aspergillus spp.* in the different feed. Conditions for all of feeds were similar in all the treatments (p>0.05). Significant differences were observed in the mean aflatoxin (ppb) levels among feeds (p>0.05) (Table 3, 4).

Results showed that aflatoxin concentrations increased as the levels of *Aspergillus sp.* contamination increased in the feed. It was observed that feeds contaminated with *Aspergillus sp.* gave higher levels of aflatoxin (6.82 ppb) at warm season and lower levels of aflatoxin (1.1 ppb) at cold seasons. The decrease in aflatoxin level may have been the result of the deteriorating growth of *A. flavus* as time progressed.

Table 1: Aflatoxin levels in the different feed in warm seasons (T = 18.4°C ±5.94)

Feed samples	Mean of aflatoxins(ppb)			
	B ₁	B ₂	G ₁	G ₂
FFT	1.46	5.16	0	0
SFT	0.0	2.7	0	0
BFT	0.0	1.21	0	0
GFT	0.0	0.0	0	0
SFT	0.0	0.0	0	0

Table 2: Aflatoxin levels in the different feed in cold seasons (T = 3.6°C ±7.5)

Feed samples	Mean of aflatoxins (ppb)			
	B ₁	B ₂	G ₁	G ₂
FFT	1.46	2.91	0	0
SFT	0.0	0.0	0	0
BFT	0.0	0.0	0	0
GFT	0.0	0.0	0	0
SFT	0.0	0.0	0	0

Table 3: B₁ and B₂ levels in the different seasons

Seasons	Mean of aflatoxins produced (ppb)	
	B ₁	B ₂
Warm	0.832±1.42	0.381±0.654
Cold	0.292±0.65	1.814±2.175

Table 4: B₁+B₂ levels in the different seasons

Seasons	Aflatoxins produced (ppb)
	B ₁ +B ₂
Warm	1.053±1.713
Cold	0.607±1.09

Aflatoxin production is the consequence of a combination of species, substrate and environment. The factors affecting aflatoxin production can be divided into three categories: environment, nutritional and biological factors. Physical factors include temperature, pH, moisture, light, aeration and level of atmospheric gases. Aflatoxins are produced only between temperatures of 12 and 14°C and the optimal temperatures is 25 to 35°C (Asis *et al.*, 2002).

Result in this study showed in second stage, fall and winter were negative, but of the samples of the first stage, spring and summer there were 5 positive samples because temperature in the first stage was $3.6^{\circ}\text{C} \pm 7.5$ but in the second phase was $18.4^{\circ}\text{C} \pm 5.94$. The total concentration of toxin (B_1 , B_2 , G_1 , G_2) was between 1.21 to 6.62 ppb. The sample has been concentration of 6.62 ppb highly exceeded the allowed level. During these examinations, it was revealed that, the farms which had executed the hygienic principals of stocking, showed lower levels of toxin in the diet and vice versa. The toxin levels detected between spring and summer are higher than those of fall and winter due to the high heat and humidity of the warehouse.

Mycotoxin producing fungi are responsible for significant financial losses encompassing a broad spectrum of food and farm animals and extending through the food chain to the consumer. Every year a significant percentage of the world's grain and oil seed crops are contaminated with hazardous mycotoxins, such as aflatoxin. Unfortunately, discontinuing the feeding of aflatoxin contaminated grain is not always practical, especially when alternative feedstuffs are not readily available or affordable. Thus, these toxins frequently are detected in animal feed (Sanders *et al.*, 1968; Koehler, 1938; Kiermeier, 1977; Russo and Yanong, 2002).

Aflatoxins are poisons produced by naturally occurring molds. These molds can grow in grains and prepared feeds intended for fish production when storage conditions are suboptimal: temperatures of 27°C (80°F) or warmer and moisture at levels greater than 14%. These conditions are frequently seen in tropical and subtropical aquaculture.

To prevent aflatoxicosis, follow manufacturer's recommendations regarding shelf life and try to determine the feed manufacture date. Avoid using feeds that appear discolored, lump together and smell musty. Clean feed storage bins and automatic feeders regularly.

Aflatoxins lower production efficiency of cultured fish by reducing growth rates, impairing immunity and in some cases, causing mortality. Storing feed properly (in a cool, dry area on pallets and at least one foot away from any walls) can prevent unnecessary economic losses.

The moisture content of the substrate and temperature are the main factors regulating fungal growth and mycotoxin formation (Jarvis, 2008).

Koehler (1938) established that a moisture content of 18.3% on a wet weight basis, was the lower limit for the growth of *A. flavus* in shelled corn. Extensive studies under precisely controlled conditions (Sanders *et al.*, 1968; Taniwaki, 2001; Davis and Diener, 1970) established a moisture content in equilibrium with a relative humidity of 85% (or water activity (a_w) = 0.85) as the lower limit for growth of *A. flavus* and for the production of aflatoxins. In starchy, cereal grain such as wheat, oats, barley, rice, sorghum and maize, the lower limit is a moisture content of 18.3-18.5% on a wet weight basis and in groundnuts, Brazil nuts, other nuts, copra and sunflower and safflower seeds, all of which have a high oil content, it is a moisture content of 9-10%. The minimum, optimum and maximum temperatures for aflatoxin production are 12, 27°C and $40-42^{\circ}\text{C}$, respectively (Davis and Diener, 1970). Northolt *et al.* (1976) studied the effect of water activity and temperature on the growth and aflatoxin production of *A. parasiticus* and came to the conclusion that no detectable quantities of aflatoxin B_1 were formed at an a_w value below 0.83 and at temperatures below 10°C . In studies by Strzelecki and Gasiorowska (1974), aflatoxins occurred in 12.7% of 306 samples of animal feed and feed components in Poland, 4.2% of the samples containing more than $100 \mu\text{g kg}^{-1}$ and 2.6% of the samples containing more than $1000 \mu\text{g kg}^{-1}$. Feed components, mainly groundnut meals, were contaminated by aflatoxins more frequently and with higher levels. On the other hand, aflatoxin was detected in only one sample (2.7%) of cattle and sheep feeds ($300 \mu\text{g kg}^{-1}$) and in one sample (1.7%) of poultry feeds ($30 \mu\text{g kg}^{-1}$).

Swine feeds contained aflatoxins in 11.4% of samples, with 6 samples (5.7%) exceeding $250 \mu\text{g kg}^{-1}$. Two recent surveys of mixed feeds in the Federal Republic of Germany revealed that 1 in 60 samples contained aflatoxin B₁ levels exceeding $20 \mu\text{g kg}^{-1}$ (Seibold and Ruch, 1977); 45 out of another 105 samples contained levels of between 7 and $300 \mu\text{g kg}^{-1}$ (Kiermeier, 1977). Similar results were obtained in the United Kingdom (Patterson, personal communication) where, 95/172 samples of dairy feed were contaminated with aflatoxin B₁ levels of 1-350 $\mu\text{g kg}^{-1}$ and 92.4% contained no more than $30 \mu\text{g kg}^{-1}$.

MANAGEMENT AND CONTROL

Purchase of feeds that have been recently prepared and properly stored is recommended. Debris must be removed from feed ingredients and grains should be stored in clean bins or buildings. Where possible, complete fish feeds should be stored in an air-conditioned building for temperature and humidity control. Otherwise, feed should be stored off the ground, on pallets and at least one foot away from any walls (to avoid condensation) in a cool, dry area and for no longer than three months. If feed is held in bins outside, storage for longer than two weeks is not recommended.

When feeds are stored for long periods or under poor conditions, fish health problems may arise, not only from molds, but also from loss of vitamins and rancidity of oils in the feed. Control of rodents and insects is also important in maintaining nutrient quality and aflatoxin-free feeds.

Feeds that have the manufacturer's date stamped on the bags will prevent the purchasing of old feed. It is also a good idea to be familiar with when the feed was bought and how the feed was being stored by the feed supplier prior to purchasing feed.

Feeds stored for a long time and probably contaminated with molds appear stale, are discolored lump together and smell musty. Stale foods are often saturated with moisture and appear to sweat. Any containers that are used to store food (bins, automatic feeders) should be cleaned thoroughly on a regular basis to prevent mold growth on their surfaces (which may be hidden by newly placed fresh feed).

Regular testing for aflatoxins is a good idea. Simple on-farm inspection can be done visually (look for the presence of blue/grey mold on feed) or with a black light which may cause a bright greenish/yellow fluorescence if *A. flavus* is present. While the black light method is a rapid procedure, it is only a potential indicator of the presence of *A. flavus* and it may not work in all cases.

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