EFFECT OF AFLATOXIN-CONTAMINATED FEEDS IN NILE TILAPIA (*Oreochromis niloticus* L.)

Arsenia G. Cagauan[1], Roselyn H. Tayaban[1], Joselito R. Somga[2] and Rodora M. Bartolome[1]

[1]College of Fisheries and Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

[2]Fish Health Section, Bureau of Fisheries and Aquatic Resources, Arcadia Bldg., Quezon City, Philippines

Abstract

Aflatoxin-contaminated feeds at different levels (good feed, 10% moldy feed, 50% moldy feed and 100% moldy feed) were evaluated on the growth, survival and histology of liver of Nile tilapia (*O. niloticus)* reared under aquarium conditions for 120 days. The aflatoxin content ranged from <5 -115.34 ppb, with 100 % moldy feed having the highest and that of good feed having the lowest.

Results showed that the different levels of aflatoxin contamination did not significantly affect the final average length, weight and gain in weight of fish ($P>0.05$). However, percent survival of fingerlings was significantly influenced by aflatoxin ($P<0.001$). As the level of aflatoxin contamination increased, the percent survival correspondingly decreased to as low as 33%. Good feed or 0 % molds resulted to highest survival of 100%, consequently giving the highest biomass of 233.83 g. Fish fed with 100% moldy feed consistently gave the lowest biomass of 93.77 g ($P<0.05$). External manifestations in fish fed with aflatoxin-contaminated feeds were eye opacity leading to cataract and blindness, lesions on the body surface, fin and tail rot, yellowing of the body surface, abnormal swimming, feeble and stationary on one place, and reduced appetite. Histological analyses of the liver of fish fed with aflatoxin-contaminated feeds revealed alterations of the liver. Some fish fed with aflatoxin-contaminated feeds may survive and their growth unaffected but could cause immediate damage or gradual deterioration of the liver. Indeed, the study confirms that the yellowing of tilapia experienced by farmers in Central Luzon was due to aflatoxin contamination in the feed.

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 feeds in many parts of the world. These toxins have been incriminated as the cause of high mortality in livestock

and in some cases of death in human beings (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, 1992; Tacon, 1992; Wu, 1998; Chavez *et al*., 1994; Murjani, 2003) and *Penaeus monodon* (Bautista *et al*., 1994). There are very few researches regarding the effect of aflatoxin on Nile tilapia *Oreochromis niloticus* (Chavez *et al*., 1994; Diab *et al*., 1998; Tuan, 2001).

The yellowing of tilapia was widespread in the province of Pampanga in the wet season of 2002 and 2003. 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. Inaccessibility of farms due to bad roads caused by the heavy rains during the wet season resulted to non-delivery of feeds to farm houses. Feeds were left on the road near the farm for the farmers to pick up but due to inavailability of transport, feeds were left for days and they got wet by the rain. Nevertheless, farmers had to use these feeds since they have been allocated by the feed company as credit for the farmers. Farmers perception showed that moldy feed is safe to feed the tilapia since they have seen this in the pig industry where moldy feed is also fed. Fish mortalities due to the yellowing of tilapia were experienced by farmers. Moreover, some tilapias survived and were sold in the market but at a lower farm gate price because of their yellow color. This happening in the seat of tilapia production in the country was only described as jaundice in tilapia but the cause of it was not determined.

The purpose of this study was to assess the effect of aflatoxin-contaminated feeds on the growth and survival of Nile tilapia including external manifestations and effect on the liver. Results from this research will answer some of the questions of tilapia farmers on their experience on the yellowing of tilapia.

Methodology

Treatments and preparation of feeds

Four treatments were employed as follows: I = control (0% moldy feed) good feed; II = 10 % moldy feed + 90% good feed; III = 50 % moldy feed + 50% good feed; and IV = 100 % moldy feed. Commercial feed was sprinkled with tap water and infected with 10 µl of cultured *Aspergillus flavus* acquired from the Bureau of Post Harvest Research and Extension, Central Luzon State University, then the feed mixture was covered with a plastic sack. Contaminated feed was subjected to conditions favorable for the growth of molds such as moist condition and high temperature. Required amounts of good feeds and moldy feeds for each treatment were weighed carefully before mixing them thoroughly. Each of the feed mixture was analyzed for aflatoxin concentration at the Bureau of Animal Industry. Aflatoxin levels in the feeds were analyzed 7 and 14 days after contamination. The preparation of aflatoxin-contaminated feeds was done every week to avoid loss of the efficacy of the toxic compound due to aging.

Experimental set up

Twelve rectangular glass aquaria measuring 24" x 12" x 12", each equipped with aeration and filtration and filled up with 20 liters of water, were used in the experiment.

Fish

A total of two hundred mixed sex *O. niloticus* with weights ranging from 30-40 grams were used. Fish were stocked at a density of four fish per aquarium. The upper lips of the fish were cut to avoid cannibalism. The fish were fed a day after stocking and daily thereafter, two times a day, at 7:00 A.M. and at 5:00 P.M. at a feeding rate of 3% of the body weight. A total feeding period of 90 days was employed. Adjustment of feed ration was based from the monthly sample weight. Individual length and weight of fish were measured initially and monthly thereafter.

Gross external manifestations of the aflatoxin were observed. After three months of feeding, the liver and spleen of fish were preserved and examined based on the prescribed histological procedure in Herrera (1996) with the assistance of the Fish Health Section, Bureau of Fisheries and Aquatic Resources (BFAR).

Data gathered were initial and final individual length and weight, final total biomass, absolute gain in weight, specific growth rate (SGR) and survival of the fish.

Water quality

Water quality parameters like dissolved oxygen (DO) (mg l^{-1}), temperature (^{o}C), and pH were measured once a week using YSI D.O. meter Model 55 and pen-type HANNA pH meter. Total alkalinity, expressed as mg l^{-1} $CaCO_3$ was analyzed initially and monthly thereafter using the prescribed procedures in Boyd and Tucker (1992).

Statistical analysis

Data on growth and survival were analyzed using analysis of variance (ANOVA) in completely randomized design with three replications. Comparison of means was done using least significance difference (LSD). The general linear model in the Statistical Package for Social Sciences (SPSS) version 9 was used.

Results and discussion

Aflatoxin levels in the different treatment feeds

Table 1 presents the aflatoxin levels in the different treatments. As expected, Treatment IV (100% moldy feed) had the highest aflatoxin level ranging from 53.02-115.34 ppb, followed by Treatment III (50% moldy feed) with a value of 28.82-72.39 ppb, and Treatment II (10% moldy feed) with a value of <5- 38.62 ppb. Results showed that aflatoxin concentrations increased as the levels of *A. flavus* contamination increased in the feed.

It was observed that feeds contaminated with *A. flavus* gave higher levels of aflatoxin (i.e. 38.62 – 115.34 ppb) at 7-days after contamination and lower levels of aflatoxin (i.e. <5 – 53.02 ppb) after 14-days. The decrease in aflatoxin level may have been the result of the deteriorating growth of *A. flavus* as time progressed. After 7-days of contamination, *A.*

Table 1. Aflatoxin content (ppb) of feed from 7 to 14 days after inoculation of *Aspergillus flavus*.

Feed	Aflatoxin (ppb)	
	7-days	14 days
10 % aflatoxin-contaminated feed + 90 % good feed	38.62	<5
50 % aflatoxin-contaminated feed + 50 % good feed	72.39	28.82
100 % aflatoxin-contaminated feed	115.34	53.02

flavus growing on the feed looked yellow-green then became yellow-brown in color (perhaps a sign of aging) towards the 14th day.

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, 1992).

Effect on the growth and survival

Table 2 summarizes the initial and final growth and survival of *O. niloticus*. Initial length, weight and total biomass were similar in all the treatments ($P>0.05$). No significant differences were observed in the mean final weight, total length and gain in weight of fish among treatments ($P>0.05$). Final mean total length ranged from 15.01-17.05 cm, weight ranged from 53.80-78.27 g and gain in weight ranged from 19.31-44.40 g. SGR of fish at different levels of aflatoxin revealed that Treatment IV had the highest SGR (0.87) while those with Treatment III had the poorest SGR of 0.49. However, analysis of variance on the SGR showed no significant differences among the treatments ($P>0.05$). Mean per cent survival in the different treatments were significantly different ($P<0.001$). Decreasing survival was observed as *A. flavus* contamination in the feed increased. The lowest percent survival (33%) was obtained in Treatment IV (100 % moldy feed) while Treatment I (good feed) gave 100% survival. Mortality increased as aflatoxin level in the feed increased. Mean total biomass of Nile tilapia at harvest was highest in Treatment I (233.83 g), followed by Treatment II (162.07 g), and lowest in Treatments IV (93.77 g) and III (91.60 g) ($P<0.05$). This suggests that the fish total biomass was affected by the percent survival.

It was observed in aflatoxin treatments that the daily feed ration given to the fish was not completely consumed, an indication of the decreased appetite of fish or perhaps due to unpalatable feed. Decreased growth and appetite were observed by Royes *et al.* (2002) when tilapia was fed with diets containing 1,800 ppb of aflatoxin for 75 days.

Table 2. Summary of growth parameters and percent survival of *O. niloticus.*

Parameters	Treatment			
	I	II	III	IV
INITIAL				
Average total length (cm)	11.73	11.78	11.70	11.69[a]
Average weight (g)	33.88	34.84	34.49	33.86[a]
Total biomass (g)	135.50	139.37	137.87	135.43[a]
FINAL				
Average length (cm)	15.01[a]	15.68[a]	15.75[a]	17.05[a]
Average weight (g)	62.21[a]	61.91[a]	53.80[a]	78.27[a]
Gain in weight (g)	28.34[a]	27.07[a]	19.31[a]	44.40[a]
Total biomass (g)	233.83[a]	162.07[b]	91.60[c]	93.77[c]
Total weight difference (g)	98.33[a]	22.70[b]	-46.27[c]	-41.67[c]
Absolute growth rate (g day^{-1})	0.32[a]	0.30[a]	0.21[a]	0.49[a]
Specific growth rate (%)	0.67[a]	0.63[a]	0.49[a]	0.87[a]
Survival (%)	100[a]	67[b]	42[c]	33[c]

Note: Means followed by the same letters are not significant at $P > 0.05$.

Chavez *et al*. (1994) reported that *O. niloticus* fingerlings were able to tolerate the immediate effect of aflatoxin but later the fish developed external and internal abnormalities.

External manifestations

Different external manifestations of abnormality in fish fed with aflatoxin-contaminated feeds were eye opacity leading to cataract and blindness, lesions on the body surface, fin and tail rot, yellowing of the body surface of the fish, abnormal swimming, feeble and stationary on one place, and reduced appetite. These manifestations became more intense as aflatoxin level in the feed increased.

Effect on the liver

Observed manifestations of aflatoxin in the liver of fish were abnormal enlargement and yellowing. Collapsed liver were found in newly dead fish in Treatments III and IV. Similar manifestations were reported by Roberts (1978), Ferguson (1989), Wu (1998) and Royes *et al.,* (2002).

Several histological alterations were recognized in the liver of the fish examined and these were chronic manifestations. The liver of fishes in Treatments II, III and IV had extensive necrosis, acute cellular swelling or ballooning necrosis, chronic granulomatous inflammation, loss of color where the necrotic tissue becomes paler than the surrounding normal tissue.

Joner (2000) described the effect of aflatoxin in the liver as follows: first, aflatoxin is absorbed from the diet in the alimentary canal and is passed to different organs. The principal target organ for aflatoxins is the liver. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and leads to necrosis or liver cell death. The main reason for this is that aflatoxin metabolites react negatively with different cell proteins, which leads to

inhibition of carbohydrate and lipid metabolism and protein synthesis. In relation with the decrease in liver function, there is a derangement of the blood clotting mechanism, jaundice, and a decrease in essential serum proteins synthesized by the liver.

Water quality

Water quality ranges were as follows: temperature - 23.61 to 27.09^{o}C, dissolved oxygen concentrations - 5.16 to 6.07 mg l^{-1}, and pH - 7.77 to 7.87. Total alkalinity concentrations were 147.35 to 226.85 mg l^{-1} $CaCO_3$. These values are all ideal for the growth of Nile tilapia based on Boyd and Tucker (1992).

Summary

This study generated information on the effect of aflatoxin-contaminated feeds on *O. niloticus*. It was proven that aflatoxin-contaminated feeds can be tolerated by the fish but later develop liver damage. The yellowing of tilapia was indeed due to the aflatoxin in the feeds.

It would be interesting to investigate the residual aflatoxin in the flesh of tilapia which has implication on the consumers.

References

Bautista, M. N., L. Pitogo, C. R. Subosa, and E.T. Begino. 1994. Response of *Penaeus monodon* juveniles to aflatoxin B1 dietary contamination. The Third Asian Fisheries Forum Society, Manila. pp. 771-775.

Boyd, C. E. and C. S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Agricultural Experiment Station, Auburn University, Auburn, Alabama, U.S.A. 183 pp.

Chavez, S., P. Martinez, M. Osorio, C. A. M. Palacios, and I. O. Mareno. 1994. Pathological effects of feeding young *Oreochromis niloticus* diets supplemented with different levels of aflatoxin. Aquaculture 127 (1): 49-61

Diab, A. S., S. M. M. Abusead, and M. M. AbouElMagd. 1998. Effect of aflatoxin B1 on reproductive traits in *O. niloticus* and *O. aureus* and its control. Proc. 5th International Symposium on Tilapia in Aquaculture.

Ferguson, A. W. 1989. Systemic Pathology of Fish. Iowa State University Press, USA.

Herrera, A. A. 1996. Histology of tilapia (*Oreochromis niloticus*). Bureau of Fisheries and Aquatic Resources, Quezon City, Philippines. pp. 23-78.

Jantrarotai, W. and R. T. Lovell. 1990. Subchronic Toxicity of Dietary Aflatoxin B_1 to Channel Catfish. J. of Aquatic Animal Health, 2: 248 – 254.

Joner, A. 2000. Mycotoxins. (http://www.ansci.cornell.edu/courses/as625/1999term/toner/aflatoxins.html)

Lovell, R.T. 1992. Mycotoxins: hazardous to farmed fish. Feed International, 13(3): 24–28.

Murjani, G. 2003. Chronic aflatoxicosis in fish and its relevance to human health. Central Institute of Freshwater Aquaculture. India.

Roberts, R. J. 1978. Fish Pathology. Casell Ltd., London.

Royes, J. B. and R.P.E. Yanong. 2002. Molds in fish and aflatoxicosis. (http://edis.ifas.ufl.edu/FAO95)

Tacon, A.G. J. 1992. Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fish Technical Paper No. 330. Rome. 75 p.

Tuan, N. A. 2001. Response of Nile tilapia fed diets containing selected mycotoxins. (http://www.egsz.or/BilogicalCurrentContent/Zoology?Comparative%20Physiology/TOXICOLOGY.html)

Wu, F.C. 1998. Retention of diet-related mycotoxins in tissues of channel catfish. (http://www.egsz.or/BilogicalCurrentContent/Zoology?Comparative%2Physiology/TOXICOLOGY.html)