EFFECT OF AFLATOXIN-CONTAMINATED FEEDS IN NILE TILAPIA *OREOCHROMIS NILOTICUS* L.

A. G. Cagauan, R. H. Tayaban and R. M. Bartolome
College of Fisheries/Freshwater Aquaculture Center
Central Luzon State University
Science City of Muñoz, Nueva Ecija

J.R.Somga
Fish Health Section
Bureau of Fisheries and Aquatic Resources
Arcadia Bldg., Quezon City

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Introduction

The problem……..

- The yellowing of tilapia was widespread in the province of Pampanga in the wet season of 2002 and 2003.
- Several farms administered moldy feeds to tilapia. Interview with farmers indicated that moldy feed was caused by high moisture content and improper storage of 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 farms.
- Farmers believe 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.
- Some tilapias survived and were sold in the market but at a lower farm gate price because of their yellow color.

Moldy feeds in sacks (August 2002)

Boatful of moldy feed applied to tilapia pond (August 2002)

Caused by aflatoxin ?????

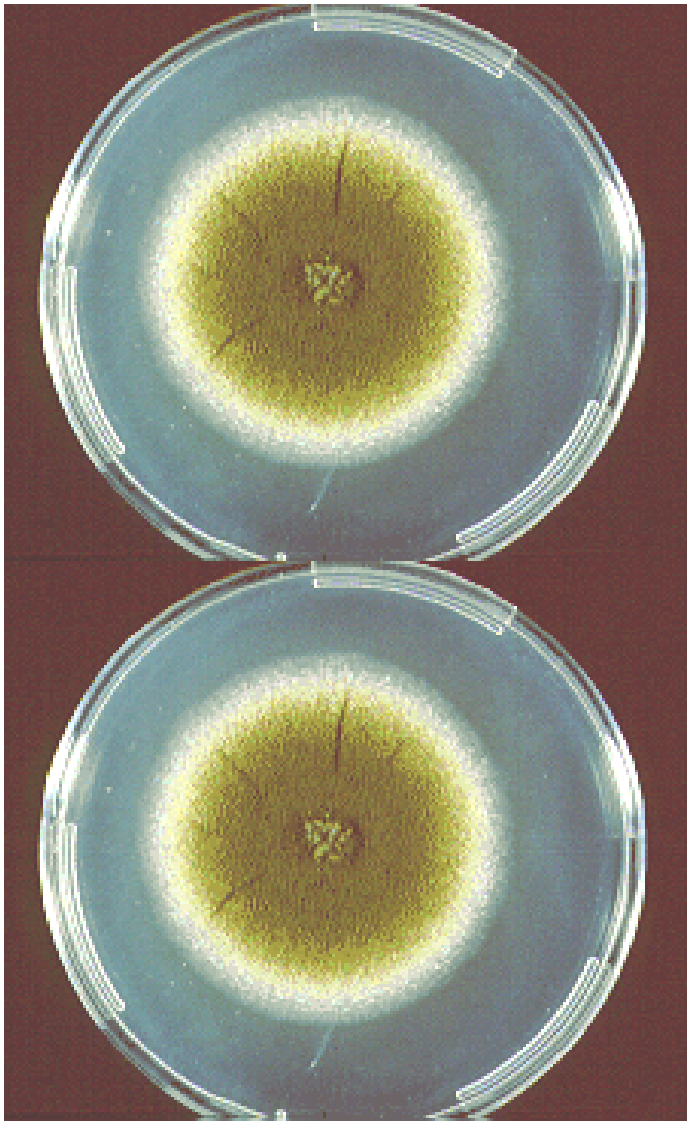
- This happening in the seat of tilapia production in the country was **described only as jaundice in tilapia** but the cause of it was not determined.

Tilapia fed with feed without molds

Photoes above taken from newly harvested Nile tilapia in Pampanga. (Photo credit to C.Tolentino)

What is aflatoxin ?

- **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.**
- **The toxin has been incriminated as the cause of high mortality in livestock and in some cases of death in human beings.**
- **The carcinogenic effect of aflatoxin B1 has been studied in fishes such as salmonid, rainbow trout, channel catfish, tilapia, guppy and Indian major carps and *Penaeus monodon*.**
- **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).**

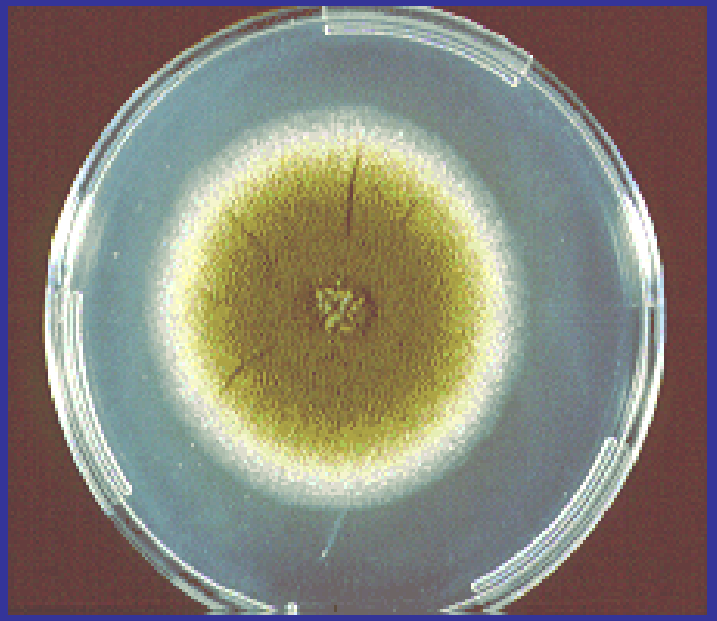


Objectives

- Generally, this study determined the effect of aflatoxin in Nile tilapia.
- Specifically, the study assessed the
  - **external manifestations of aflatoxin in Nile tilapia *O. niloticus***
  - **its effect on growth and survival**
  - **histological changes in the liver.**

METHODOLOGY

- **Preparation of fish feeds and aflatoxin analysis**
  - Commercial feed sprinkled with tap water and infected with 10 µl of cultured *Aspergillus flavus* from the BPRE, CLSU, then the feed mixture covered with a plastic sack.
  - Conditions were moist and high temperature.
  - Required amounts of good feeds and moldy feeds for each treatment were weighed and mixed thoroughly.
  - Each of the feed mixture analyzed for aflatoxin concentration at the BAI.
  - Aflatoxin levels in the feeds 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.



Culture of *A. flavus (BPRE)*

Treatments	Description
I	Good feed
II	10 % moldy feed + 90 % good feed
III	50 % moldy feed +50 % good feed
IV	100 % moldy feed

Experimental set up and design

Experimental set up. 12 glass aquaria, each equipped with aeration and filtration and with a capacity of 20 liters.

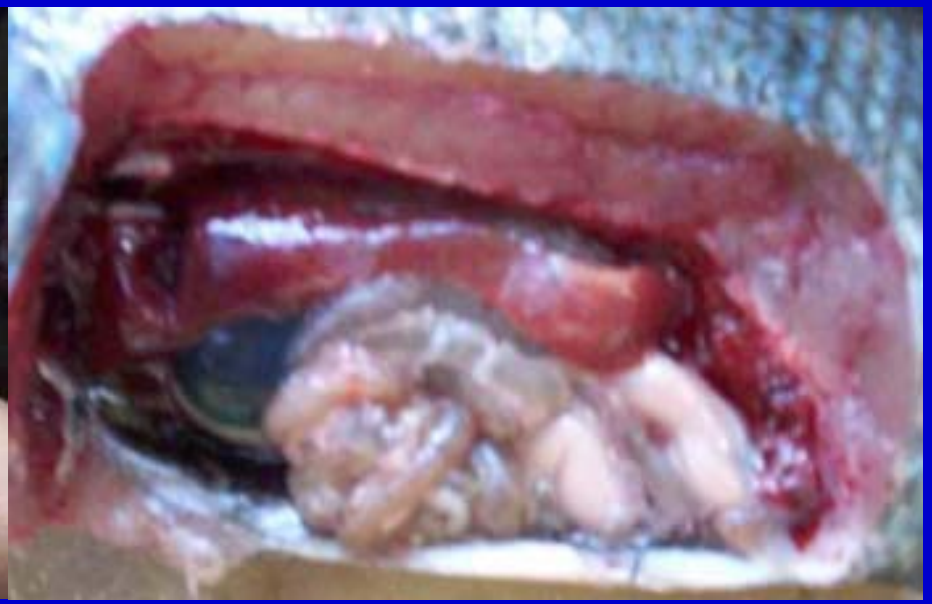
Experimental design: completely randomized design (CRD) with three replications

Test fish

- Nile tilapia (BFAR 2000), 30-40 g.
- Stocking density: 4 fish per aquarium
- Culture period: three months (90 days)
- Fish were fed at 3% of the body weight
- Adjustment of feed ration was based from the monthly sample weight.
- Individual length and weight of fish were measured initially and monthly thereafter

Manifestations of aflatoxin

- **External and internal manifestations** of the aflatoxin were observed. After three months of feeding, the liver of fish was 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

A. FISH

Parameter	Frequency of sampling	Method
➢**Individual length and weight** **-Gain weight** **- Total biomass** **-Absolute growth rate** **-Specific growth rate**	Initial and monthly thereafter	Digital weighing balance
➢**Survival**	At harvest	Counting
➢**Liver histological changes**	At harvest	Histological procedures

B. Water quality parameters

pH	Initially and biweekly thereafter	Digital pH meter
Temperature	Initially and biweekly thereafter	YSI DO meter Model 55
Dissolved oxygen	Initially and biweekly thereafter	YSI DO meter Model 55
Total alkalinity	Initially and monthly thereafter	Boyd and Tucker (1990)

Statistical Analysis

- analysis of variance in statistical Package for Social Sciences (SPSS) version10 for Windows Program (1999).
- mean comparison by least significant difference (LSD)

RESULTS AND DISCUSSION

Range of aflatoxin levels of feed from 7 to 14 days after inoculation of *Aspergillus flavus.*

Feed	Mean Aflatoxin (ppb)*	
	7-days	14 days
10 % *A. flavus* infected feed + 90% good feed	38.62	<5
50 % *A. flavus* infected feed + 50% good feed	72.39	28.82
100% *A. flavus* infected feed	115.34	53.02

* Mean of two samples.

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).

Summary of growth parameters and survival rate of Nile tilapia *O. niloticus.*

Parameters	Treatment 1	2	3	4
INITIAL				
Average length (cm)	11.73	11.78	11.70	11.69
Average weight (g)	33.88	34.84	34.49	33.86
Total biomass (g)	135.50	139.37	137.87	135.43
No. fish / aquarium	4	4	4	4
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]	3.77[c]
Total weight diff.	98.33 [a]	22.70 [b]	-46.27[c]	-41.67[c]
AGR(g/day)	0.32 [a]	0.30 [a]	0.21[a]	0.49[a]
SGR (%)	0.67 [a]	0.63 [a]	0.49 [a]	0.87[a]
Survival rate (%)	100 [a]	67[b]	42 [c]	33 [c]

Note: Similar letters are not significant at P < 0.001

External gross signs of fish on the different treatments

eye opacity leading to cataract and blindness

lesions on the body surface

Fin rot

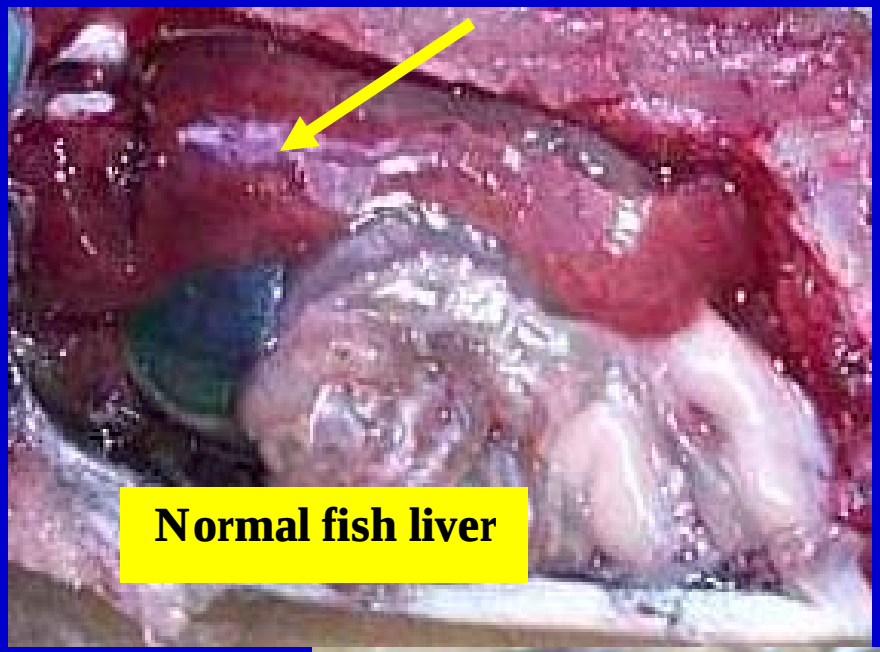
Yellowing of the body

Treatment III

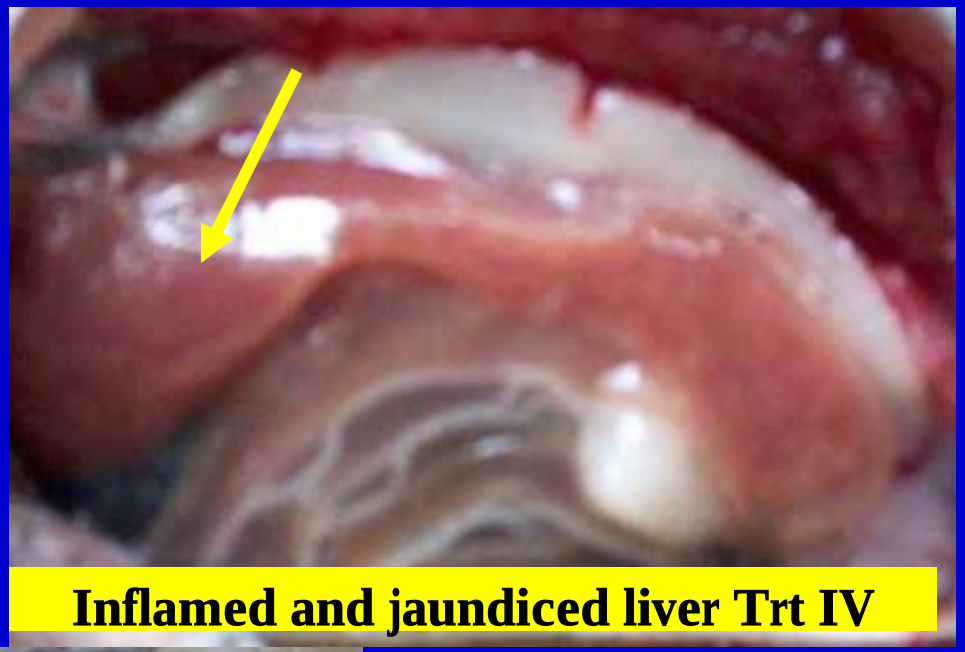
Control

Treatment IV

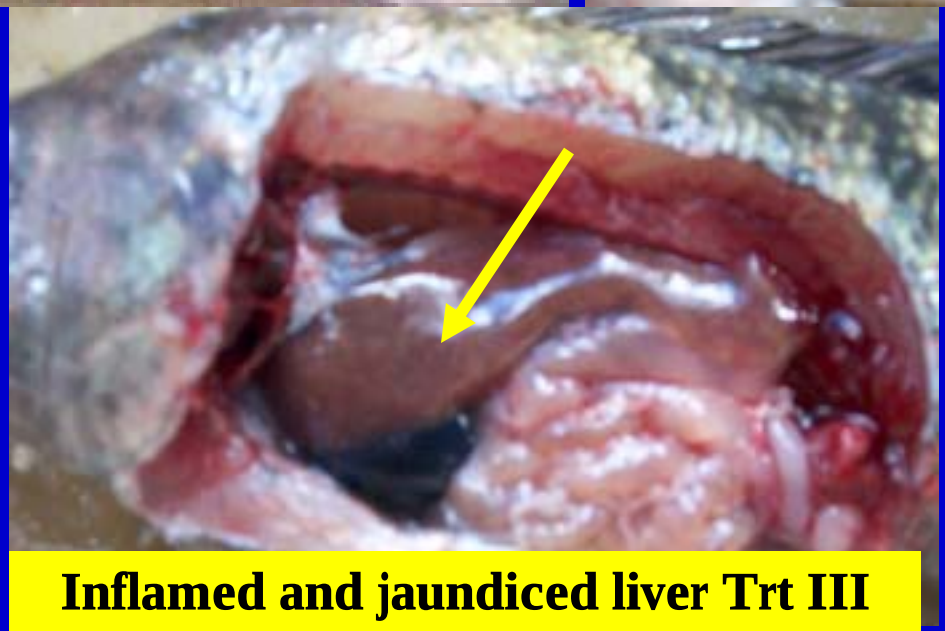
Liver manifestations



Normal fish liver

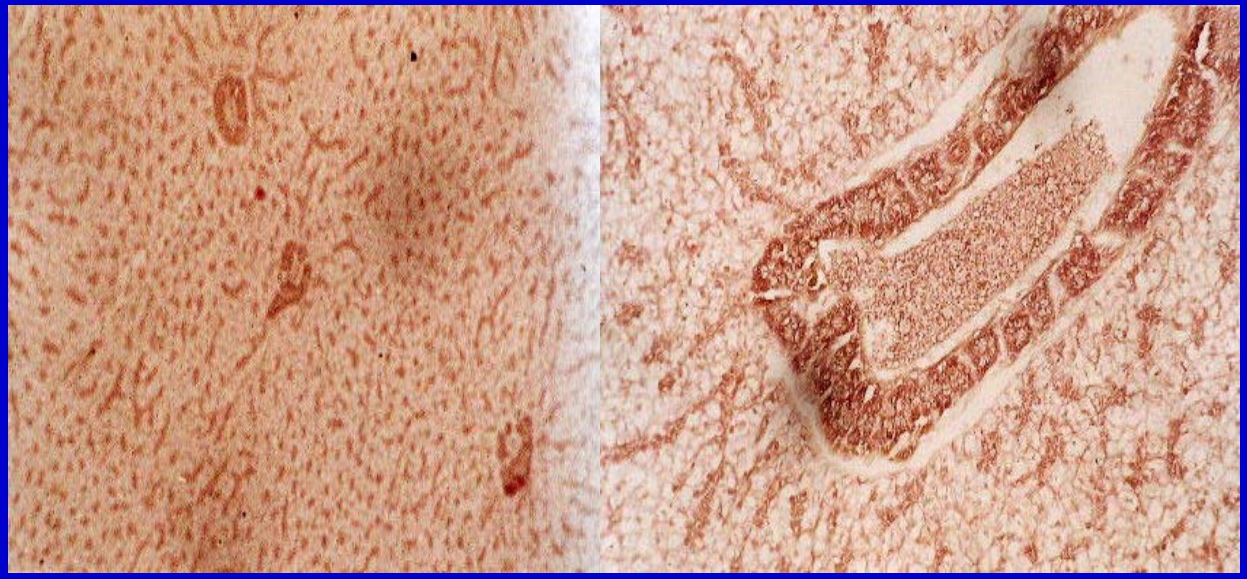


Inflamed and jaundiced liver Trt IV

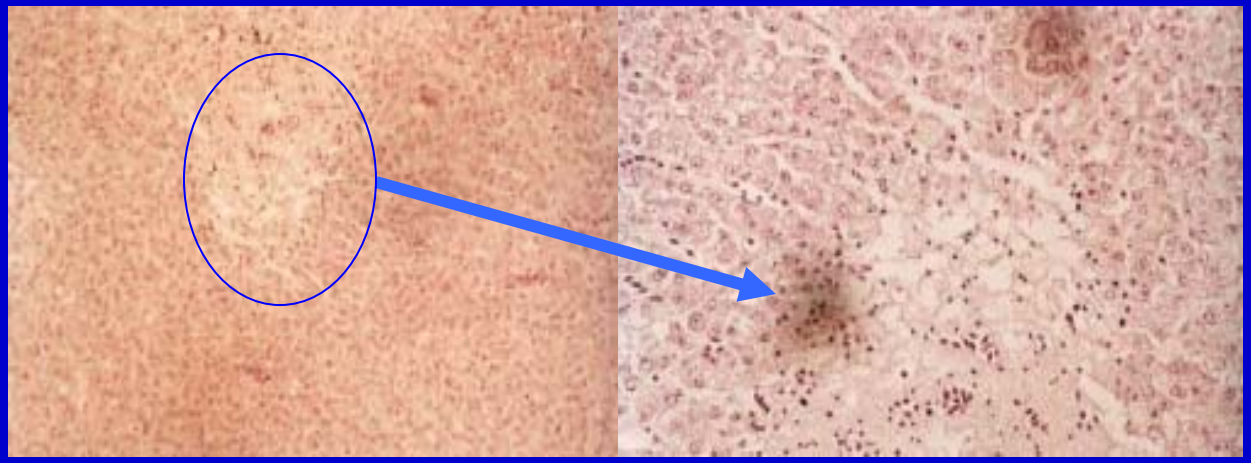


Inflamed and jaundiced liver Trt III

Histological changes in the liver

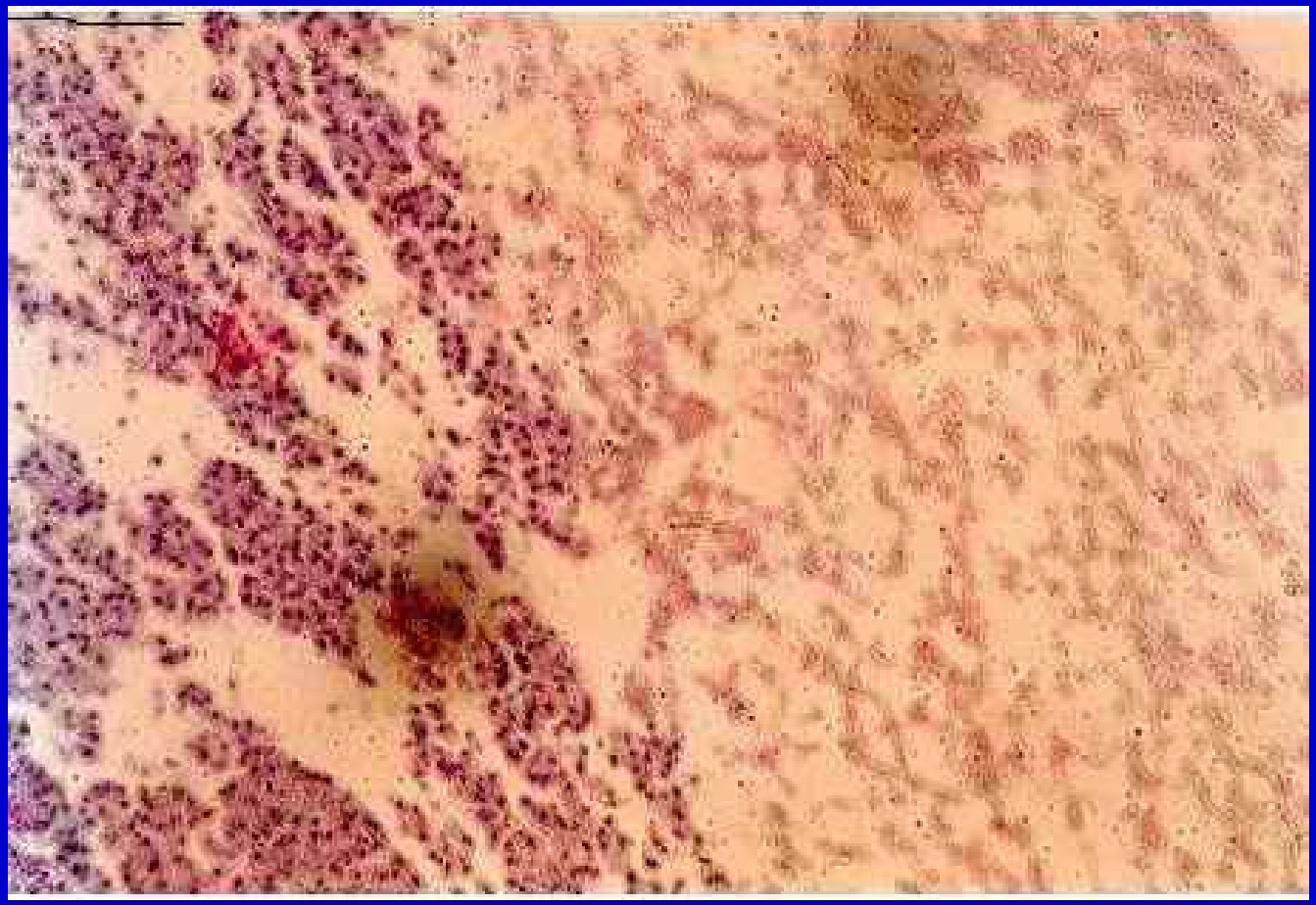


Normal liver 4x (left), 10x (right), Treatment I



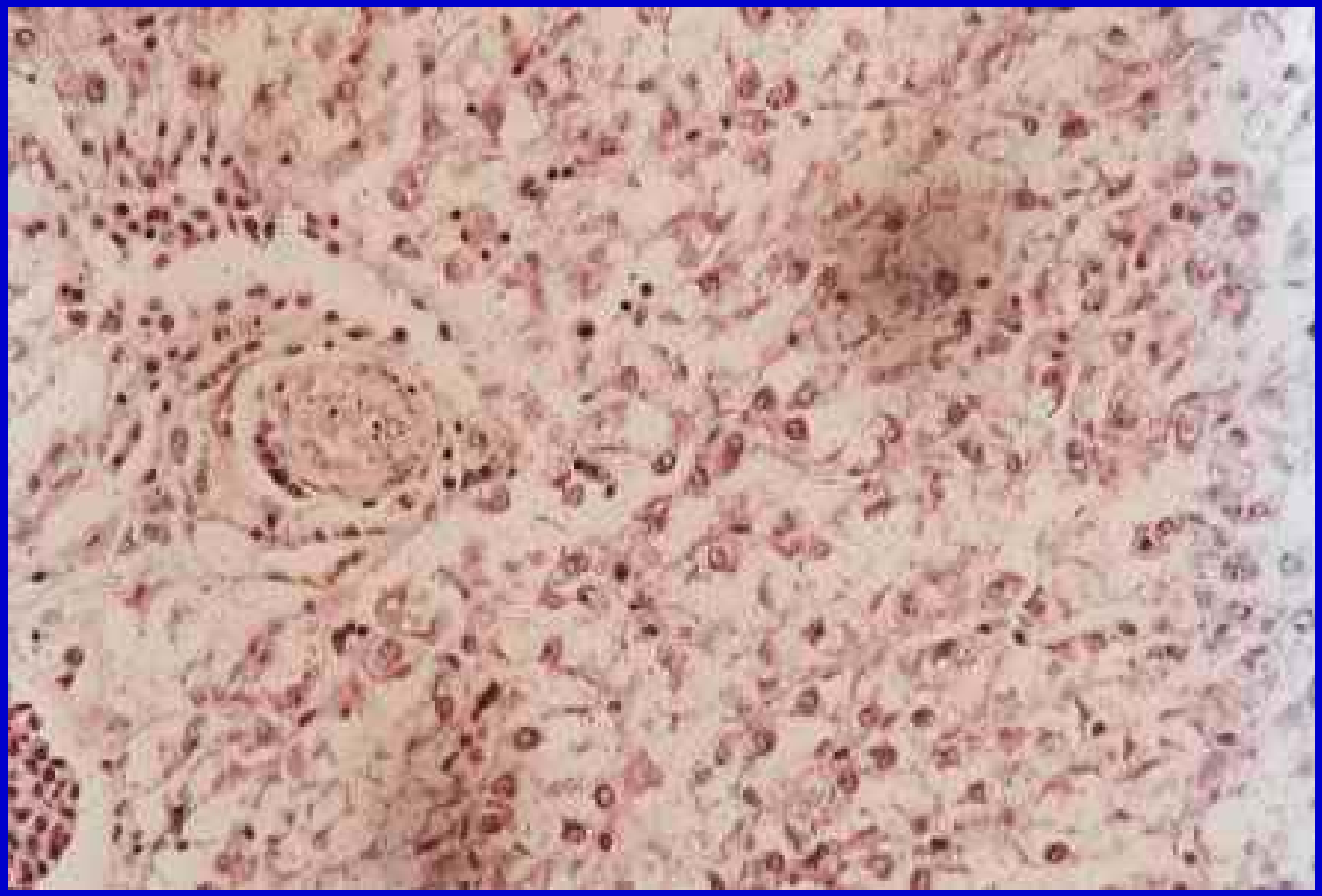
Extensive necrosis in Treatment IV

Histological changes in the liver



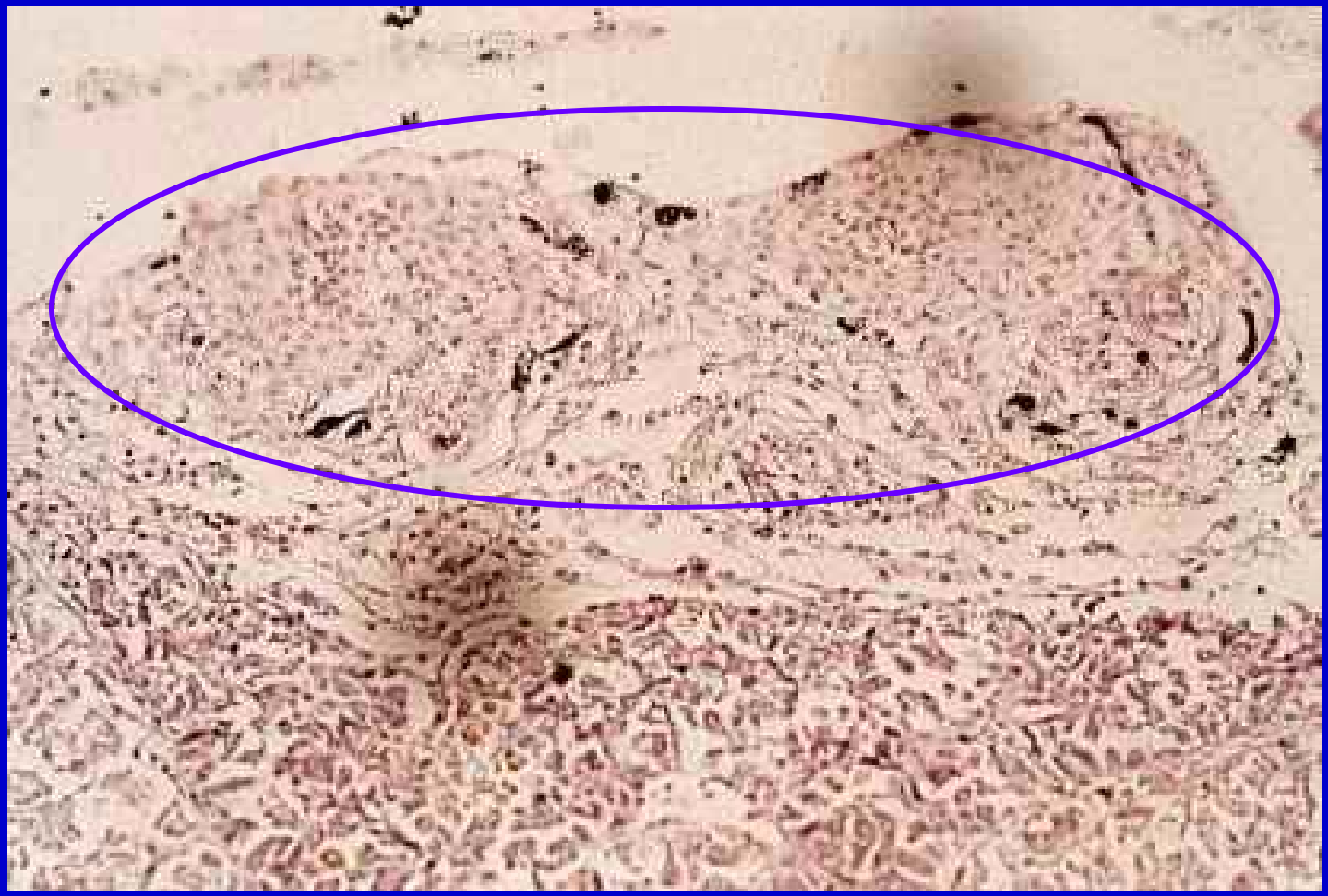
Early necrosis where the normal cells become reddish to pale brown (H.E. x 10) in Treatment IV

Histological changes in the liver



Acute cellular swelling or ballooning necrosis (H.E. x 20) in Treatment IV

Histological changes in the liver



Granuloma in Treatment III

CONCLUSIONS AND RECOMMENDATION

1. Yellowing of the body was indeed a manifestation of aflatoxin in the feed. Yellowing of tilapia was well manifested in feeds containing ≥29 ppb aflatoxin.

2. It was proven that aflatoxin-contaminated feeds can be tolerated by tilapia, particularly at low levels, but later develop liver damage.

3. Feeds containing aflatoxin levels less than 5 ppb can manifest histological changes in the liver.

4. Higher levels of aflatoxin in the feed can cause fish mortality.

5. It was proven in this study that it is not safe to feed tilapia with moldy feed (aflatoxin-contaminated feed).

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

Thank you!!

Maraming salamat po!!!