Experimental Aflatoxicosis in layer chicken (emphasizing reproductive pathology)

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1. Introduction

- Aflatoxins are toxic metabolites produced by certain fungi in/on foods and feeds v.i.z.
- *Aspergillus parasiticus and Aspergillus flavus*

a. History of Aflatoxicosis

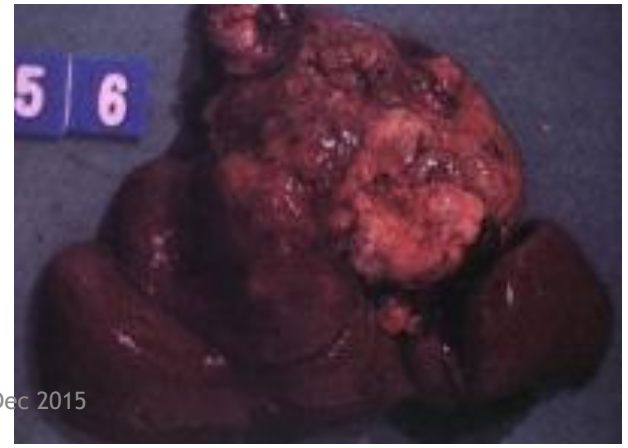
- 1960 more than 100,000 young turkeys on poultry farms in England died-Apparently the new disease was termed **"Turkey X disease"** .
- Later ducklings and young pheasants affected causing heavy mortality.
- All associated with feeds, v.i.z. Brazilian peanut meal.
- The name “Aflatoxin” derived from the name given to the toxin produced by *A. flavus* during 1962 (Sargeant *et al.*, 1963).

b. Aflatoxin

- Toxin was given the name **Aflatoxin** by virtue of its origin (A. flavis--> Afla).
- four major aflatoxins: B1, B2, G1, G2 & metabolic products, M1 and M2.
- AFs M1 and M2 isolated from milk of lactating animals (M)
- B –AFs B1 and B2 - blue fluorescence under UV-light,
- G - yellow-green fluorescence of the relevant structures under UV-light.
- Among all, AFB1 highly toxic compound of the group for almost all species of animals including poultry.
- High production of AF in feed at temperatures above 27^{o}C, with humidity levels above 62 per cent and moisture levels above 14 per cent (Royes and Yanong, 2002).

c. Aflatoxicosis and Animal Health

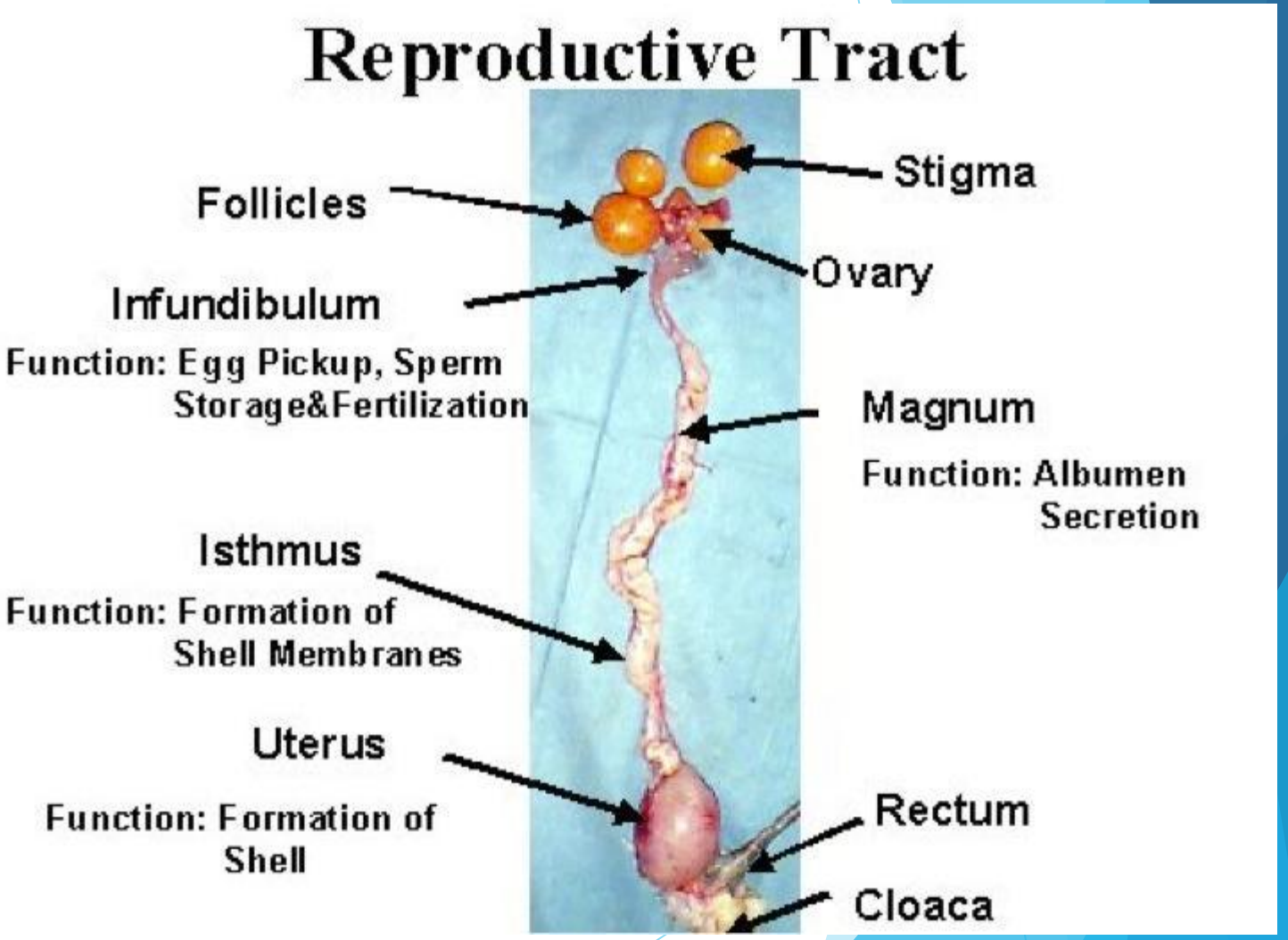
- Aflatoxicosis is primarily a hepatic disease.
- cause liver damage, decreased milk and egg production, immune suppression .
- Embryo toxicity.
- cause cancer in animal species.



d. Objectives

Unlike in broilers, very few studies conducted in layer chicken hence conducted study with following objectives:

- **Study the toxicopathological effects on laying hens.**
- **Emphasis on reproductive pathology.**



2. MATERIALS & METHODS

a. AF production

Aflatoxin was produced on rice (Shotwell *et al*., 1966)

- *A. parasiticus* NRRL 2999 - subcultured in PDA

- *A. parasiticus* NRRL 2999 - Culture on rice

b. Feeding of AF

- Layer birds –WL forsgate strain
- Layer mash –Pretested feed for mycotoxins
- Vaccine –NDV K strain
- Feeding 1 and 2 ppm of AF in the diets to laying hens for a period 4 and 6 weeks.
- Study of important parameters- feed consumption, body wt., egg production etc.

c. Experimental design

Group code	Groups	Levels of AF(ppm)
T1	Control	0
T2	Treatment	1
T3	Treatment	2

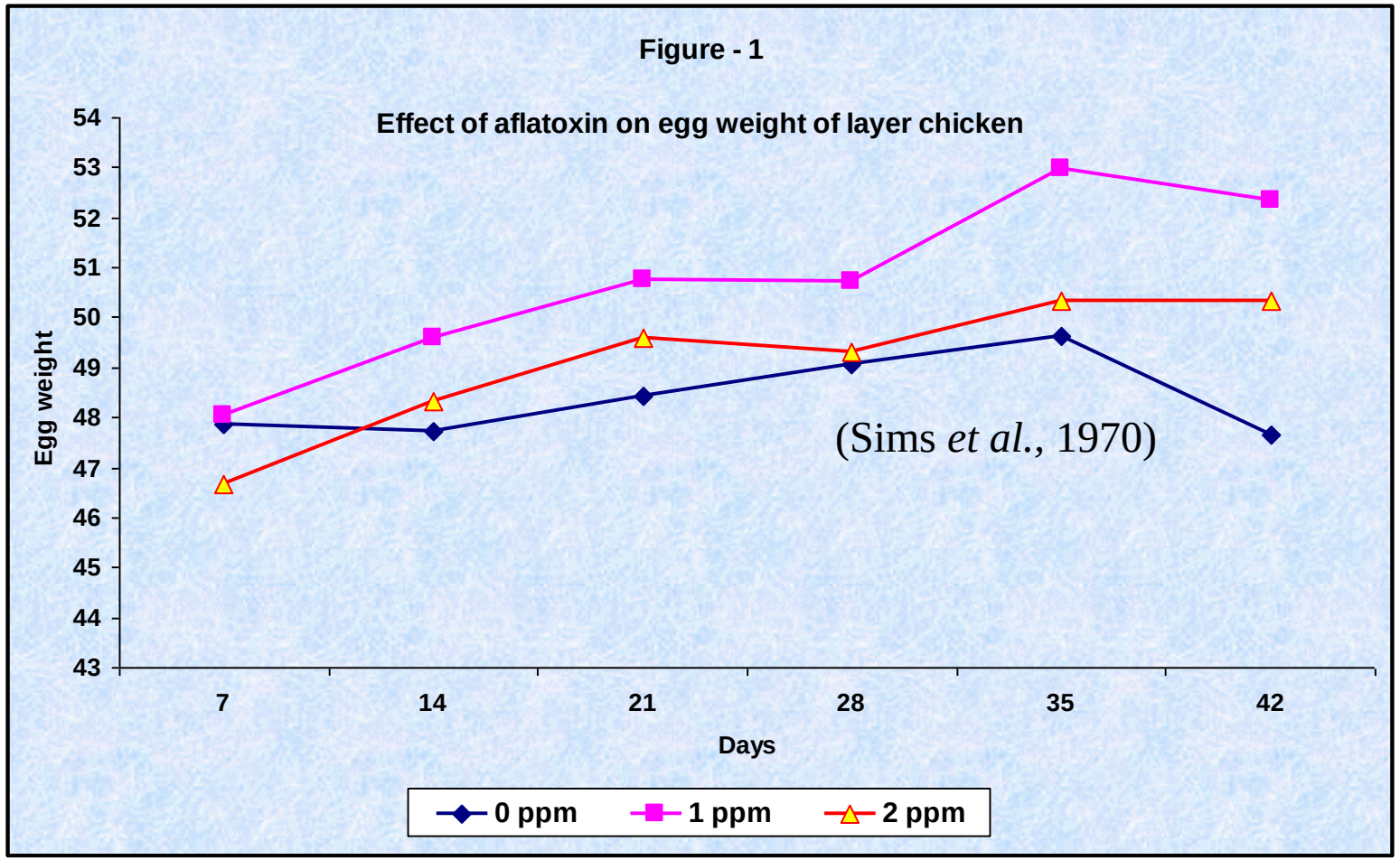
d. Statistical analysis: analysis of variance (ANOVA) test **by SPSS 10**

3. Results & discussion

a. Clinical signs and mortality

- No clinical signs or mortality were observed in layers fed with 1 and 2 ppm AF for 42 days.
- *Similar reports in laying hens fed up to 5 ppm AF for four weeks (Fernandez et al., 1995; Zaghini et al., 2005). Ghosh et al. (1990)*

b. Production parameters-Egg weights



Similar findings by feeding 2 to 10ppm (Sims *et al.*, 1970), Stephen et al. (1991), Vermal et al. (2003)

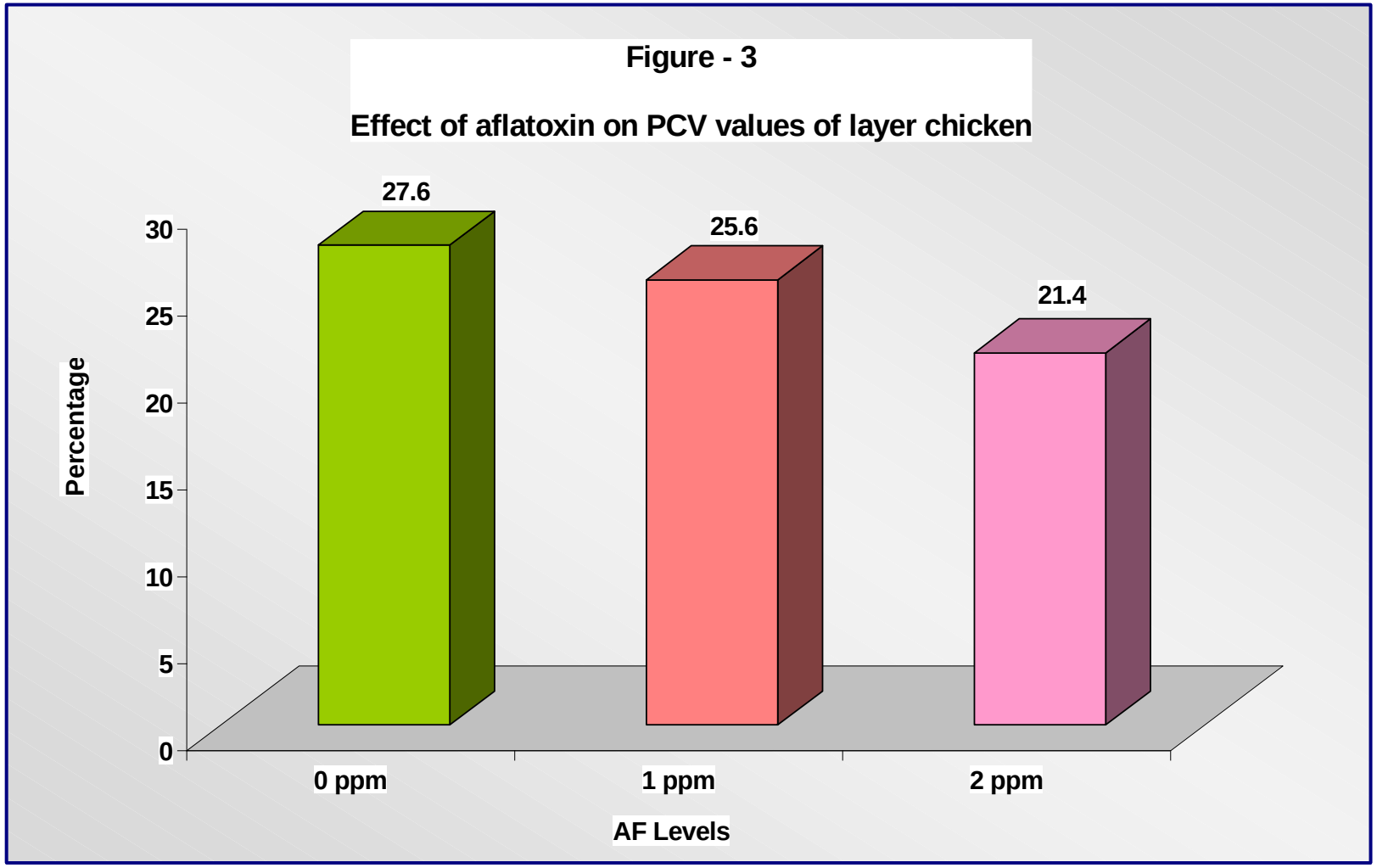
There was significant ($P<0.05$) increase in egg weight in 1 ppm fed birds when compared to control and 2 ppm fed groups.

Production parameters- Egg qualities

Photo A. Control egg (left) and pigmented shell (right) 1 ppm AF

Photo B. yellow egg yolk, blood spot- 2ppmAF

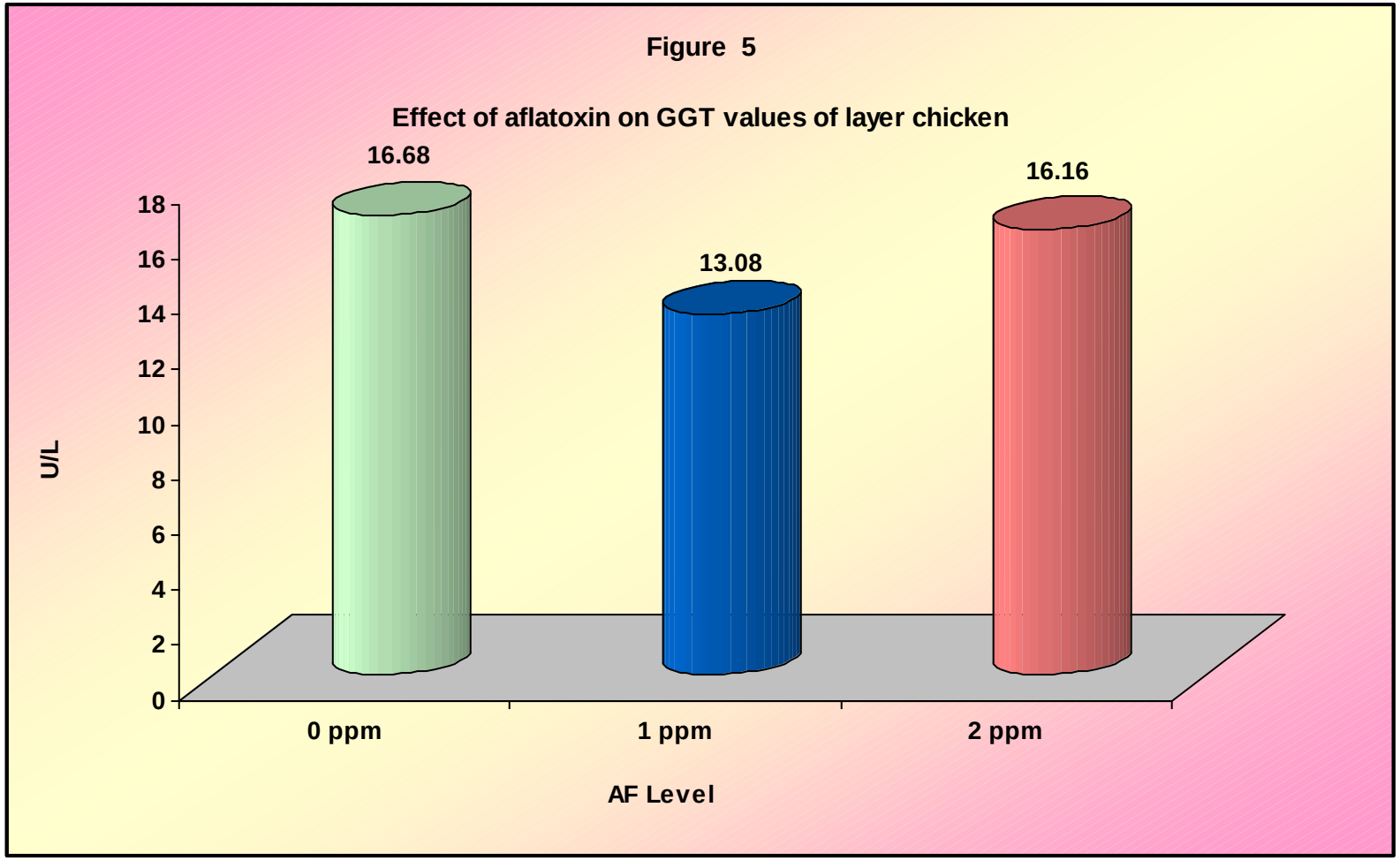
c. Effect on hematological parameters- PCV



Gounalan *et al.* (2005) reported significant reduction in PCV values in layer chicken fed with 0.5 ppm from 0 to 12 weeks age.

Significant ($P<0.05$) differences in PCV values were observed between the control and AF fed birds.

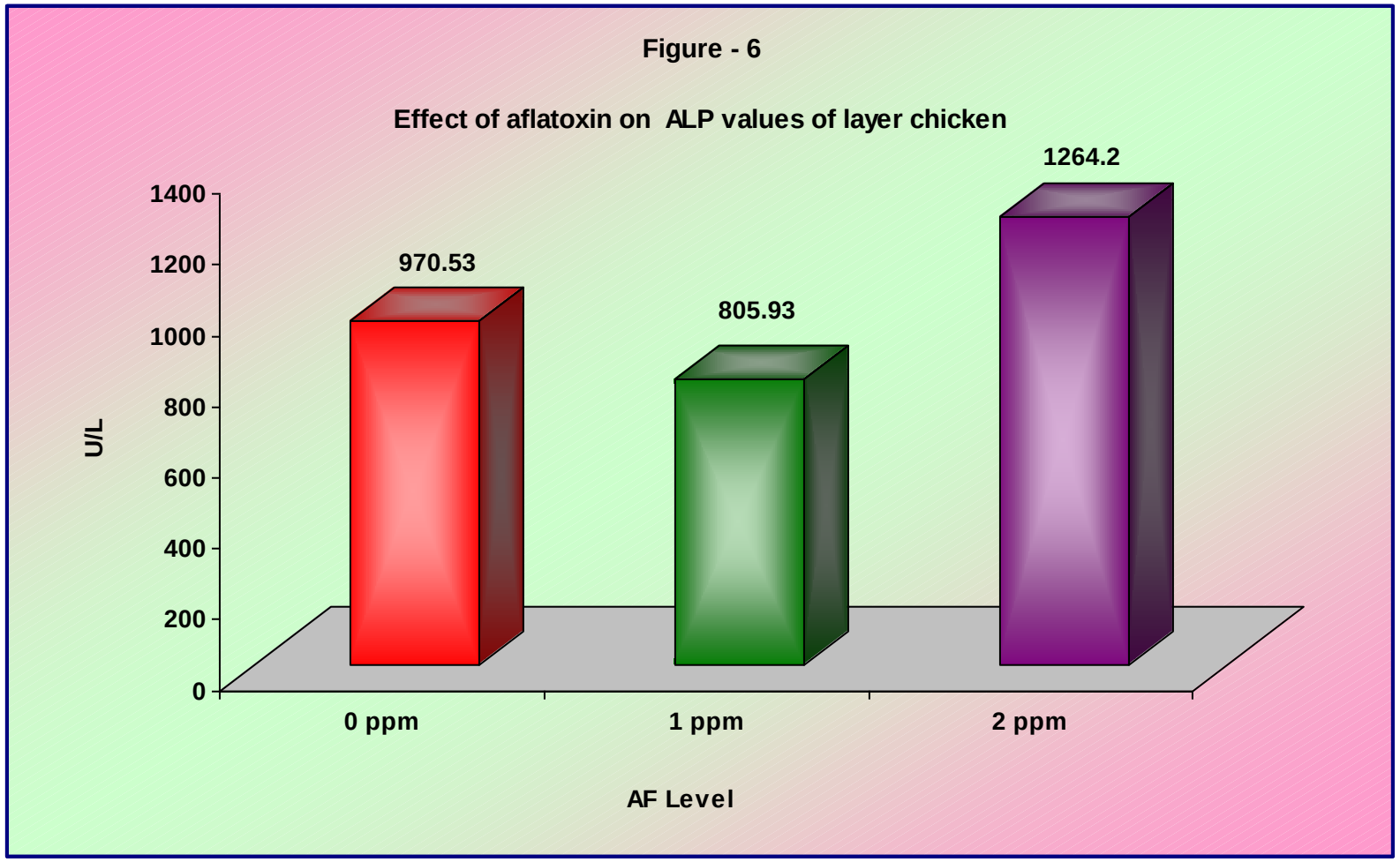
d. LFT-Serum enzymes- GGT values



Increased level of GGT in birds fed 500 ppb AF from 2 days onwards (Rao and Joshi, 1990; Kim *et al.*, 2003; Gounalan *et al.*, 2005).

GGT values differed significantly ($P<0.05$) between 1 and 2 ppm AF fed birds

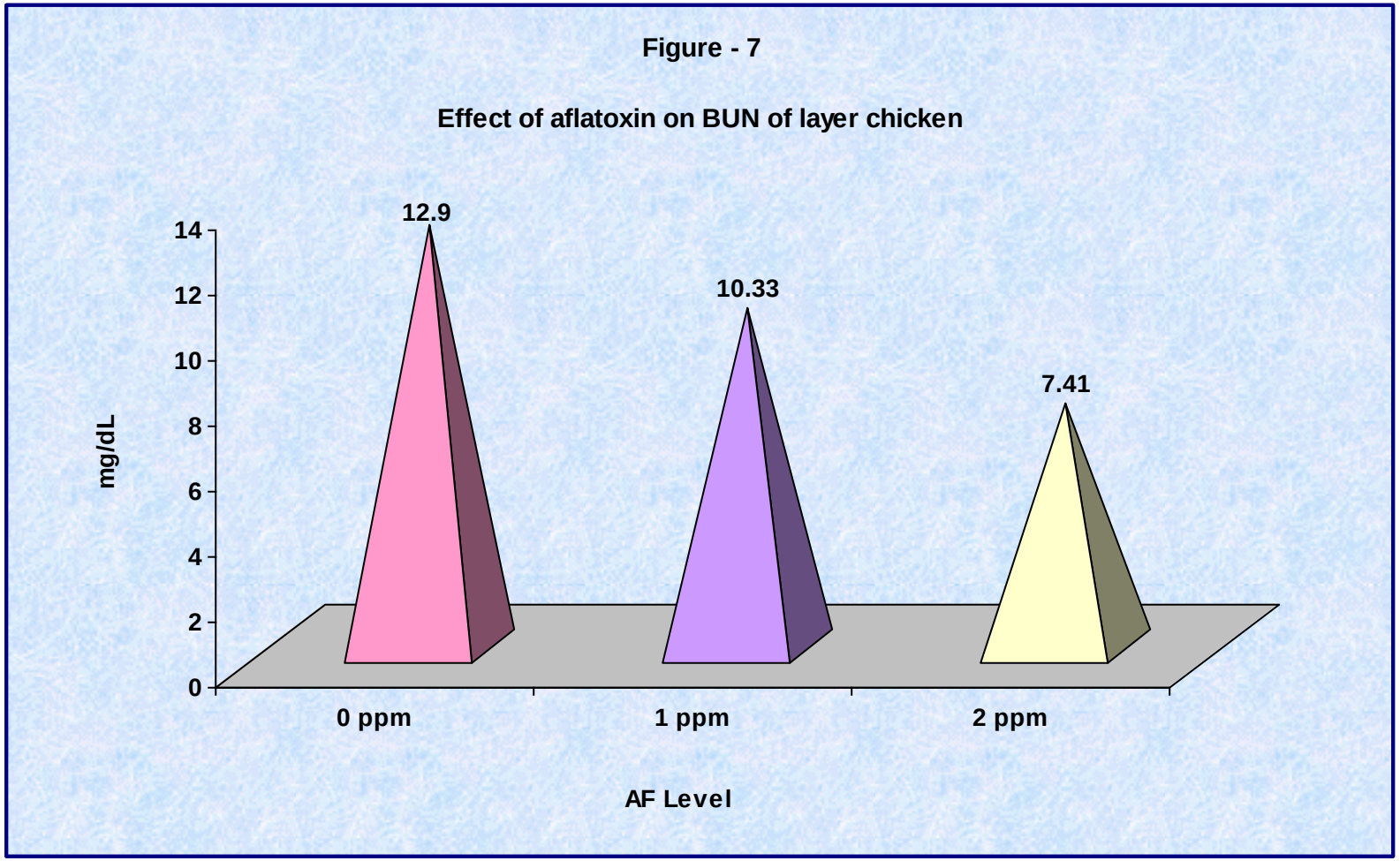
LFT-Serum enzyme- ALP values contd.



Similar findings in feeding of AF (0.5 to 2 ppm) from 0 to 60 days in broiler chicken (Jindal *et al.*, 1993; Nath *et al.*, 1996).

There was significant ($P<0.05$) increase in the level of ALP in 2 ppm AF fed layer birds when compared to the 0 and 1 ppm AF fed birds.

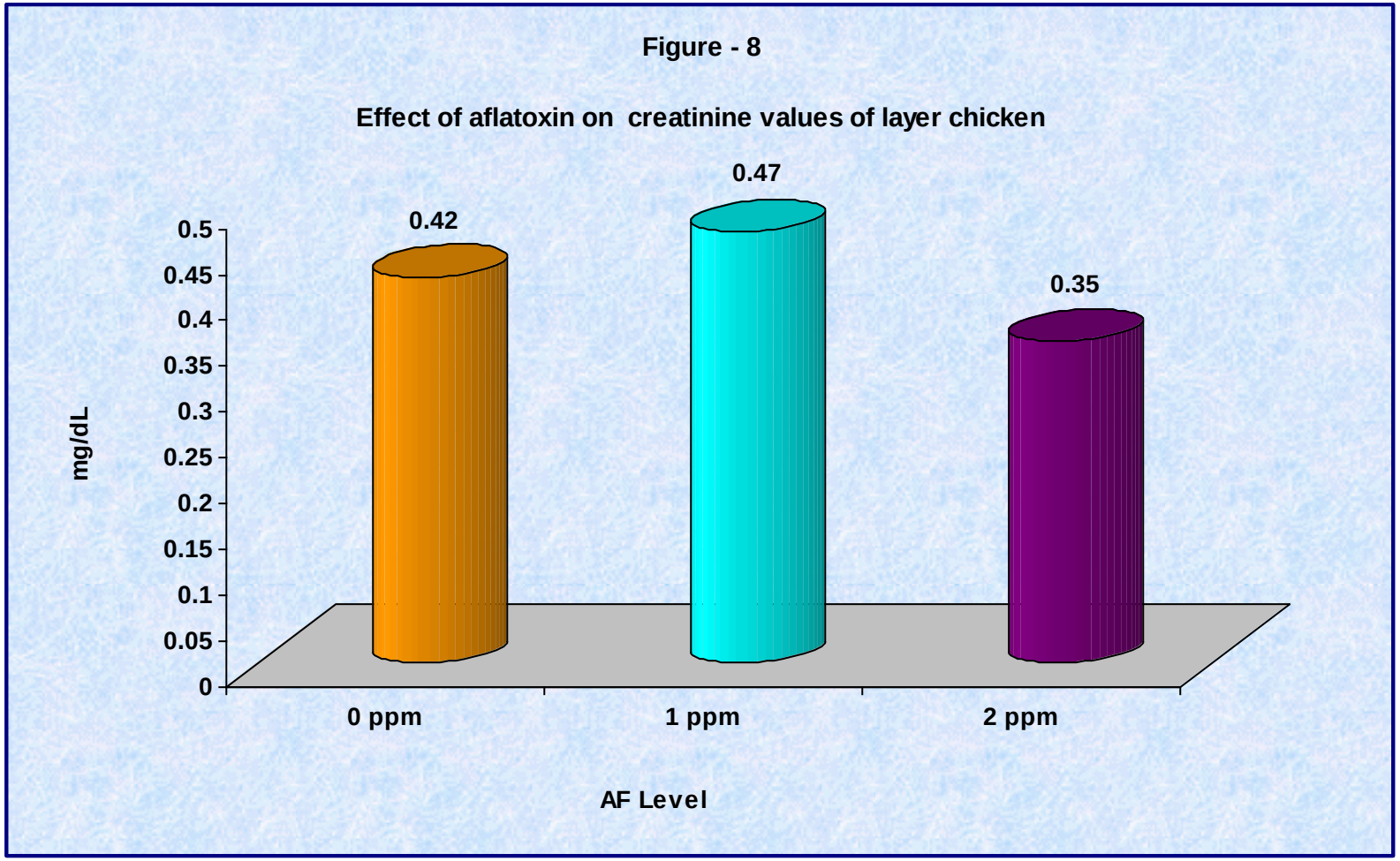
e. Kidney Function tests- BUN values



In contrary, increase in creatinine were reported by feeding 0.5 ppm AF to layer chicken up to 12 weeks of age (Gounalan *et al.*, 2005).

Significant differences ($P<0.05$) were observed between the control and AF treated birds for BU

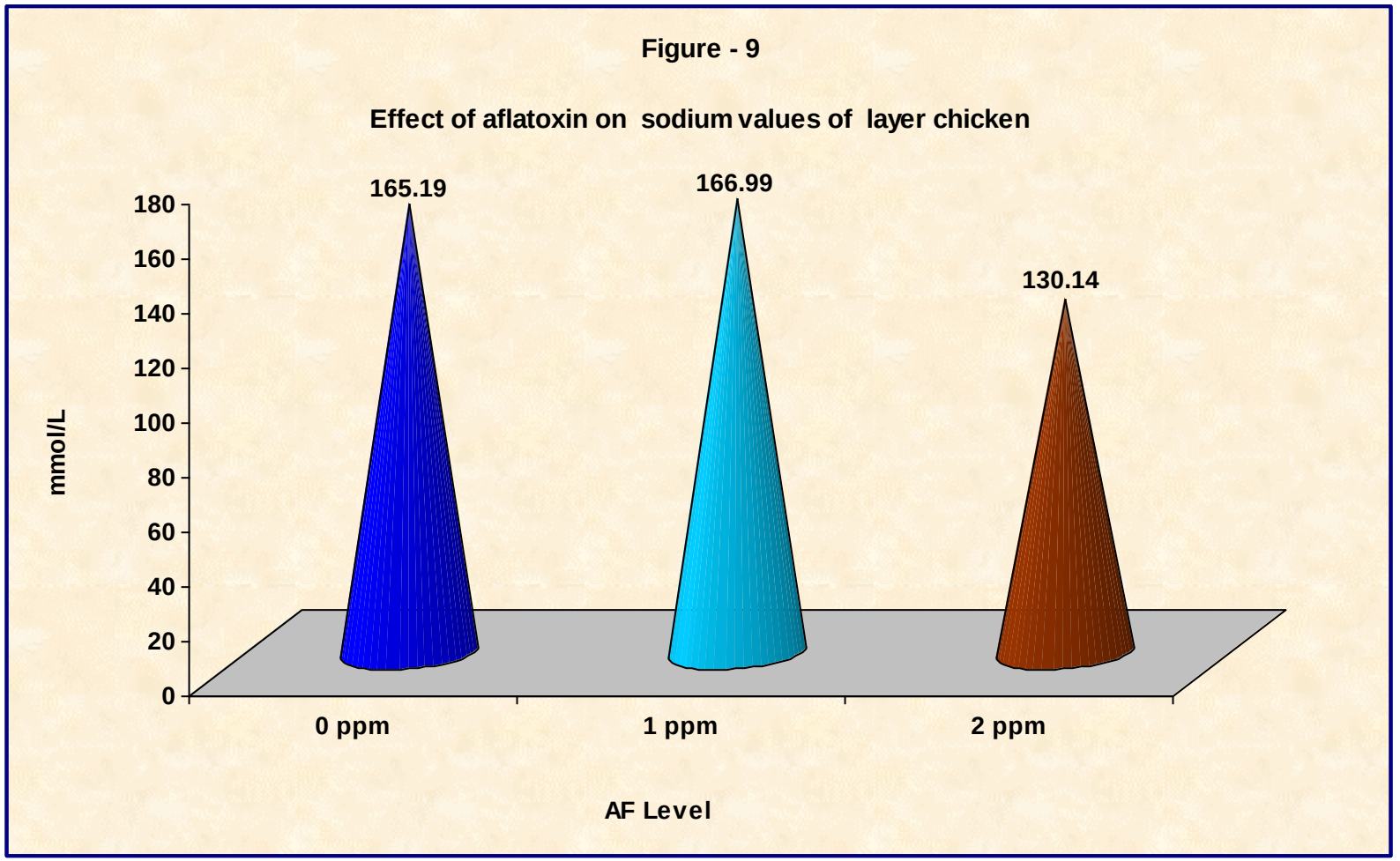
KFT- Serum creatinine values



In contrary, increase in creatinine were reported by feeding 0.5 ppm AF to layer chicken up to 12 weeks of age (Gounalan *et al.*, 2005).

variable results were observed for serum creatinine (Fig.8) when compared to the control.

KFT- Sodium values



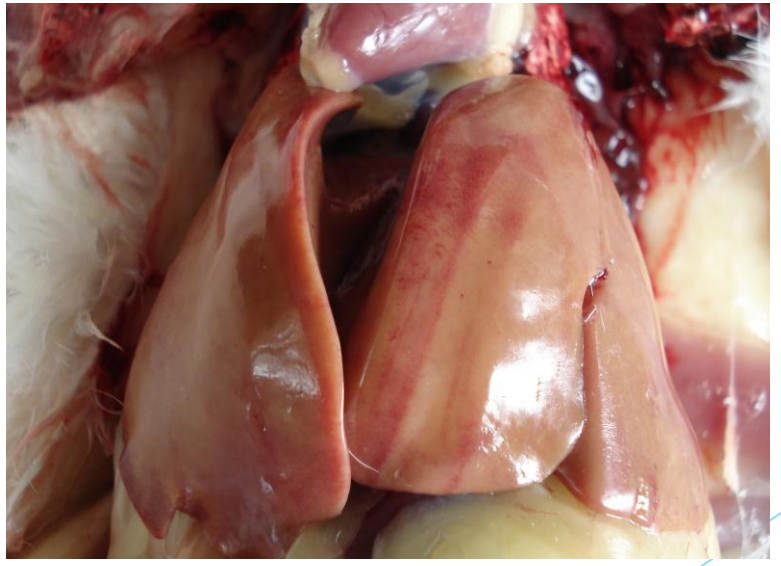
Serum sodium values decrease significantly ($P<0.05$) in 2 ppm AF fed layer chicken (Fig.9) when compared to the control and 1 ppm AF fed layer birds.

f. Pathology

Gross Lesion

Photo A. Liver-congestion & paleness 1 ppm AF

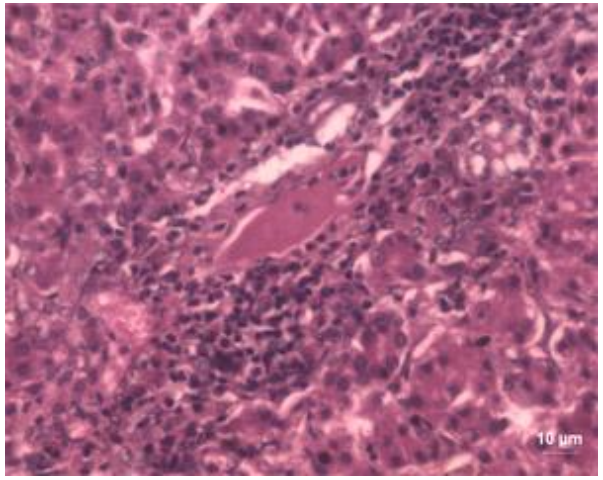
Photo B. Liver-pale & petechiae 1 ppm AF

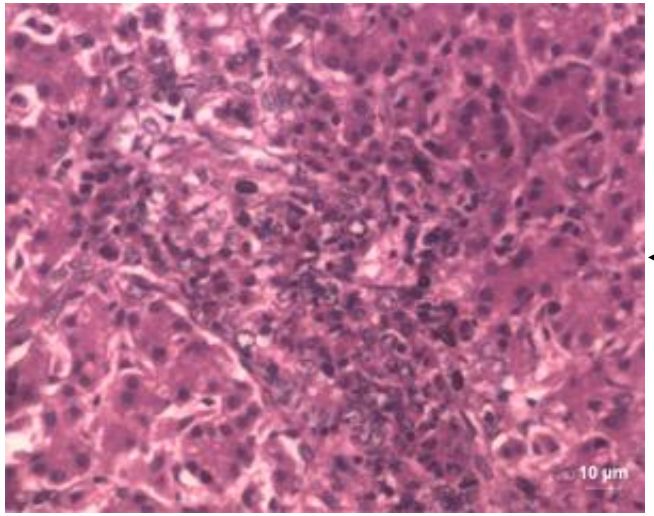


Concurred with the findings of the earlier authors (Sims *et al.*, 1970; Huff *et al.*, 1975; Howarth and Wyatt, 1976; Iqbal *et al.*, 1983; Fernandez *et al.*, 1994; Khan, 1994; Oliveira *et al.*, 1999; Mukhopadhyay *et al.*, 2000; Gounalan *et al.*, 2005; Pandey and Chauhan, 2005).

g. Histopathology-Liver-1 ppm- 42 days

Perivascular MNC infiltration →

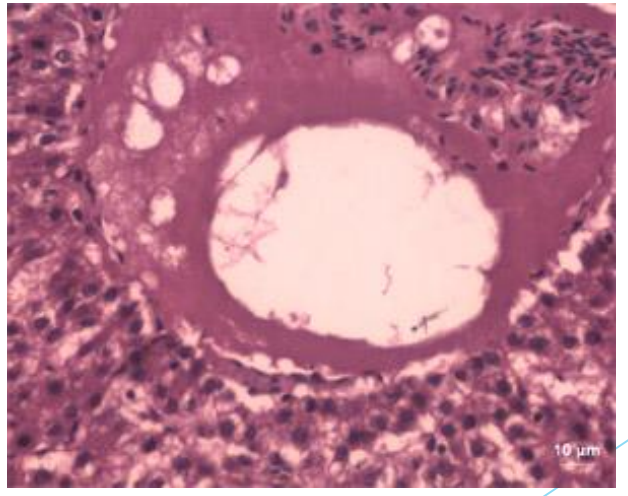




← Focal necrosis

Similar changes were reported in layer chicken treated with 120 ppb onwards for varying periods (Sims *et al.*, 1970; Fernandez *et al.*, 1994; Oliveria *et al.*, 1999; Mukhopadhay *et al.*, 2000; Kim *et al.*, 2003; Pandey and Chauhan, 2007).

DIC and endothelial damage →

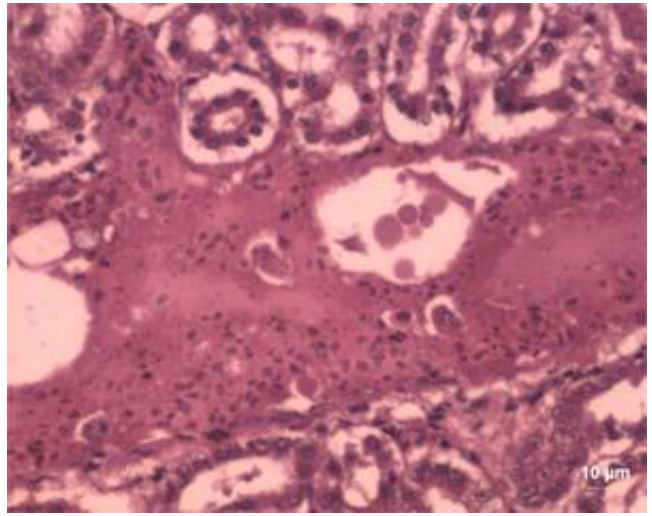


However, DIC observed in this study was not reported by earlier workers.

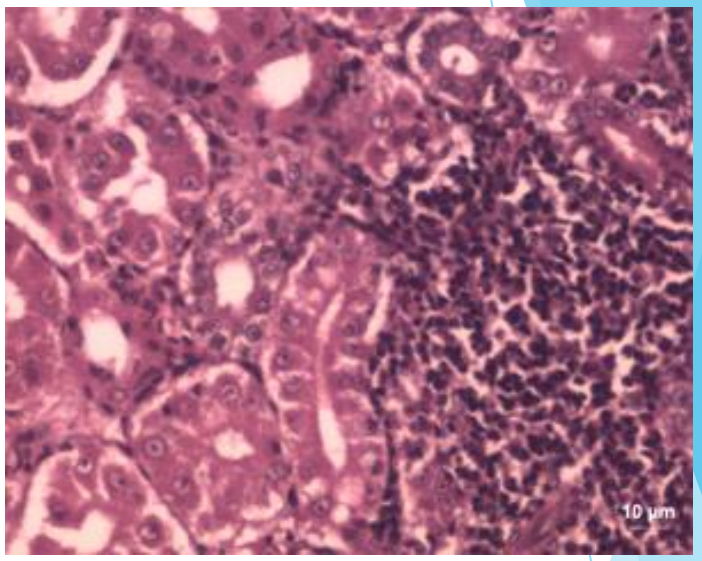
KIDNEY-1 PPM- 28 DAYS

Findings agreed with those of Fernandez *et al.* (1994) and Pandey and Chauhan (2007) at higher levels i.e. 2.5 to 3.91 ppm.

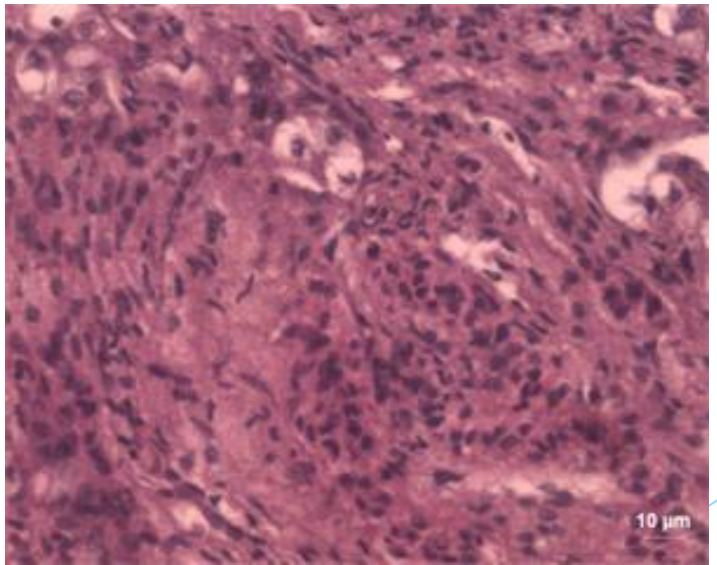
Interstitial hemorrhage



MNC infiltration



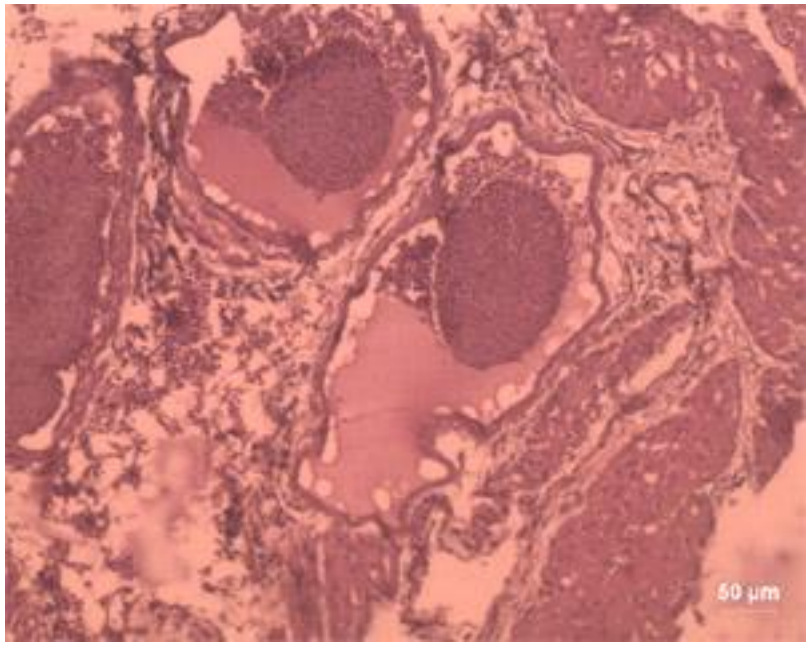
Focal fibroplasia



Kidney-1 ppm- 48 days

Ovary -1 ppm

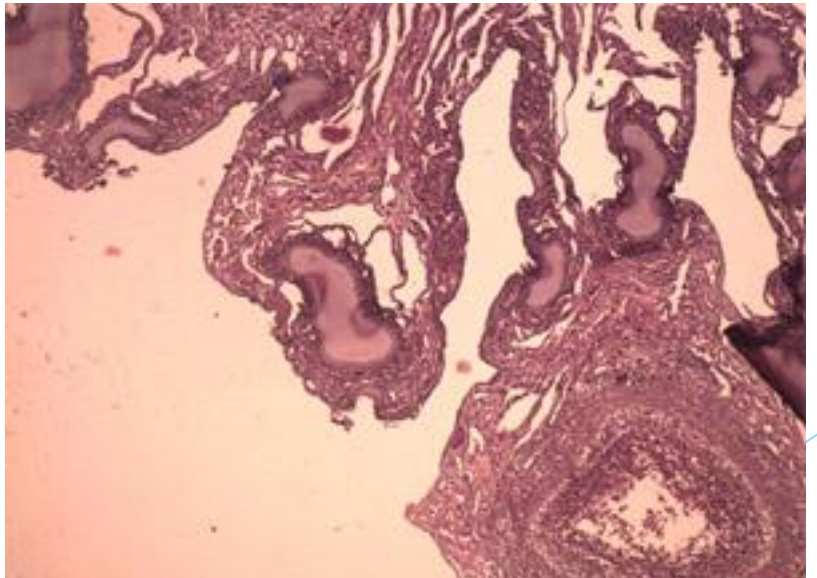
28 days



Intafollicular edema and haemorrhage

Similar observations were also made by earlier workers in higher levels of AF toxicity viz. 2.5 ppm and above (Hafez *et al.*, 1992; Pandey and Chauhan, 2007)

42 days

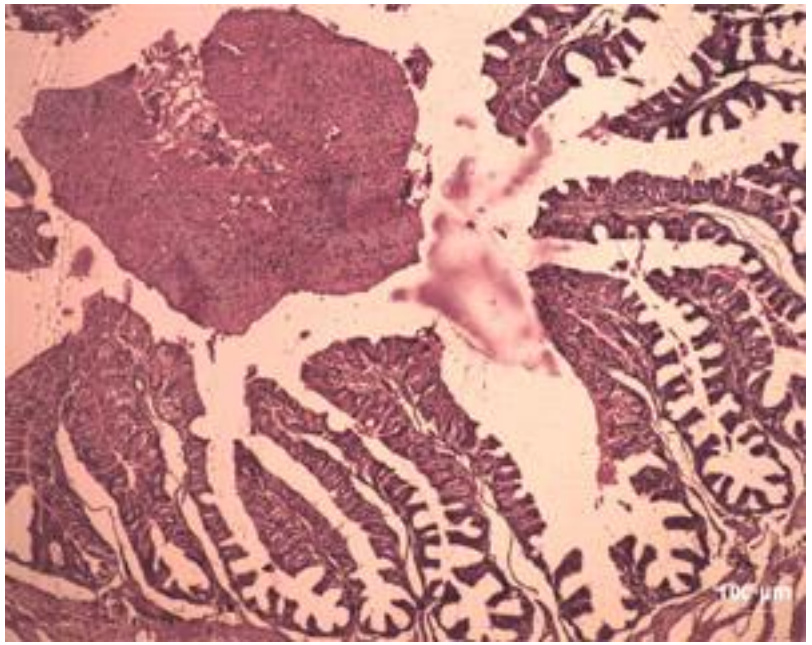


Atrophic follicles

Infundibulum- 1 ppm

42 days

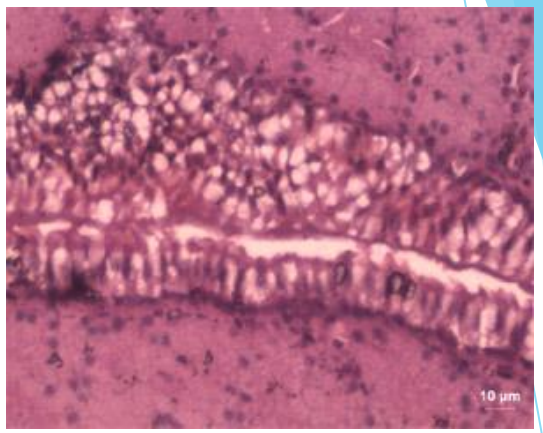
Intraluminal haemorrhage

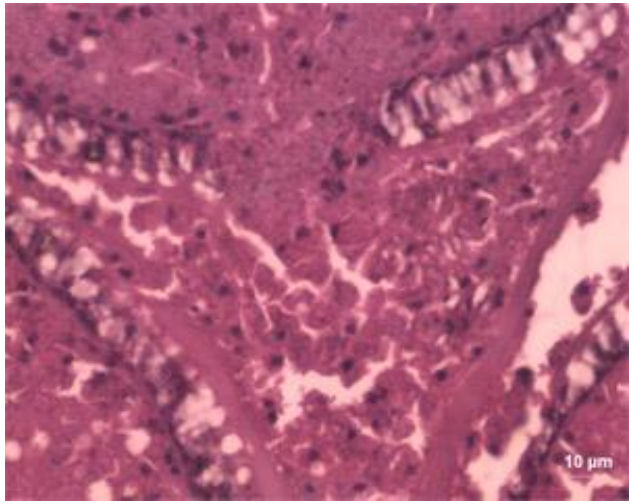


MAGNUM- 1 PPM

28 days

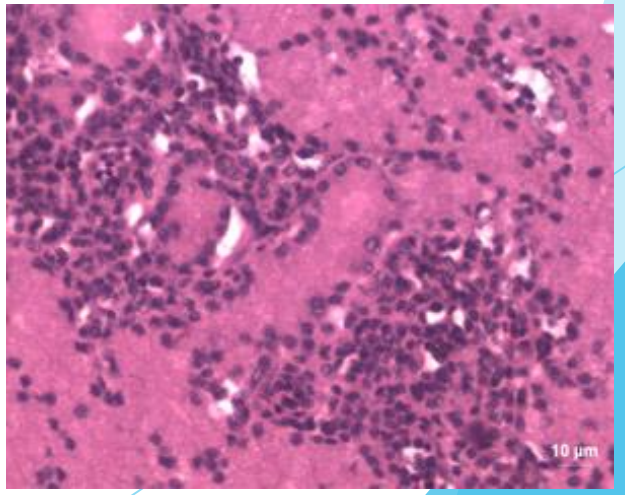
Mucosal hyperplasia of surface epithelium





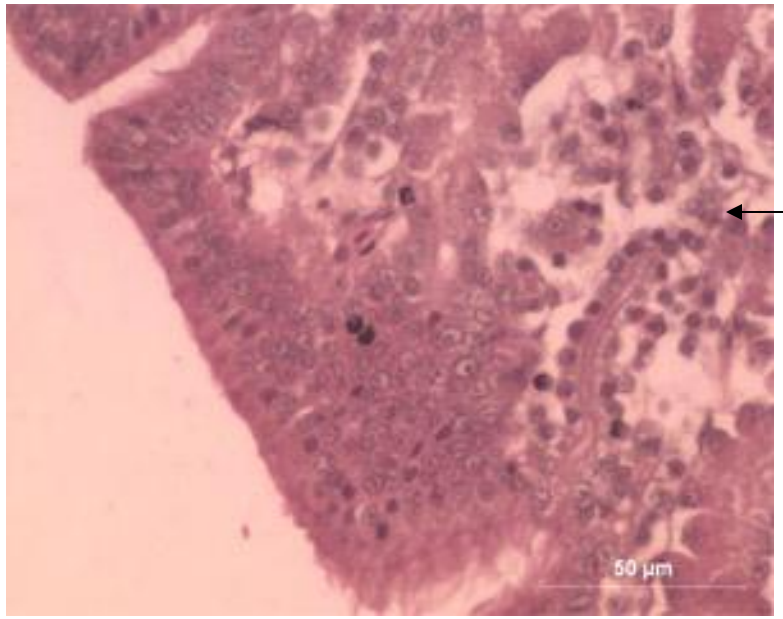
Degeneration of secretory granules \and eosinophillic materials Adhering to the surface epithelium

Loss of secretory granules and MNC infiltration



ISTHMUS

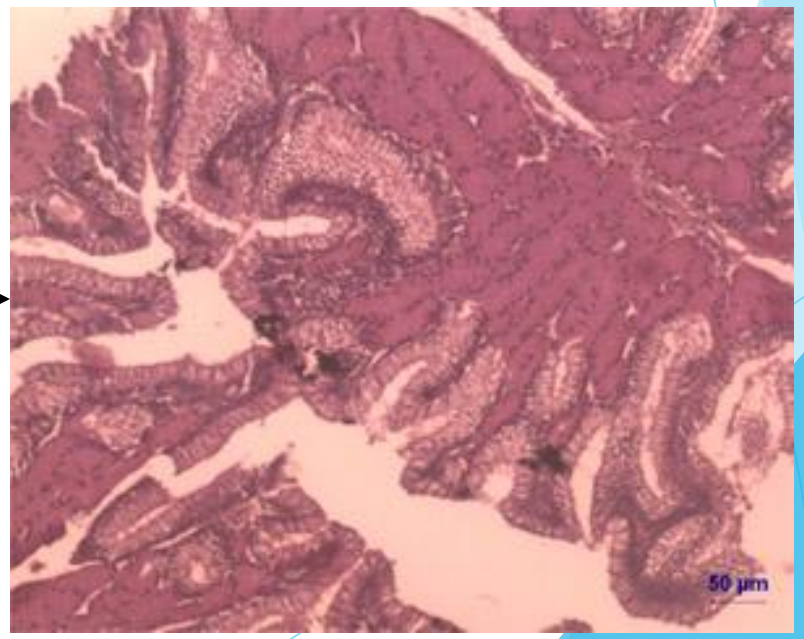
1 ppm-28 days



Focal hyperplasia of epithelial cells, degeneration of glandular cells

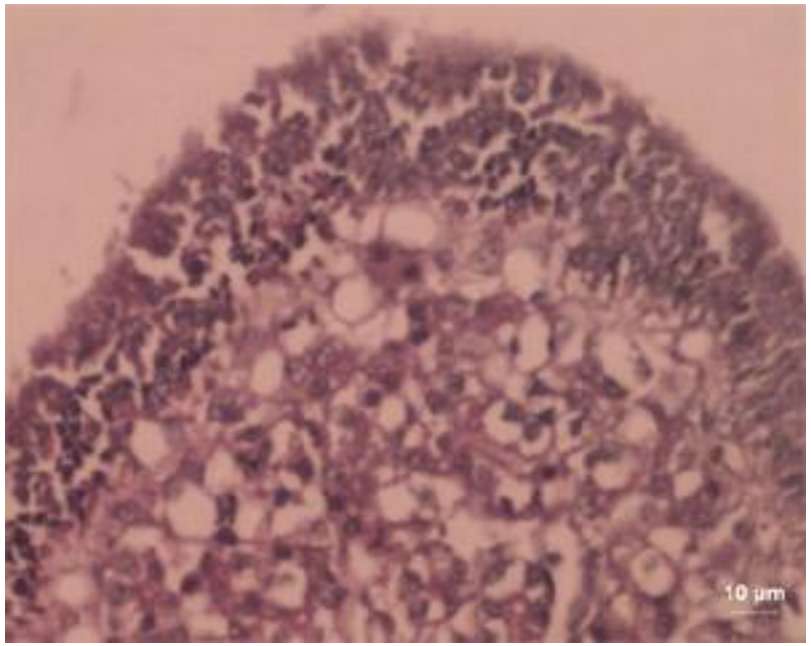
2ppm-28 days

Mucosal hyperplasia of surface epithelium



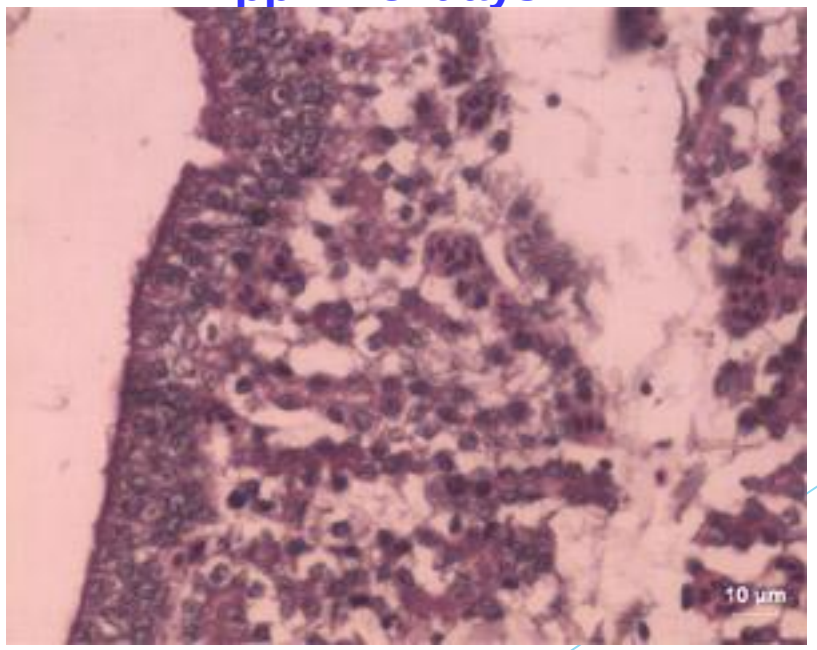
UTERUS

1 ppm-28 days



Hyperplasia of surface epithelium, degeneration of glandular cells

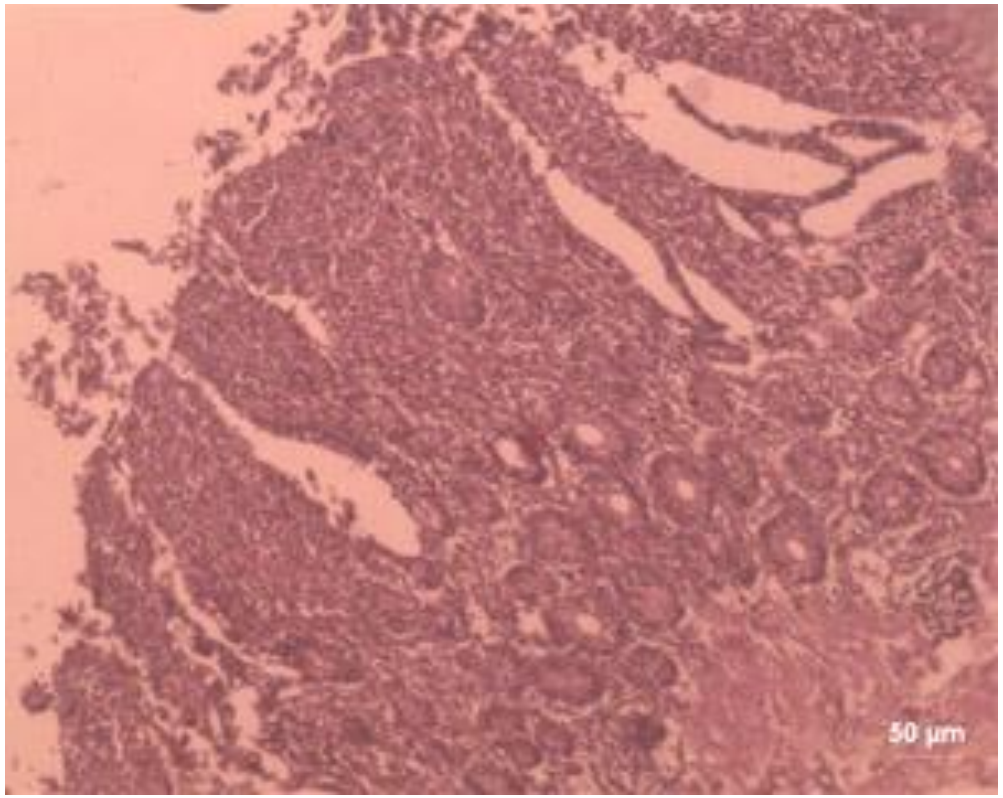
2 ppm-28 days



Mild hyperplasia of surface epithelium, degeneration of glandular cells

Similar findings in natural aflatoxicosis in layer chicken (Vijayalingam *et al.*, 2006).

Intestine- 1 ppm – 28 days



Partial fusion of villi

Concurred with the findings of Gounalan *et al.* (2005) who fed 0.5 ppm of AF to layer chicken from 0 to 12 weeks of age.

4. Conclusions

- AF fed to 27 week old layers up to 2 ppm level for 6 weeks did not show appreciable pathological changes.
- Elevation in ALP and decrease in BUN and sodium reflecting the toxic effects on liver and kidneys.
- At the end of 28 days of AF treatment, there was a decrease in the trend of egg production. Egg quality is affected even at these doses -pigmented, blood spot.
- Microscopical changes observed in the oviduct in AF fed birds suggested that there was definite damage done to the reproductive tract during the observation period.
- The forsgate strain of WL may be resistant to AF up to 2 ppm level of AF.

Thank You