DETERMINATION OF PERMISSIBLE LEVEL OF AFLATOXIN IN BROILER CHICKEN FEED

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"DETERMINATION OF PERMISSIBLE LEVEL OF AFLATOXIN IN BROILER CHICKEN FEED"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"DETERMINATION OF PERMISSIBLE LEVEL OF AFLATOXIN IN BROILER CHICKEN FEED"** is a record of research work done independently by **Ms. A. Arulmozhi**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Dedicated to God

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Introduction

INTRODUCTION

Commercial broiler production is a fast growing and popular segment in poultry industry. Low capital investment, minimum space requirement and quick returns are the advantageous factors in broiler production. The broiler meat is popular because of the low fat content and the relative ease in cooking. Broiler rearing being a highly profitable sector, it has encouraged the enterprising farmers to set up several commercial broiler units in the country.

The major expenditure incurred in commercial broiler rearing is towards the poultry feed. The quality and quantity of the feed play an important role in determining the overall performance of the flock. The profit margin in the industry depends to a great extent on the quality of the feed. As the demand of feed increases the quality may decrease, while there is steep rise in prices. The drop in quality can be either to use of substandard feed ingredients or due to higher moisture content in the mixed feeds.

Feed ingredients may acquire higher moisture content due to improper storage and exposure to humidity. This leads to congenial environment for the growth and multiplication of certain species of fungi. Usually the fungal species produce various toxins into the substrate thereby feed becomes toxic and

deleterious to health. Such fungal growth and toxin production in feed ingredients is related to the harvesting season and the time of storage.

Toxic contamination of animal and poultry feed with mycotoxin first came into picture with a study conducted on "Turkey x disease" in England in 1961 (Asplin and Carnaghnn, 1961). More than one lakh turkey poults died due to consumption of feed contaminated with mycotoxin subsequently identified as aflatoxins. Contamination of feed and feed ingredients with various mycotoxins, particularly aflatoxin is a major threat to the livestock and poultry industry especially in the tropical countries, where the climate is favourable for the fungus growth.

Aflatoxin even in the lowest level can be deleterious to the performance of birds. But in a tropical country like India, it is a difficult task to maintain our livestock and poultry feeds totally free from aflatoxin. Sudarshansingh and Singh (1987) reported that the overall content of aflatoxin in poultry feed was 30.76 per cent in Andhra Pradesh. In Kerala, 50.1 per cent of poultry feed samples were reported to be contaminated with aflatoxin (Rajan *et al.*, 1991). It will not be an economically viable proposition to discard feed or feed ingredients based on the presence of aflatoxins. Hence, it has become necessary to determine a permissible level of aflatoxin in the feed to various livestock and poultry, as there is variation in the susceptibility among species.

The safe level suggested at present by various agencies in foreign countries and India varies from 20 to 400 ppb for poultry and this is a wide range to be accepted as a standard. These standards may not be applicable and practical in situations prevailing in India, particularly in Kerala. Moreover, the climatic conditions, the feed ingredients used in poultry feed and the susceptibility to aflatoxins between strains of birds vary from place to place.

Of late, there is an increase in the awareness among farmers regarding the quality of feed which they purchase from the market. As a result a number of legal issues have comeup in the consumer redressal forums demanding compensation from feed companies in lieu of losses due to feeding aflatoxin contaminated feed. In such forums, the level of aflatoxin present in the feed has always been a matter of controversy where the veterinary experts havemet with many difficulties to draw valid conclusions.

Under the circumstances, the determination of maximum permissible level of aflatoxin in poultry feed and feed ingredients under the climatic conditions prevailing in Kerala has been a strong felt need of the farmers. Once the permissible level is fixed, the quality control and standardization of feed can be more effective and loss of production due to aflatoxicosis can be reduced considerably.

Hence the present study was undertaken with the following objectives.

1. To assess the effect of various levels of aflatoxin on the performance of broiler birds and to establish the permissible level of aflatoxin in broiler feed.

2. To quantify the residual aflatoxin in the muscle, liver and kidney of broilers fed with various levels of aflatoxin.

Review of Literature

REVIEW OF LITERATURE

2.1 Incidence of aflatoxin in poultry feed

Khan and Ahamed (1987) noted that about 80 per cent of feed sample were contaminated by *Aspergillus flavus* and that 16.79 per cent of samples were positive for aflatoxin B_1. Groundnut cake was the only major ingredient contaminated with aflatoxin as observed by Sudarshan Singh and Singh (1987) and the overall incidence of aflatoxin in the feed stuffs in Andra Pradesh was 30.76 per cent. Selvasubramanian *et al.* (1987) reported that more than 40 per cent of the feed samples in Tamil Nadu contained moderate to high level of aflatoxin. Rajan *et al.* (1991) evaluated that 50.1 per cent of the feed samples available in the market of Kerala were contaminated with aflatoxin.

2.2 Influence of aflatoxin on the body weight and feed intake

A significant reduction in weight gain was observed by San-Gabriel (1971) when aflatoxin was added at 450 ppb level to the feed in broiler chicken. Chicken fed with ration containing 2500 or 5000 ppb of aflatoxin for a period of three weeks showed depressed body weight gain (Lanza *et al.*, 1980; Doerr *et al.*, 1981; Huff *et al* 1986; and Giroir *et al* 1991). Decreased body weight gain was also recorded by

Stanely *et al.* (1993) in broilers which were on a diet containing aflatoxin at the rate of 5000 ppb for four weeks.

Graded levels of aflatoxin in the feed at the rate of 0, 75, 225 and 675 ppb fed to chicken from day old to seven weeks gave body weight viz., 2256 ± 21, 2028 ± 20, 1989 ± 20 and 2047 ± 24 g respectively which showed a decrease in body weight corresponding to the increased levels of aflatoxin (Doerr *et al.*, 1983). Broiler chicks dosed per os with 50 and 100 µg of aflatoxin per kg body weight daily resulted in decreased body weight gain (Maurice *et al.*, 1983). A significant reduction in body weight gain and feed consumption was observed in broiler chicken at 750 ppb level for four weeks (Reddy *et al.*, 1984). Hubbard broiler strain when fed with 50 and 100 ppb of aflatoxin in the feed for a period of five weeks showed a reduction in body weight gain (Mashaly *et al.*, 1986).

Aflatoxicosis induced experimentally in quails by feeding 200, 300, 500 and 750 ppb of aflatoxin from 6 to 35 days of age resulted in decreased bodyweight by 129 ± 2.35, 122 ± 2.36, 116 ± 2.36 and 106 ± 2.5 g respectively compared to 133 ± 2.55 g in control (Panda *et al.*, 1987). Graded levels of aflatoxin given in feed to quails resulted in growth inhibitory effect at all levels starting from 500 ppb (Anandkumar *et al.*, 1993).

A reduction in body weight gain and feed conversion ratio was recorded in broiler chicken fed with 500 and 1000 ppb of aflatoxin in feed from day old to six weeks (Devegowda *et al.*, 1994). Reduced body weight gain and lowered

antibody titre against IBD were the effects of aflatoxin at the level of 100 and 200 ppb in the feed (Azzam and Gabal, 1997). Broiler chicks maintained on a ration containing 0, 750 and 1500 ppb of aflatoxin from 0 to 5 weeks of age resulted in a proportionate reduction in body weight gain (1854 ± 24, 1558 ± 20 and 1425 ± 20 g); feed consumption (4474, 4270 and 4203 g) and feed efficiency (2.42, 2.74 and 2.95 respectively) with the increase in the toxin level (Mani *et al.*, 1997).

Muthiah *et al.* (1998) observed that the daily feed intake were reduced from 109.13 g to 100.25 g and 97.66 g as the toxin level in the feeds increased from 0 ppb to 500 and 1000 ppb respectively in egg type breeders.

Feed containing graded levels of aflatoxin (0, 250, 500 and 1000 ppb) given to broilers for four weeks resulted in a proportionate reduction in body weight gain (756.7 ± 13.1, 582.3 ± 9.3, 528.7 ± 11.8 and 412.1 ± 2 g); feed consumption (1473 ± 37, 1138 ± 34, 1093 ± 90 and 919 ± 39 g) and feed conversion ratio (2.07 ± 0.05, 2.11 ± 0.04, 2.20 ± 0.03 and 2.5 ± 0.08 respectively) (Vasan *et al.*, 1998).

2.3 Haematology

The alteration in the haematological parameters due to aflatoxicosis has been well documented by Mohiuddin *et al.*, 1986; Mani *et al.*, 1993; Fernandez *et al.*, 1995 and Vasan *et al.*, 1998.

2.3.1 Erythrocyte sedimentation rate (ESR)

Significant increase in the mean values of ESR and blood clotting time has been reported by Suryanarayan Murthy *et al.* (1984) in calves treated with AFB_1 at the rate of 70 µg/kg body weight and have attributed it to increase in the ESR to the low levels of serum protein. Anbiah (1996) reported a significant increase in ESR values in ducks fed aflatoxin at the rate of 15 µg/kg body weight.

2.3.2 Packed cell volume (PCV)

Decrease in the PCV as low as 11 per cent, was observed in chicks given feed containing aflatoxin at the rate of 2500 and 5000 ppb (Lanza *et al.*, 1977; Doerr and Huff, 1980). Broiler chicks fed aflatoxin at the rate of 0 and 2500 ppb in the feed for three weeks showed a reduction in PCV by 27.4 ± 0.1 and 24.9 ± 0.5 per cent respectively (Campbell *et al.* (1983).

A reduction in PCV was reported by Mohiuddin *et al.* (1986) in cockerels given a feed containing 20,000 ppb of aflatoxin. Broiler chicks fed on a diet containing aflatoxin at the rate of 0, 750 and 1500 ppb resulted in a reduction in PCV by 31.25 ± 0.25, 27.75 ± 0.25 and 24.75 ± 0.25 per cent respectively (Mani *et al.*, 1993).

A reduction in PCV was also recorded by Fernandez *et al.* (1995) in broilers given AFB_1 at 2500 and 5000 ppb level in the feed. Feed containing graded levels of aflatoxin at the rate of 0, 250, 500 and 1000 ppb when fed to

broiler chicken for four weeks resulted in a decrease in the PCV. The respective values were 40.5 ± 2.1, 35.8 ± 2.9, 33.3 ± 1.6 and 30.3 ± 0.3 per cent (Vasan *et al.*, 1998).

2.3.3 Haemoglobin

Haemolytic anaemia has been observed as a clinical feature of aflatoxicosis in broiler chicken and the haemoglobin values recorded were 8.7 ± 0.1 and 7.6 ± 0.2 g% when aflatoxin was fed at the rate of 0 and 2500 ppb in the feed (Campbell *et al.*, 1983). Feed containing aflatoxin above 750 ppb levels when given to birds resulted in significant reduction in haemoglobin values (Reddy *et al.*, 1984).

Mohiuddin *et al.* (1986) opined that the decrease in the mean values of PCV, haemoglobin and RBC in birds treated with aflatoxin was indicative of an inhibitory effect of aflatoxin on the haemopoietic tissue.

Graded levels of aflatoxin at 0, 200, 300, 500 and 750 ppb in the feed given to quails resulted in significant reduction in the heaemoglobin values (Panda *et al.*, 1987).

Mani *et al.* (1993) recorded significant reduction in haemoglobin values in chicks viz., 8.25 ± 0.25 and 7.50 ± 0.29 when feed containing aflatoxin was fed at the rate of 750 and 1500 ppb respectively when compared to 9.00 ± 0.00 g% in control. Similar observations were also noticed by Fernandez *et al.* (1995) at 2500

and 5000 ppb levels and Vasan *et al.* (1998) at 250, 500 and 1000 ppb levels in the feed.

2.3.4 Total leucocyte and differential count

Tung *et al.* (1975) reported leucocytosis with heterophilia and lymphopenia, when male broilers were given a ration containing 10,000 ppb of aflatoxin. Heterophilia and lymphopenia were also observed by Campbell *et al.* (1981) in broilers which were given a diet containing 0 and 2500 ppb of AFB_1. The lymphocyte counts were 62.1 ± 2.2 and 57.5 ± 3.8 per cent and the heterophil counts were 30.3 ± 2.5 and 34.7 ± 3.2 per cent.

Mohiuddin *et al.* (1986) observed a mild increase in total leucocyte and heterophil count with a decrease in lymphocytes and basophils in cockerels fed with 20,000 ppb aflatoxin in the feed. Such leucocytosis with heterophilia was attributed to the inflammatory response elicited by the toxin and the lymphopaenia to the immunosuppressive action of aflatoxin.

However, Nageswar Rao *et al.* (1988) reported that the total leucocyte count and the differential count in chicken were not affected by the aflatoxin fed at 500 and 1000 ppb level in the feed for nine weeks.

Leucocytosis with neturophilia were also observed in four month old ducks treated with aflatoxin at the rate of 15 µg/kg body weight (Anbiah, 1996).

2.4 Serum chemistry

2.4.1 Total serum protein

Alterations in the serum protein values due to aflatoxicosis in poultry have been well documented (Campbell *et al.*, 1983; Fernandez *et al.*, 1995 and Vasan *et al.*, 1998).

Wyatt *et al.* (1973) reported a decrease in total serum protein in broiler breeder roosters maintained with feed containing aflatoxin at a level of 20,000 ppb for four weeks.

A decreased serum protein was considered as a sensitive indicator in experimental aflatoxicosis in broilers (Lanza *et al.*, 1980; Campbell *et al.*, 1983 and Huff *et al.*, 1988). Aflatoxin has been shown to limit protein synthesis through inhibition of RNA synthesis (Campbell *et al.*, 1983).

Reddy *et al.* (1984) observed a decrease in serum protein level in broiler chicken fed aflatoxin at the rate of 750 ppb in the feed. Feed containing aflatoxin at the rate of 0, 200, 300, 500 and 750 ppb when fed to quails resulted in a reduction in the serum protein content were 3.166 ± 0.012, 2.988 ± 0.108, 2.666 ± 0.057, 2.016 ± 0.08 and 1.883 ± 0.026 g/dl respectively. They viewed that aflatoxin has primary locus of action on protein metabolism.

Feeding of graded levels of aflatoxin at 500 and 1000 ppb level in the feed to chicken resulted in decreased serum protein values viz., 5.3 and 4.8/dl

respectively compared to 5.7 g/dl in control (Rao *et al.*, 1988). A reduction in serum protein value was recorded even at the level of 100 ppb aflatoxin when fed to chicks from day 7 to day 49. Raina *et al.* (1991) opined that the decrease in total serum protein may be due to degeneration of endoplasmic reticulum and inhibition of protein synthesis. However, Singh *et al.* (1992) observed an increased serum total protein when day old broiler chicks were kept on a diet containing 500 ppb of aflatoxin for 45 days.

Feed containing graded levels of aflatoxin at 750 and 1500 ppb in the feed when fed to broilers from day one to five weeks of age resulted in a reduction in serum protein values by 3.56 ± 0.02 and 3.26 ± 0.00 /dl respectively compared to 3.77 g/dl in control (Mani *et al.*, 1993). They viewed that the poor absorption of amino acid from intestine, inactivation of amino acid, the inhibitory effect of AFB_1 on protein synthesis and blocking of RNA synthesis in the nucleolus, were some of the factors responsible for the low serum protein levels during aflatoxicosis.

Fernandez *et al.* (1995) reported a decreased levels of serum protein in chicken maintained on a feed containing aflatoxin at the rate of 2500 and 5000 ppb. Similar observations were made by Jassar and Singh (1993) at 3000 and 6000 ppb levels of aflatoxin.

Diet containing graded levels of aflatoxin when fed to chicks at the rate of 250, 500 and 1000 ppb for four weeks resulted in a decrease in total serum protein

values as 2.21 ± 0.11, 1.81 ± 0.08 and 1.56 ± 0.05 g/dl respectively compared to 2.24 ± 0.07 g/dl in control (Vasan *et al.*, 1998).

2.4.2 Serum albumin and globulin

A reduction in serum albumin was reported by Maurice *et al.* (1983) in broiler chicks treated with AFB_1 at the rate of 50 and 100 µg per kg body weight. Similar finding was reported by Huff *et al.* (1988) in chicken given feed containing 2500 ppb of aflatoxin. Ghosh *et al.* (1990) observed a decrease in serum albumin and globulin in broilers reared on a diet containing aflatoxin at the rate of 300 and 1000 ppb from day one to six weeks. A reduction in serum albumin was reported in broilers fed diet containing 5000 ppb aflatoxin (Stanely *et al.*, 1993).

Broiler chicken when fed with aflatoxin at the rate of 0, 2500, 5000 ppb in the feed did not show any variation in serum albumin values (2.45, 2.38 and 2.52 g/dl respectively) whereas significant reduction in serum albumin was observed in laying hens fed with the same dose level of aflatoxin (Fernandez *et al.*, 1995).

Shukla and Pachauri (1995) recorded a significant decrease in the total serum protein and albumin with a significant increase in the globulin fraction in birds fed aflatoxin mixed feed at 2500, 5000 and 10000 ppb levels. Such an increase in the serum globulin and decrease in serum albumin led to a decrease in albumin:globulin ratio.

Anbiah (1996) detected a fall in serum albumin and globulin levels in ducks dosed with aflatoxin at the rate of 15 µg/kg body weight from fourth to eighth month of age. A reduced serum albumin value was also noted by Kubena *et al.* (1997) in broilers fed diet containing 3500 ppb of toxin.

Broilers maintained on a diet containing aflatoxin in graded levels 0, 250, 500 and 1000 ppb, for four weeks showed decreased albumin values viz., 1.14 ± 0.05, 0.93 ± 0.08, 0.73 ± 0.05 and 0.62 ± 0.02 g/dl and increased albumin:globulin ratios of 1:0.96, 1:1.38, 1:1.48 and 1:1.52 respectively (Vasan *et al.*, 1998).

Suresh (1999) observed a reduction in the values of serum protein, albumin and globulin in quails treated with aflatoxin twice a week for 60 days at the rate of 0.5 µg/g body weight.

2.4.3 Enzymology

2.4.3.1 Serum alkaline phosphatase (ALP)

Chiang *et al.* (1986) observed an increased serum alkaline phosphatase activity in chicks given feed mixed with aflatoxin at 500 ppb level. Panda *et al.* (1987) reported that quails which received graded levels of aflatoxin at 0, 200, 300, 500 and 750 ppb showed a proportionate increase in serum ALP viz., 4.37 ± 0.873, 9.863 ± 0.629, 12.673 ± 1.226, 12.295 ± 1.580 and 20.231 ± 2.137 IU/L respectively.

Amer *et al.* (1988) viewed that the serum and liver alkaline phosphatase values can be considered as very sensitive indicators in aflatoxicosis.

Raina *et al.* (1991) noted that the serum ALP values were generally increased during mycotoxicosis. However, Singh *et al.* (1992) reported a significant reduction in alkaline phosphatase and serum aspartate amino transferase levels in broilers fed with a ration containing 500 ppb of aflatoxin.

Elevation of ALP was observed in quails dosed aflatoxin at the rate of 500, 1000, 1500 and 2000 ppb in the feed (Anandakumar *et al.*, 1993). They viewed that such an elevation of ALP was a result of hepatic injury induced by aflatoxin.

Jassar and Singh (1993) noted an elevation of serum ALP in broilers fed with a diet containing 3000 and 6000 ppb of aflatoxin and opined that the degeneration of hepatic cells and consequent leakage of enzymes into tissue might have caused the increase in serum alkaline phosphatase values.

Feed containing aflatoxin in graded levels 0, 2500, 5000 and 10000 ppb fed to cockerels resulted in proportionate increase in serum ALP level viz., 17.08 ± 1.05, 27.83 ± 1.78, 28.60 ± 1.70 and 30.49 ± 1.22 IU/L respectively (Shukla and Pachauri, 1995).

2.4.3.2 Gamma Glutamyl Transferase (GGT)

Rosalki (1975) stressed the importance of elevated serum and tissue GGT, in hepatic diseases and stated that GGT is the most sensitive enzyme in a variety

of liver diseases. Brawn *et al.* (1987) observed an increase in serum GGT values following administration of various drugs and chemicals.

Ducks treated with aflatoxin at the rate of 0.04165 mg/kg body weight resulted in an increase in serum GGT level (Leenadevi, 1992). An increase in serum GGT activity was observed by Devagowda *et al.* (1994) in birds fed with diet mixed with aflatoxin at 500 ppb level. Similar changes were also observed by Fernandez *et al.* (1994) at 2500 and 5000 ppb level.

2.5 Organ weight

2.5.1 Liver

Chicken maintained on feed containing graded levels (400, 600, 800 and 3200 ppb) of aflatoxin showed a decrease in liver weight at lower doses and an increased weight at higher doses (Schroeder *et al*, 1972). Increased liver weight compared to body weight was also reported by Doerr and Huff (1981) at levels of 625, 1250 and 2500 ppb of aflatoxin in the feed. Similar observations were also made by Aletor *et al* (1981).

Increased liver weights were also recorded in birds given a feed mixed with aflatoxin at 750 and 5000 ppb levels (Reddy *et al.*, 1982 and Chiang *et al.*, 1986). Broiler chicken fed a diet 250 ppb aflatoxin in the feed showed significant decrease in liver weight (Khan *et al.*, 1989).

Espada *et al.* (1992) observed an increased liver weight in chicken treated with 0.2 and 3 µg of AFB_1/g body weight. This increase in the liver weight was attributed to the increased accumulation of lipids in the liver during aflatoxicosis.

Increased liver weight due to consumption of aflatoxin contaminated feed was also recorded in broilers by Mani *et al.* (1993) at 500 and 750 ppb; Devagowda *et al.* (1994) at 500 ppb and Edrington *et al.* (1997) at 4000 ppb level.

2.5.2 Bursa

A significant reduction in the size of bursa of Fabricius was observed in chicken given feed containing aflatoxin at 2500 ppb level (Campbell *et al.*, 1983). The weight of the bursa was unaltered in broilers given a diet containing aflatoxin at the level of 750 ppb for 28 days (Reddy *et al.*, 1984).

The bursa and thymus in toxin fed birds showed a reduction in the size and weight compared to control, in both acute (4000 ppb for 4 weeks) and chronic (1000 ppb for 12 weeks) aflatoxicosis (Ram *et al.*, 1988). Similar findings were reported by Mani *et al.* (1993) at 750 ppb and Devagowda *et al.* (1994) at 100 and 200 ppb levels of aflatoxin in the feed given to chicken.

2.5.3 Spleen

The weight of the spleen was unaltered (Reddy *et al.*, 1984) when feed mixed with 750 ppb of aflatoxin was fed to birds for 28 days. Splenic enlargement

and increase in the weight was noted by Sadana *et al.* (1992) in quails fed on a diet mixed with 500 ppb of aflatoxin. They related this enlargement to reticulo-endothelial cell hyperplasia. Mani *et al.* (1993) observed an increased spleen weight in chicken given feed containing 750 ppb of aflatoxin.

2.6 Residual aflatoxin in tissues

Blaha (1982) observed residues of aflatoxin in the liver, but not in the muscle of broiler chicken treated with 340 µg aflatoxin for a period of 42 days.

Residues of aflatoxin were detected in the liver, heart, breast muscle and kidney of broiler chicken, after two weeks of feeding with a diet containing graded levels (100, 250, 500 and 750 ppb) of aflatoxin (Teleb and Fakhry, 1988).

Maryamma *et al.* (1992) detected aflatoxin residues in the liver (10 to 20 µg/kg) and kidney (10 and 80 µg/kg) of White Leghorn chicks fed 100 µg/kg body weight for 30 days.

Kryukov and Krupin (1993) reported higher levels of residual aflatoxin in the liver and muscle of broiler chicken, fed on a diet containing AFB_1 at 250, 500 and 1000 ppb levels from day 4 to 49 days.

Yadav *et al.* (1995) assessed the deposition and clearance of aflatoxin from the tissues of broilers fed with aflatoxin mixed diet at 500 ppb level for 45 days.

They observed that the highest values of residual aflatoxin B_1 in liver and muscle were 4.7 and 2.0 ppb respectively at the 45th day.

Toxic residues were detected in the eggs (10 to 14 ppb) of White Leghorn chicken maintained on a diet containing 200 ppb of aflatoxin for 22 weeks (Azzam and Gabal, 1997).

2.7 Gross pathology

2.7.1 Liver

Schroeder *et al* (1972) observed icterus and massive necrosis in the liver of chicken given a diet with 3200 ppb of aflatoxin. Administration of graded levels of aflatoxin (75, 225, 675 ppb) resulted in fatty livers in broiler by Doerr *et al.* (1983). Balachandran and Ramakrishnan (1987) noticed enlarged, pale livers with moroccan leather appearance in the first few weeks and yellow discolouration of livers at a later stages in chicken fed with a diet mixed with 1000 ppb of aflatoxin. Enlargement of liver with increased friability was also observed by them at the dose of 3000 ppb in the feed.

Aflatoxin fed broiler chicken and quails revealed enlargement and extensive degeneration of liver with distended gall bladder (Borisova *et al.*, 1987; Panda *et al.*, 1987 and Ghosh *et al.*, 1990) Paleness, friability, greyish white nodularity and distended gall bladder were the lesions observed by Maryamma *et al.* (1990) in ducks given a ration containing 1000 ppb of aflatoxin.

Broilers fed with 0.2 and 3 µg of aflatoxin/g of body weight revealed diffuse, pale yellowish discolouration of liver and enlargement of gall bladder (Espada *et al.*, 1992). Similar lesions were also observed by Anjaneyulu and Rao (1993) in chicken given 2000 ppb and by Anandkumar *et al.* (1993) in quails fed 500 and 2000 ppb of toxin mixed with the diet.

Bakshi *et al.* (1995) found enlarged livers with patechiae haemorrhages in broilers fed on rations with graded levels of aflatoxin (750, 1500 and 3000 ppb) for six weeks.

Livers of ducks given aflatoxin at the rate of 15 µg/kg body weight were enlarged, pale yellow with occasional subcapsular haemorrhages and they usually contained yellow nodules discretely distributed in the parenchyma (Anbiah, 1996).

2.7.2 Kidney

Chicken which received graded doses of dietary aflatoxin showed enlargement of kidney (Tung *et al.*, 1973). Kidneys were pale and friable in the chicken fed with aflatoxin mixed diet at 1000 ppb level for six weeks (Ghosh *et al.*, 1989).

Sadana *et al.* (1992) did not observe any appreciable changes, except for slight congestion in the kidneys of quail chicks which received a feed containing 500 ppb aflatoxin in the feed for a period of two weeks.

Broilers fed with a ration containing 2000 ppb AFB_1 for a period of nine weeks revealed enlargement of kidneys with mild to severe haemorrahges (Anjaneyulu and Rao, 1993).

Anandkumar *et al.* (1993) noticed enlargement of kidneys with petechial haemorrhages in quails which received aflatoxin in graded levels for a period of 30 days.

Gabal and Azzam (1998) opined that aflatoxin was a potent nephrotoxin and the continued exposure to it resulted in tremendous kidney damage.

2.7.3 Bursa

Thaxton *et al.* (1974) noticed atrophy of the bursa of Fabricius and thymus in chicken fed with high doses of aflatoxin. The bursa was either regressed or atrophied in quails fed with graded levels of aflatoxin (Panda *et al.*, 1987 and Anandkumar *et al.*, 1993). Ram *et al.* (1988) viewed that the reduction in size of bursa and thymus in toxin fed birds might be due to lymphoid depression during acute and chronic aflatoxicosis. Atrophy of bursa and thymus was also reported by Ghosh *et al.* (1989) at the levels of 300 and 500 ppb of AFB_1 in the feed.

Sadana *et al.* (1992) observed enlargement of bursa by the 21st day, in quails fed with a diet mixed with aflatoxin at 500 ppb level and opined that the enlargement of bursa was due to congestion and presence of serofibrinious exudate in the interfollicular connective tissue. Enlargement of bursa was also

noticed by the seventh week in chicken maintained on a diet mixed with aflatoxin at 2000 ppb level (Anjaneyulu and Rao, 1993). However, Bakshi *et al.* (1995) reported regression of bursa and thymus in chicken fed with graded levels of aflatoxin for a period of six weeks.

2.7.4 Spleen

Ghosh *et al.* (1989) observed splenomegaly in chicken which received a diet containing aflatoxin at 300 and 1000 ppb levels for six weeks. Similar observation was also made by Sadana *et al.* (1992) in quails kept on a diet mixed with 500 ppb of aflatoxin from day old to six weeks. They opined that the enlargement was a result of reticuloendothelial cell hyperplasia. Splenomegaly in experimental aflatoxicosis was also reported by Anjaneyulu *et al.* (1993) at 2000 ppb level in the feed and Bakshi *et al.* (1995) at graded levels in chicken.

2.8 Histopathology

2.8.1 Liver

Butler (1964) reported sequential histopathological lesions in the liver of Khaki Campbell ducklings exposed with aflatoxin at 15 µg/kg body weight. He observed oval cell proliferation with stray mitotic cells throughout the portal system and fatty infiltration at the periphery of the lobule. San Gabriel (1971) recorded diffuse fatty degeneration, nodular hyperplasia, discrete hyperplasia of

portal spaces, occlusion of hepatic sinusoids and enlarged nuclei in the livers of broiler chicken fed aflatoxin at 450 ppb level in the feed.

Histopathological lesions in experimental aflatoxicosis observed by Giambrone *et al* (1978) were hepatocytomegaly occasional necrosis of parenchymal cells and biliary hyperplasia. Mohiuddin *et al.* (1986) reported periportal fatty changes and necrosis, loss of normal hepatic architecture and an increased Kupffer cell reaction in chicken maintained on a feed containing 20000 ppb of aflatoxin. Moorthy *et al.* (1986) noted bile duct proliferation, lipidosis and regenerating hepatic cells which were moderately circumscribed by thin connective tissue in experimental aflatoxicosis in chicken maintained on rations mixed with 3120 ppb and 6250 ppb of aflatoxin.

Microscopic lesions observed by Balachandran and Ramakrishnan (1987) in chicken given diets containing 1000 and 3000 ppb of aflatoxin were hyperemia, cloudy swelling, mild hydropic degeneration, mild to moderate fatty changes and necrosis of hepatic cells as well as bile duct hyperplasia, connective tissue proliferation, haemorrhages around portal triads, multifocal collections of heterophils and lymphocyte and periportal fibrosis.

Chicken fed with diets containing aflatoxin at 300 and 1000 ppb levels exhibited moderate fatty changes, individualization and necrosis of hepatocytes, lymphocytic infiltration in portal area, hypertrophy of Kupffer cells, hepatocytomegaly and hepatokaryomegaly, sinusoidal dilatation, phlebosclerosis

and fibroblastic proliferation in portal areas (Ghosh *et al.*, 1989). Degenerative changes and deposition of lipids in hepatocytes were shown to cause hepatocytomegaly in aflatoxicosis (Raina *et al.*, 1991).

Hepatic lesions reported by Espada *et al.* (1992) in chicken dosed with 0.2 and 3 μg aflatoxin/g b.w were vacuolations of hepatocytes due to fatty metamorphosis and bile duct proliferation.

The livers of chicks fed on a diet containing 2000 ppb of aflatoxin revealed regenerating hepatocytes which assumed a tubular pattern in later stages of experiment (Anjaneyulu and Rao, 1993).

Microscopic picture observed by Sridevi and Sriraman(1996) were mild to moderate fatty changes, numerous hyperplastic nodules, periportal megalocytes, hypertrophied Kupffer cells, mild periportal bile duct and connective tissue proliferation and periductular lymphoid aggregates. They attributed these hyperplastic and hypertropic changes to the regenerative stimulus consequent to degenerative changes in the liver.

2.8.2 Kidney

San-Gabriel (1971) observed glomerular hyperplasia, discrete glomerulosclerosis, vacuolar degeneration of tubules, hyaline deposits in the lumen, increase of mucous in distal tubules and desquamation of tubular epithelium in broilers given a diet containing 450 ppb of aflatoxin.

Degeneration of tubular epithelial cells, haemorrhages and mild interstitial fibroblastic proliferation were the lesions noticed in the kidney of broiler chicken given feed mixed with 1000 and 3000 ppb of aflatoxin (Balachandran and Ramakrishnan, 1987). Histopathological picture of kidney in experimental aflatoxicosis were thickening of glomerular basement membrane along with desquamation of endothelial cells in capsular spaces, necrosis of epithelial linings of proximal convoluted tubules and albuminous casts in the lumen (Ghosh *et al.*, 1989). Irregular dilatation of some proximal tubules without evidence of cell degeneration was reported by Espada *et al.* (1992).

Microscopically, kidney revealed parenchymatous degeneration of tubules, presence of cast, swollen glomerular tufts, inter tubular haemorrhages and atrophied glomeruli with interstitial fibrosis in birds fed with a diet containing aflatoxin at 2000 ppb level (Anjaneyulu and Rao, 1993).

Subcapsular haemorrhages, swelling of tubular epithelial cells, pockets of regenerating renal cells, desquamated epithelial cells in the lumen, and hyperplasia of glomerular epithelium with swelling of glomerular tufts, thickened Bowman's capsule in isolated glomerulus and regenerating renal cells were reported by Sridevi and Sriraman (1996) in quails given feed mixed with aflatoxin at 5000 ppb level.

2.8.3 Bursa

Moderate to marked depletion of lymphocytes from the lymphoid follicles of the bursa of Fabricius was observed by Giambrone *et al.* (1978); Rao *et al.* (1988) and Ghosh *et al.* (1989) in chicken maintained on diets containing aflatoxin at 1000 and 2500 ppb levels.

Chicken dosed 0.2 and 3 µg of AFB_1 per gram of body weight daily for 21 days revealed a reduction in number of bursal follicles, pyknosis and karyorrhexis of lymphoid cells and less frequently formation of varying sized cysts on the surface epithelium (Espada *et al.*, 1992)

The histopathological lesions in bursa of chicken fed on a diet containing aflatoxin at 2000 ppb level were mild interfollicular infiltration and hyperplasia of reticulo-endothelial cells by the fifth week and lymphoid depletion from follicles with interfollicular haemorrhages by ninth week of experiment (Anjaneyulu and Rao, 1993).

Anandkumar *et al.* (1993) noticed lymphoid depletion resulting in the atrophy of bursal follicles and intrafollicular fibrosis in young quails fed graded levels of dietary aflatoxin at 500 and 2000 ppb levels for a period of 30 days. Interfollicular edema, mild depletion of lymphocytes and intrafollicular cyst formation were the microscopic lesions in quails kept on a diet containing aflatoxin at 5000 ppb level for four weeks (Sridevi and Sriraman, 1996). Similar

observations were also made by Suresh (1999) at a dose rate of 0.5 µg/g body weight for 60 days.

2.8.4 Spleen

San-Gabriel (1971) reported the absence of any significant microscopic lesion in the spleen of chicken fed with a ration containing aflatoxin at 450 ppb level. Giambrone, *et al* (1978) noticed occasional areas of lymphoid depletion and necrosis in the germinal centers and white pulp of spleen in chicken given a diet containing 2500 ppb of aflatoxin from day one to seven weeks. Marked lymphoid depletion and proliferation of reticular cells in the malphigian corpuscles of spleen were the lesions observed by Balachandran and Ramakrishnan (1987) in chicken fed with a diet containing aflatoxin at 3000 ppb level for four weeks and Ghosh *et al.* (1989) at 300 and 1000 ppb levels for six weeks. Feed mixed with graded levels of aflatoxin at 750, 1500 and 3000 ppb when fed to broilers for six weeks resulted in the degeneration and depletion of lymphoid cells of spleen (Bakshi *et al.*, 1995).

Mild depletion of lymphocytes from splenic white pulp and hypertrophy of the wall of the blood vessels were the lesions noticed by Sridevi and Sriraman (1996) in quails fed on a ration containing 2000 and 5000 ppb of aflatoxin for 12 weeks. Suresh (1999) noticed focal necrosis of lymphocytes, vascular sclerosis and diffuse lymphoid depletion in the spleen of quails treated with aflatoxin at the rate of 0.5 µg/g body weight twice weekly for 60 days.

2.9 Ultrastructural pathology of liver

Ultrastructural changes reported by Novi (1977) in the Wistar female rats treated with 25 μg AFB_1 for a period of eight weeks were proliferation of smooth endoplasmic reticulum, dilatation of rough endoplasmic reticulum, the loss of ribosomes and mitochondrial swelling with loss of granules and cristolysis. He also noted focal disruption and fragmentation of nuclear membrane, dilatation of secretory vesicles, abundance of glycogen particles and increased number of microbodies.

An electron microscopic study by Mollenhauer *et al.* (1989) on the liver of male broiler chicks fed with diet containing aflatoxin at 1250, 2500 and 5000 ppb levels for 21 days revealed hepatocellular lipidosis, enlargement of bile canaliculi and reduction in mitochondrial size.

2.10 Safe level of aflatoxin for chicken

Allocraft (1969) recommended 20 ppb as the safe level of aflatoxin in the feed of chicken. The recommended safe level of aflatoxin as per the EEC (European Economic Community, 1976) was also 20 ppb in chicken.

The PAG (Protein Advisory Group, 1987) of the United Nations recommended 30 ppb as the lowest limit of aflatoxin in the feed rich in protein.

The safe level of AFB_1 in the animal feed in Belgium is 40 ppb, while that in USA it is 20 to 25 ppb. Rhodesia has set a limit of 50-400 ppb whereas in UK it is 20 ppb in the feed (FAO, 1976 - Food and Agricultural Organisation, UN).

The tolerance levels of aflatoxin in pure bred broiler chicks is 200 ppb and for cross bred broiler chicks is 400 ppb as determined by Johri and Panda (1988) at CARI (Central Avian Research Institute).

Twenty ppb has been fixed as the safe level of aflatoxin in the feed for broiler chicken as per the "Package of Practices Recommendations" (1994) followed in Kerala Agricultural University.

Arora (1995) observed that the birds can tolerate aflatoxin at levels as high as 500 ppb, if the feed is supplemented with extra protein and the amino acid methionine.

Materials and Methods

MATERIALS AND METHODS

3.1 Materials

3.1.1 Experimental birds

One hundred and eighty (n=180) healthy day-old Hubbard broiler chicks were obtained from the Coastal Krishna Hatcheries, Thrissur, Kerala. The experiment was carried out during the period from February 3rd to March 9, 1999.

3.1.1.1 Experimental groups

The broiler chicks were randomly divided into six groups of thirty birds each and were numbered using wing-bands. All the six groups were kept in separate rooms under deep litter system throughout the experiment. Ideal brooding conditions were provided upto four weeks of age. Each group was given scheduled experimental feed. Water was given *ad libitum*.

3.1.1.2 Management

The experimental rooms were thoroughly cleaned with 2.5 per cent phenol and fumigated with formaldehyde gas (35 ml of commercial formalin plus 17.5 g potassium permanganate per 100 cubic feet area).

3.1.2 Experimental feed

Broiler chicken feeds, both starter and finisher were procured from the market. The toxin level in the feed was analysed by modified Pons method (Pons *et al.*, 1966) and grouped into six categories according to the aflatoxin level as follows:

1. 0 ppb
2. 20 ppb
3. 40 ppb
4. 60 ppb
5. 80 ppb
6. 100 ppb

The level of aflatoxin in each category of feed was adjusted by adding aflatoxin free feed to the feed containing excess toxin level.

All the six groups of birds were reared on the above formulated ration from day-old age as per the following schedule.

Group	Number of birds	Aflatoxin content of feed
I	30	0 ppb
II	30	20 ppb
III	30	40 ppb
IV	30	60 ppb
V	30	80 ppb
VI	30	100 ppb

3.2 Methods

The following parameters were employed for the study.

1. Weight gain at weekly intervals
2. Daily feed intake and feed conversion ratio at weekly intervals.
3. Haematology - ESR, Hb, PCV, DLC, TLC
4. Serum protein, albumin, globulin and albumin-globulin ratio (A/G)
5. Serum alkaline phosphatase (ALP)
6. Serum Gamma glutamyl transferase (GGT)
7. Organ weight - liver, spleen, bursa
8. Estimation of residual aflatoxin in muscle, liver and kidney
9. Gross pathology
10. Histopathology of liver, kidney, spleen and bursa of Fabricius.
11. Electronmicroscopy of liver
12. Economic performance
13. Incidence of aflatoxin in poultry feed
14. Statistical analysis

3.2.1 *Experimental design*

Ten birds from each group were disposed on 15th, 30th and 45th day. Blood and tissues from these birds were collected for haematological, serological,

histopathological and ultrastructural studies. Gross lesions were evaluated and recorded.

3.2.2 Body weight

Body weight of the chicks at day-old were recorded before the commencement of the experiment and thereafter at weekly intervals in all the groups.

3.2.3 Feed intake and feed conversion ratio

Daily feed intake and average weekly feed intake of the control and experimental birds were recorded. Feed conversion ratio was calculated from the weekly feed intake to the weekly body weight gain and the overall FCR were worked out based on the mean body weight and cumulative feed intake.

$$\text{FCR (weekly)} = \frac{\text{Weekly feed intake}}{\text{Weekly body weight gain}}$$

$$\text{Overall feed conversion ratio (FCR)} = \frac{\text{Cumulative feed intake}}{\text{Mean body weight}}$$

3.2.4 Haematological parameters

The study included the determination of Haemoglobin (Hb) concentration, Erythrocyte sedimentation rate (ESR), Packed cell volume (PCV), Differential leucocyte count (DLC) and Total leucocyte count (TLC).

Blood samples were collected from the wing vein at fifteen days interval. Two millilitre of blood was collected for the haematological studies using dipotassium salt of Ethylene diamine tetraacetic acid (EDTA) at the rate of 1 mg/ml of blood as the anticoagulant.

Haemoglobin concentration was estimated by the cyanmethaemoglobin method described by Miale (1967) and the final readings were taken in an Erma Photometer.

For determination of PCV, microhaematocrit method of Cohen (1967) was used. ESR was estimated by Wintrobe and Landsburg method (1964).

The TLC was determined as per the method described by Sastri (1976). The DLC was done with the copper peroxidase method of Sato and Sekiya (1965).

3.2.5 Total serum protein

Five millilitre of blood was collected separately in a sterile test tube without adding anticoagulant for serum separation.

The total protein content in the serum was estimated by Biuret method as described by Inchiosa (1964).

The albumin content in the serum was estimated by the BCG (Bromo Cresol Green dye) binding method described by Doumas *et al.* (1971).

Serum globulin value was determined by deducting values of serum albumin from total serum protein.

Albumin-globulin ratio was calculated by dividing albumin values with globulin level.

3.2.6 Serum enzymes

Blood (5 ml) was collected without adding anticoagulant and serum was separated out.

3.2.6.1 Serum alkaline phosphatase (ALP)

ALP was estimated by using commercially available kits (M/s Merck India Ltd., Mumbai). The final readings were taken using spectrophotometer at 405 nm.

3.2.6.2 Gamma glutamyl transferase (GGT)

GGT was also determined by using commercially available kits (Sigma Diagnostics, USA). The final readings were taken using spectrophotometer at 405 nm.

3.2.7 Organs weight

Weight of organs like liver, spleen and bursa of Fabricius recorded at fortnightly intervals.

3.2.8 Toxin residues in tissues

Toxin residues in muscle, liver and kidney were determined by modified Pons method (Pons *et al.*, 1966) using thin layer chromatography (TLC). For quantification, the plate was scanned fluorimetrically using Hitachi-3000 model computerized fluroscence spectrophotometer at a wavelength for excitation 365 nm and emission 420 nm.

Results obtained from the TLC scanning were randomly checked by analysing the samples with high performance liquid chromatography (HPLC) using pump Hitachi L-7100 model (Merck Pvt. Ltd.). For this, the extract from the Pon's method were further purified through column chromatography using chloroform as the eluting solvent. The aliquot from this purified extract were applied onto C-18 column. Methanol acetonitrile and water (23:22:55) system was used as the mobile phase. Aflatoxin content in the effluent was scanned by fluroscent spectrophotometer with computer attachment.

3.2.9 Gross and histopathology

Birds which were disposed at fortnightly intervals and those died during the course of the experiment were subjected to detailed autopsy examination. Gross lesions in different organs were recorded. Representative pieces of liver, kidney, bursa of Fabricius and spleen were collected for histopathological studies.

Tissues were fixed in 10 per cent neutral buffered formalin and were processed by routine paraffin embedding technique (Armed Forces Institute of Pathology, 1968). Sections were cut at five micron thickness and stained with Harri's haematoxylin and eosin as described by Sheehan Hrapchak (1980).

3.2.10 Ultrastructural studies

Liver tissues from all the six groups at fortnightly intervals were also collected for ultrastructural studies.

Fresh tissues were immediately fixed in 2.5 per cent glutaraldehyde and the fixed tissues were washed three times in phosphate buffer (pH 7.4). Post fixation was done with one per cent buffered osmium tetraoxide (Sigma, USA) at 4°C for 2 hrs. They were then dehydrated in ascending grades of acetone and embedded in spurr low viscosity embedding resin (Bio-Rad, Microsciences Division, USA). Sections were cut on a Reichter Jung Ultracut-R microtome. Ultra thin sections were taken on copper grids and stained with Uranyl acetate and lead citrate (both

Sigma Products, USA) and examined in an Electron microscope (Hitachi-600A) at 75 kv and the electron micrographs were taken.

3.2.11 Economic performance

The cost of chicks, feed, miscellaneous items as well as the returns from adult birds, manure and gunny bags were estimated based on the prevailing market rate at the time of experiment. The net returns and the economic loss per bird in each group were calculated.

3.2.12 Incidence of aflatoxin contamination in poultry feed

One hundred and four poultry feed samples were randomly collected from different farms of the state during the period January-July (1999). The samples were assayed for the presence of aflatoxin by Modified Pons Method (Pons *et al.*, 1966) using thin layer chromatography.

3.2.13 Statistical analysis

The data obtained from various parameters were subjected to statistical analysis and analysis of variance (ANOVA) was conducted as per Snedecor and Cochran (1985).

Results

RESULTS

The results obtained in the study on "Determination of permissible level of Aflatoxin in Broiler chicken feed" are presented in this chapter.

4.1 Daily feed intake

Birds fed with aflatoxin showed a dose related decrease in the daily feed intake throughout the experimental period compared to control. The difference was not statistically significant upto fourth week. Thereafter aflatoxin fed birds exhibited a significant decrease ($P<0.01$) in the daily feed intake compared to that of the control (Table I and Fig.1).

4.2 Body weight

All the birds in the control as well as experimental groups showed a gradual increase in body weight throughout the experimental period. However, the experimental groups recorded highly significant reduction ($P<0.01$) in the weekly body weight compared to control (Table II; Fig.2). The reduction in the body weight was in correlation with the level of toxin in the feed and the birds in Group V and VI gave the lowest body weight at the sixth week.

Table I. Average daily feed intake (g) at weekly intervals

Group	Toxin level (ppb)	Age in weeks					
		1	2	3	4	5	6
I	0	24.000 ± 5.407	70.143 ± 5.366	102.286 ± 1.766	119.714 ± 2.490	134.286 ** ± ^{a} 1.698	146.857** ± ^{a} 1.650
II	20	24.000 ± 5.407	68.286 ± 5.060	101.857 ± 1.577	116.286 ± 1.909	128.714 ± 1.300	141.571 ± 1.890
III	40	24.000 ± 5.407	68.000 ± 5.011	99.286 ± 2.000	115.286 ± 2.240	128.571 ± 1.430	140.714 ± 1.698
IV	60	22.143 ± 4.721	68.286 ± 5.160	97.714 ± 2.320	114.571 ± 2.540	128.000 ± 0.850	134.286 ± 1.698
V	80	20.571 ± 4.410	65.857 ± 5.400	96.857 ± 1.910	114.429 ± 2.280	127.571 ± 0.950	134.286 ± 1.698
VI	100	20.143 ± 4.310	61.714 ± 4.630	94.857 ± 2.510	113.714 ± 2.750	125.714 ± 2.530	129.286 ± 1.302
	F value					3.42**	14.492**
	CD					4.49	4.79

Means differed significantly from the Control (**P<0.01)

FIG.1 AVERAGE DAILY FEED INTAKE AT WEEKLY INTERVELS

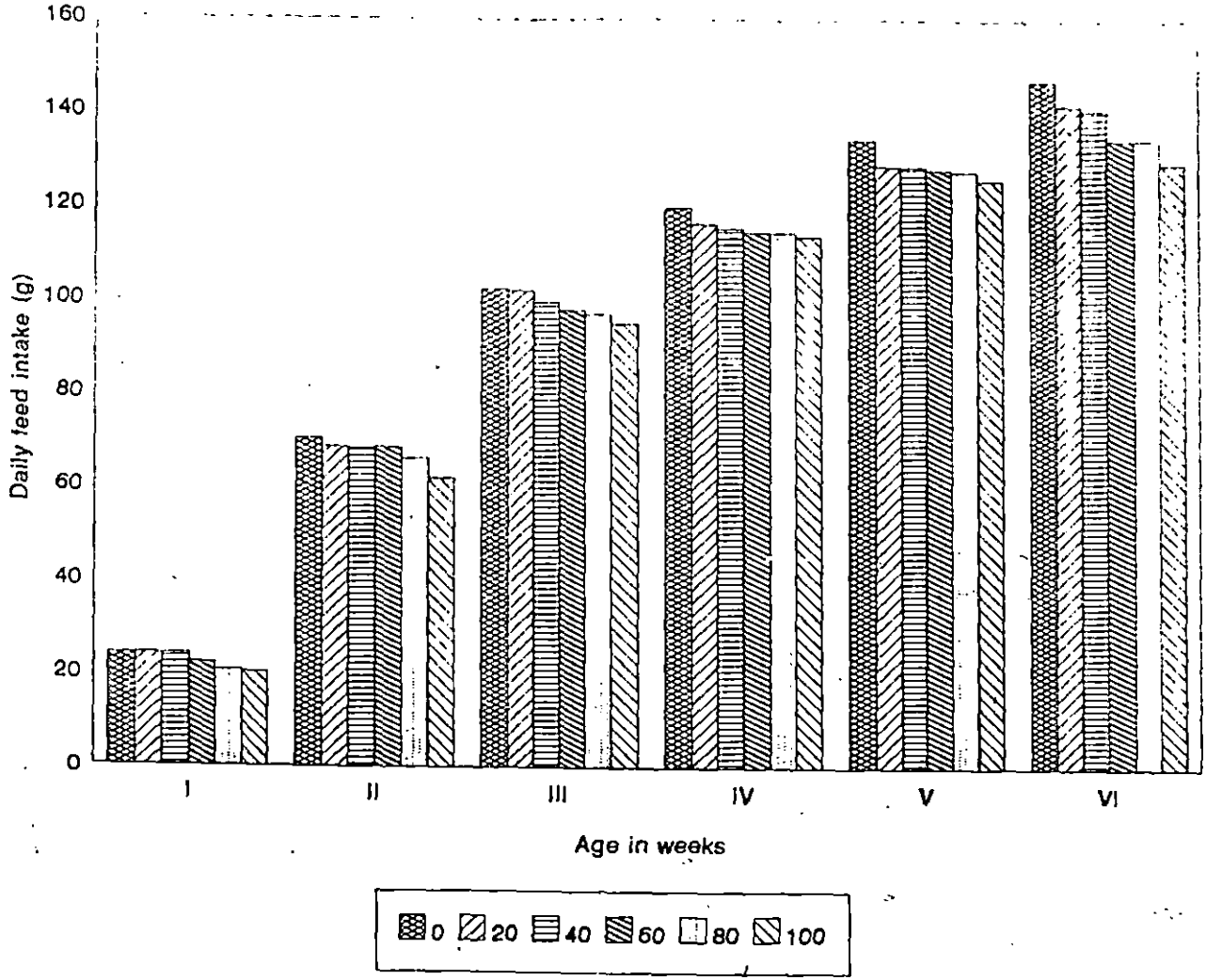
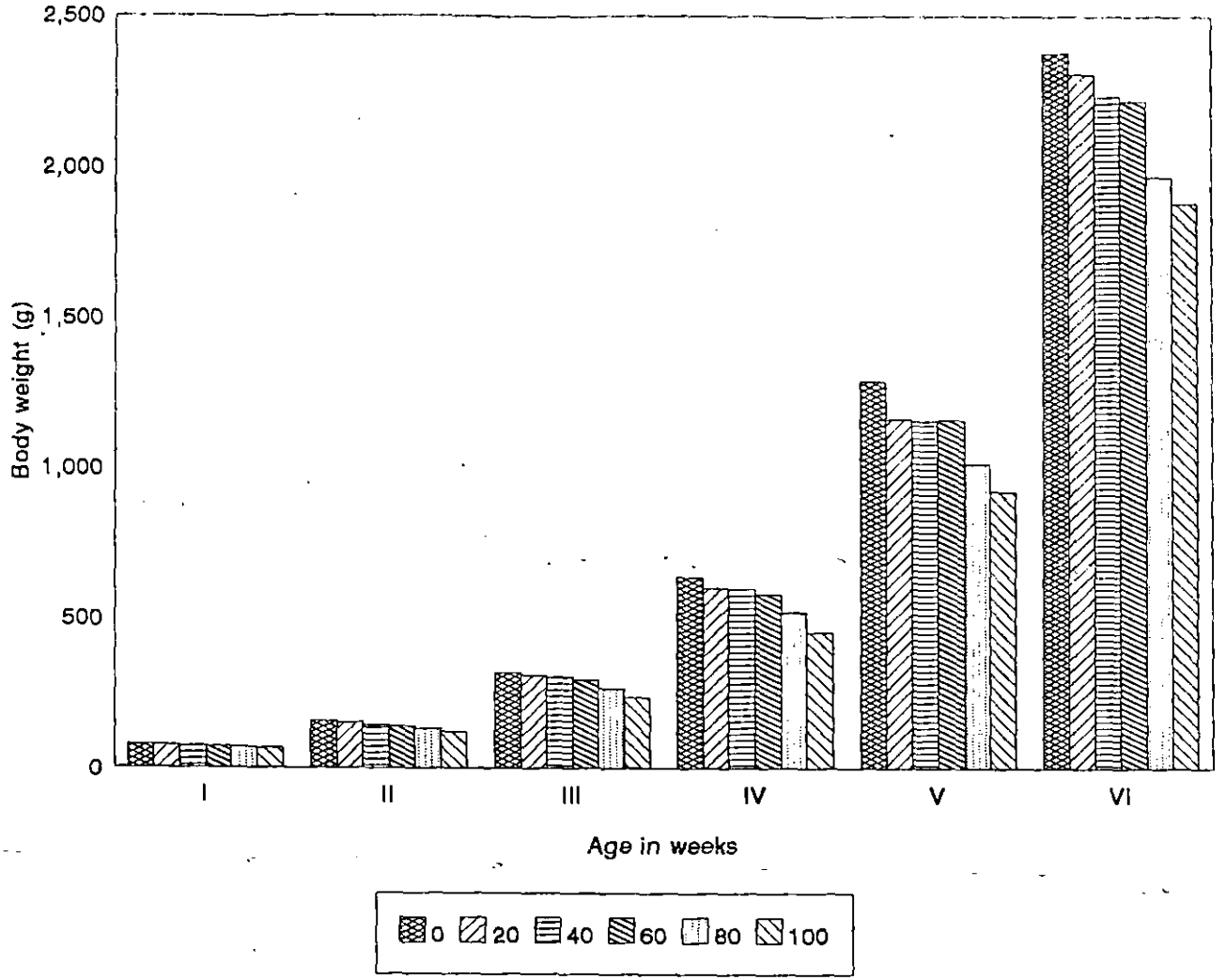


Table II. Mean body weight (g) at weekly intervals

Group	Toxin level (ppb)	Age in weeks						
		1	2	3	4	5	6	
I	0	77.0053 ± [a] 3.490	157.3241 ± [a] 4.80	317.9471 ± [a] 10.500	636.3005 ± [a] 85.040	1288.173 ± [a] 101.060	2377.534 ± [a] 110.720	100
II	20	75.8398 ± [a] 1.587	151.769 ± 7.354	308.7215 ± [a] 9.160	598.728 ± 19.250	1162.876 ± 120.721	2307.405 ± [a] 132.940	97
III	40	71.9683 ± 4.45	142.6308 ± 9.06	304.0933 ± [a] 25.600	596.3953 ± 52.900	1156.044 ± 163.721	2232.604 ± [a] 149.28	94
IV	60	72.5168 ± 2.634	140.0892 ± 8.850	293.7193 ± 28.12	579.9012 ± 43.92	1260.052 ± 146.187	2218.954 ± [a] 156.78	93
V	80	69.3533 ± 2.29	131.681 ± 6.548	266.889 ± 27.137	518.6686 ± 56.75	1013.659 ± 162.281	1968.013 ± 172.241	83
VI	100	67.5852 ± 2.29	121.368 ± 6.48	236.133 ± 46.40	452.598 ± 181.63	921.3662 ± 196.81	1880.53 ± 201.76	79
	F value	17.2439**	68.2149**	28.317**	33.9295**	14.2313**	7.0575**	
	CD	2.55	4.64	18.538	36.189	109.364	242.7813	

Means having the same superscript did not differ significantly from that of the control (**P<0.01)

FIG.2 MEAN BODY WEIGHT AT WEEKLY INTERVELS



4.3 Feed conversion ratio (FCR)

There was decrease in weekly feed conversion ratio (FCR) in all the treatment groups at all ages particularly from second to sixth week compared to control (Table IIIa and Fig.3a). The cumlative FCR, at sixth week of age was more or less same upto 60 ppb level and it was extremely poor at levels 80 and 100 ppb (Table IIIb and Fig.3b).

4.4 Haemogram

A dose and time related response in all the haematological parameters were recorded in the toxin fed groups compared to control throughout the experimental period.

4.4.1 Erythrocyte sedimentation rate (ESR)

There was a significant increase ($P<0.05$) in the ESR in Group V and VI compared to control on the 30th and 45th day of experiment (Table IVa, b, c and Fig.4).

4.4.2 Packed cell volume (PCV)

A significant reduction ($P<0.05$) in the PVC was noticed in Group VI compared to control on the 30th day of experiment (Table IVa, b, c and Fig.5).

Table IIIa. Feed conversion ratio at weekly intervals (FCR)

Group	Toxin level (ppb)	Age in weeks					
		1	2	3	4	5	6
I	0	2.18	4.18	4.32	3.48	2.45	1.75
II	20	2.24	4.26	4.40	3.62	2.64	1.76
III	40	2.33	4.52	4.40	3.59	2.63	1.81
IV	60	2.14	4.52	4.48	3.65	2.59	1.78
V	80	2.08	4.59	4.80	4.01	2.94	1.99
VI	100	2.09	4.72	5.24	4.49	3.16	2.03

Note: Higher numerical values indicate lower FCR

FIG.3a FEED CONVERSION RATIO AT WEEKLY INTERVELS

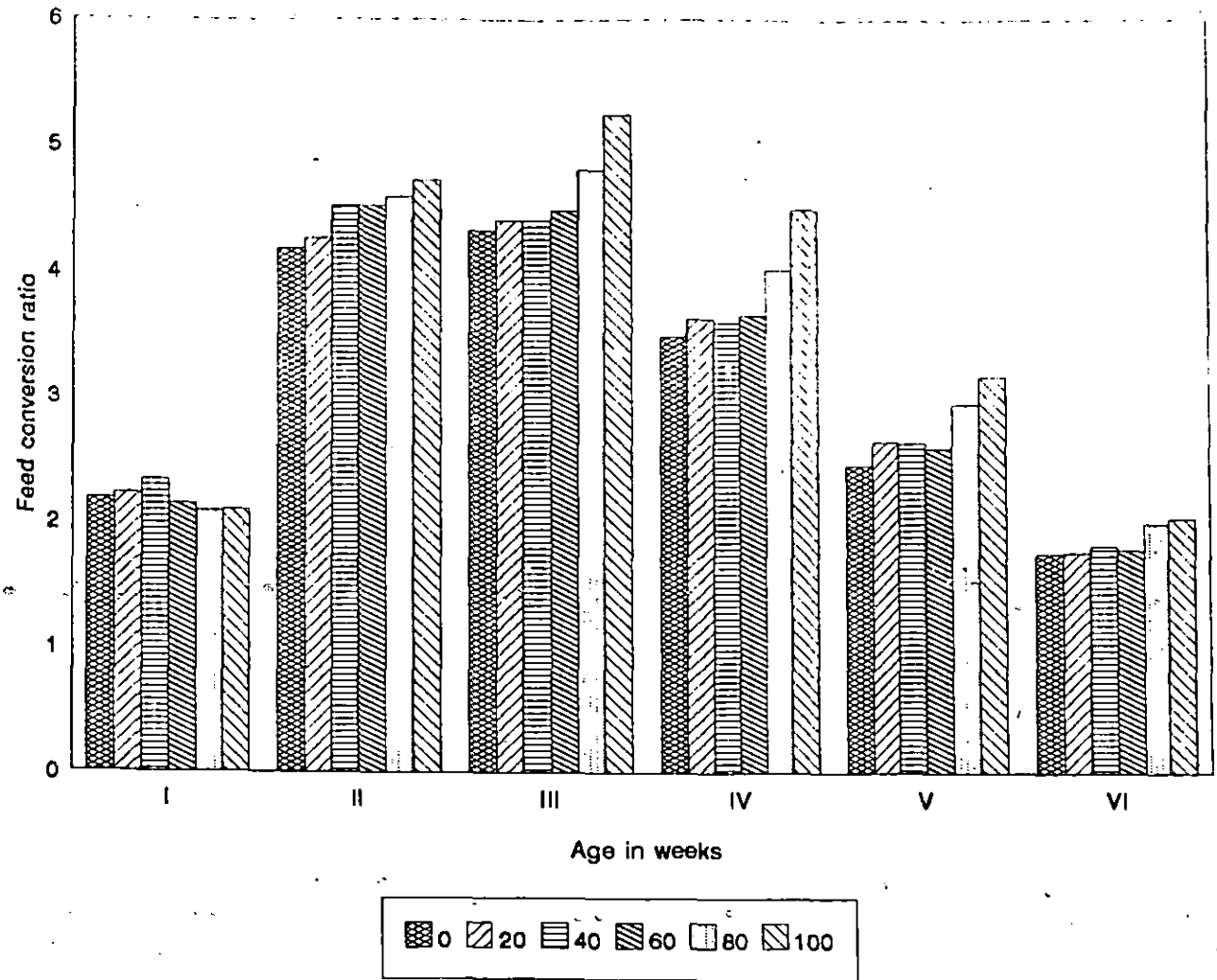
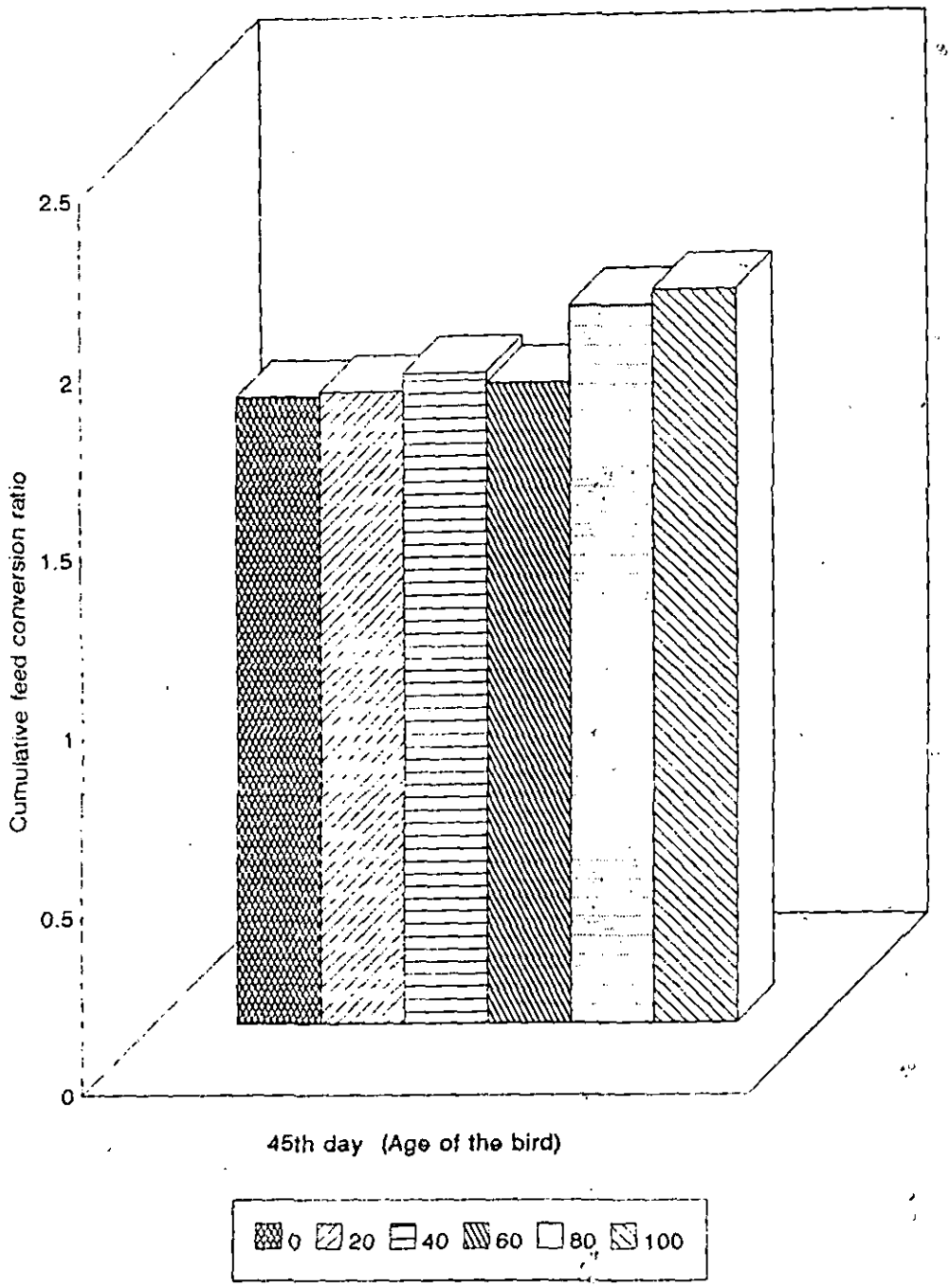


Table IIIb. Cumulative FCR at sixth weeks of age

Group	Toxin level (ppb)	Cumulative feed intake upto sixth week (g)	Mean body weight at sixth week (g)	FCR = Cum. Feed intake / Mean body weight
I	0	4180.98	2377.534	1.75
II	20	4064.97	2307.405	1.76
III	40	4030.98	2232.604	1.81
IV	60	3954.98	2218.954	1.78
V	80	3916.97	1968.013	1.99
VI	100	3817.98	1880.53	2.03

Note: Higher numerical values indicate lower FCR

FIG.3b CUMULATIVE FEED CONVERSION RATIO



4.4.3 Haemoglobin (Hb)

The haemoglobin values were significantly reduced in Group IV, V and VI on the 30th ($P<0.01$) and 45th day ($P<0.05$) of experiment (Table IVa, b, c and Fig.6).

4.4.4 Total leucocyte count (TLC)

Eventhough a mild dose related decrease in the TLC was noticed in all toxin fed groups, statistical significance was observed only on the 45th day in Groups IV, V and VI (Table IVa, b, c and Fig.7).

4.4.5 Differential leucocyte count (DLC)

Heterophil counts were significantly increased in Groups IV, V and VI on the 15th day ($P<0.01$) and 30th day ($P<0.05$). While the lymphocyte counts were decreased in Group V and VI on 15th ($P<0.01$), and 45th day and in Group III to VI on the 30th day ($P<0.05$) of experiment. No appreciable variation was seen in the monocyte, eosinophil and basophil counts (Table IVa, b, c and Fig.8a and 8b).

4.5 Serum profile

4.5.1 Total serum protein

A dose dependent decrease in the total serum protein values was recorded throughout the experimental period with significant decrease ($P<0.05$) in all the treatment groups on the 45th day of experiment (Table Va, b, c and Fig.9).

Table IVa. Haemogram – 15th day of experiment

Group	Toxin level (ppb)	ESR (mm/h)	PCV (%)	Hb (g%)	TLC ($x10^3$/cumm)	Differential leucocyte count (per cent)				
						H	L	M	E	B
I	0	2.250 ± 0.25	30.0 ± 0.815	10.275 ± 0.335	21.15 ± 1.30	33.75 ± [a] 0.48	61.5 ± [a] 0.865	2.5 ± 0.289	1.5 ± 0.289	0.75 ± 0.250
II	20	2.600 ± 0.22	28.5 ± 0.924	9.060 ± 0.278	19.70 ± 1.50	35.60 ± [a] 0.601	60.00 ± [a] 0.731	2.7 ± 0.213	1.6 ± 0.221	0.50 ± 0.167
III	40	3.50 ± 0.467	26.9 ± 0.560	8.990 ± 0.528	19.68 ± 1.34	35.80 ± [a] 0.474	60.1 ± [a] 0.623	2.7 ± 0.153	1.3 ± 0.153	0.30 ± 0.153
IV	60	3.50 ± 0.37	27.6 ± 1.766	8.050 ± 0.456	18.76 ± 0.862	36.6 ± 0.427	60.1 ± [a] 0.566	2.6 ± 0.163	1.3 ± 0.153	0.200 ± 0.133
V	80	3.5 ± 0.37	26.9 ± 2.092	8.0 ± 0.629	17.44 ± 0.656	37.7 ± 65.2	58.3 ± 0.845	2.6 ± 0.163	1.5 ± 0.167	0.3 ± 0.153
VI	100	3.5 ± 0.269	25.2 ± 1.528	8.05 ± 0.335	17.08 ± 0.567	38.5 ± 0.636	57.4 ± 0.62	2.6 ± 0.163	1.4 ± 0.163	0.5 ± 0.167
	F value					6.395**	3.634**			
	CD					2.08	2.55			

Means having the same superscript did not differ significantly from the control (**P<0.01)

Fig.4 ERYTHROCYTE SEDIMENTATION RATE

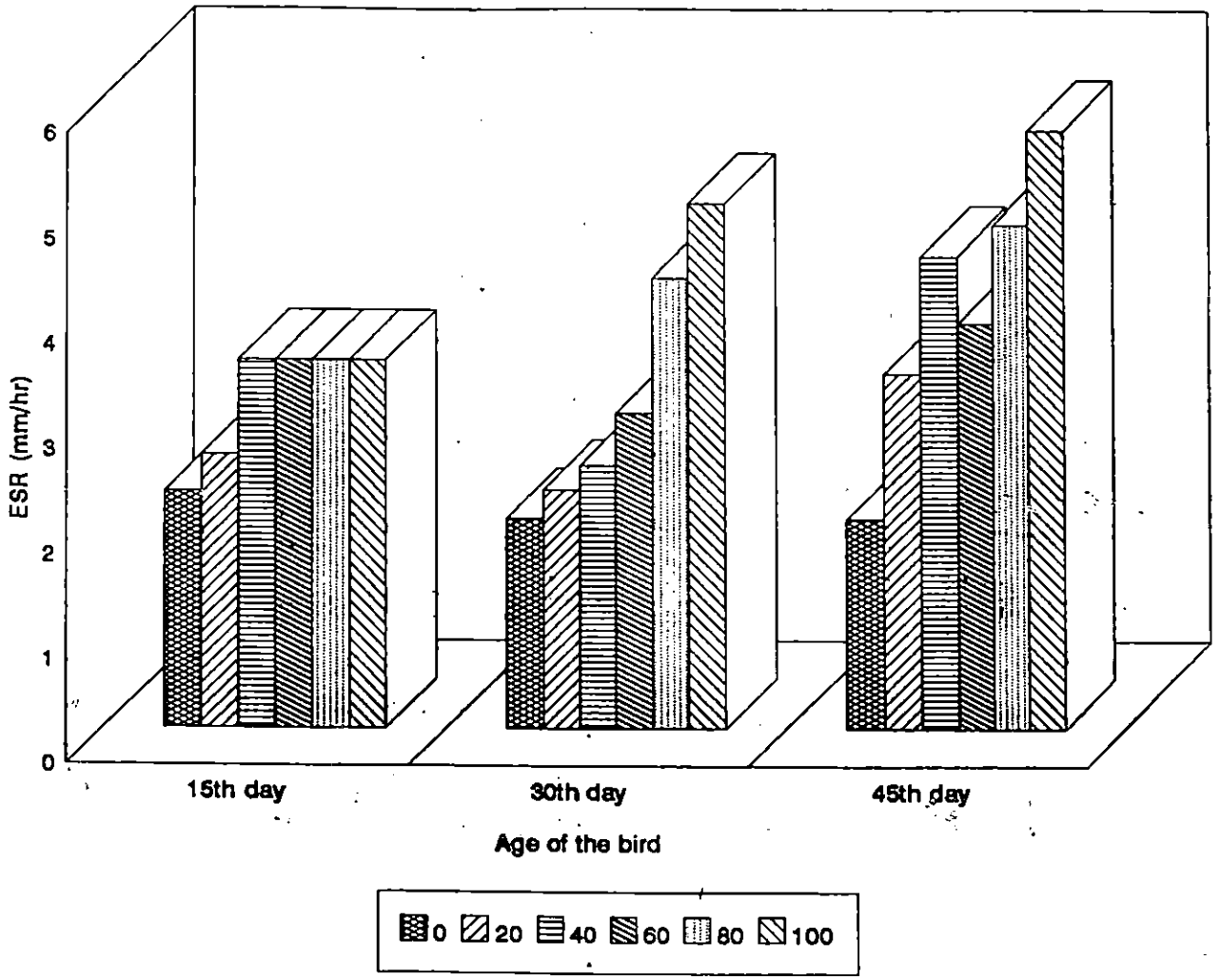


Table IVb. Haemogram – 30th day of experiment

Group	Toxin level (ppb)	ESR (mm/h)	PCV (%)	Hb (g%)	TLC ($x10^3$/cumm)	Differential leucocyte count (per cent)				
						H	L	M	E	B
I	0	2.000 ± [a] 0.41	30.250 ± [a] 0.855	10.975 ± [a] 0.65	19.70 ± 1.175	33.50 ± [a] 1.560	62.50 ± [a] 1.325	2.250 ± 0.250	1.250 ± 0.250	0.500 ± 0.289
II	20	2.286 ± [a] 0.185	29.571 ± [a] 0.969	9.100 ± [a] 0.294	19.40 ± 0.72	34.00 ± [a] 1.027	60.857 ± [a] 0.958	2.714 ± 0.286	1.857 ± 0.261	0.571 ± 0.202
III	40	2.500 ± [a] 0.224	27.833 ± [a] 2.302	8.617 ± 0.453	18.36 ± 0.665	35.667 ± [a] 0.882	59.00 ± 0.967	3.167 ± 0.401	1.667 ± 0.211	0.500 ± 0.224
IV	60	3.000 ± [a] 0.309	27.857 ± [a] 0.985	7.857 ± 0.358	17.24 ± 0.991	35.857 ± [a] 0.883	58.857 ± 0.857	3.00 ± 0.218	1.714 ± 0.286	0.571 ± 0.202
V	80	4.286 ± 0.521	24.571 ± [a] 1.769	7.714 ± 0.539	17.38 ± 0.661	37.143 ± 0.702	57.429 ± 0.781	2.857 ± 0.261	1.857 ± 0.261	0.714 ± 0.184
VI	100	5.00 ± 1.234	22.714 ± 2.460	6.543 ± 0.808	16.22 ± 1.040	38.000 ± 0.532	57.286 ± 0.521	2.571 ± 0.220	0.571 ± 0.202	0.571 ± 0.202
	F value	3.449*	2.690*	6.28**		3.656*	4.544*			
	CD	2.078	5.704	1.754		2.845	2.825			

* P<0.05
** P<0.01
Means having the same superscript did not differ significantly from the control

Fig.5 PACKED CELL VOLUME

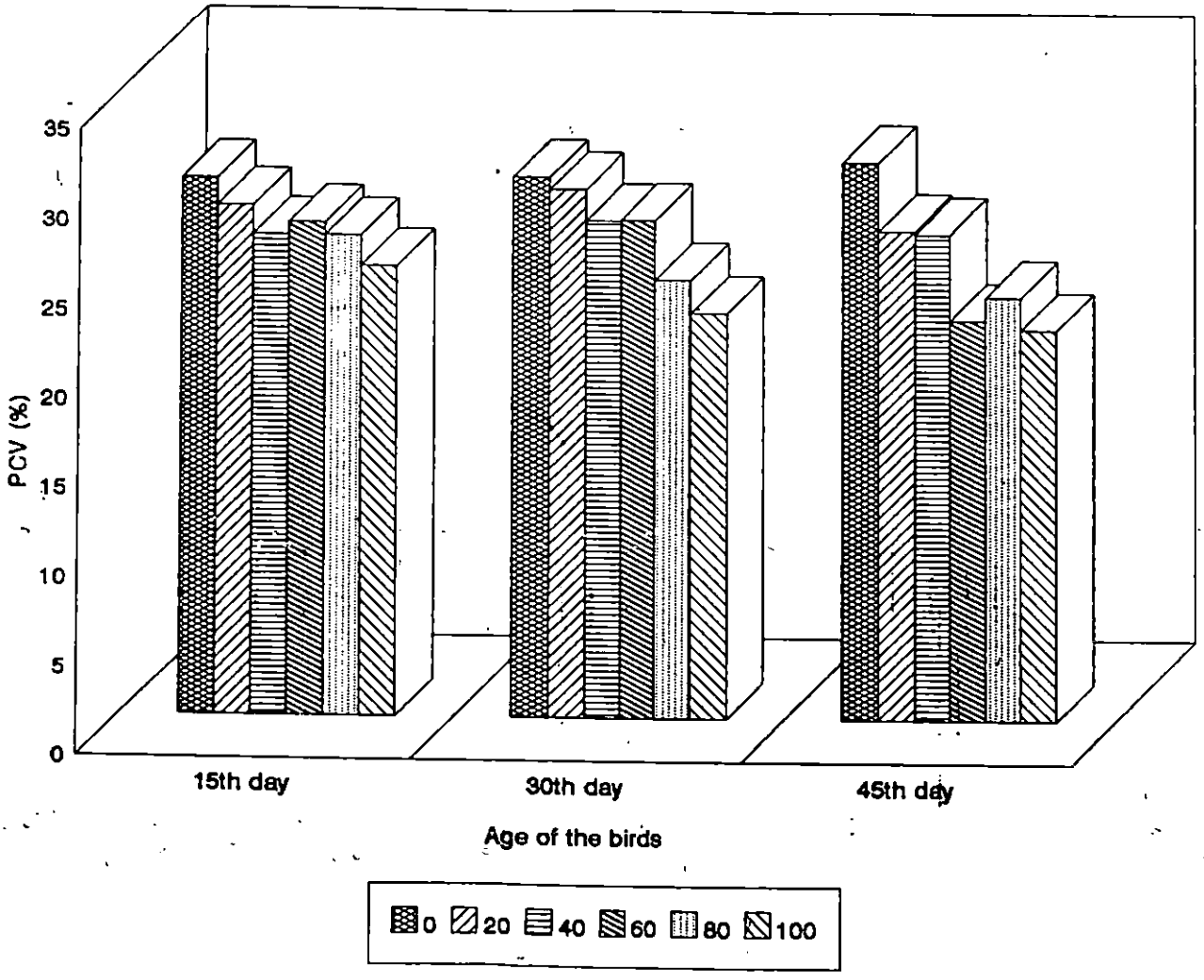


Fig.6 HAEMOGLOBIN

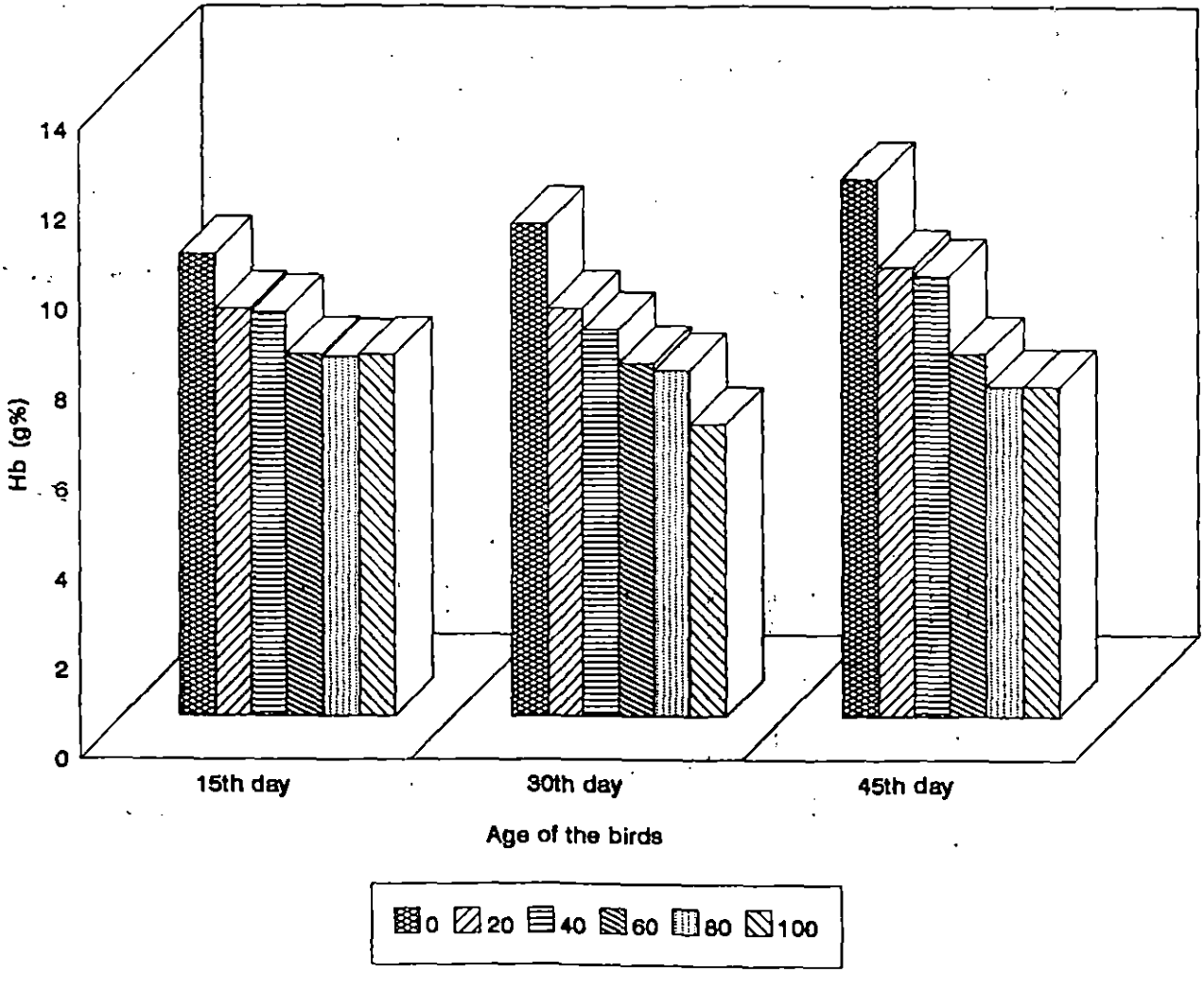


Fig.7 TOTAL LEUCOCYTE COUNT

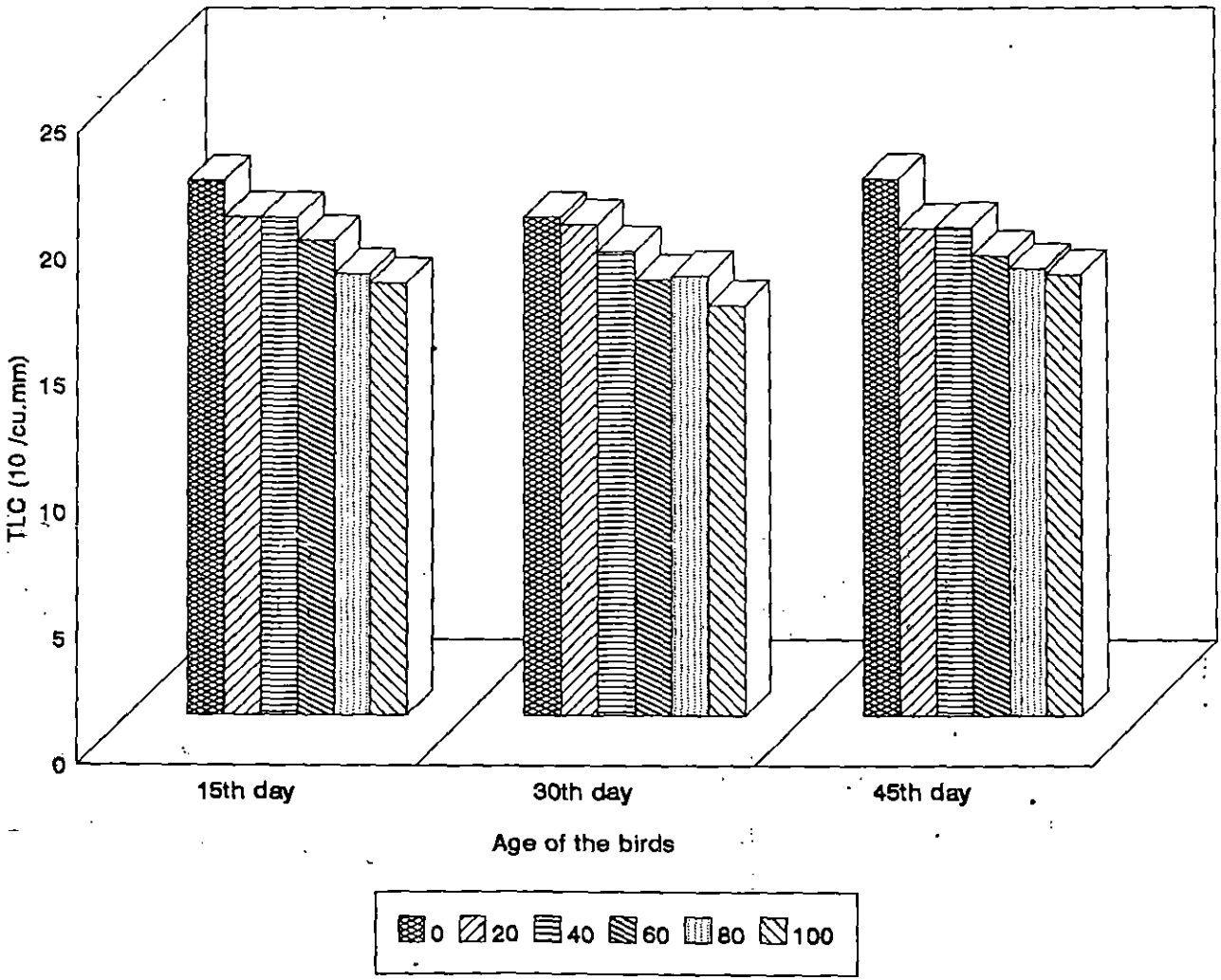


Fig.8a DIFFERENTIAL LEUCOCYTE COUNT - HETEROPHIL

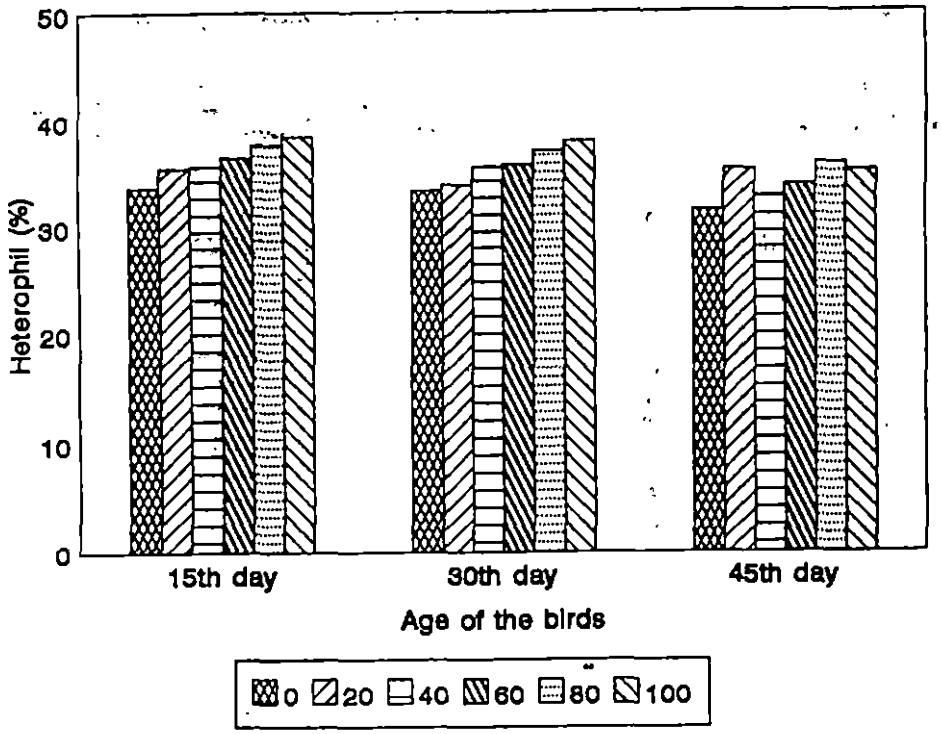


Fig.8b DIFFERENTIAL LEUCOCYTE COUNT - LYMPHOCYTE

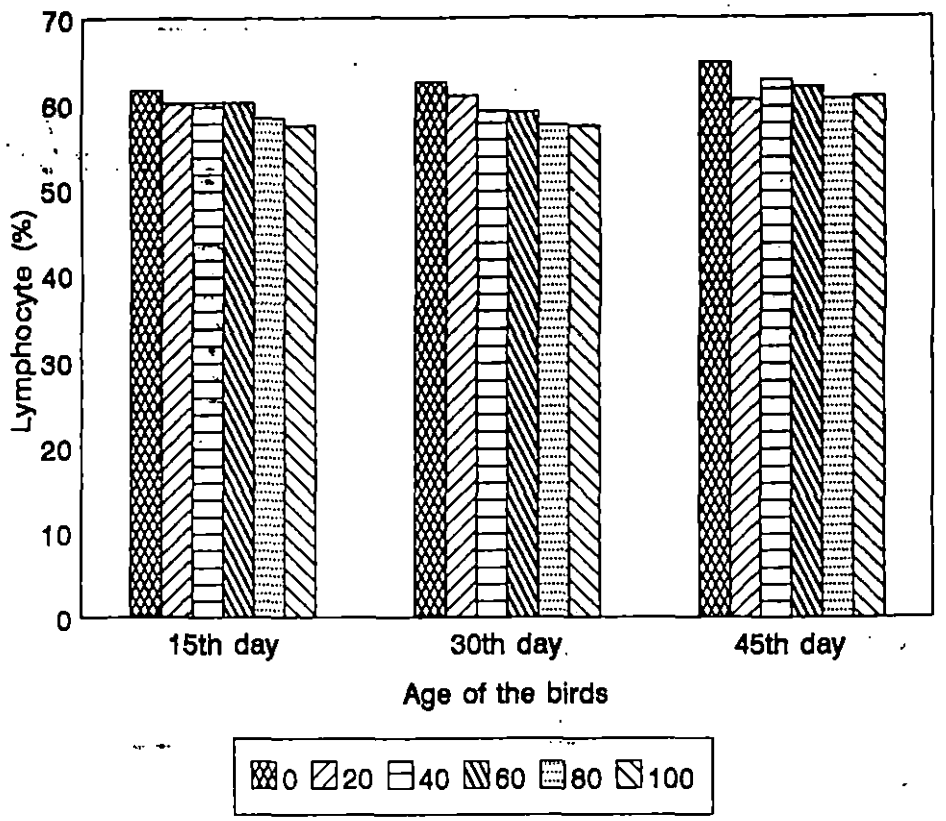


Table IVc. Haemogram – 45th day of experiment

Group	Toxin level (ppb)	ESR (mm/h)	PCV (%)	Hb (g%)	TLC ($x10^3$/cumm)	Differential leucocyte count (per cent)				
						H	L	M	E	B
I	0	2.000 ± [a] 0.317	31.200 ± 2.263	11.980 ± [a] 1.071	21.220 ± [a] 1.125	31.600 ± 0.679	64.800 ± [a] 0.857	2.200 ± 0.374	1.000 ± 0.316	0.400 ± 0.245
II	20	3.375 ± [a] 0.597	27.375 ± 3.332	10.025 ± [a] 1.396	19.260 ± [a] 1.147	35.375 ± 1.534	60.375 ± [a] 1.336	2.625 ± 0.324	1.250 ± 0.250	0.500 ± 0.267
III	40	4.500 ± 0.373	27.200 ± 2.569	9.830 ± [a] 1.158	19.320 ± [a] 0.915	32.900 ± 0.689	62.800 ± [a] 0.728	2.600 ± 0.267	1.400 ± 0.267	0.300 ± 0.153
IV	60	3.875 ± [a] 0.579	22.375 ± 2.463	8.087 ± 0.993	18.200 ± 0.549	34.000 ± 0.707	61.875 ± [a] 0.717	2.250 ± 0.313	1.375 ± 0.263	0.500 ± 0.189
V	80	4.800 ± 0.573	23.700 ± 2.427	7.360 ± 0.668	17.700 ± 0.437	36.000 ± 1.316	60.500 ± 0.946	2.200 ± 0.389	1.100 ± 0.314	0.200 ± 0.133
VI	100	5.700 ± 0.896	21.900 ± 2.060	7.360 ± 0.617	17.460 ± 0.348	35.300 ± 1.022	60.800 ± 0.953	2.400 ± 0.221	1.200 ± 0.200	0.300 ± 0.15
	F value	3.23*		2.787*	2.870*		2.463*			
	CD	2.13		3.35	2.40		3.22			

Means having the same superscript did not differ significantly from the control (* P<0.05)

4.5.2 Serum albumin

Significant decrease in serum albumin values were recorded in Group IV, V and VI throughout the experiment. But a dose related significant decrease in the albumin values were recorded in all the toxin fed groups on the 45th day of experiment (Table Va, b, c and Fig.10a).

4.5.3 Serum globulin

There was no significant variation in the globulin values between control and experimental groups. However a dose related mild decrease in globulin values were observed on the 45th day of experiment (Table Va, b, c and Fig.10b).

4.5.4 A/G ratio

A/G ratio did not show much variations between groups except in Group IV and V ($P<0.05$) on 30th day (Table Va, b, c).

4.5.5 Serum enzymes

All the aflatoxin treated birds showed a dose related increase in the serum Alkaline phosphatase (ALP) and serum Gamma glutamyl transferase (GGT) compared to control throughout the experiment. A statistically significant increase in serum ALP was recorded in Group IV, V and VI throughout the experiment. Serum GGT was significantly increased in Group IV, V and VI on 15th day

Table Va. Serum profile – 15th day of experiment

Group	Toxin level (ppb)	TP (g/dl)	AI (g/dl)	GI (g/dl)	A/G	ALP (IU/l)	GGT (IU/l)
I	0	2.990 ± 0.18	1.570 ± 0.100 [a]	1.420 ± 0.14	1.142 ± 0.155	34.12 ± 1.78 [a]	8.21 ± 1.68 [a]
II	20	2.553 ± 0.193	0.968 ± 0.032	1.585 ± 0.171	0.663 ± 0.057	36.13 ± 1.24 [a]	10.12 ± 0.98 [a]
III	40	2.502 ± 0.148	1.246 ± 0.127 [a]	1.272 ± 0.193	1.249 ± 0.237	37.14 ± 1.08 [a]	10.24 ± 1.11 [a]
IV	60	2.414 ± 0.146	1.184 ± 0.108	1.232 ± 0.104	1.069 ± 0.149	40.17 ± 1.94	11.42 ± 1.26
V	80	2.267 ± 0.136	1.116 ± 0.117	1.151 ± 0.044	0.977 ± 0.107	45.47 ± 1.18	14.12 ± 1.42
VI	100	2.267 ± 0.136	1.011 ± 0.028	1.137 ± 0.187	1.188 ± 0.228	45.48 ± 1.28	16.12 ± 1.74
	F value		3.237*			3.426**	3.92**
	CD		0.343			2.420	2.240

* P<0.05

** P<0.01

Means having the same superscript did not differ significantly from the control

Fig.9 SERUM TOTAL PROTEIN

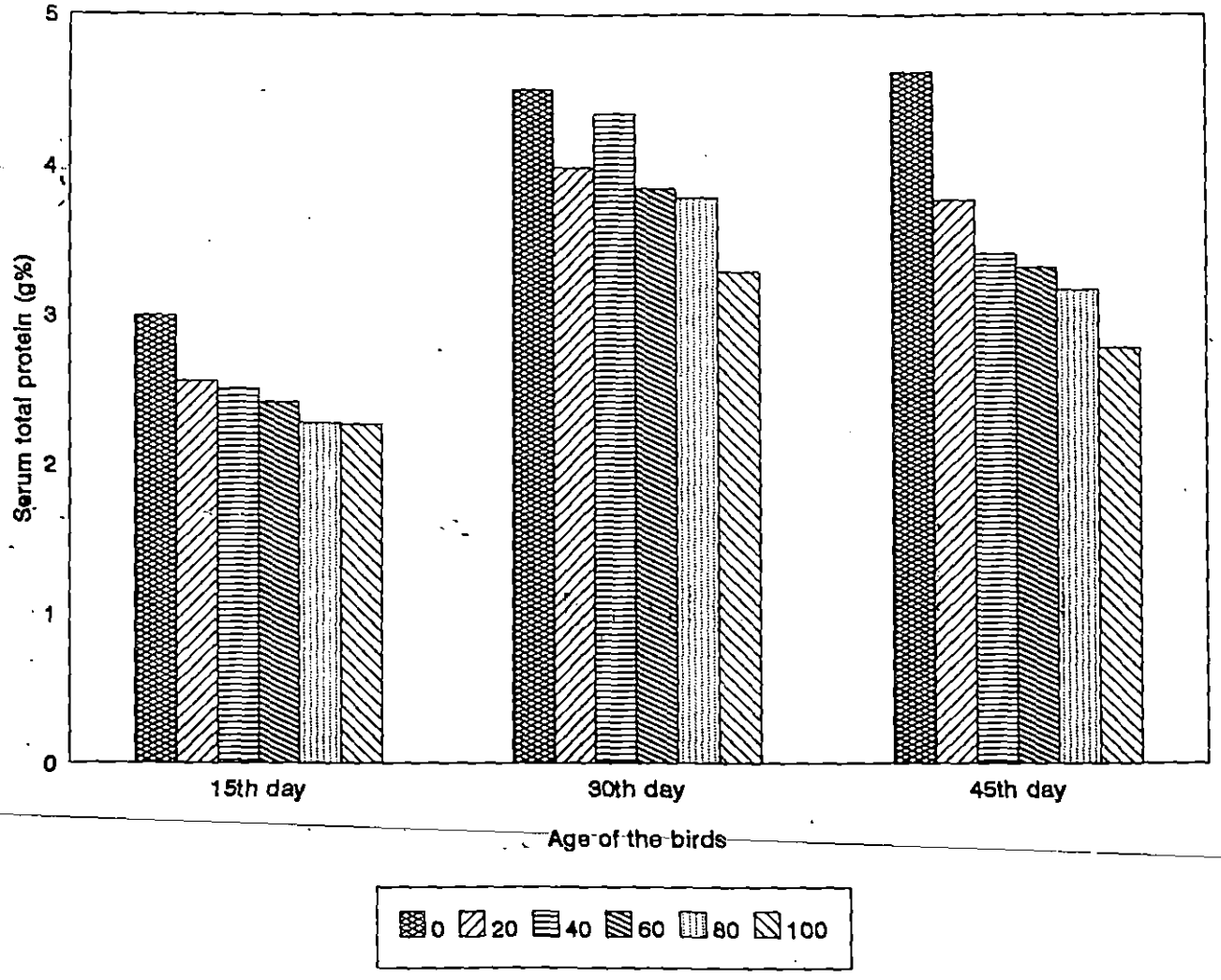


Table Vb. Serum profile – 30th day of experiment

Group	Toxin level (ppb)	TP (g/dl)	AI (g/dl)	GI (g/dl)	A/G	ALP (IU/l)	GGT (IU/l)
I	0	4.515 ± 0.205	2.397 ± 0.095 [a]	2.115 ± 0.170	1.155 ± 0.100 [a]	32.12 ± 2.48 [a]	9.12 ± 0.84
II	20	3.975 ± 0.298	1.926 ± 0.313 [a]	2.049 ± 0.102	0.947 ± 0.185 [a]	33.13 ± 1.28 [a]	9.14 ± 1.12
III	40	4.344 ± 0.302	2.073 ± 0.241 [a]	2.270 ± 0.192	0.928 ± 0.159 [a]	34.92 ± 1.42 [a]	9.29 ± 1.05
IV	60	3.836 ± 0.257	1.143 ± 0.075	2.693 ± 0.208	0.431 ± 0.030	37.18 ± 1.84	10.12 ± 1.95
V	80	3.783 ± 0.336	1.520 ± 0.094	2.263 ± 0.325	0.649 ± 0.053	40.84 ± 0.94	10.56 ± 1.21
VI	100	3.282 ± 0.204	1.389 ± 0.245	1.891 ± 0.068	0.760 ± 0.169 [a]	42.12 ± 1.05	10.94 ± 1.47
	F value		4.360**		3.234*	4.221**	
	CD		0.683		0.435	3.240	

*-P<0.05
** P<0.01
Means having the same superscript did not differ significantly from the control

Fig.10a SERUM ALBUMIN

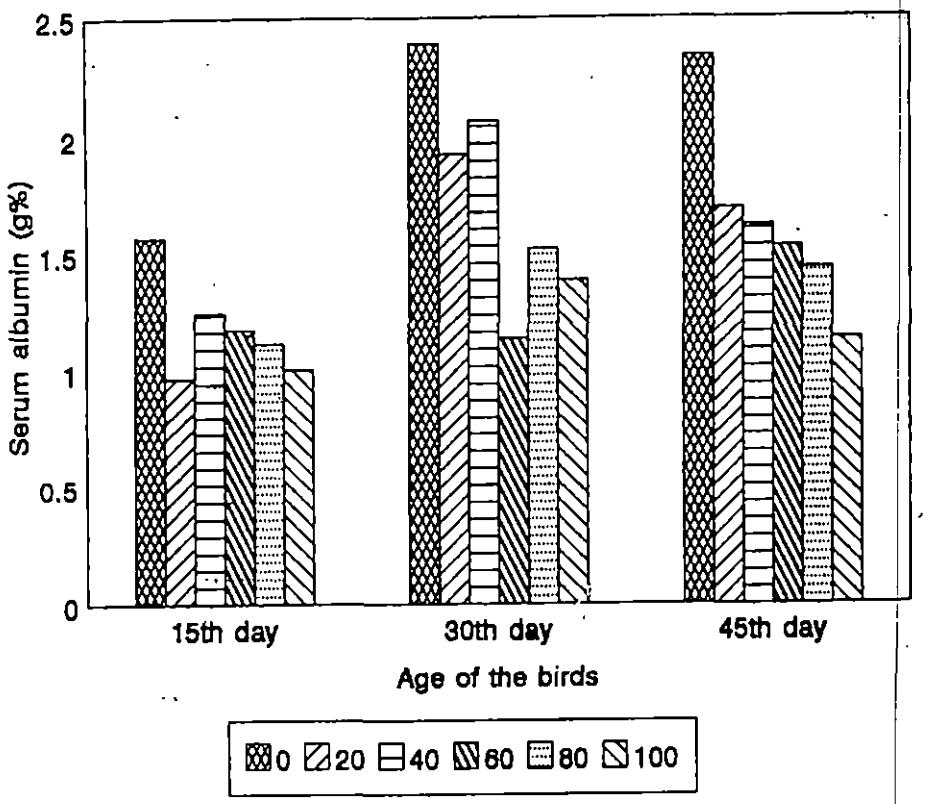


Fig.10b SERUM GLOBULIN

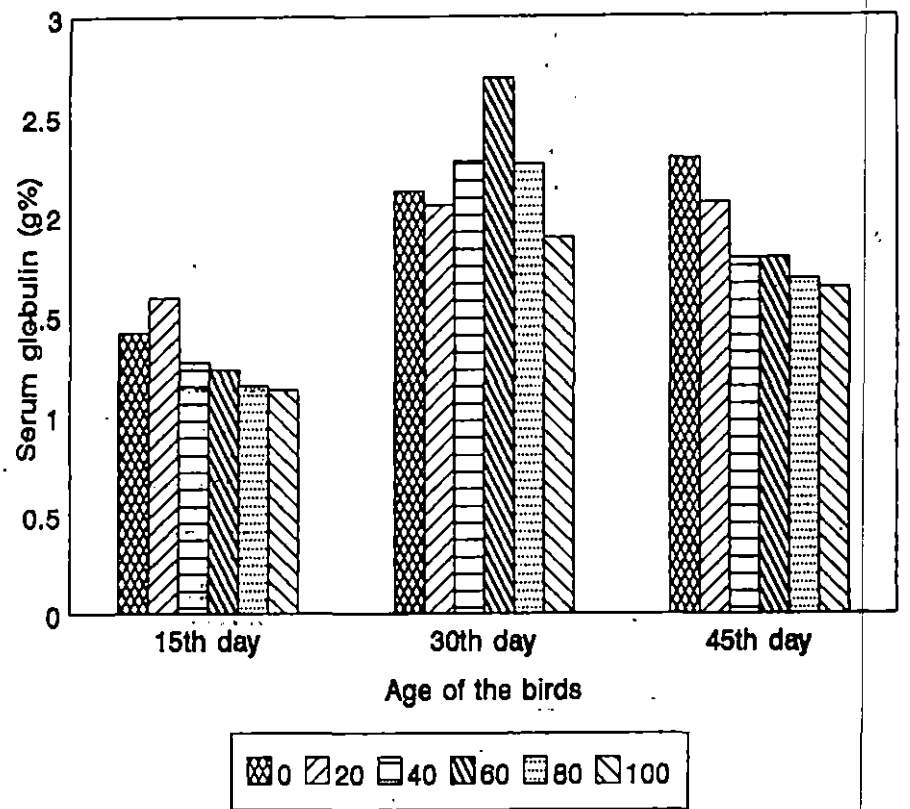


Table Vc. Serum profile – 45th day of experiment

Group	Toxin level (ppb)	TP (g/dl)	AI (g/dl)	GI (g/dl)	A/G	ALP (IU/l)	GGT (IU/l)
I	0	4.624 ± 0.179 a	2.338 ± 0.103[a]	2.286 ± 0.080	1.021 ± 0.0089	31.12 ± 1.12 [a]	9.42 ± 1.24 [a]
II	20	3.771 ± 0.375	1.700 ± 0.064	2.071 ± 0.413	1.241 ± 0.332	34.08 ± 1.21 [a]	9.47 ± 1.12 [a]
III	40	3.411 ± 0.149	1.624 ± 0.073	1.794 ± 0.152	0.963 ± 0.089	36.12 ± 1.41 [a]	9.52 ± 0.98 [a]
IV	60	3.322 ± 0.336	1.532 ± 0.074	1.790 ± 0.370	1.001 ± 0.134	37.14 ± 1.82	10.04 ± 1.24 [a]
V	80	3.116 ± 0.168	1.435 ± 0.076	1.682 ± 0.120	0.886 ± 0.0736	37.16 ± 1.92	10.49 ± 0.82 [a]
VI	100	2.780 ± 0.225	1.137 ± 0.057	1.644 ± 0.212	0.932 ± 0.222	38.16 ± 1.07	12.72 ± 0.96
	F value	5.013**	23.061**			4.220**	2.642*
	CD	0.840	0.240			5.528	1.520

* P<0.05
** P<0.01
Means having the same superscript did not differ significantly from the control

Fig.11a SERUM ALKALINE PHOSPHATASE

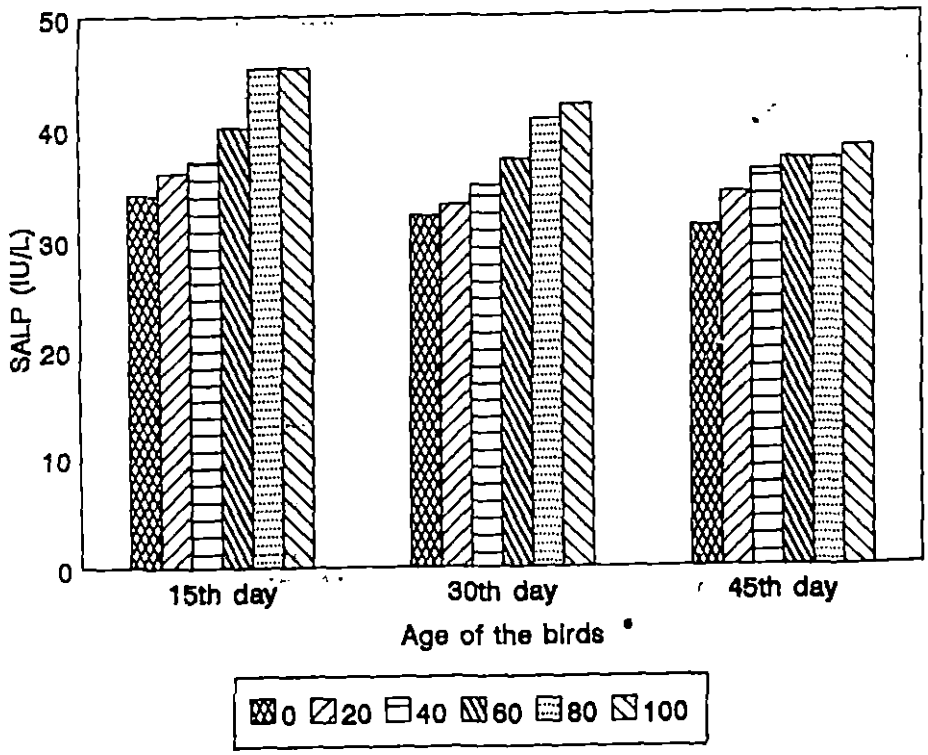
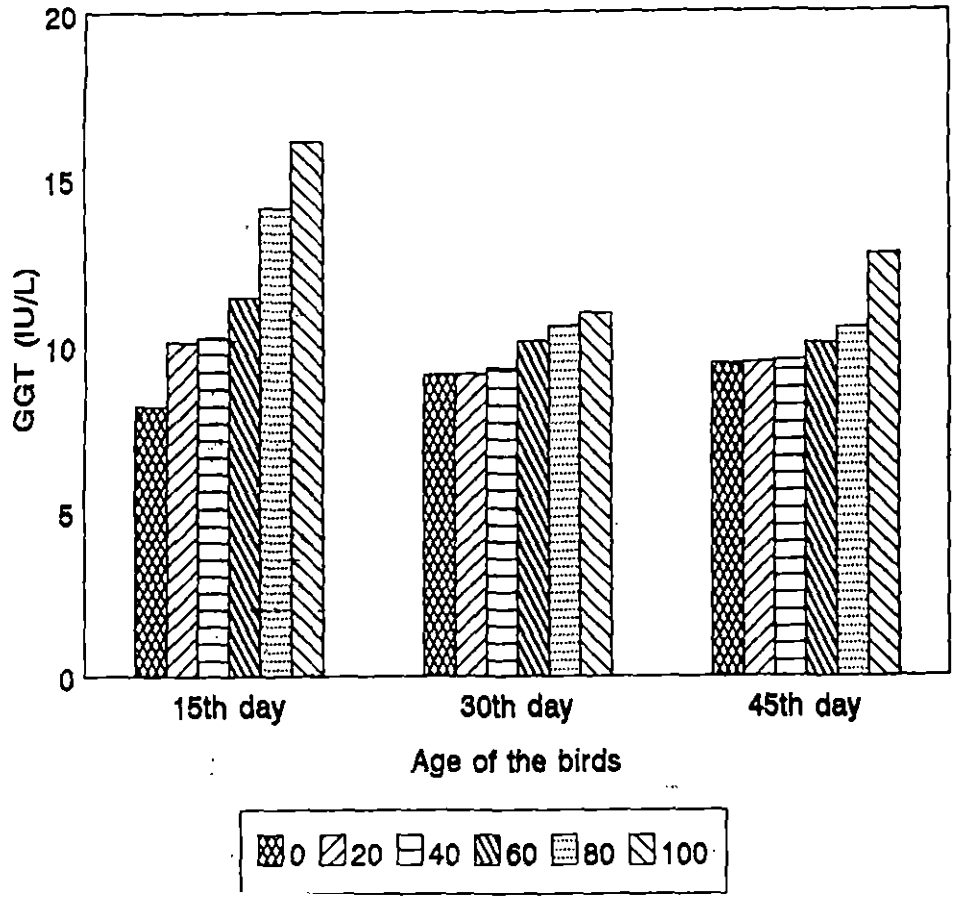


Fig.11b SERUM GAMMA GLUTAMYL TRANSFERASE



(P<0.01) and Group VI on 45th day (P<0.05) of experiment (Table Va, b, c and Fig.11a and b).

4.6 Organ weight

4.6.1 Liver

Weight of liver showed dose related variations. The increase (P<0.01) in liver weight recorded in Group II III, IV and V was statistically non significant while Group VI showed a significant reduction (P<0.01) in the liver weight on 15th day of experiment compared to that of the control. On the 30th day, Groups II, III and IV recorded increased (P<0.01) liver weight but Group V and VI showed a significant decrease in liver weight (Table VI and Fig.12a). At 45th day of age, liver weights recorded in groups III and IV were significantly higher whereas that in group VI was significantly lower in comparison to the values recorded in the groups fed with 0 and 20 ppb levels of aflatoxin.

4.6.2 Spleen

Although the aflatoxin fed birds showed a dose related decrease in spleen weights at 15th and 30th day of age in all treatments, a statistically significant decrease was observed only in Group VI on 15th day (P<0.01) and 30th day (P<0.05) of experiment. On the 45th day of experiment a significant increase in spleen weight (P<0.01) in Group V and VI and a decrease in Group II, III and IV were noticed (Table VI and Fig.12b).

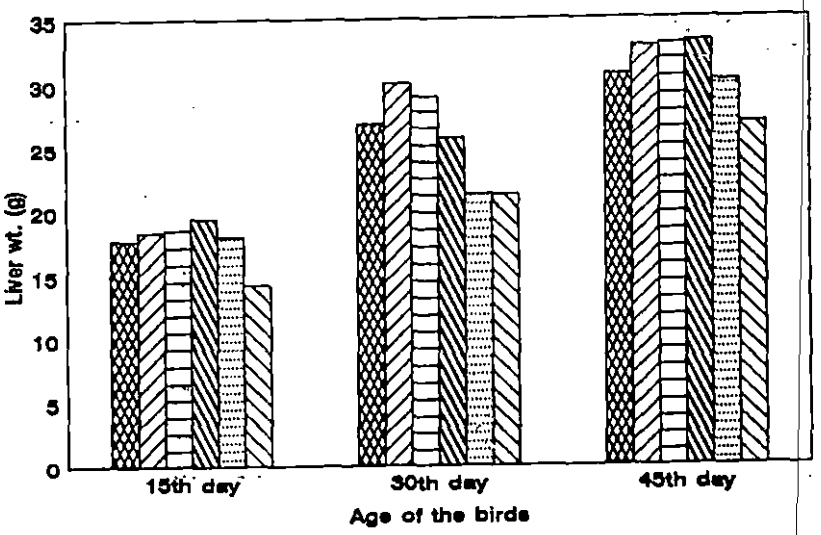
Table VI. Weight of the organs (liver, spleen and bursa)

Group	Toxin level (ppb)	15th day			30th day			45th day		
		Liver (g)	Spleen (g)	Bursa (g)	Liver (g)	Spleen (g)	Bursa (g)	Liver (g)	Spleen (g)	Bursa (g)
I	0	17.590 ± [a] 0.510	1.427 ± [a] 0.040	2.440 ± 0.180	26.850 ± [a] 0.685	1.558 ± [a] 0.105	3.958 ± [a] 0.180	30.660 ± [a] 0.9018	2.770 ± [a] 0.183	5.236 ± 0.1563
II	20	18.301 ± [a] 0.554	1.396 ± [a] 0.018	2.440 ± 0.085	30.084 ± [a] 3.019	1.459 ± [a] 0.053	3.061 ± [a] 0.169	32.836 ± [a] 1.279	2.394 ± 0.0848	7.115 ± 0.456
III	40	18.478 ± [a] 0.611	1.369 ± [a] 0.032	2.669 ± 0.165	28.943 ± [a] 3.155	1.498 ± [a] 0.053	4.275 ± [a] 0.335	33.002 ± 1.348	2.458 ± 0.729	8.209 ± 0.234
IV	60	19.356 ± [a] 0.785	1.297 ± [a] 0.025	2.877 ± 0.158	25.709 ± [a] 1.453	1.469 ± [a] 0.079	5.257 ± 0.453	33.097 ± 1.216	2.394 ± 0.085	4.477 ± 0.244
V	80	17.950 ± [a] 0.465	1.288 ± [a] 0.025	2.645 ± 0.196	21.210 ± 0.801	1.376 ± [a] 0.049	5.530 ± 0.479	30.139 ± [a] 1.601	3.072 ± 0.085	3.361 ± 0.759
VI	100	14.210 ± 0.794	1.183 ± 0.123	2.178 ± 0.177	21.207 ± 0.804	1.354 ± 0.049	2.099 ± 0.253	26.771 ± 1.208	3.234 ± 0.263	3.299 ± 0.310
	F value	7.812**	4.186**		4.220**	2.642*	9.389*		4.240**	
	CD	2.407	0.216		6.528	0.202	1.152		0.201	

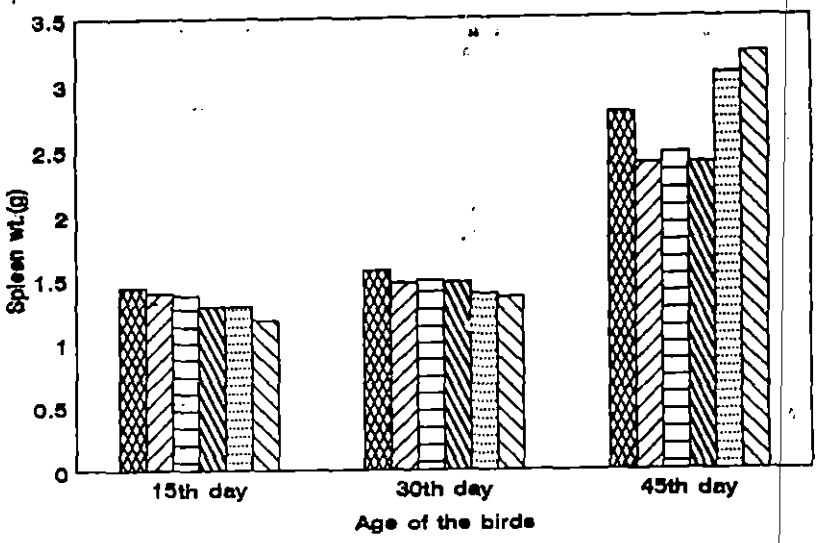
* P<0.05
** P<0.01
Means having the same superscript did not differ significantly from the control

FIG.12 WEIGHT OF THE ORGANS

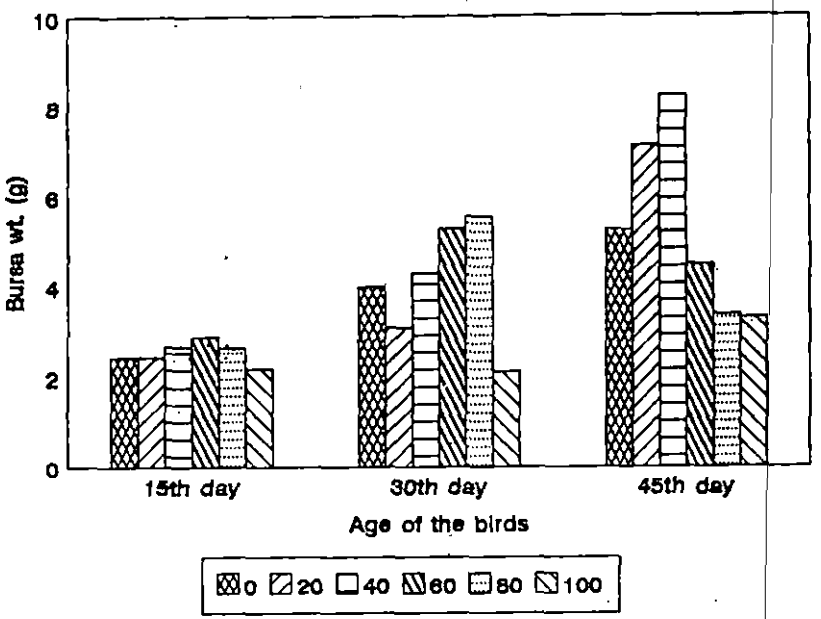
(a) LIVER WEIGHT



(b) SPLEEN WEIGHT



(c) BURSA WEIGHT



4.6.3 Bursa

There was no significant difference in the weight of bursa between aflatoxin fed groups and control group on the 15th day. A significant increase in ($P<0.05$) bursa weight was noticed in Group IV, and V, whereas Group VI showed a significant decrease ($P<0.05$) on 30th day when compared to the control. On 45th day, Groups IV, V and VI revealed a decrease in bursa weight while Group II and III showed an increase in comparison to control (Table VI and Fig.12c).

4.7 Residual aflatoxin in tissues

Residual aflatoxin was not detected in liver and muscle on the 15th and 30th day of experiment except for the trace residues in liver (2.35 ng/g) and muscle 2.02 ng/g) in Group VI on the 30th day. On the 45th day all the toxin treated groups recorded residual AFB_1, in liver and muscle. However, statistically significant ($P<0.01$) results were recorded only in Group V and VI in liver and Group VI in muscle (Table VII and Fig.13).

Pooled kidney samples showed residues of AFB_1, in Group IV, V and VI viz., 2.29, 2.7 and 3.10 ng/g respectively on the 30th day. On the 45th day of experiment, the corresponding values in the above groups were 1.18, 2.19 and 3.21 ng/g.

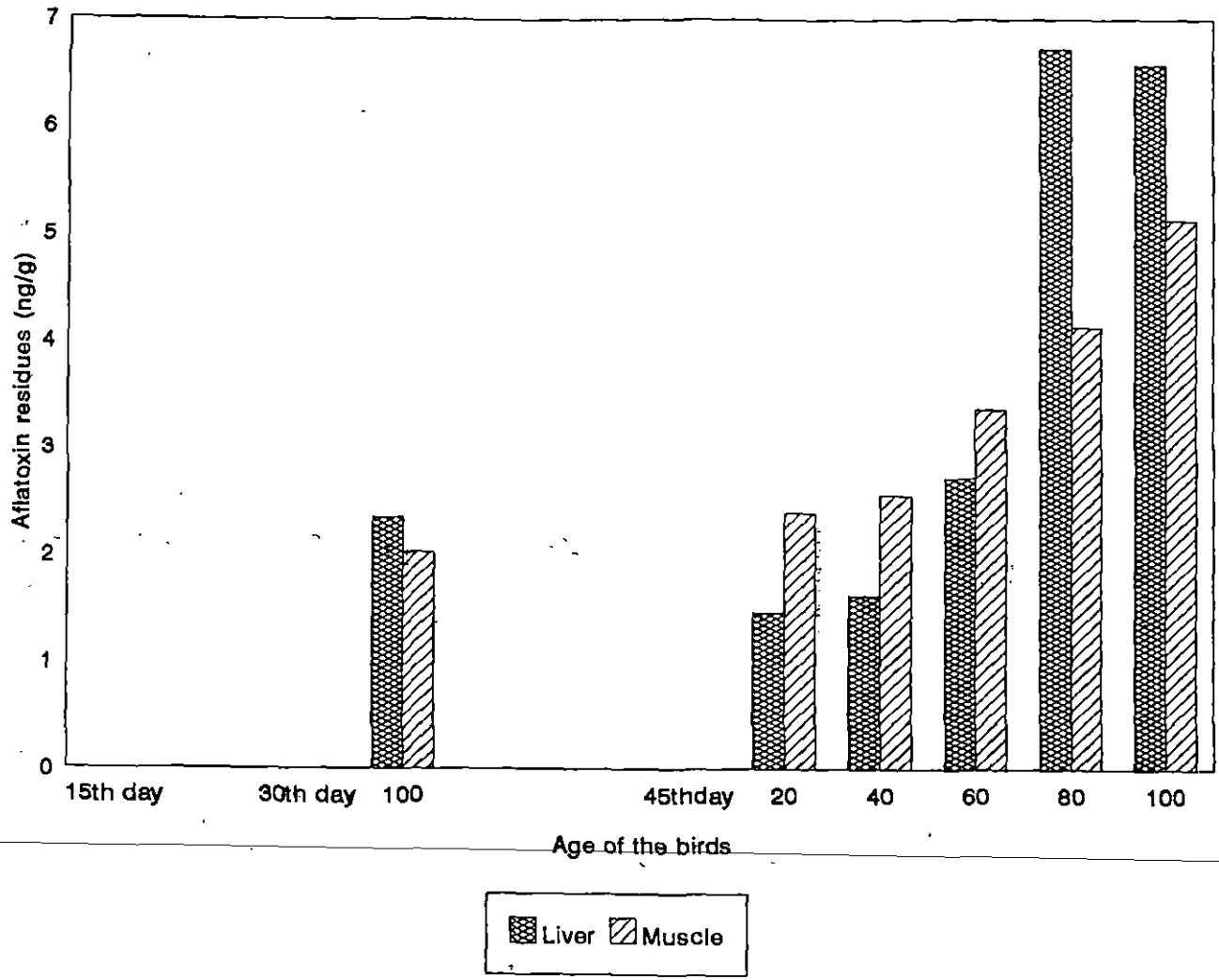
The results of the random samples cross-checked by the HPLC method were similar to the corresponding TLC values.

Table VII. Aflatoxin residues in tissues (ng/g)

Group	Toxin level (ppb)	15th day			30th day			45th day		
		Liver	Muscle	Kidney	Liver	Muscle	Kidney	Liver	Muscle	Kidney
I	0	-	-	-	-	-	-	0	0	0
II	20	-	-	-	-	-	-	1.463 ± [a] 0.439	2.391 ± [a] 0.502	-
III	40	-	-	-	-	-	-	1.618 ± [a] 0.336	2.560 ± [a] 0.865	-
IV	60	-	-	-	-	-	2.29	2.720 ± [a] 0.147	3.360 ± [a] 0.847	1.18
V	80	-	-	-	-	-	2.70	6.740 ± 0.762	4.131 ± [a] 0.552	2.19
VI	100	-	-	-	2.35	2.016	3.10	6.571 ± 1.659	5.129 ± 0.502	3.21
	F value							9.405**	5.533**	
	CD							2.518	1.985	

Means having the same superscript did not differ significantly from the control (** P<0.01)

FIG.13 AFLATOXIN RESIDUES IN TISSUES



4.8 Clinical symptoms

In general, the toxin fed birds were dull in appearance particularly during the initial stages of the experiment. During the fifth and sixth week of the experiment they showed greenish diarrhoea, anorexia, reduced weight gain and depressed appearance. The birds in the control group were apparently healthy and active.

4.9 Gross pathology

The birds in control groups did not show any apparent gross lesions. The lesions seen in the toxin fed birds are detailed below.

4.9.1 Fifteenth day

The livers of the toxin treated birds in Groups II, III and IV showed mild to moderate enlargement on the 15th day of experiment, while those in Group VI showed marked reduction in size. This trend continued till the 30th and 45th days of experiment and the reduction in size was marked in group V and VI.

Livers were generally yellow in colour (Fig.14 and 15) with some showing congested appearance. The surface was smooth and glistening with rounded borders. Subcapsular haemorrhage was seen in focal areas.

Kidneys were slightly enlarged and pale yellow in appearance. Bursa and spleen did not show any gross lesions at the 15th day. A few birds in the toxin

treated groups revealed petechial haemorrhages in the muscles of thigh and breast region.

4.9.2 Thirtieth and forty fifth day

The gross lesions observed on the 30th and 45th day of experiment were more or less similar and are summarised as follows.

The livers of chicks at the 30th and 45th days of experiment showed reduction in size in groups V and VI (Fig.16). Enlargement of liver in groups III and IV were marked on the 45th day of experiment.

Enlarged pale yellow livers with rounded borders, subcapsular haemorrhage (Fig.17) and focal areas of necrosis were noticed in the toxin fed birds. But the livers of birds in Group VI were mostly congested and reduced in size. Pinpoint haemorrhages were also seen in focal areas.

Kidneys were enlarged and pale in appearance. Bursa was generally enlarged in the toxin fed birds and the lumen contained greyish white mucoid substance. However, the birds in Group VI on the 30th and 45th days showed bursal atrophy.

The spleen appeared normal except for mild congestion on the 30th day of experiment. Splenomegaly was noticed in Group V and VI on the 45th day of experiment.

4.10 Histopathology

Histopathological lesions of varying intensity were seen in the organs of all the toxin fed groups starting from 20 ppb onwards throughout the experimental period. The severity of the lesions were in correlation with the levels of toxin and the period of exposure. No apparant histopathological lesions were seen in the control groups. They are summarised in Table VIIIa, b, c and d).

4.10.1 Liver

4.10.1a Fifteenth day

The livers of all the toxin treated birds on the 15th day of experiment exhibited moderate to severe vacuolar degeneration of hepatocytes (Fig.18). Isolated cells with pyknotic nuclei (Group V and VI) were seen scattered in the parenchyma. Disruption of the cord like arrangement of the hepatocytes (Group IV to VI), Megalocytosis and mild to moderate megalokaryosis (Group III to VI) were also visible. Mild biliary hyperplasia, periportal fibrosis and Kupffer cell reactions were the reactive changes (Group III to VI). Moderate to severe congestion of portal and central vessels with perivascular edema were noticed in all the groups. Mild focal infiltration of mononuclear cells in the form of micronodule were seen mostly in the portal areas and occasionally in the parenchyma in groups III to VI.

4.10.1b Thirtieth day

The pathological alterations were more or less similar to those noticed on the 15th day but were of higher intensity. Besides that, phlebosclerosis, periportal fibrosis, mononuclear infiltration (Fig.19), Kupffer cell reactions (Fig.20) and regeneration of hepatocytes were well pronounced on the 30th day. Hepatocytes, in isolated cases, were seen arranged in a ductular or circumscribed pattern (Fig.21) disrupting the cord like arrangement of the hepatocytes. Diffuse vacuolar degenerative changes and occasional pyknotic cells were also prominant. The changes were more pronounced in Group IV to VI.

4.10.1c Forty fifth day

The microscopic picture of the liver in toxin treated groups on the 45th day were of a higher intensity, especially in Group IV, V and VI and consisted of biliary hyperplasia (Fig.22), phlebosclersis, periportal fibrosis (Fig.23) and Kupffer cell reaction. Hepatocytes in general showed enlarged vesicular nucleus and faint blue cytoplasm (Fig.24). The cord like arrangement of hepatocytes in many areas were disrupted and showed a ductular or acinar pattern. Vacuolar degenerative changes and isolated cells showing pyknotic nuclei were also observed. Severe congestion of portal and central vessels and subcapsular haemorrhage were also noticed. Mononuclear cell accumulation in the periportal area was well pronounced.

Table VIIIa. Histopathological lesions in the liver of Aflatoxin fed broilers

Hitopathological lesions	15th day (ppb)					30th day (ppb)					45th day (ppb)				
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
Degenerative changes	+++	+	++	++	++	+++	+	++	++	++	+	+	++	++	+
Necrosis				+	+	+	+	++	+	+	+		+	+	+
Regeneration of hepatocytes		+	+	+	+	+	+	+	++	++	+	+	++	++	+
Biliary hyperplasia		+		+	+			+	+	+	+	+	+	++	++
Portal fibrosis				+	+	+	++	++	+	++		++	+	++	+
Kupffer cell reaction			+	+		+	+	++	+	++	+		++	+	+
Vascular changes	+++	++	+++	+++	++	+++	++	++	+++	+++	+++	+++	+++	+++	++
Mononuclear infiltration		+	+	+	+	+	++	+	+	+		++	+	++	+++

4.10.2 Kidney

4.10.2a Fifteenth day

Moderate to severe vacuolar degeneration of proximal, distal and collecting tubules were noticed in all the aflatoxin treated groups on the 15th day of experiment. Mild to moderate necrotic changes with desquamation of epithelium were also noticed. Tubules with epithelial cells showing karyomegaly were seen diffusely spread in the parenchyma. Focal collection of regenerating tubules were also common. Mild to severe hyperplasia of mesangial cells of glomeruli showing enlarged vesicular nucleus was a prominant finding in all the treatment groups. Occasionally, dilatation and partial occlusion of Bowman's space were visible. Moderate congestion with occasional haemorrhages in the interstitium and calyces were also seen (Fig.25).

4.10.2b Thirtieth day

Moderate to severe vacuolar degeneration, necrotic changes (Fig.26) and vascular changes like congestion and haemorrhages (Fig.27) were well appreciated in the kidney on the 30th day of experiment. Tubules showing regenerating epithelium were seen in clusters amidst the necrotic tubules.

4.10.2c Forty fifth day

Degenerative changes were well pronounced as in previous days of observation. However, the necrotic changes were of lesser intensity. Tubular

Table VIIIb. Histopathological lesions in the kidney of Aflatoxin fed broilers

Histopathological lesions	15th day (ppb)					30th day (ppb)					45th day (ppb)				
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
Tubular degeneration	+++	+++	+++	++	+++	++	++	++	+++	+++	+++	+++	++	++	+++
Tubular necrosis	+++	+	++	+	+++	++	+++	++	+++	+++	++	+	+	+	++
Regeneration of tubular epithelium		++	++	++	++	++	++	++	+++	++	++	++	+++	+++	+++
Mesangial cell proliferation	++	++	+++	+	+	++	+	+	++	+	++	+	++	+	++
Congestion	+	++	+	++	++	++	++	+	+++	++	+++	++	+	++	+++
Haemorrhage	+	+	+	+	+	+	+	+	++	-	++	+	+	+	++

regeneration was well appreciated.Tubular epithelial cells showed enlarged nuclei with vesicular appearance and faint bluish cytoplasm. Focal collection of regenerating cells were also seen occasionally (Fig.28). Hyperplasia and karyomegaly of the mesangial cells of glomeruli (Fig.29) were seen as earlier. Interstitial capillaries and venules were severely congested. Haemorrhages in the interstitium and calices were also observed.

4.10.3 Bursa

4.10.3a Fifteenth day

The bursa of all the toxin treated groups on the 15th day revealed moderate to severe lymphoid depletion from the medulla and to a lesser extent from the cortex of the follicle (Fig.30). Degenerative changes, necrosis and lysis of the follicle were seen in focal areas. Intrafollicular cyst formation was seen in the isolated follicles, where degeneration and lysis were noticed (Fig.31). Mild to moderate fibrous tissue proliferation in the inter follicular area and edema of the follicular sinuses were seen. Mild reticuloendothelial hyperplasia was noticed in the corticomedullary junction of the follicle. Follicular epithelium showed proliferation and thickening with the presence of small isolated cysts.

4.10.3b Thirtieth day

All the changes observed in the bursa of toxin treated birds on the 15th day were seen in a higher intensity on the 30th day also, particularly in Group IV, V and VI (Fig.32).

Table VIIIc. Histopathological lesions in the bursa of Aflatoxin fed broilers

Hitopathological lesions	15th day (ppb)					30th day (ppb)					45th day (ppb)				
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
Lymphoid depletion	++	+	+	++	++	++	+	++	++	++	+	+	++	++	++
Degeneration, lysis and cyst formation in the follicle	++	++	+	++	++	+	++	+	+	+	++	+	+	+	+
Edema in the follicular sinuses	+	++	+	+	+	++	+	+	+	++	+	+	+	+	+
Reticulo endothelial hyperplasia	-	+	+	+	+	+	-	++	+++	++	+	+	+++	+++	+++
Fibrous tissue proliferation	+	+	+	++	++	+	+	++	+	+++	+	++	+++	+++	++
Surface epithelial changes	+	+	++	+	+	+	+	++	++	++	+	+	++	++	++

4.10.3c Forty fifth day

In addition to the changes described on 15th and 30th day, the bursa showed intense fibrous tissue proliferation, and presence of sheet like cells in the corticomedullary junction of follicles (Fig.33). The changes in the surface epithelium were more pronounced in the toxin fed birds.

4.10.4 Spleen

4.10.4a Fifteenth day

Mild lymphoid depletion from the periarteriolar lymphoid sheath (PALS) and germinal centers were appreciable in all the groups. Mild reticulo-endothelial proliferation was seen in the ellipsoid in Groups V and VI.

4.10.4b Thirtieth day

Spleen of all the toxin treated birds showed mild to moderate lymphoid depletion from PALS on the 30th day of experiment (Fig.34). Peri Ellipsoidal Lymphoid Tissue (PELT) showed the presence of many large macrophages containing phagocytic materials, blast cells and plasma cells (Group III, IV, V and VI). PELT also showed patchy areas of degeneration and lysis giving a moth eaten appearance (Group IV).

4.10.4c Fortyfifth day

In addition to the above mentioned lesions, spleen of the toxin fed birds showed pronounced reticulo-endothelial cell proliferation in the ellipsoid and

Table VIIId. Histopathological lesions in the spleen of Aflatoxin fed broilers

Histopathological lesions	15th day (ppb)					30th day (ppb)					45th day (ppb)				
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
Lymphoid depletion	+	+	+	+	++	++	+	++	+	++	++	+	+	+	++
Degenerative changes								+					+	+	+
Reticuloendothelial hyperplasia in the ellipsoid and PELT	+	+	++	++	++	+	+	+	+	++	++	+++	+	++	+
Congestion	+	+	+	+	++	+	+	+	++	++	+	+++	+	++	+

PELT (Fig.35). These cells were arranged in sheets, like a syncytium with indistinct cytoplasmic borders and enlarged vesicular nucleus.

Mild to moderate vascularsclerosis (Fig.36) congestion of trabecular vessels and sinusoids were also seen throughout the experimental period in the toxin fed birds.

4.11 Ultrastructural pathology

Hepatocytes of the birds in all the treatment groups showed moderate to severe ultrastructural changes indicating degeneration and necrosis.

Dilatation and vesiculation of rough endoplasmic reticulum with mild to moderate degranulation of ribosomes was a prominant lesion (Fig.37 and 38). Mitochondrial swelling with loss of cristae was also noticed. Membrane bound electronlucent globular structures of varying sizes were seen within the cytoplasm (Fig.39). Nuclei of the hepatocytes showed various stages of necrobiosis.

4.12 Economic performance

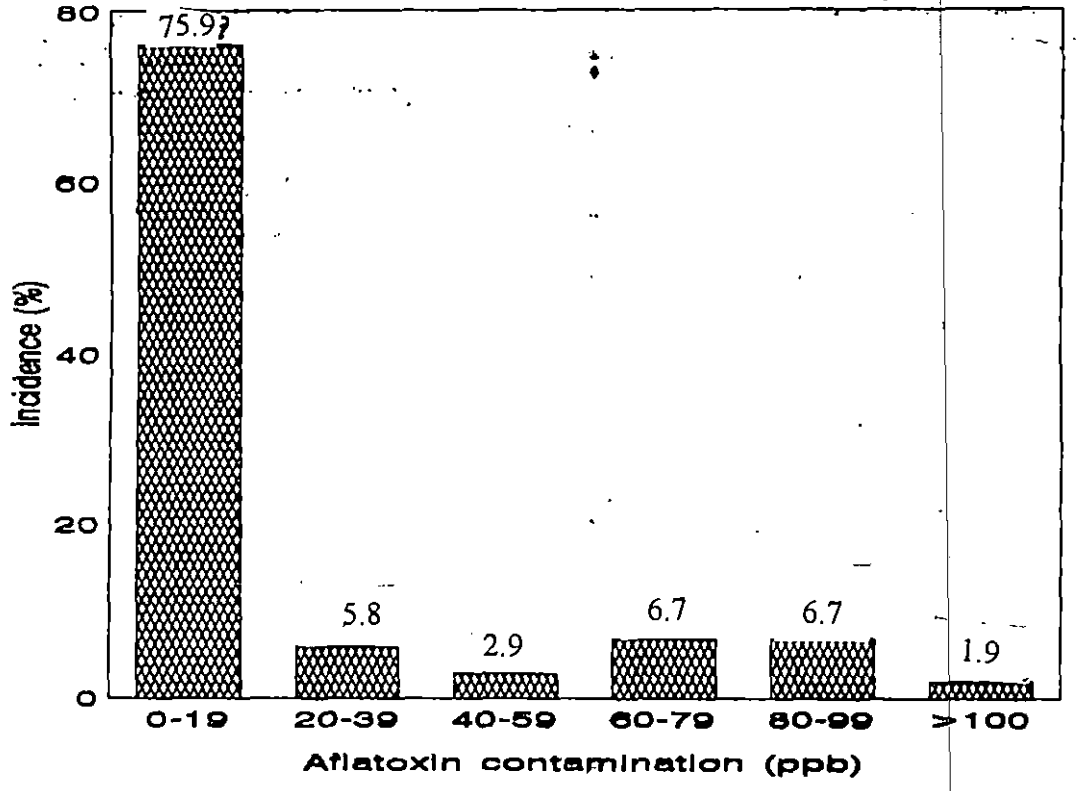
The dose related decrease in the feed consumption and body weight resulted in a corresponding reduction in the expenditure as well as total receipts. The net return per bird from Group I to Group VI were Rs.16.82, 15.58, 13.33, 13.63, 5.25 and 3.24 respectively (Table IX). The loss in profit compared to

Table IX Economic loss due to dose related aflatoxicosis in broiler chicken

Sl.No	Items	Toxin level in the feed (ppb)					
		0	20	40	60	80	100
I.	**Recurring expenditure**						
(i)	Cost of the birds (Rs.15.50/bird)	15.50	15.50	15.50	15.50	15.50	15.50
(ii)	Feed cost (Rs.10.50/kg of feed)	43.89	42.67	42.31	41.53	41.13	40.08
(iii)	Miscellaneous cost (Rs.10/bird)	10.00	10.00	10.00	10.00	10.00	10.00
	Total expenditure in Rupees	69.39	68.17	67.81	67.03	66.63	65.58
	% difference in expenditure over control	-	-1.76	-2.28	-3.40	-3.98	-5.49
II.	**Receipts**						
(i)	Sale of birds (Rs.35/kg)	83.21	80.75	78.14	77.66	68.88	65.82
(ii)	Sale of manure (Rs.2/bird)	2.00	2.00	2.00	2.00	2.00	2.00
(iii)	Sale of gunny bags (Re.1/bird)	1.00	1.00	1.00	1.00	1.00	1.00
	Total receipts in Rupees	86.21	83.75	81.14	80.66	71.88	68.82
	% difference in receipts over control	-	-2.85	-5.88	-6.44	-16.62	-20.17
III.	Net return (in Rupees)	16.82	15.58	13.33	13.63	5.25	3.24
IV.	Loss in profit						
	In Rupees	0	1.24	3.49	3.19	11.57	13.58
	In Per cent	0	7.3%	20.7%	18.9%	68.8%	80.7%

Note: (i) Recurring expenditures are not taken into account for this calculation
(ii) The above table shows economic loss per bird

FIG.40 INCIDENCE OF AFLATOXIN CONTAMINATION IN POULTRY FEED



control in Group II to Group VI were calculated as 7.3, 20.7, 18.9, 68.8 and 80.7 per cent respectively.

4.13 Incidence of aflatoxin contamination in poultry feed

Out of the 104 poultry feed samples, 24 per cent samples showed the presence of AFB_1 in the range of 20 to 200 ppb. Seventy six per cent samples were either negative or had less than 20 ppb of AFB_1 level which was considered as toxin free feed. The higher incidence of aflatoxin was noted in the range of 60 to 79 ppb (6.7%), 80 to 99 ppb (6.7%) and 20 to 39 ppb (5.8%), 1.9 per cent samples showed AFB_1 at levels above 100 ppb (Fig.40).

Fig.14. Liver - 15th day - 20 ppb. Mild hepatomegaly and pale yellow discolouration

Fig.15. Liver - 15th day - 40 ppb. Diffuse yellow discolouration and moderate hepatomegaly


40 ppb

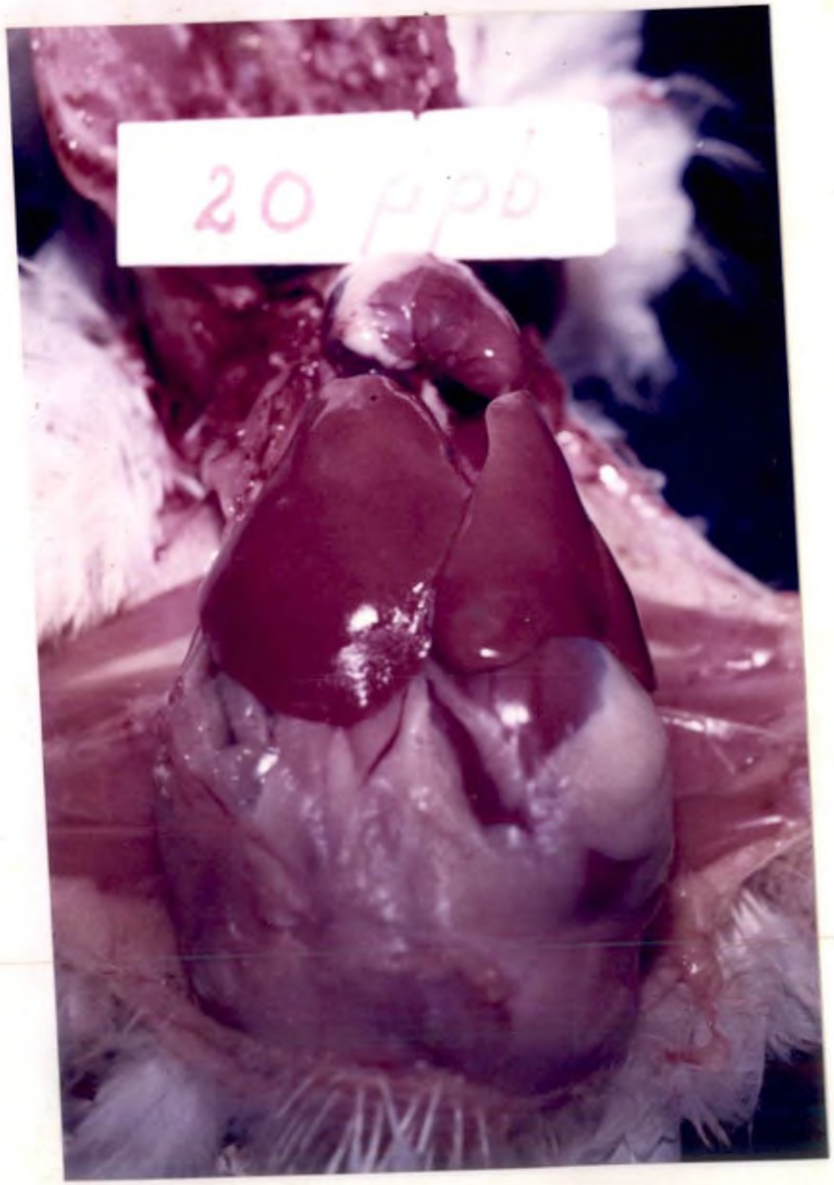

20 ppb

Fig.16. Liver, Bursa and spleen - 45th day - 60 and 80 ppb. Congestion and reduction in size of liver in 80 ppb compared to that of 60 ppb. Enlargement of bursa in both the groups.

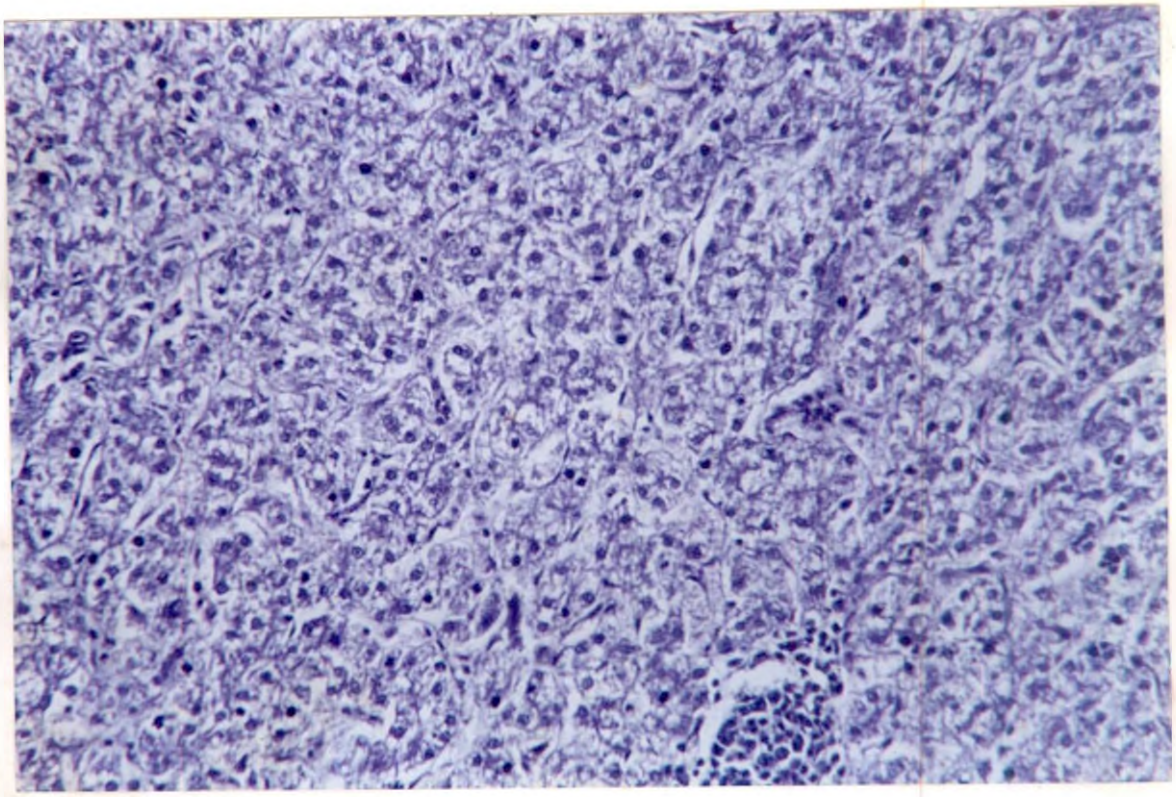
Fig.17. Liver - 30th day - 40 ppb. Subcapsular haemorrhage and hepatomegaly


80ppb
60ppb
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24


40 ppb

Fig.18. Liver - 15th day - 60 ppb. Vacuolar degeneration of hepatocytes and diffuse nuclear pyknosis - H&E x 400

Fig.19. Liver - 30th day - 40 ppb. Periportal mononuclear infiltration and mild fibrosis. H&E x 250



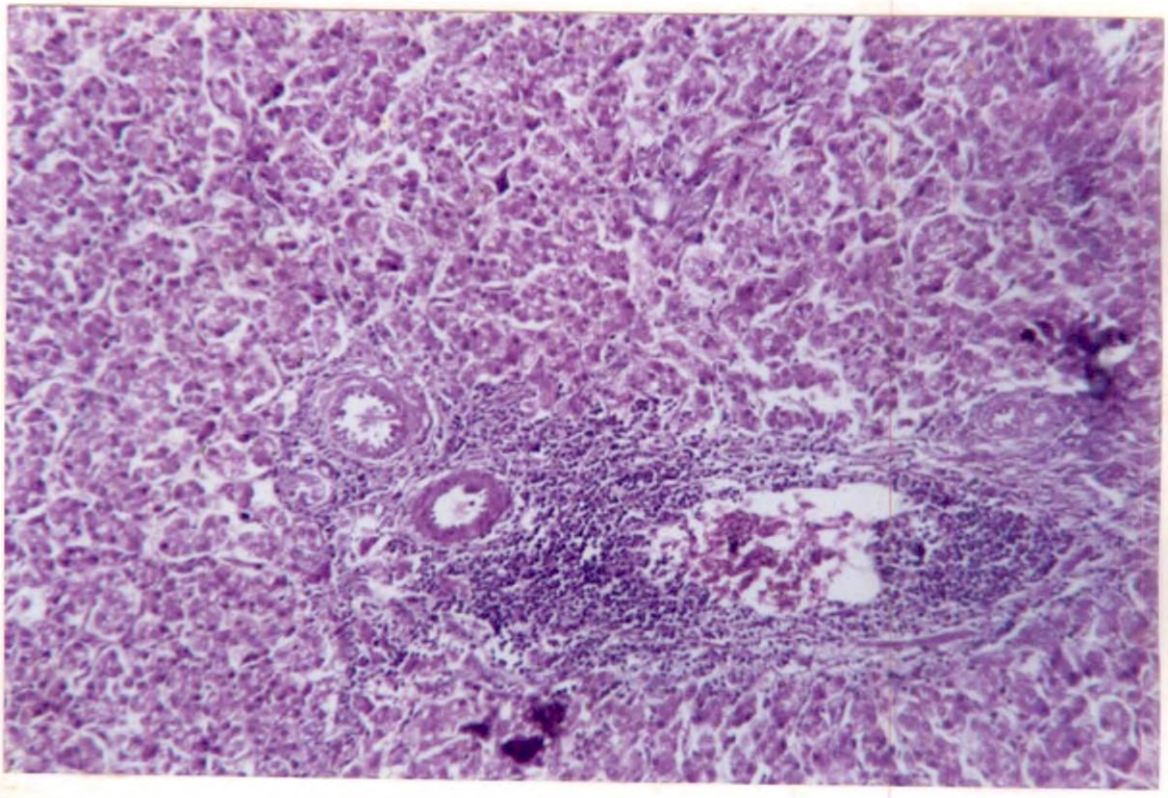
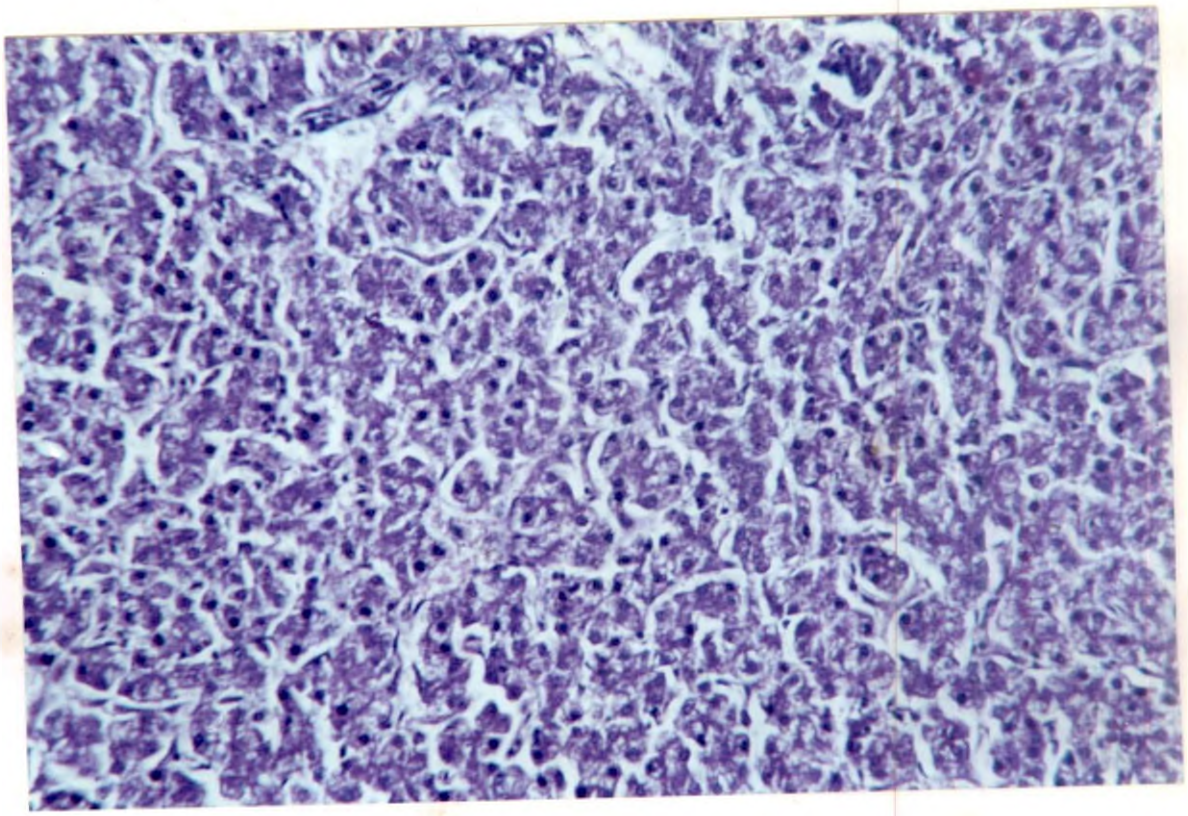


Fig.20. Liver - 30th day - 60 ppb. Kupffer cell reaction and disruption of hepatic cords. H&E x 400

Fig.21 Liver - 30th day - 60 ppb. Ductular pattern of arrangement of hepatocytes and vacuolar changes. H&E x 400



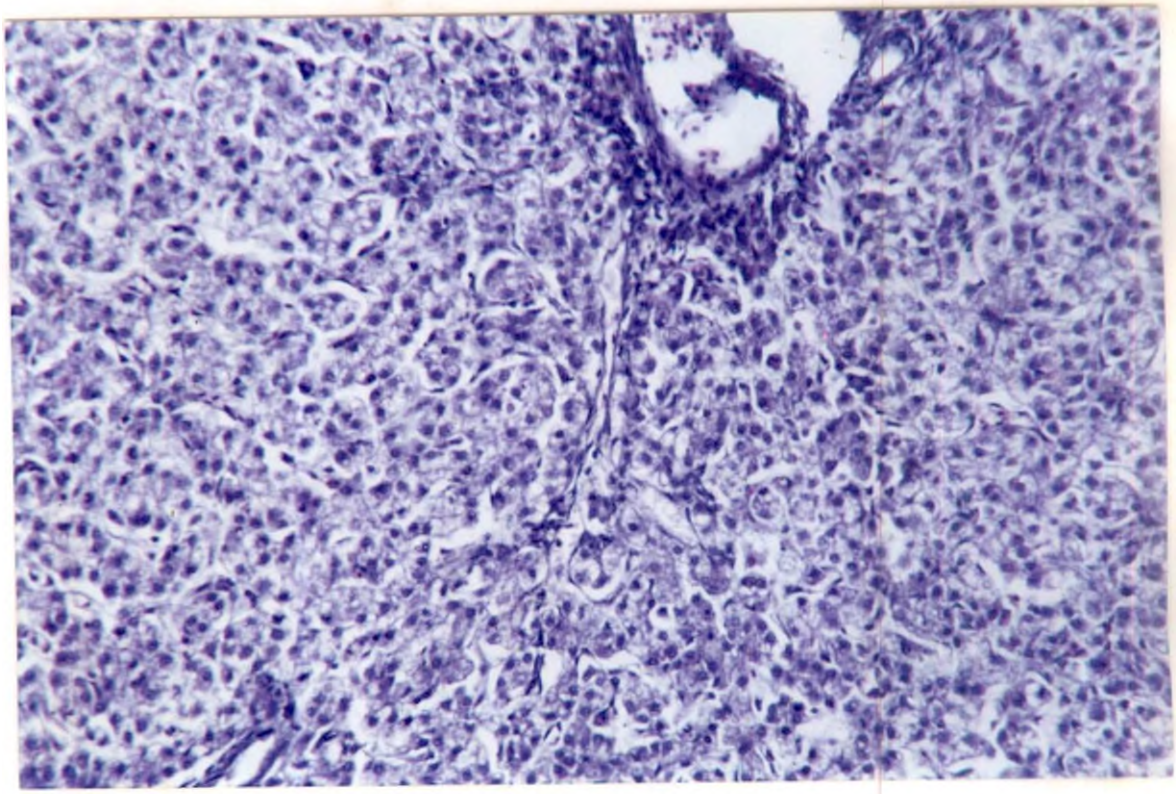
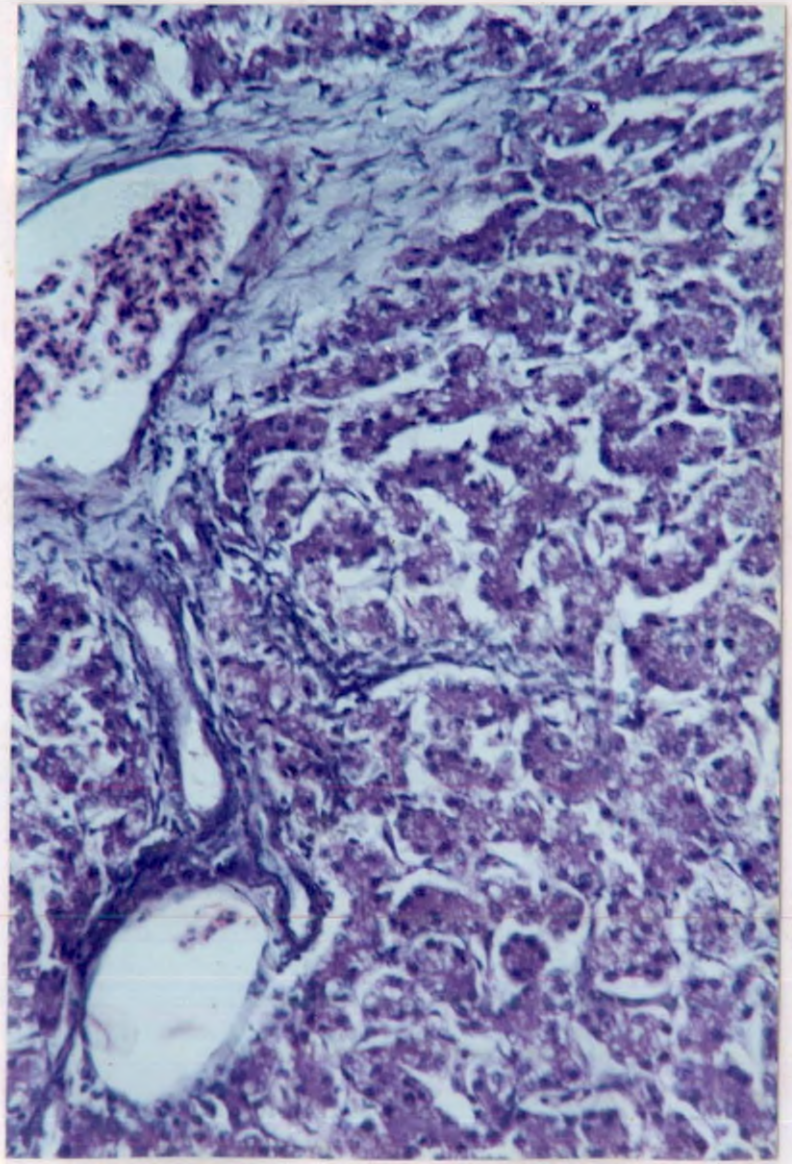


Fig.22. Liver - 45th day - 80 ppb. Biliary hyperplasia and congestion of portal vessels. H&E x 400

Fig.23. Liver - 45th day - 40 ppb. Phlebosclerosis, periportal fibrosis and congestion of portal vessels. H&E x 400



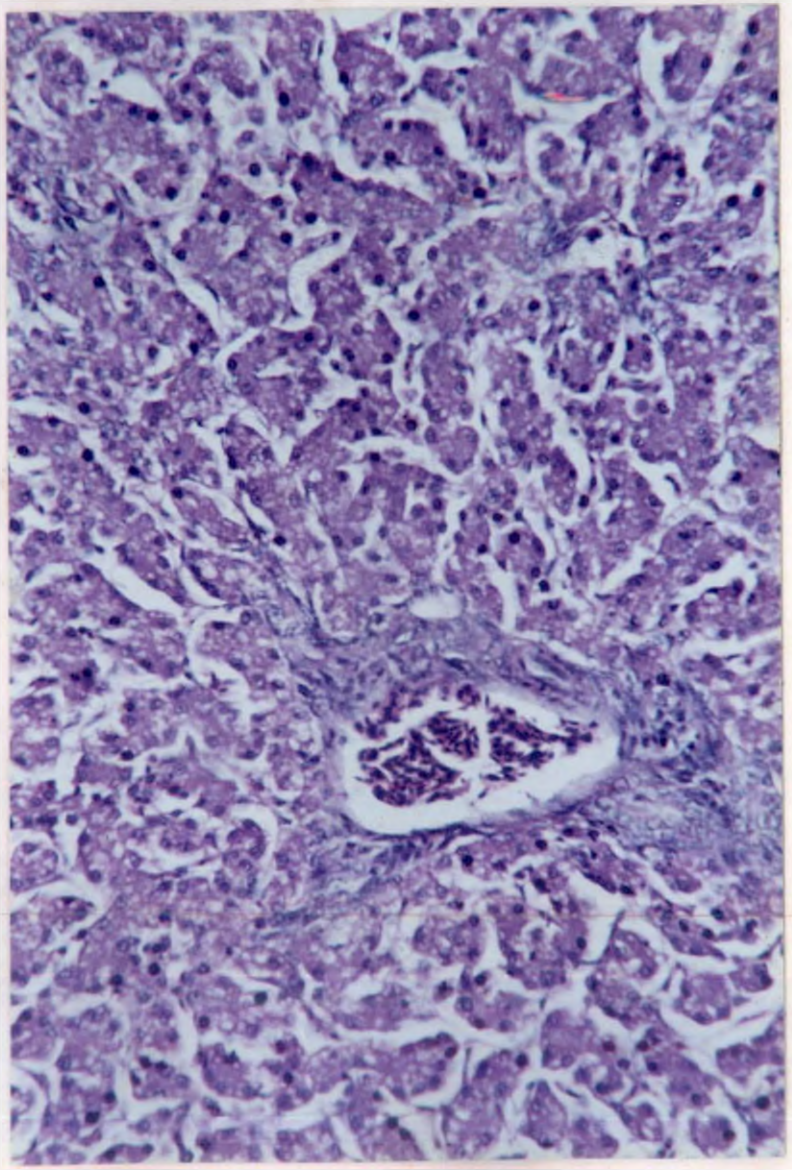
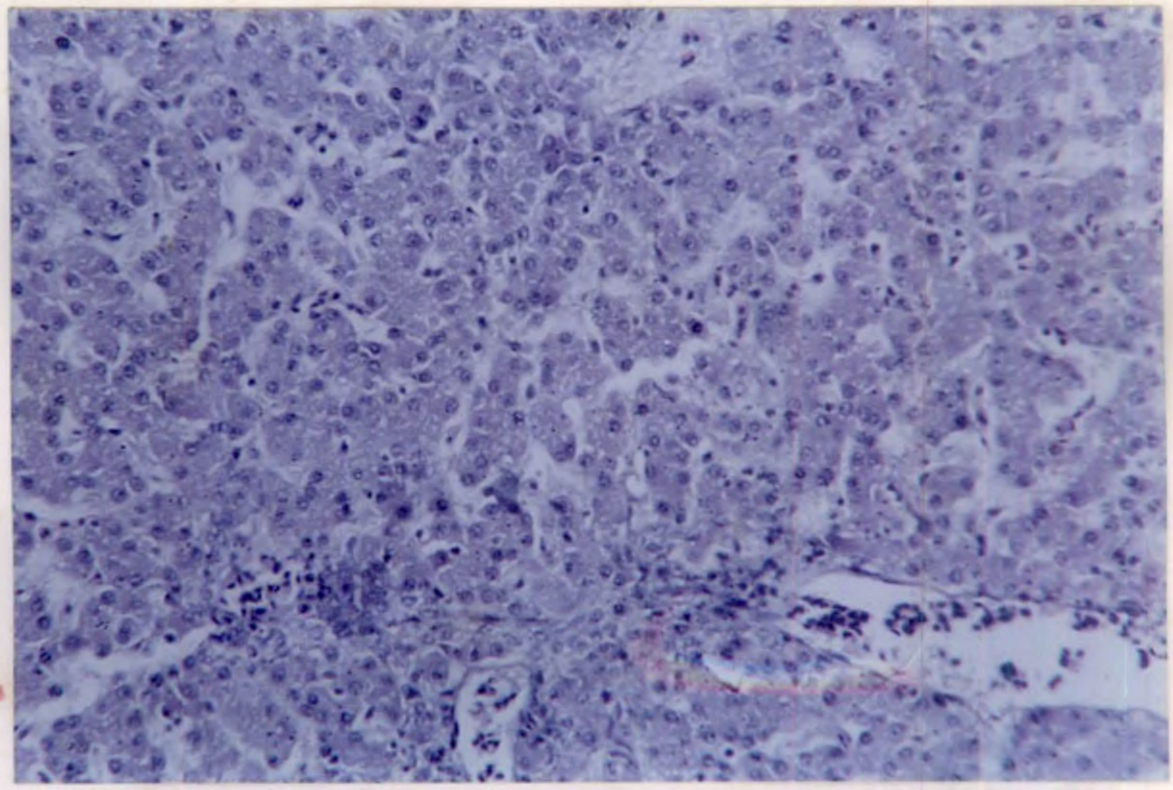


Fig.24. Liver - 45th day - 100 ppb. Hepatocytes with enlarged vesicular nucleus. H&E x 400

Fig.25. Kidney - 15th day - 20 ppb. Degeneration, necrosis of tubular epithelium and haemorrhages in the renal calyces. H&E x 250



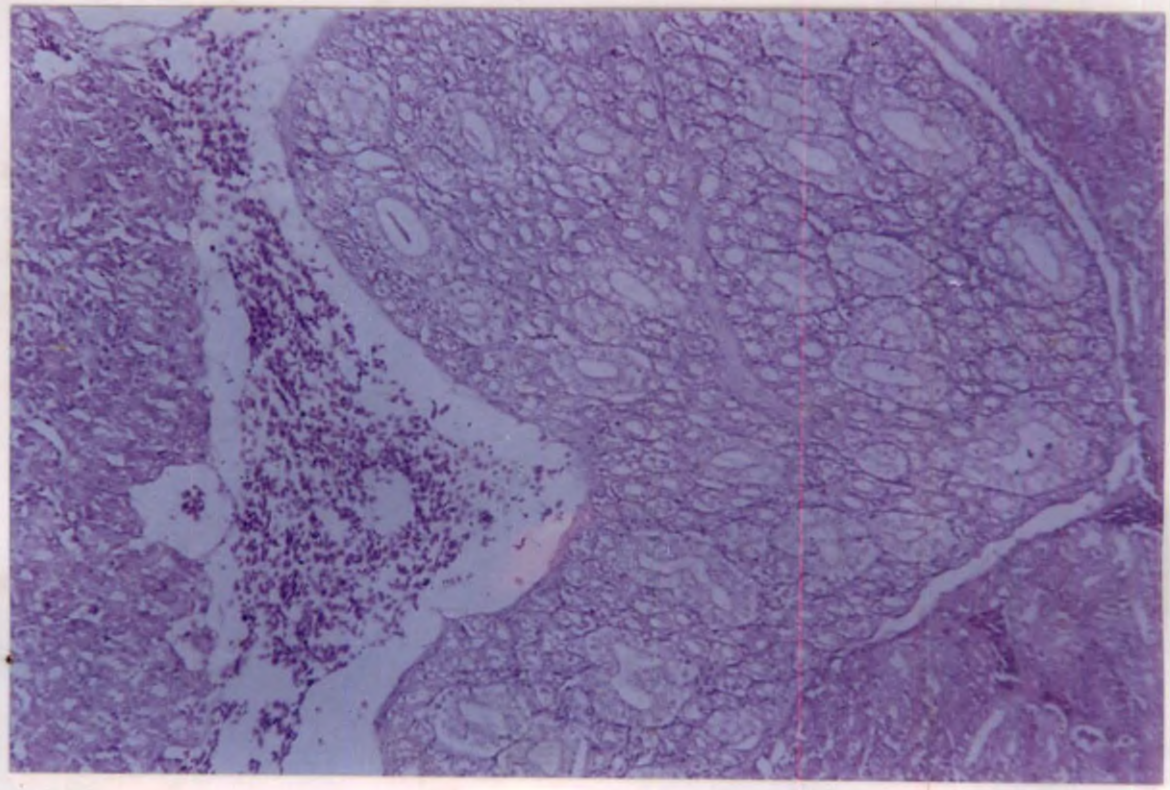
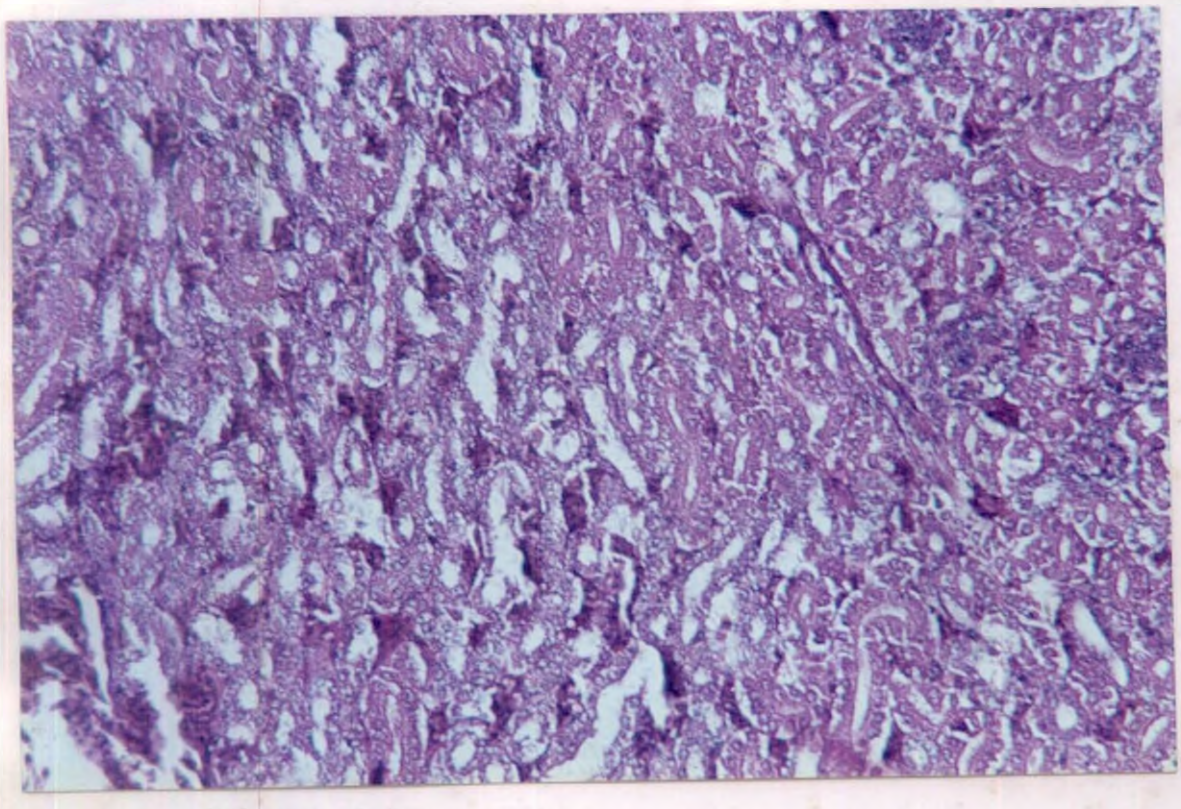


Fig.26. Kidney - 30th day - 80 ppb. Degeneration and necrosis of tubular epithelium. H&E x 250

Fig.27. Kidney - 30th day - 80 ppb. Severe congestion of the inter tubular capillaries. H&E x 160



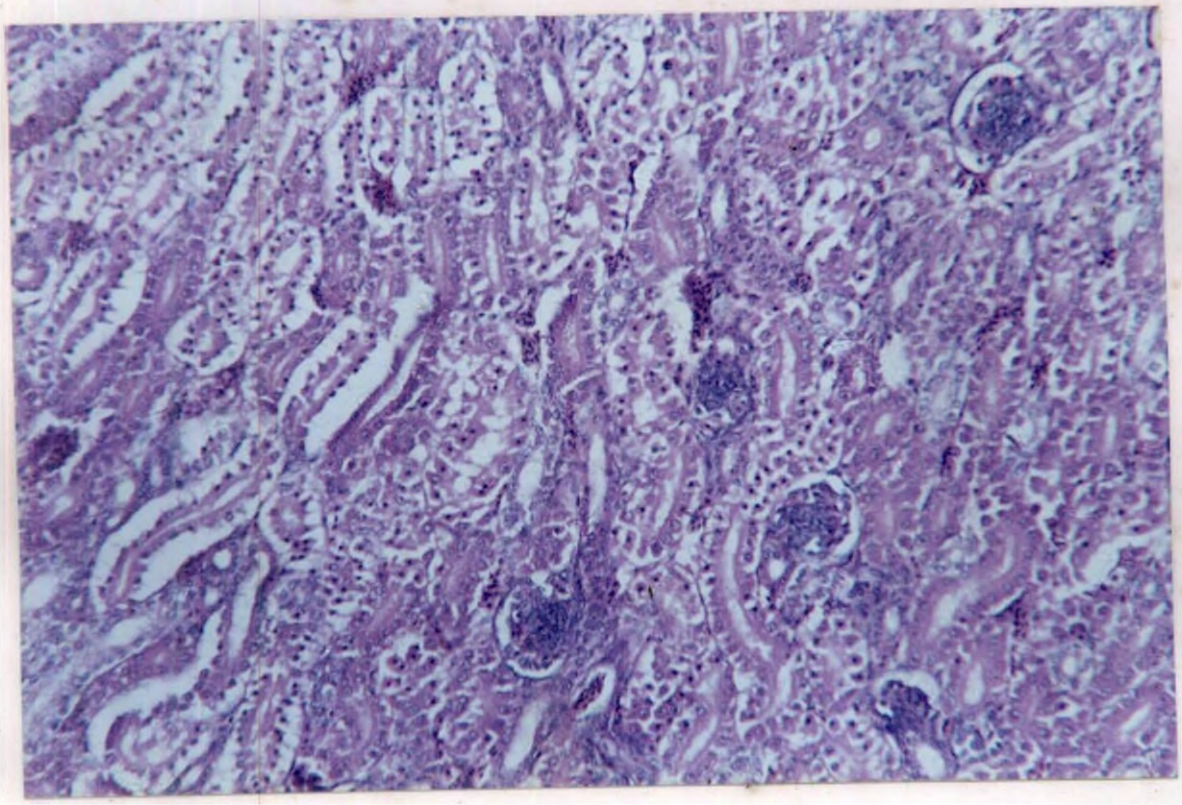
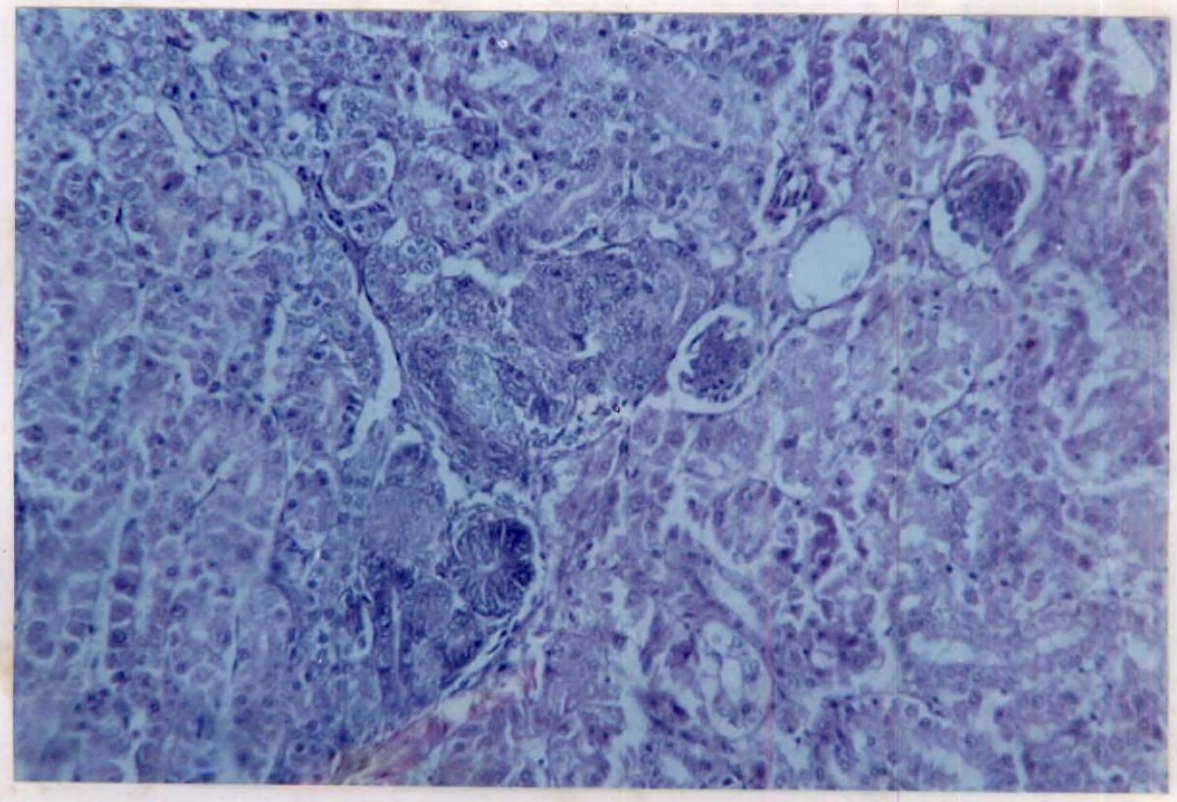


Fig.28. Kidney - 45th day - 60 ppb. Regenerating epithelial cells in clusters showing enlarged vesicular nucleus and faint blue cytoplasm along with tubules showing degeneration and necrosis. H&E x 400

Fig.29. Kidney - 45th day - 100 ppb. Glomeruli showing mesangial cell proliferation with partial occlusion of Bowman's space. Tubular epithelium showing enlarged vesicular nucleus and vacuolar degenerative changes. H&E x 400



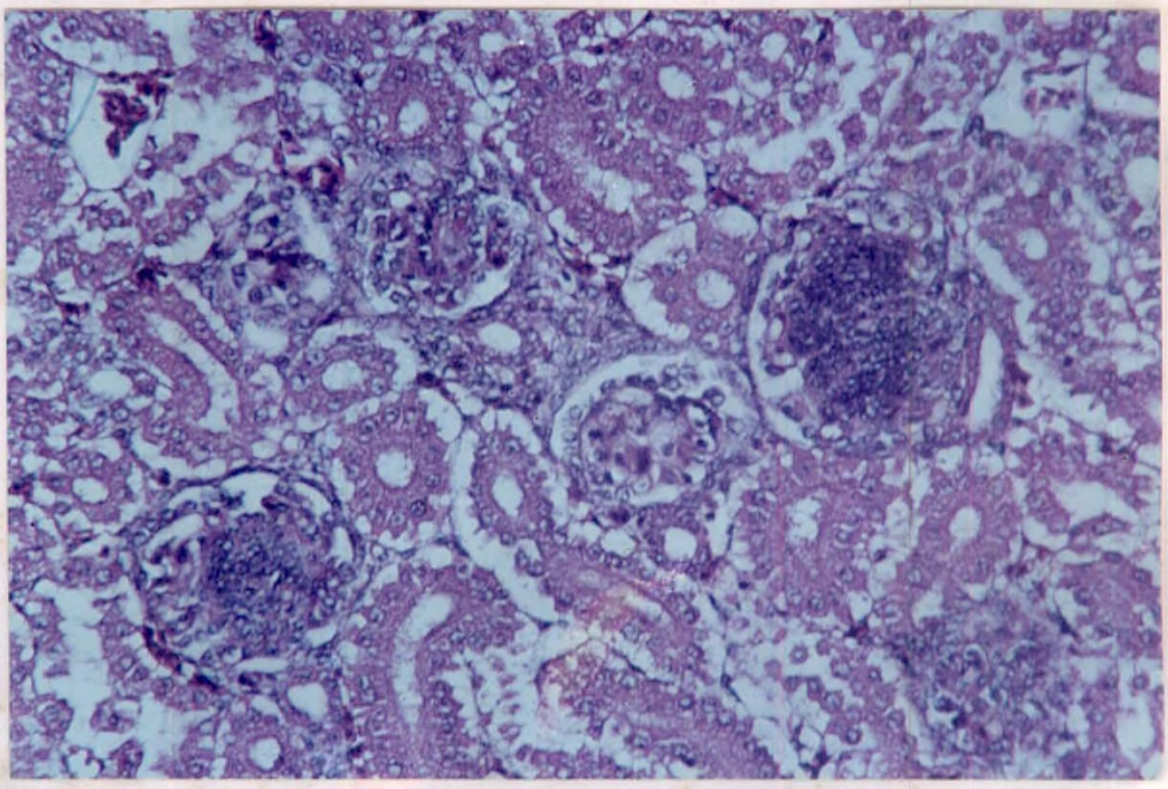
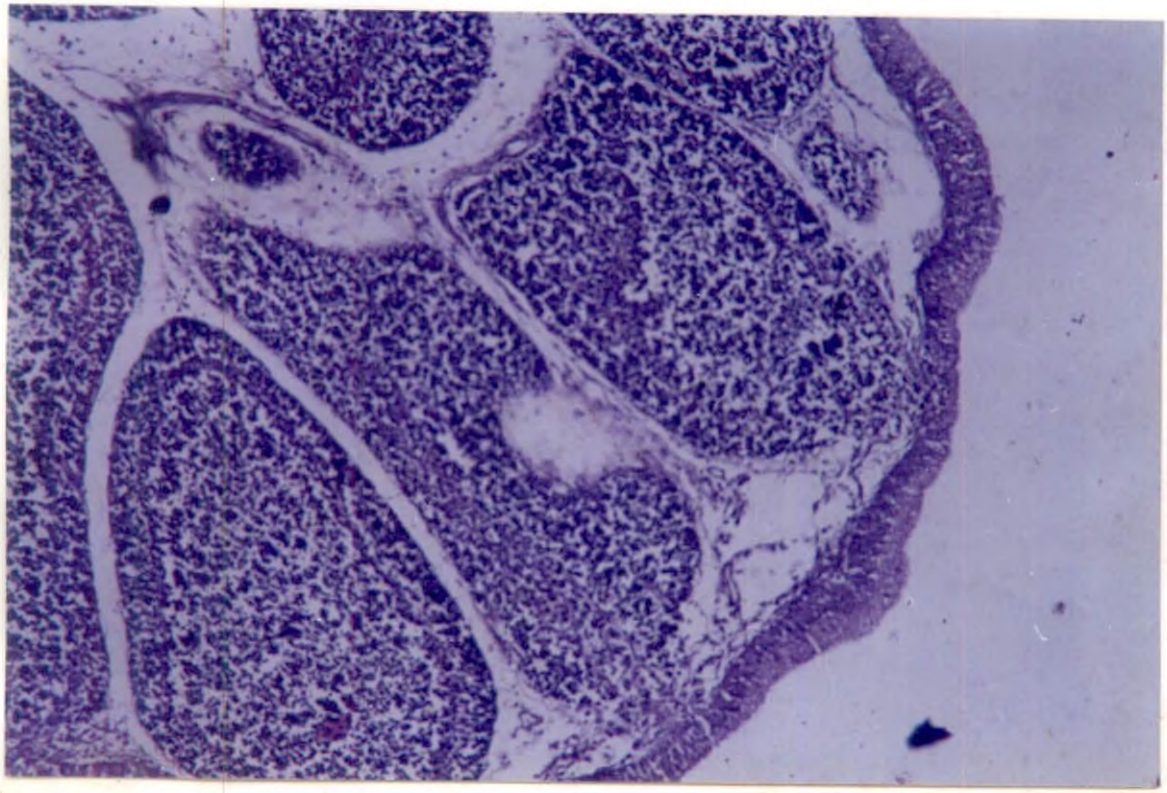


Fig.30. Bursa - 15th day - 20 ppb. Moderate lymphoid depletion from the bursal follicle. H&E x 160

Fig.31. Bursa - 15th day - 40 ppb. Follicle showing lymphoid depletion, degeneration and lysis with cyst formation. Inter follicular edema. H&E x 250



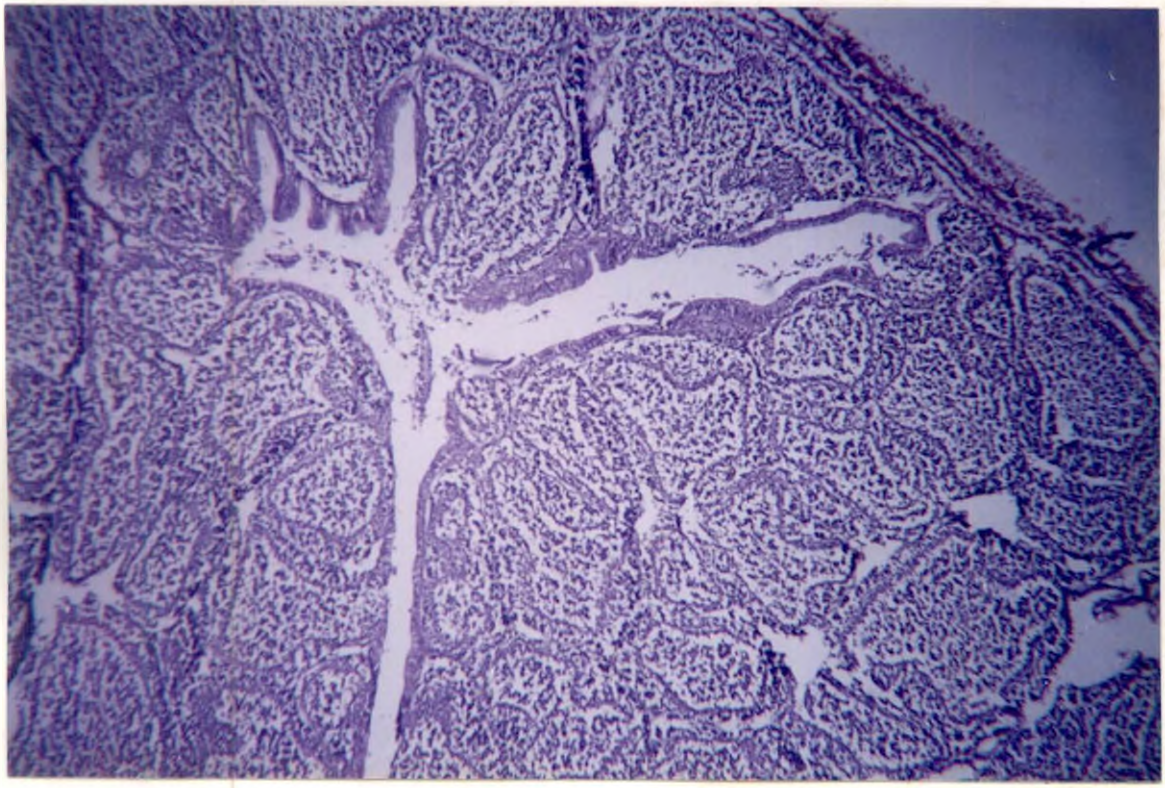
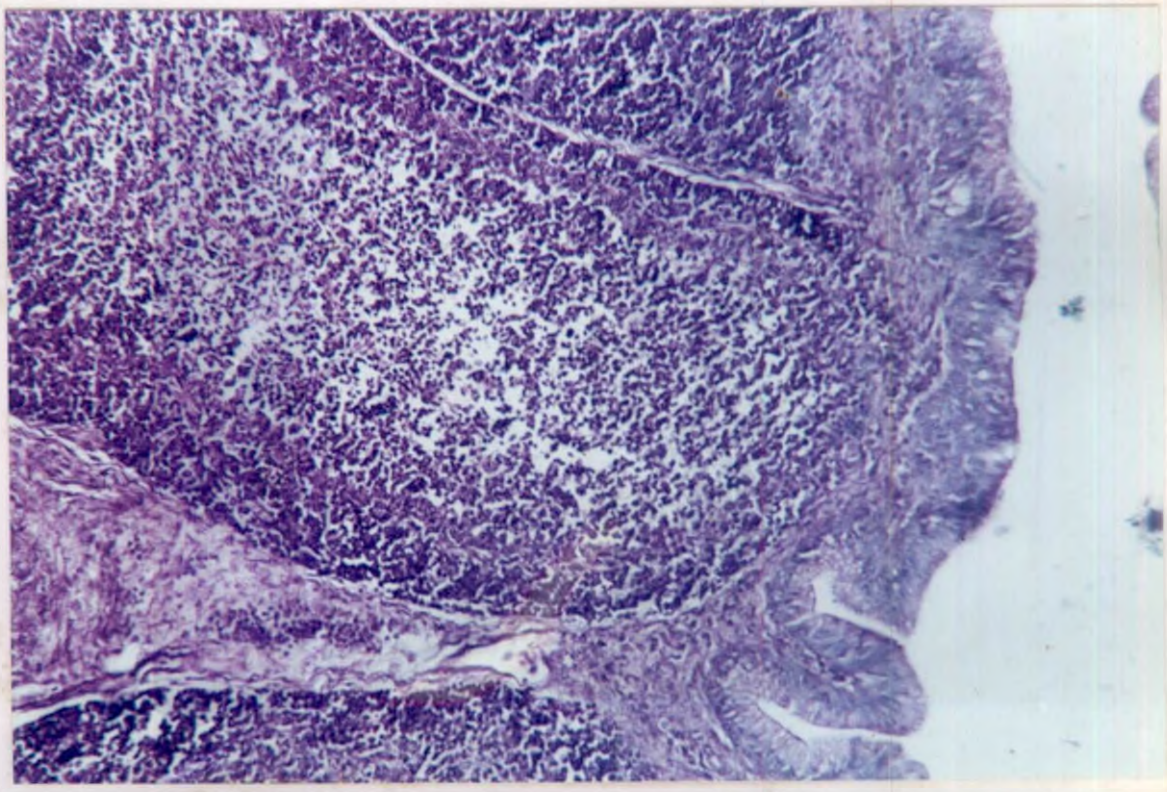


Fig.32. Bursa - 30th day - 60 ppb. Interfollicular fibrosis. Mild lymphoid depletion from the medulla of the follicle. Mild epithelial tissue proliferation. H&E x 400

Fig.33. Bursa - 45th day - 60 ppb. Degeneration and lysis of the lymphoid cells in the follicle. Mild reticulo-endothelial hyperplasia in the cortico medullary junction of the adjacent follicle. H&E x 400



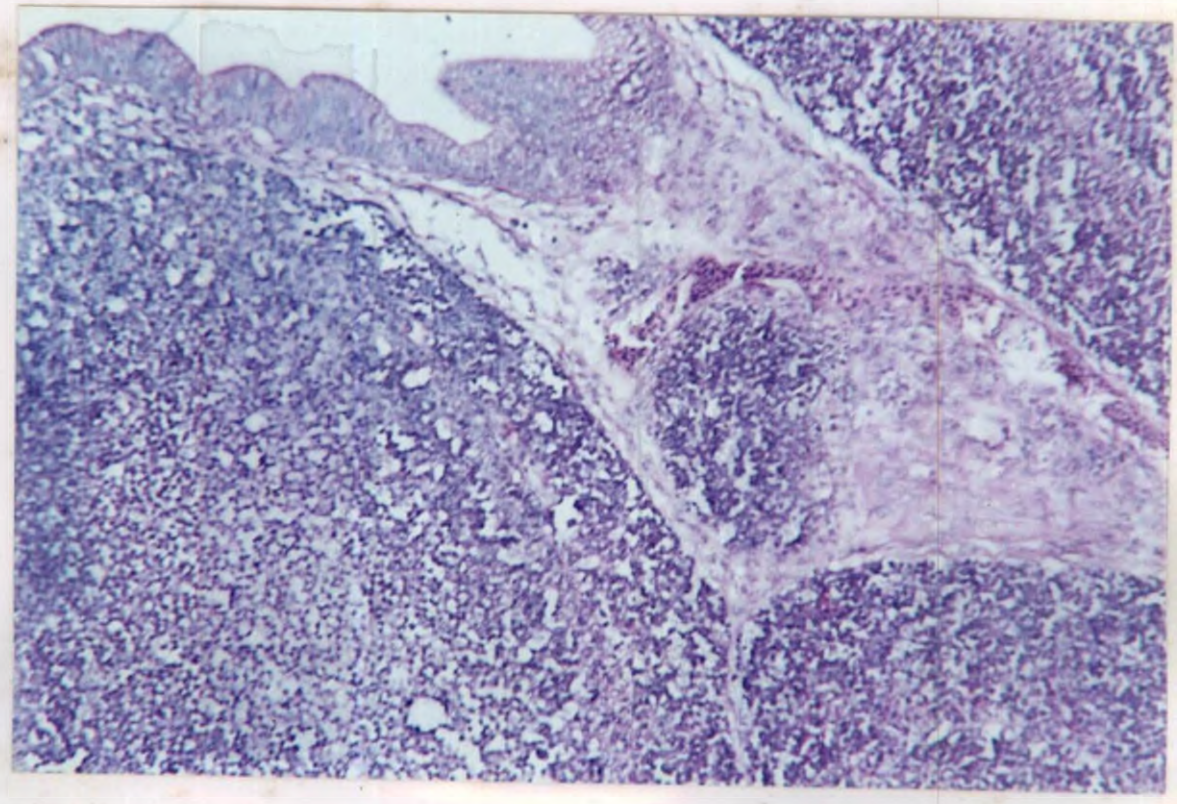
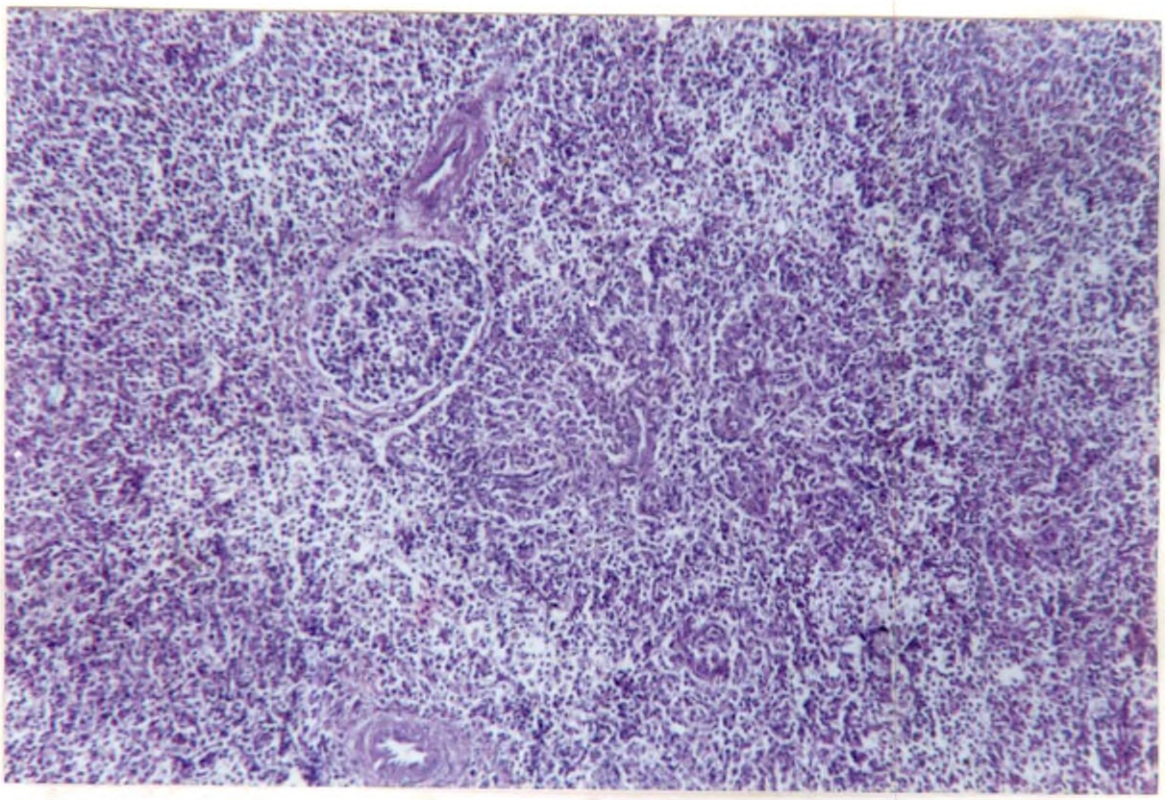


Fig.34. Spleen - 30th day - 80 ppb. Mild lymphoid depletion from the peri arteriolar lymphoid sheath (PALS). H&E x 400

Fig.35. Spleen - 45th day - 40 ppb. Reticulo endothelial hyperplasia in the ellipsoids and peri ellipsoidal lymphoid tissue (PELT). H&E x 250



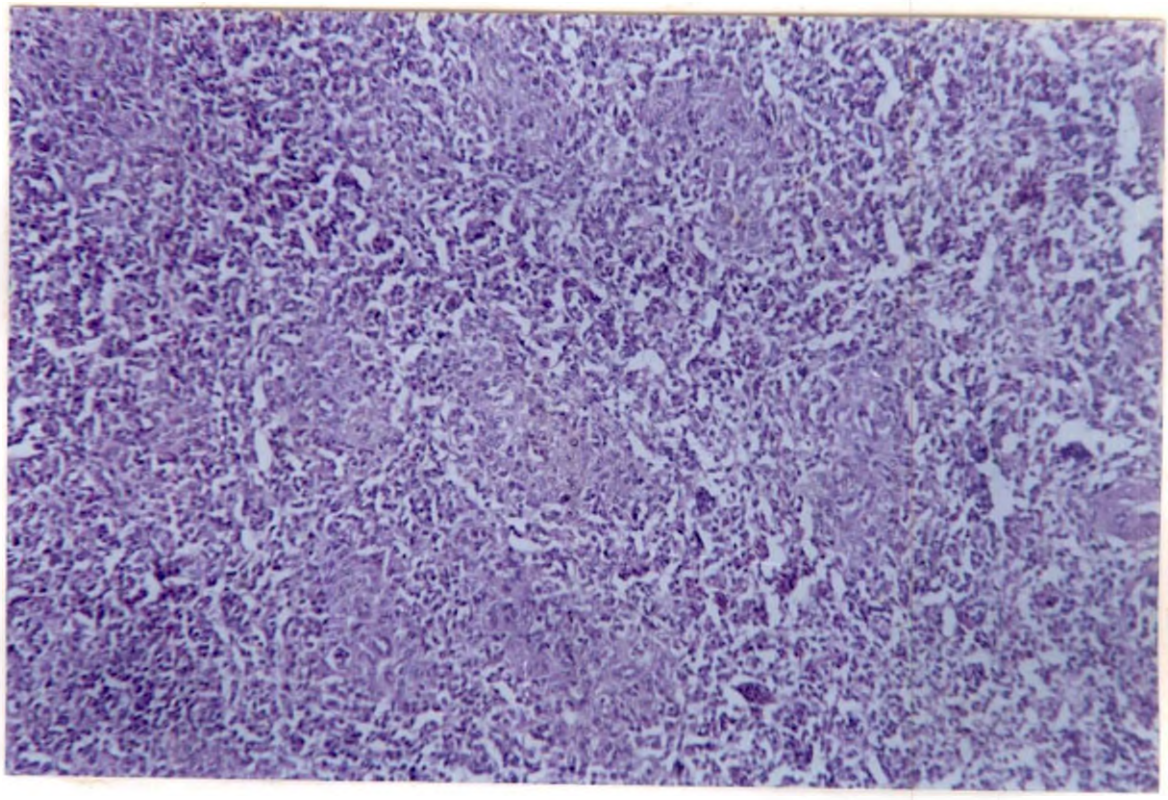
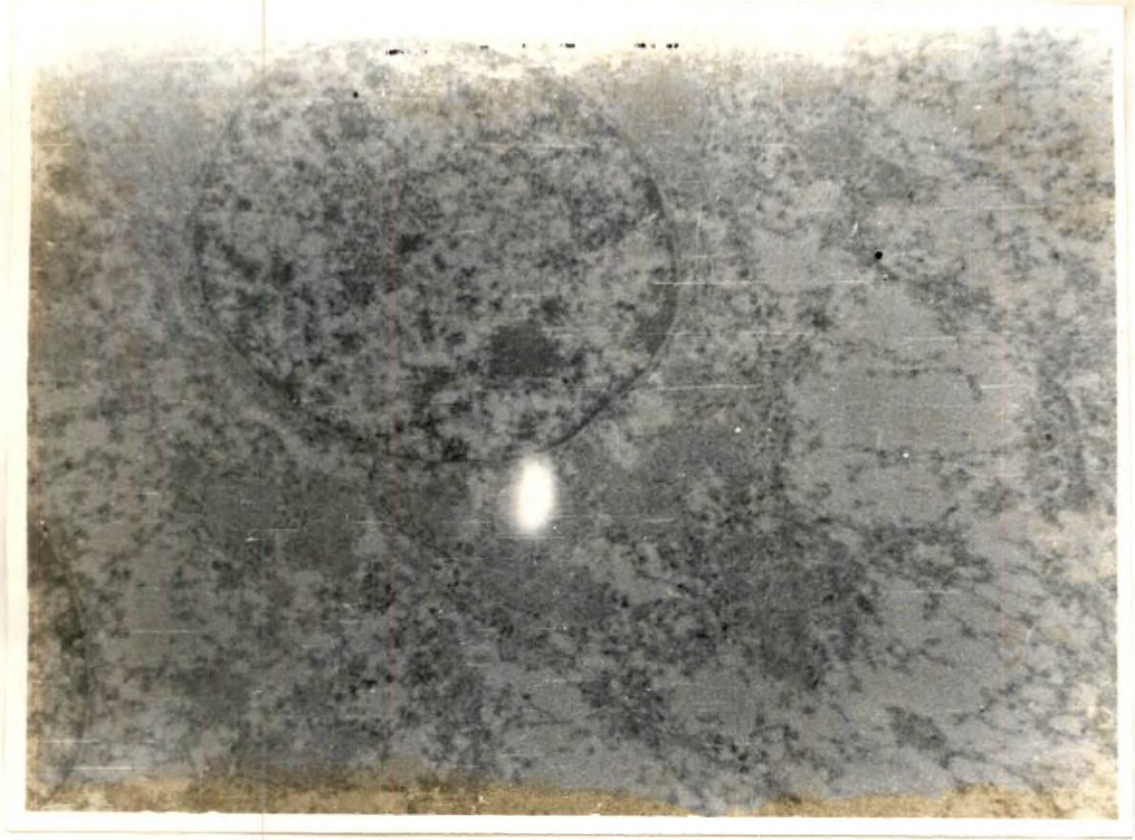


Fig.36. Spleen 45th day - 100 ppb. Vasculosclerosis and mild lymphoid depletion. H&E x 250

Fig.37. E/M Liver - 15th day - 20 ppb. Dilatation and vesiculation of rough endoplasmic reticulum, nucleus showing necrobiosis. 2.5 x 1000



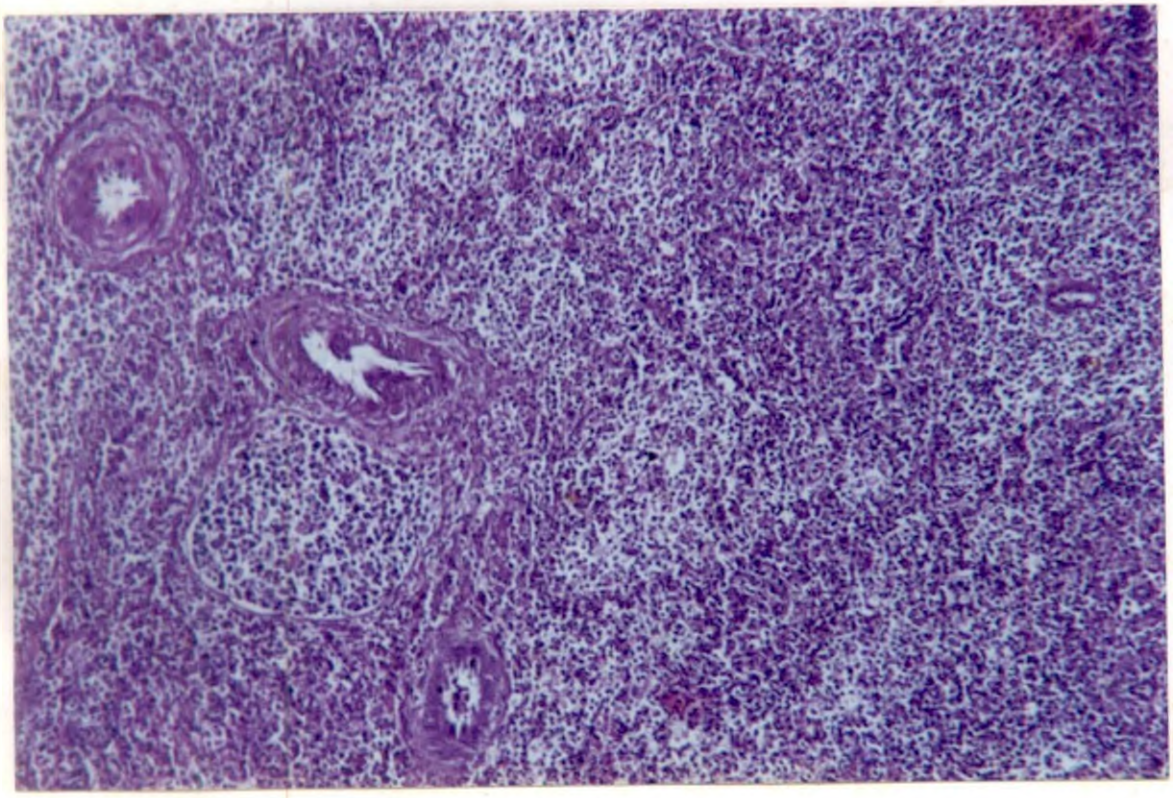
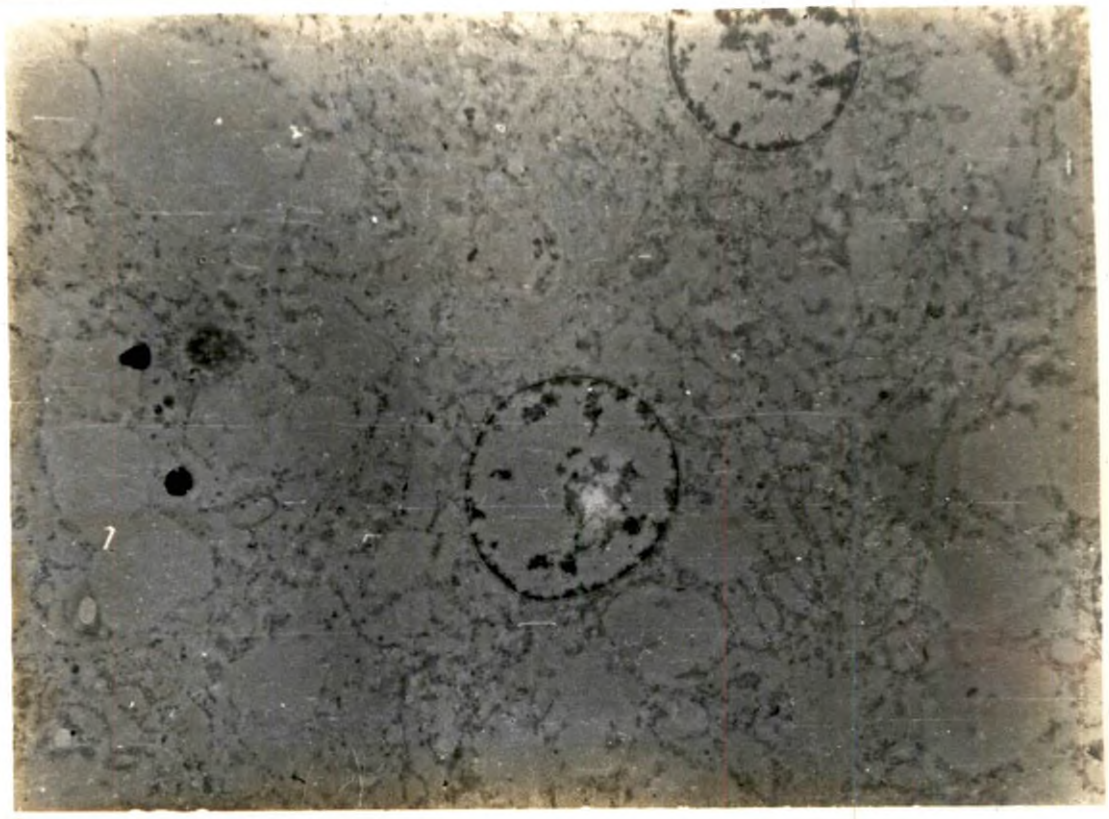


Fig.38. E/M Liver - 15th day - 20 ppb. Dilatation and vesiculation of rough endoplasmic reticulum, nucleus showing necrobiosis. 2.5 X 1000

Fig.39. E/M Liver - 45th day - 40 ppb. Lipid globules of varying sizes within the hepatocytes. 5.3 X 1000



Discussion

DISCUSSION

The present study was undertaken to determine the permissible level of aflatoxin in broiler chicken feed. Feed containing aflatoxin in graded levels at 0, 20, 40, 60, 80 and 100 ppb was given to broilers for six weeks. The pathological and biochemical parameters as well as the production performance of the birds were studied at fortnightly intervals to evaluate the dose related effects of aflatoxin. The parameters included feed intake, feed efficiency, body weight, haemogram, serum profile, organ weight and residual aflatoxin in tissues. The gross, histological and ultrastructural pathology of visceral organs were also studied.

Weight gain and feed efficiency were considered as the most sensitive indicators in aflatoxicosis (Armbrecht *et al.*, 1971). The birds in all the toxin treated groups in the present study showed a dose-associated reduction in the feed intake and feed efficiency. Significant reduction in the feed intake was seen by the fifth and sixth weeks when compared to control. This observation is in agreement with the earlier findings reported by Reddy *et al.*, 1984; Mani *et al.*, 1997 and Vasan *et al.*, 1998. This can be attributed to the toxic injury inflicted by aflatoxin on the alimentary system particularly the liver, pancreas and intestine leading to inappetence, poor metabolism and lowered absorption of nutrients as reported by Hamilton (1989).

A dose related reduction in the body weight was recorded in all the groups throughout the experimental period with the most severe reduction in Groups V and VI to the extent of 17 and 21 per cent respectively compared to control at the sixth week of experiment. This indicated that a reduced feed intake coupled with poor utilization of feed adversely affected the growth in broilers fed with higher levels of aflatoxin. Similar observations were made by Devegowda *et al.* (1994); Mani *et al.* (1997); Muthiah *et al.* (1998) and Vasan *et al.* (1998).

A significant increase in the ESR was observed in the broilers dosed with aflatoxin at 80 and 100 ppb level. A loss of suspenstion stability of erythrocytes as a result of altered serum protein has been reported to cause increased erythrocyte clumping and rapid sedimentation (Schalm, 1975). The decreased serum protein and albumin noticed in the present study might have contributed to the increase in the ESR.

The dose related decrease in the PCV and Hb values clearly indicated the inhibitory effect of aflatoxin on the haemopoietic tissue and these observations are in line with the findings of Lanza *et al.* (1977); Panda *et al.* (1987); Fernandez *et al.* (1995) and Vasan *et al.* (998). The reduction in the haemoglobin and PCV noticed in the toxin fed broilers resulted in anaemia which might have contributed to the increase in ESR values.

Leucopenia with lymphopenia observed in the present study might be due to the inhibitory effect of aflatoxin on the haemopoietic and lymphoid tissues. The

heterophilia could be due to inflammatory reaction consequent to the degenerative changes in the hepatic parenchyma as revealed microscopically. However, Nageswar Rao *et al.* (1989) reported that the total leucocyte count were not affected in chicken fed with aflatoxin.

The decreased levels of total serum protein and albumin noticed in the present study can be imputed to the hepatic damage. The gross, histological and ultrastructural observations in the toxin fed birds clearly indicated severe hepatic injury. Aflatoxin has been shown to limit the protein synthesis through the inhibition of RNA synthesis (Clifford and Rees, 1967 and Yu, 1981) and to cause inactivation of amino acids in the liver and poor absorption of amino acids from the intestine (Mani *et al.*, 1993).

The increase in the serum ALP and GGT values can be correlated with the pathological changes observed in the hepatobiliary system in the toxin fed birds. Aflatoxin induced hepatocellular damage results in increased cellular permeability and release of enzymes into the serum (Jassar and Singh 1993 and Shukla and Pachauri, 1995). The serum enzyme profile which displayed an increase in ALP and GGT values on the 15th day showed a decline towards the latter part of the experiment. The repair mechanisms as indicated by the regenerative changes in the liver and kidney seen towards the latter part of the experiment may be the reason for the decline in enzyme values. The findings are in agreement with those reported by Jassar and Singh (1993).

All the birds in the toxin treated groups starting from 20 ppb onwards showed gross and histopathological lesions throughout the experimental period. However, the intensity of the lesions varied in accordance with the level of toxin and the period of exposure.

Enlargement and pale yellow discolouration of liver with an increase in weight was observed in birds fed with aflatoxin upto 60 ppb level, at all fortnightly intervals. Liver of 80 and 100 ppb groups were congested and showed reduction in the size and weight. Aflatoxin has been found to cause defective phosphorylation of fatty acids resulting in accumulation of fat in the hepatocytes (Moorthy *et al.*, 1986; Balachandran and Ramakrishnan, 1987; Ghosh *et al.*, 1989 and Espada *et al.*, 1992). Diffuse vacuolar degenerative change with necrosis of isolated hepatocytes was a predominant microscopic lesion throughout the experimental period. Fat globules were also evident ultrastructurally in the hepatocytes. The enlarged pale yellow livers noticed in the present study might be due to the accumulation of lipids in the hepatocytes. The reduction in the size and weight of liver seen in Group V and VI might be due to necrosis and loss of hepatic parenchyma at higher dose levels. Khan *et al.* (1989) has also reported reduction in the size and weight of the liver in broilers fed aflatoxin at 250 ppb levels.

Eventhough aflatoxin caused degeneration of hepatocytes, the more resistant connective tissue and biliary tissue reacted to the toxic injury with proliferative changes. This was evident by phlebosclerosis, periportal fibrosis,

Biliary hyperplasia and Kupffer cell reactions. These lesions were well pronounced in the 30th and 45th day of experiment. Bile duct proliferation, which is a pathognomonic lesion in aflatoxicosis, has been considered as a regenerative change (Siller and Ostler, 1961; Gimeno and Martins 1983 and Espada *et al.*, 1992). Regeneration of hepatocytes was well pronounced in the latter part of the experiment indicating repair mechanism. These findings were in agreement with those of Balachandran and Ramakrishnan (1987); Anjaneyulu and Rao (1993) and Sridevi *et al.* (1996).

Enlarged, pale appearance of kidneys in the aflatoxin fed birds were consequent to degenerative and necrotic changes of tubular epithelium. The vacular degenerative changes observed in the tubular epithelium can be imputed to direct toxic injury on the cell membrane. In severe cases, the degenerative changes were to the extent that there was disruption and desquamation of epithelium leaving only the basement membrane. These findings correspond with those of San-Gabriel (1971) and Ghosh *et al.* (1989). Regeneration of tubular epithelium was a consistent feature in the treatment groups throughout the experiment. These regenerating tubules indicated the repairative process and is in line with the finding of Sridevi and Sriraman (1996).

Enlargement of glomeruli with mesangial cell proliferation was a prominant feature throughout the experiment in all the dose levels. Rarely there was glomerulosclerosis and thickening of glomerular basement membrane. These changes can be attributed to the stimulatory effect of the toxin on the more

resistant supporting tissue. These findings mirror those of Ghosh *et al.* (1989); Anjaneyulu and Rao (1993) and Sridevi and Sriraman (1996). The severity of lesions observed in the kidney implies that aflatoxin is not only a hepatotoxin but also a potent nephrotoxin.

The enlargement and increased weight of the bursa noticed in the birds fed with aflatoxin upto 60 ppb level may be due to bursal edema and presence of greyish white colloid material in the lumen. Lymphoid depletion from the follicle particularly in the medullary region was a characteristic and consistent feature in all the groups throughout the experiment. The atrophy along with lymphoid depletion of the bursa (Merkley *et al.*, 1987), thymus (Gimeno and Martins 1983 and Venkata *et al.*, 1988) and spleen (Bakshi *et al.* (1995) suggest that these lesions in the immune system is a reliable indicator of aflatoxicosis. Degeneration, necrosis and lysis of lymphoid cells in focal areas of bursal follicles resulted in intrafollicular cyst formation. The atrophy and reduction in the weight of bursa seen in Group V and VI may be the result of such lesions. The above findings are in line with those reported by Sridevi and Sriraman (1996) and Suresh (1999). Interfollicular connective tissue proliferation and reticulo endothelial hyperplasia observed in the toxin treated birds especially on the 30th and 45th day of experiment may be due to the stimulatory effect of the toxin on the reticulo endothelial tissue as opined by Anjaneyulu and Rao (1993) or due to the repair and replacement of degenerated and lysed follicles with fibrous tissue.

A significant decrease in the spleen weight was observed in the toxin fed groups throughout the experiment except for a moderate increase in splenic size and weight in Group V and VI on the 45th day of experiment. Mild to moderate lymphoid depletion from the Peri Arteriolar Lymphoid Sheath (PALS) and to a lesser extent from the Peri Ellipsoidal Lymphoid Tissue (PELT) and the Germinal Centers, was the prominant lesion in all the toxin treated groups throughout the experiment. This clearly implies the immunosupressive action of aflatoxin. Suppression of lymphoid tissue and reduction in the lymphocyte production as a result of diminished protein synthesis mightbe the reason for the reduced spleen weight (Balachandran and Ramakrishnan, 1987; Bakshi *et al.*, 1995 and Suresh, 1999).

Splenomegaly and the increased splenic weight seen in Group V and VI by the 45th day of experiment may be due to the reticulo endothelial (RE) hyperplasia. The sheet like arrangement of lightly stained cells with indistinct cytoplasmic borders and enlarged vesicular nucleus seen in the ellipsoid and PELT region could be due to the formation of syncytium by the macrophages and reticular cells which were activated in the toxin treated groups. Similar observations were also made by Balachandran and Ramakrishnan (1987). Vasculosclerosis as reported by Sridevi and Sriraman (1996) and Suresh (1999) was also a well pronounced feature in the present study and may be due to stimulatory effect of aflatoxin on the connective tissue.

Residual toxin was not detected in the tissues on the 15th and 30th day of experiment except for trace residual toxins detected in the liver (2 ng/g) and muscle (2.4 ng/g) of birds in group VI. However dose related increase in the toxic residues was detected in the muscle (2.4 to 5ppb) liver (1.5 to 6.7) and kidney (1 to 3 ppb) by the 45th day. Maryamma *et al.* (1992) estimated toxic residues ranging from 10-20 µg/kg in the liver and 10-80 µg/kg in the kidney of White Leghorn chicks fed with 100 ppb of aflatoxin for 30 days. Similarly, Yadav *et al.* (1995) recorded 4.7 and 2.0 ppb of toxic residues in the liver and muscle respectively by the 45th day of experiment in broilers fed 500 ppb aflatoxin.

The residual aflatoxin in the meat and other edible tissues is a matter of concern to human health. Aflatoxin has been known to play a role in many disorders like liver carcinoma, infertility, Reye's syndrome and Kwashiorkir disease in human beings (Alpert *et al.* (1971) Wilkinson *et al.* (1993) Eaton and Groopman (1994) and Ibeh *et al.* (1994). FDA (1969) has recommended 20 ppb as the acceptable limit of AFB_1 in foods for human consumption and FAO (1976) has set 30 ppb as the safe limit in the food items in India. The results obtained in the present study showed that the residual aflatoxin present in the muscle and liver of broilers even at 100 ppb level on the 45th day is well below the standards set by FDA and FAO.

The dose related decrease in the feed consumption and body weight resulted in a decrease in the total expenditure as well as total receipts. The percentage difference in expenditure over control showed a marginal reduction.

Whereas the percentage difference in receipt over control revealed a substantial reduction. This trend is fully reflected in the net returns which showed significant reduction with increasing aflatoxin levels. The loss in profit at 20 ppb level was 7.3 per cent, while the losses at higher toxic levels were in the range of 18 to 80 per cent. The economic loss at 20 ppb level can be considered as marginal.

The incidence of aflatoxin contamination in the poultry feed available in the market of Kerala is very high. The hot and humid climatic conditions prevailing in the state are conducive for the fungal growth. In this situation it is a difficult task to maintain poultry with a feed totally free from aflatoxin. A preliminary study conducted to estimate the aflatoxin levels in the poultry feed showed that about 24 per cent of the poultry feed samples available in the market of Kerala were contaminated with toxic levels of aflatoxin with a range of 20 to 200 ppb. Rajan *et al.* (1991) reported that 50.1 per cent of the feed samples in the market of Kerala were contaminated with aflatoxin. This indicates that there is no proper quality control over the marketed feed with regard to aflatoxin level.

Twenty ppb has been recommended as the safe level of aflatoxin in the poultry feed as per the package of practice recommendations of KAU as well as the EEC standards. The level suggested by PAG was 30 ppb. If these limits are strictly implemented in Kerala, a considerable portion of the poultry feed in the market has to be rejected. This necessitates fixing a permissible level of aflatoxin in the broiler chicken feed which in suitable to the climatic conditions prevailing

in Kerala. There is also a need to implement strict quality control measures at the production unit itself.

While considering a permissible level in addition to the pathological effects, the production performance of the bird, the economic loss to the farmer and residual toxin in the meat and edible portions also has to be looked into. It may be noted that the permissible level can vary with different situations like method of estimation, susceptibility of strain, nutritional status of the bird, type of feed ingredients used, environmental condition, etc.

In this investigation, all these factors have been analyzed. The results of the present study show that even at 20 ppb there was cellular and subcellular damage to the tissues. The intensity of histological lesions were less at 20 and 40 ppb levels compared to higher levels of toxin.Likewise, the haematological and biochemical parameters also showed dose associated variations from 20 ppb onwards although these variations were not significant at 20 and 40 ppb levels compared to control. In spite of the toxico-pathological lesions, the production performance of the birds and the economic loss at 20 ppb level was marginal. A level upto 20 ppb may not be of much influence on the production barring the least toxicopathological effect under experimental conditions. Also it may be noted that the aflatoxin residues in various tissues even at 100 ppb levels, were well below the permissible levels fixed by FAO and FDA.

To conclude, the present study makes it clear that even 20 ppb cannot be considered as a safe level, since there were lesions at the cellular and subcellular levels in all the tissues studied. However, for practical purposes, 20 ppb can be taken as the permissible level of aflatoxin in broiler feed, as the reduction in body weight and economic loss at this level were minimum. Implementation of strict quality control measures at all levels starting from production unit, will reduce the incidence of aflatoxin contamination in the feed and feed ingredients. Also, it is necessary to impart awareness on aflatoxin hazards among feed manufacturers and poultry farmers by proper extension education.

Summary

SUMMARY

The study was undertaken to assess the permissible level of aflatoxin in broiler chicken feed. Day old broiler chicks (n=180) were divided into six groups of 30 birds each and were given graded levels of aflatoxin at 0, 20, 40, 60, 80 and 100 ppb in the feed from first day to 45th day of age. Feed intake, weekly body weight gain, feed efficiency, haemogram, serum profile, organ weight, residual aflatoxin in tissues and pathological changes of liver, kidney, bursa and spleen were studied at fortnightly intervals to evaluate its dose related effects.

A significant decrease in the daily feed intake in the aflatoxin fed birds was noticed at fifth and sixth weeks when compared to control. All the experimental groups recorded a highly significant reduction in the body weight in relation to the level of toxin in feed throughout the experiment. Feed efficiency was extremely poor at 80 and 100 ppb levels.

Haematological studies indicated that the toxin at all levels caused a decrease in haemoglobin, PCV, total leucocyte and lymphocyte counts while an increase in the ESR and heterophil counts was noticed at the 15th, 30th and 45th day of age.

Serum profile revealed a significant decrease in the total serum protein and albumin in toxin treated birds by the 45th day. The serum enzymes GGT and ALP were elevated in all the AFB_1 fed broilers, throughout the experiment. The higher enzyme values seen on the 15th day subsequently showed a declining tendency by the 30th and 45th day.

In general the toxin fed birds, were dull in appearance and showed anorexia, greenish diarrhoea and reduced weight gain. The control birds were apparantly healthy and active.

The birds fed with aflatoxin upto 60 ppb had pale yellowish enlarged livers with increased weight and rounded borders. Whereas at 80 and 100 ppb levels, the livers were reduced in size and weight and were congested. Kidneys were enlarged and pale in appearance. Bursa was enlarged and its lumen contained greyish white mucoid substance while at 100 ppb level bursa showed reduction in size. A dose associated decrease in the spleen weight was observed in all toxin treated groups throughout the experiment except for a mild splenomegaly in 80 and 100 ppb groups on the 45th day of experiment.

The severity of the histopathological lesions were progressive with the levels of toxin and the period of exposure. No apparent histopathological lesions were observed in the control groups.

The prominant lesions in the liver of toxin fed birds were diffuse vacuolar degeneration of hepatocytes with isolated pyknotic cells scattered in the

parenchyma. Regenerative changes in the hepatocytes were seen particularly in the 30th and 45th day of experiment. Bile duct hyperplasia, periportal fibrosis, phlebosclerosis, Kupffer cell reactions and mononuclear infiltrations were prominant in Group IV, V and VI by the 30th and 45th day. Moderate to severe congestion of the portal and central vessels and perivascular edema were seen in all the groups throughout the experimental period. Ultrastructurally, the hepatocytes showed dilatation and vesiculation of the rough endoplasmic reticulum with mild to moderate degranulation of ribosomes. Membrane bound electronlucent globular structures of varying sizes identified as fat globules were also seen.

The prominant histopathological lesions in the kidney of toxin fed birds were vacuolar degeneration, necrosis as well as regeneration of the tubular epithelium, which was seen throughout the experimental period. Proliferation of mesangial cells of glomeruli, congestion and haemorrhage into the interstitium and calyces were also seen.

Mild to moderate lymphoid depletion with degeneration, lysis and cyst formation in occasional follicles were the microscopic lesions in the bursa of Fabricius in the toxin fed birds throughout the experiment. Reticulo endothelial hyperplasia in the cortico-medullary junction of the follicles was seen by the 30th and 45th day. Lymphoid depletion was also evident in white pulp of spleen throughout the experiment. Reticulo endothelial hyperplasia in the ellipsoid and PELT also was characteristic by the 45th day.

Residual aflatoxin was not detected in liver and muscle on the 15th and 30th day of experiment except for traces in Group VI on the 30th day. On the 45th day, all the toxin treated groups showed residual AFB_1 in liver and muscle. Pooled kidney samples showed residues of AFB_1 in Group IV, V and VI on the 30th and 45th day of experiment. The residual toxin even at 100 ppb level is well below the safe limits recommended by the FAO and FDA.

The net returns from the toxin treated birds showed a dose related decline compared to control, with a substantial reduction at 80 and 100 ppb levels. The loss in profit at 20 ppb level was marginal.

The incidence of aflatoxin contamination in the poultry feed samples analysed during the period from January to July -1999, was 24 per cent with a range of 20 to 200 ppb levels.

The results of the present study shows that even at 20 ppb, there was cellular and subcellular damage to the tissues. The haematological and biochemical parameters also revealed a dose related variations from 20 ppb onwards. However, the intensity of these changes were less at this level. Inspite of these changes, the production performance and the economic loss at 20 ppb level were marginal. Hence 20 ppb can be considered as permissible level of aflatoxin in the broiler feed.

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DETERMINATION OF PERMISSIBLE LEVEL OF AFLATOXIN IN BROILER CHICKEN FEED

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ABSTRACT OF A THESIS

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ABSTRACT

The study was undertaken to assess the effect of various levels of aflatoxin on the performance of broilers and to establish the permissible level of aflatoxin in the broiler feed. One hundred and eighty day old broiler chicks were divided into six groups of 30 birds each and were given feed containing graded levels of aflatoxin at 0, 20, 40, 60, 80 and 100 ppb for a period of 45 days. Feed intake, weekly body weight gain, feed efficiency, haemogram, serum profile, organ weight, residual aflatoxin in tissues and pathological changes in liver, kidney, bursa and spleen were studied at fortnightly intervals to evaluate its dose related effects.

A dose dependent decrease in the daily feed intake and mean body weights were noticed in all the groups. The cumulative FCR was extremely poor at 80 and 100 ppb levels at sixth week of age.

The toxicopathological changes in the birds varied in its intensity in relation with the level of aflatoxin in the feed, with most severe changes being at higher dose levels. The values of haemoglobin, PCV, total leucocyte count, lymphocytes, total serum protein and albumin showed a decrease whereas the ESR, heterophil and serum enzyme were increased.

The aflatoxin, even at 20 ppb levels caused degenerative and necrotic changes in liver and kidneys and the intensity of the lesions increased with higher levels of toxin. Attempt for regeneration and repair processes were well pronounced by the 30th and 45th day. Lymphoid depletion was a characteristic feature in bursa and spleen. Degeneration and lysis of lymphocytes with cyst formation was also seen in bursal follicles.

Residual aflatoxin was detected in the liver and muscle in all groups by the 45th day. The net returns from the toxin treated birds showed a dose related decline compared to control and the loss in profit at 20 ppb level was marginal

About 24 per cent of the poultry feed samples tested during the period from January to July 1999 were contaminated with aflatoxin quantitatively ranging from *20 to 200 ppb.*

The results of the present study shows that even at 20 ppb aflatoxin causes cellular and subcellular damage to the tissues. However, the economic loss at this level was marginal when compared to higher dose levels. Hence 20 ppb can be considered as permissible level in broiler chicken feed.