IMMUNOPATHOLOGICAL RESPONSE OF THE JAPANESE QUAIL *(Coturnix coturnix japonica)* IN EXPERIMENTAL AFLATOXICOSIS

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**IMMUNOPATHOLOGICAL RESPONSE OF THE JAPANESE QUAIL (*Coturnix coturnix japonica*) IN EXPERIMENTAL AFLATOXICOSIS**" 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 "**IMMUNOPATHOLOGICAL RESPONSE OF THE JAPANESE QUAIL (*Coturnix coturnix japonica*) IN EXPERIMENTAL AFLATOXICOSIS**" is a record of research work done independently by **Sri. M. Sureshkumar**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Loving Parents and Brother

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INTRODUCTION

Japanese quails (*Coturnix coturnix japonica*) are migratory game birds belonging to the same family as the domestic fowl (*Gallus domesticus*). They were initially domesticated in Japan. These birds are now being reared commercially for meat and egg production. Their eggs are highly nutritious and are found to be effective against certain ailments like asthma. Realising the quality of meat and the value of eggs, quail farming has been taken up on a large scale by farmers all over the state of kerala and it has now become a full fledged business enterprise in many parts of the state. Its multiple advantages like ease of handling, inexpensive maintenance and their physiological resemblance to chicken combined with a great egg laying capacity have made them acceptable for profitable large scale farming. Furthermore, cell cultures derived from various organs of quails have been utilised effectively for virological research and as experimental birds, they are becoming highly popular all over the world.

Adult Japanese quails are quite resistant to most of the diseases. But their domestication and artificial feeding have made them vulnerable and they have become as susceptible as chicken to many disease processes. Under the intensive system

of management they are subjected to a number of stress factors and a variety of pathogens, chemicals and toxins. These cause diverse deleterious effects like depression of the productive performance and immunosuppression, leading to many secondary diseases and increased mortality.

Mycotoxins produced on the food commodities by a variety of fungi pose a great threat to the health of the birds. Among the mycotoxins, aflatoxin B_1 producedby *Aspergillus flavus* is of prime importance. The toxin is hepatotoxic, teratogenic, mutagenic, carcinogenic and antimitotic in activity. Although Japanese quails are resistant to the toxic effects of aflatoxins, it continues to remain a problem of considerable reckoning in younger birds. Since quails are raised in the same environment and are fed with the same artificial feeds as other poultry, the problem of mycotoxicosis seems to be on the higher side. The secondary effects particularly immunosuppression is important as it leads to increased susceptibility to infections with increased mortality and loss of production. There are very few reported studies on the effect of aflatoxins in quails. In the light of these facts an experimental study utilising pure aflatoxin B_1 has been designed to evaluate the following:

1. The pathological changes in the birds associated with low level feeding of aflatoxin B_1.

2. Haemogram and serum protein profiles in aflatoxicosis.

3. The effect of aflatoxin B_1 to the development of immunity against RD vaccination.

Review of Literature

REVIEW OF LITERATURE

2.1 Species susceptibility

Large difference in species susceptibility to acute and subacute poisoning by aflatoxin has been recognized in domestic animals (Allcroft, 1965; Newberne and Butler, 1969). The duckling is reported to be the most sensitive, followed by the turkey poult, pheasant and chicken (Allcroft, 1965; Asplin and Carnaghan, 1961). Variation in the degree of susceptibility also exist among different breeds. New Hampshire breed of chicken showed unique sensitivity to aflatoxicosis (Brown and Abrams, 1965; and Gumbmann *et al.*, 1970). Quails are comparatively more resistant to aflatoxin than chicken (Chang *et al.*, 1979; Marks and Wyatt, 1979). The acute oral LD_{50} in young Japanese quail to aflatoxin was estimated to be 19.5 ± 4.8 mg/kg (Chang and Hamilton, 1982) as compared to 6.3 mg/kg in chicken and 0.3 mg/kg in ducklings (WHO, 1979).

2.2 Effect of aflatoxin on growth rate and body weight

2.2.1 Japanese quails

The effect of aflatoxin on the body weight of Japanese quails was studied by Gumbmann *et al.* (1970). They did not

observe any change in the body weight after the toxin was fed at the rate of 800 ppb in the diet for a period of six weeks. However, signs of toxic liver damage were evident which included a significant reduction in the liver weight and lowered liver RNA concentration.

Sawhney *et al.* (1973) reported that the presence of two and four microgram of aflatoxin per gram of feed improved the ratio of body weight to feed consumption. The ratios were lower than the controls when six microgram of aflatoxin per gram of feed was fed, except in the second and third week of treatment.

Doerr and Ottinger (1979) noticed a significant depression of growth rate and body weight of the birds fed aflatoxin at levels of five and ten microgram/gram in the feed from 7 to 21 days. The minimal growth inhibitory dose of aflatoxin for Japanese quails was found to be five microgram per gram of feed (Chang and Hamilton, 1982).

The comparative responses of genetically resistant and non-selected Japanese quails to dietary aflatoxin were studied by Pegram *et al.* (1985). They observed a significant growth depressing effect of aflatoxin in the non-selected lines at the rate of 10 microgram/gram in feed from two weeks of age.

Panda *et al.* (1987) reported a significant depression in body weight gain by aflatoxin at dose levels of 0.5 ppm and above in the feed from 6 to 35 days of age. A similar growth inhibitory effect was observed in young quail chicks fed aflatoxin at graded levels of 0.5 ppm and above in the diet (Kumar *et al.*, 1993).

2.2.2 Other avian species

Butler (1964) observed a depression in weight gain for two days after aflatoxin treatment. The birds recovered fully from this effect later.

Gumbmann *et al.* (1970) in a comparative study with different avian species found that aflatoxin at the rate of 800 ppb in the diet from two to six weeks of age generally depressed growth rate and body weight for all avian species except Australop and Barred Rock strain of chicken, Japanese quails and guinea fowls. The growth inhibitory dose of aflatoxin in chicken was estimated to be 2.5 ppm (Smith and Hamilton, 1970).

Arafa *et al.* (1981) reported that in goslings, 0.7 mg of aflatoxin per kilogram caused some increase in body weight as compared with control birds.

Maurice *et al.* (1983) studied the effect of purified aflatoxin B1 at the rate of 100 μg/kg/day in chicken from second day to three weeks of age and reported that there was no effect on the feed intake and body weight gain. Aflatoxin at the rate of 2.5 μg/g in the feed for two weeks was found to be highly growth depressing (Campbell *et al.*, 1983). They found the growth depressing effect to be synergistic with ochratoxin treatment at a level of 2.5 μg/g in the feed.

Huff *et al.* (1984) reported a reduction in the body weight of broilers by Ochratoxin A and a combination of Ochratoxin A and aflatoxin treatments on a long term basis.

Dafalla *et al.* (1987) reported depressed body weight gain in chicks fed 0.5 ppm aflatoxin in the diet for four weeks. Similar findings in chicken were also reported by many other workers (Ghosh *et al.*, 1989; Rao *et al.*, 1988; Rizvi and Shakoor, 1992) in aflatoxicosis.

2.3 Organ weights

2.3.1 Japanese quails

Gumbmann *et al.* (1970) observed considerable reduction in the liver weights when the birds were given aflatoxin at the rate of 800 ppb in the feed. The weight reduction was evident from two to six weeks of age. A significant increase in liver

weight relative to body weights, along with a reduction in testes weights was noticed in aflatoxin fed quails at a dose level of five microgram per gram in the feed from one to three weeks of age (Doerr and Ottinger, 1979).

Chang and Hamilton (1982) reported a significant depression in the weights of liver, pancreas, gizzard and proventriculus by graded levels of aflatoxin in the feed. They also observed a significant reduction in bursal weights at a dose of five microgram per gram and above in the feed whereas liver and pancreas were affected by even 1.25 μg/g of aflatoxin in the feed.

2.3.2 Other avian species

Carnaghan (1964) reported a significant increase in liver weight and body weight ratios in RIR chicks.

Gumbmann *et al.* (1970) in a study to evaluate the aflatoxin susceptibility in various breeds of poultry reported a significant decrease in liver body-weight ratios in the New Hampshire chicken and Broad breasted white turkeys at a dose level of 800 ppb in the diet.

Campbell *et al.* (1983) observed a significant depression in bursal weights of chicken exposed to combined aflatoxin and Ochratoxin A.

Rao *et al.* (1988) reported a significant depression in bursal weights by aflatoxin at 0.5 ppm and above in chicken at 12 weeks of age along with a significant increase in spleen weights at both 12th and 18th week of age.

Mani *et al.* (1993) observed a significant reduction in bursal weight in broiler chicken by aflatoxin at a dose level of 0.75 ppm from 0-8 weeks of age. An increase in liver and spleen weights was also noticed in the treated chicken.

Shiang *et al.* (1994) reported a significant decrease in liver weights in Taiwan country chicken to aflatoxin treatment at a dose rate of 800 ppb from one to five weeks of age.

2.4 Haemogram

The alteration in the haematological parameters due to aflatoxicosis has been well documented by many workers in various species of birds and farm animals.

2.4.1 Erythrocyte sedimentation rate (ESR)

Yaman *et al.* (1989) observed a significant increase in the mean ESR values in aflatoxin treated chicks at the rate of five microgram daily in the feed for two months. An increase in ESR in laying hens was reported by Fernandez *et al.*, 1995.

Anbiah (1996) reported a significant increase in ESR values in aflatoxin treated ducks at the rate of 15 μg/kg body weight from that of controls.

2.4.2 Packed cell volume (PCV)

Decreased PCV was observed by Tung *et al.* (1975) when male broiler chicks were given aflatoxin at the rate of 2.5 μg/g. Reddy (1981) reported lowered PCV in birds fed with 0.5 ppm of aflatoxin. Day old broiler chicks which received aflatoxin at 0.5 ppm dose level showed a decrease in packed cell volume (Mohiuddin *et al.*, 1986) from that of controls.

A significant reduction in PCV was observed in broiler chickens by Anjaneyulu and Rao (1993) when fed with one ppm of aflatoxin for three weeks. A similar reduction in PCV was also reported in aflatoxicosis in eight weeks old chicks by Mani *et al.* (1993).

2.4.3 Haemoglobin

Anaemia has been observed as a clinical feature of aflatoxicosis in ducks and chicken. Brown and Abrams (1965) observed moderate anaemia in ducklings and New Hampshire chicks dosed with aflatoxin at the rate of 0.5 ppm for six days. Lowering of hemoglobin concentration was recorded by

Tung *et al.* (1975) in male broiler chicks at the dose of 1.25 µg/g of aflatoxin and above.

Feeding of aflatoxin at 0.75 ppm to birds resulted in a significant reduction in hemoglobin level (Reddy *et al.*, 1980; Reddy, 1981). Similar findings were reported by Mohiuddin *et al.* (1986) in day old broiler chicks fed with 0.5 ppm aflatoxin.

Panda *et al.* (1987) reported that there was no significant effect by aflatoxin on the hemoglobin levels in young Japanese quails fed graded levels of aflatoxin from 0 to 0.75 ppm in the feed for 30 days.

Balachandran and Ramakrishnan (1987) attributed the findings of anaemia in aflatoxicosis in chicken to a combination of hemolytic and haemorrhagic type. A highly significant reduction in hemoglobin values in broiler chicks fed one ppm of aflatoxin for three weeks was reported by Anjaneyulu and Rao (1993). Comparable reduction in hemoglobin values was also observed in aflatoxin treated ducks (Anbiah, 1996).

2.4.4 Erythrocyte count

Wannop (1961) while studying the 'X ' diseases of birds observed a significant decrease in erythrocyte count as a

prominent feature of the disease. A reduction in the erythrocyte count was also observed by Tung *et al.* (1975) in male broiler chicks by administering aflatoxin at a dose of 0.625 μg/g. Reddy *et al.* (1980) and Reddy (1981) also recorded a reduction in the erythrocyte count in chicken fed with one ppm of aflatoxin. Mohiuddin *et al.* (1986) recorded a decrease in total erythrocyte count when aflatoxin at 0.5 ppm dose level was fed to day old broiler chicks. A significant reduction in the erythrocyte counts was noticed in ducks treated with 15 μg/kg body weight of pure aflatoxin B1 on alternate days for four months (Anbiah, 1996).

2.4.5 Total leucocyte and differential count

Wannop (1961) observed a marked increase in leucocyte counts in birds affected with `X' diseases as a result of increase in the number of heterophils and monocytes. The heterophil and monocyte counts were four and eight times higher in the affected ones than the healthy birds with a concurrent reduction in the lymphocyte count.

Tung *et al.* (1975) recorded a marked increase in leucocyte number when the experimental male broiler chicks were fed aflatoxin at a dose rate of 10 μg/g. An accompanying increase in heterophils and a reduction in basophils were also observed.

Mohiuddin *et al.* (1986) reported a slight increase in total leucocyte counts with an increase in the percentage of heterophils associated with decreased total thrombocyte and basophilic counts in cockerels fed aflatoxin at 20 ppm levels in the feed for a period of four months.

Heterophilia and lymphopenia were observed by Sova *et al.* (1991) in experimental aflatoxicosis in broilers at 25 mg level with five per cent zeolite in the feed. Leucocytosis with neutrophilia was observed in experimental aflatoxicosis in four months old ducks by Anbiah (1996).

2.5 Serum chemistry

2.5.1 Total serum protein

Alteration in serum protein has been reported in aflatoxicosis by many workers.

Reddy *et al.* (1982) reported a decrease in total serum protein in aflatoxin fed birds. Similar findings were observed in young broiler chicken during simultaneous aflatoxicosis and ochratoxicosis (Campbell *et al.*, 1983).

Chang and Hamilton (1982) observed a decrease in serum protein levels in Japanese quails even at a very low level of 1.25 μg/g of aflatoxin in the feed. Pegram *et al.* (1986)

reported a significant reduction in the total protein content in young non selected Japanese quails in acute aflatoxicosis at 2.5 mg/kg body weight orally. A gradual depression in serum protein level proportionate to increasing aflatoxin concentration in the diet of quails was reported by Panda *et al.* (1987) and Kumar *et al.* (1993).

Similar decrease in serum protein levels in aflatoxicosis was reported by many other workers (Harvey *et al.*, 1989; Jassar *et al.*, 1993; Shukla and Pachauri, 1995).

2.5.2 Serum albumin and globulin

Pegram *et al.* (1986) observed significant reduction in serum albumin levels following a single oral aflatoxin treatment at a dose of 2.5 mg per kg body weight in a non-selected population of young Japanese quails. A reduction in serum albumin levels was recorded by Harvey *et al.* (1989) in broilers fed aflatoxin B1 at the rate of three milligrams per kilogram of feed.

Ghosh *et al.* (1990) in an experimental study with broilers noticed a reduction in albumin and globulin levels in aflatoxicosis. Feeding of day old cockerels with aflatoxin at the rate of 2.5 mg and 10 mg per g of feed resulted in decreased serum albumin-globulin ratios (Shukla and Pachauri, 1995).

Anbiah (1996) reported a fall in serum albumin and globulin levels in aflatoxin dosed ducks at 10 μg/kg body weight on alternate days from four to eight months of age.

2.6 Gross pathology

2.6.1 Japanese quails

Sawhney *et al.* (1973) observed that the liver was the only organ showing gross pathological changes in experimental aflatoxicosis in the laying Japanese quails fed aflatoxin at two microgram/gram and above in the feed. The liver either appeared normal in size or highly enlarged with varying colouration of brown to tan combined with surface granularities. Doerr and Ottinger (1979) reported similar tan to yellow discolouration of livers of aflatoxin treated juvenile Japanese quails at the rate of 5 and 10 μg in the feed from one to three weeks of age. Smaller and less developed gonads were the main lesions observed by them in the reproductive system of the toxin treated birds.

Panda *et al.* (1987) reported enlarged, pale and fragile liver and kidneys together with regressed or atrophied bursa of Fabricius in experimental aflatoxicosis in young Japanese quails at 0.75 ppm level in the feed.

Sadana *et al.* (1992) reported gross enlargement of liver and kidneys, initial enlargement followed by atrophy of the bursa and ecchymotic haemorrhages in young Japanese quail at 0.5 ppm level.

Kumar *et al.* (1993) observed pale, enlarged livers, distended gall bladder and enlarged kidney with petechial haemorrhages, pale musculature and atrophy of the bursa in quail fed aflatoxin at graded levels from 0.5 to 2.0 ppm in feed for 30 days from 15 to 45 days of age.

Sridevi and Sriraman (1996) reported varying degrees of gross liver lesions ranging from mild enlargement with pin point whitish areas to pale large brownish yellow areas. These changes were proportionate to dose and duration of aflatoxin treatment. The males were more severely affected than females in quails fed two and five ppm in feed from 4 to 12 weeks of age.

2.6.2 Other avian species

Butler (1964) observed various changes in livers of ducklings fed 8.5 μg/g of aflatoxin in the feed. These changes ranged from slight paleness to putty colouration with a few small areas of haemorrhage. There was no conspicuous enlargement at dose levels from 25 μg to 100 μg/g in the feed. Putty coloured livers with numerous yellowish white lesions of

1-2 mm in diameter and nodular lesions of 1-2 mm diameter were also reported in Khaki Campbell ducklings fed seven ppm of aflatoxin B1 in the feed (Carnaghan, 1964).

Thaxton *et al.* (1974) reported atrophy of the bursa and thymus in chicken fed high doses of aflatoxin. Mohiuddin (1982) observed that aflatoxin at the rate of 100-200 μg/day in the diet for 35 days resulted in congestion of liver and atrophy of testes.

Moorthy *et al.* (1986) fed aflatoxin to chicken at the level of 6.25 ppm in the diet and reported development of nodular lesions in the livers of treated birds. Balachandran and Ramakrishnan (1987) observed enlarged, pale discoloured livers with moroccan leather appearance in chicken at one ppm level of aflatoxin in the first two weeks followed by yellow colouration in the next two weeks. At three ppm, enlargement of livers with increased friability was noticed from the first week onwards. Other reported gross lesions were pale, enlarged congested kidneys with a few petechial haemorrhages, spleenomegaly and petechial haemorrhages in the thigh and leg musculature. Ghosh *et al.* (1989) reported mottled livers, pale kidneys, petechiae on heart, spleenomegaly and atrophy of thymus and bursa of Fabricius in chicken fed one ppm aflatoxin B1 in feed for six weeks.

Maryamma *et al.* (1990) fed aflatoxin to ducks at the dose rate of one ppm and observed extensive hepatic lesions of paleness, friability, greyish white nodularity over the parenchyma and distension of gall bladder.

2.7 Histopathology

2.7.1 Japanese quails

Gumbmann *et al.* (1970) in a study to compare the aflatoxin susceptibility in various breeds of poultry observed proliferation of bile duct epithelium and hepatic cells after three weeks of feeding aflatoxin at the rate of 800 ppb, followed by a degree of recovery after five weeks of trial.

Sawhney *et al.* (1973) reported hyperplasia of bile ducts and fatty changes as the only significant changes in the liver of quails fed dietary aflatoxin at levels of two, four or six microgram per gram of feed from 40 days of age upto 72 days.

The livers of aflatoxin sensitive Japanese quail of six to eight weeks administered 0.3 mg/kg body weight by oral intubation were analysed by Dashek *et al.* (1983). They found the abnormalities of bile duct proliferation, cellular vacuolization, collapsing of sinusoids and hepatocyte swelling within five days of toxin administration.

Panda *et al.* (1987) observed various histopathological changes in the quails fed dietary aflatoxin at graded levels of 0 to 0.75 ppm from 6th to 35th day of age. They noticed degeneration of hepatic cells at 0.1 ppm aflatoxin level, hydropic degeneration of a few scattered hepatic cells at 0.3 ppm aflatoxin, confluent areas of hydropic degeneration of hepatic cells at 0.3 ppm and widespread degeneration of hepatic cells with lymphocytic and heterophilic infiltration into sinusoids at 0.75 ppm aflatoxin.

Sadana *et al.* (1992) reported that feeding of aflatoxin B_1 at the rate of 0.5 ppm to young quail chicks resulted in hepatocytomegaly with acidophilic cytoplasm, fatty changes and lymphoid aggregation in hepatic parenchyma with prominent bile duct epithelium. Lesions in kidneys included varying degrees of congestion and swelling of tubular epithelium. Haemorrhages in thigh muscles, mild enteritis characterised by mononuclear cell infiltration and necrosis of superficial epithelium were also evident in a few birds.

Kumar *et al.* (1993) reported fatty changes in hepatic cells, bile duct hyperplasia, degeneration of lining epithelial cells of proximal tubules, haemorrhages in kidneys, pancreas, heart and a lymphoid cell depletion resulting in atrophy of follicles and interfollicular fibrosis in bursa of Fabricius as the histological lesions in young quails fed

graded levels of dietary aflatoxin from 0.5 ppm to 2.0 ppm level for a period of 30 days.

Sridevi and Sriraman (1996) reported fatty changes of varying degrees, numerous hyperplastic nodules, periportal megalocytosis, Kupffer cell hyperplasia, connective tissue proliferation and lymphoid aggregation in the livers of quails dosed with aflatoxin at the rate of two ppm and five ppm for 12 weeks. Subscapular haemorrhages, swelling of tubular epithelial cells, hyperplasia of glomerular epithelium followed by atrophy of glomeruli and renal cell regeneration were the lesions observed in the kidney. The effect was more pronounced in males than females. The lesions in the bursa consisted of a depletion of lymphocytes in the follicular cortex and inter follicular connective tissue proliferation at two ppm level and interfollicular oedema, mild depletion of lymphocytes and intrafollicular cyst formation at five ppm level. In the spleen, a mild depletion of lymphocytes and hypertrophy of blood vessel wall were the lesions encountered.

2.7.2 Other avian species

Butler (1964) observed sequential histopathologcal lesions in Khaki Campbell ducklings exposed to 15 μg of aflatoxin. He reported that by 24 hours after exposure, there was oval cell proliferation throughout the portal system with few mitoses. There was increase in fat at the periphery of

the lobules where many of the cells had pyknotic nuclei. After two days, the oval cell proliferation was more marked with extension into the lobules between the parenchymal cells.

Similarly, Carnaghan (1967) reported that in ducks during aflatoxicosis there was focal lymphoid aggregation in the liver parenchyma comprising mainly of mature lymphocytes surrounded by degenerating hyperplastic bile duct epithelium. Large nodules in the liver encountered in some ducks were hepatomas comprising of hypertrophic ballooned cells occasionally containing giant vesicular nuclei with very prominent nucleoli and cholangiomata, composed of dense masses of well differentiated bile ducts surrounded by a thin fibrous capsule.

Muller *et al.* (1970) observed hepatic cell degeneration in ducklings with aflatoxicosis. Cytoplasmic vacuolisation of hepatic cells and formation of hepatic cells into cylindrical duct-like structures were the typical changes.

Moorthy *et al.* (1986) observed lipidosis, sinusoidal congestion, perivascular haemorrhage, bile duct hyperplasia, phlebitis, pseudo-lobulation and fibrosis around portal areas in the livers of chicken in experimental aflatoxicosis.

Balachandran and Ramakrishnan (1987) conducted an experimental study with one ppm and three ppm level aflatoxin

in chicken for a period of four weeks from 0 to 28 days. They observed hyperemia, cloudy swelling, mild to moderate fatty changes, extensive portal haemorrhages, necrosis of hepatic cells, bile duct hyperplasia, connective tissue proliferation around portal triads, hepatocytomegaly, multifocal collection of heterophils and lymphocytes and periportal fibrosis. The intensity of changes were proportionate to the dose level and duration of exposure to aflatoxin. Tubular degeneration of epithelial cells, haemorrhages and moderate fibroblastic proliferation were the lesions observed in the kidneys. In the spleen, there was lymphoid depletion and reticular hyperplasia at four weeks at three ppm level.

Rao *et al.* (1988) reported lymphoid depletion of spleen and bursa of Fabricius in experimental aflatoxicosis in chicken at 0.5 ppm and 1.0 ppm levels from 0-18 weeks of age.

Ghosh *et al.* (1989) conducted an experimental study with three months old chicken with 0.3 ppm and 1.0 ppm aflatoxin for six weeks. The changes included fatty changes involving the whole lobules of liver, necrosis of heaptocytes, lymphoid cell infiltration in portal areas and hypertrophy of Kupffer cells by 30th day, followed by megalocytosis, sinusoidal dialation, phlebosclerosis and fibroblastic proliferation by 43rd day at 1 ppm level. Lymphoid organs like spleen, bursa of Fabricius and thymus in general exhibited moderate to

marked depletion of lymphocytes, focal haemorrhage in the cortex of thymus and mild proliferation of reticular cells in Malphigian corpuscles of spleen.

Similar histopathological lesions in liver were also reported in aflatoxicosis in chicken by Fukal *et al.* (1990) and Sova *et al.* (1991).

2.8 Effect of aflatoxin on the immune system

2.8.1 Humoral immunity

Brown and Abrams (1965) observed aflatoxin to be an immunosuppressive agent in ducklings producing hypoproteinemia and low globulin levels, making them more prone to salmonella infection.

Pier and Heddleston (1970) reported that aflatoxin at 0.25 to 0.5 ppm level during or after the period of immunization against *Pasteurella multocida* manifested an impaired resistance in 20-67 per cent of turkey poults and chicks. Thaxton *et al.* (1974) noticed impairment of reticulo endothelial system in aflatoxicosis of chicken which resulted in decreased ability to form haemagglutinins.

Turkeys fed 0.5 ppm aflatoxin in the feed before, during and after vaccination with New Castle disease virus vaccine

showed no difference in antibody titre but a lag in interferon was noticed during the first 24 hours in aflatoxin fed birds (Pier *et al.*, 1971).

Tung *et al.* (1975) observed decreased serum immunoglobulin levels in chicken fed 2.5 μg of aflatoxin B_1 per gram of diet, particularly IgG and IgA fraction. Chenchev *et al.* (1978) reported impaired antibody production in response to New Castle disease (Lasota) vaccination in chicks fed 560 μg aflatoxin per kilogram of feed for 20 days.

Iglaz (1983) reported that in chicks aflatoxin caused suppression of immune response to ND vaccination (Lasota strain) either by feeding aflatoxin four days before vaccination or simultaneously with vaccination but not in the birds which were treated with aflatoxin three days after vaccination.

Campbell *et al.* (1983) reported severe hypoproteinemia, lymphocytopenia, decreased relative weight of the bursa of Fabricius and depressed complement activity as indicators of immunosuppression.

Rao *et al.* (1988) reported a proportionate decrease in the serum protein levels in chicks fed aflatoxin in the diet at the rate of 0.5 ppm or more and the immune response against Ranikhet disease virus was decreased. Similar

immunosuppressive effects in aflatoxicosis in chicken as manifested by decreased HI titres and leucocyte counts to Ranikhet disease vaccination were reported by Ozer *et al.*, 1989).

Padmanabhan (1989) put forth the view that the immunosuppressive effect of aflatoxin was antigen specific and aflatoxins produced RNA polymerase inhibition together with increased lysosomal activity resulting in inhibition of both the reticulo endothelial system and the specific immune system.

Rao *et al.* (1990) reported that in Japanese quails aflatoxin caused depletion of lymphocytes in the bursa of Fabricius. A significant reduction in bursal weights and immune status to New Castle disease in aflatoxin treated chicken at graded levels from 0 to 2.0 ppm in diet from zero to eight weeks were reported by Mani *et al.* (1993).

2.8.2 Cell mediated immunity

Pier *et al.* (1972) observed thymic involution and depression of delayed hypersensitivity in turkeys treated with 0.5 ppm aflatoxin. Chicken fed a diet containing 2.5 μg of aflatoxin/gram of feed from 0 to 84 weeks were deficient in cell mediated immunity, as evidenced by poor graft versus host reaction (Giambrone *et al.*, 1978).

A reduction in chemotaxis and poor phagocytic activity of heterophils and monocytes was observed in chicken fed a diet containing 2.5 μg AFB_1 (Chang and Hamilton, 1979).

Giambrone *et al.* (1985) noticed that in turkeys crude aflatoxin at a low dose of 20 ppb was sufficient to suppress the CMI whereas in broiler chicken the same level of suppression of CMI was attained at a higher dose level of 200 ppb, indicating a species variability in the degree of immunosuppression.

Aflatoxin had more pronounced effect on the CMI and complement system than humoral immunity in poultry and cattle (Pier *et al.*, 1971).

Mohiuddin *et al.* (1986) reported a marked depression of the phagocytic activity by heterophils and Kupffer cells in aflatoxicosis in chicken. A similar reduction in phagocytosis was observed in aflatoxicosis in a comparable study in chicken by Kadian *et al.* (1988).

Rao *et al.* (1988) also observed reduced CMI in chicks in experimental aflatoxicosis as measured by lymphocyte count, cutaneous reaction to 2,4 dinitrochlorobenzene and graft 26 versus host reaction. A decrease in ANAE positive cells in chicken was noticed in experimental aflatoxicosis by Ghosh *et al.* (1990).

Materials and Methods

MATERIALS AND METHODS

3.1 Experimental design

One hundred and five clinically healthy, one month old Japanese quails procured from the University Poultry Farm, Mannuthy, Kerala, were used for the study. The quails were obtained from batches which had been administered Lasota vaccine on the 7th day in the University Poultry Farm itself. The birds were randomly divided into three groups viz., Group A, B and C of thirty five birds each and were banded with numbers for proper identification. The birds were maintained in deep litter in separate rooms and were given commercial quail feed tested and found free of aflatoxin. Water was given *ad libitum*. The birds were maintained for a period of two months.

Group A

This group served as the control group. All the birds in this group were administered Ranikhet booster vaccine (R_2B strain) at the age of 46 days. They were maintained for a maximum period of two months.

Group B

This group consisted of thirty five birds which were given purified aflatoxin B_1 at the dose rate of 0.5ppm body

weight (i.e. 0.5 μg/g body weight) twice weekly throughout the experimental period starting from the first week of the experiment.

Group C

This group consisted of thirty five birds which were given purified aflatoxin B_1 at the dose rate of 0.5ppm body weight twice weekly throughout the experimental period. These birds were also administered Ranikhet booster vaccine (R_2B strain) at the age of 46 days.

Seven birds from each group were sacrificed at fortnightly intervals starting from 0 day till the end of the experimental period (i.e. 0 day, 15th day, 30th day, 45th day, 60th day).

3.1.1 Aflatoxin B_1

Pure aflatoxin B_1 was obtained from the M/s Sigma Chemical Co., St. Louis, U.S.A. Fifty milligram of the toxin was dissolved in five ml of rectified spirit to make a stock solution of the toxin. Each millilitre of the stock solution was further reconstituted to 100 ml with distilled water for administration. This reconstituted solution contained 0.05 mg and 0.1 mg of pure toxin in 0.5 ml and one ml respectively. Quails in the group B and C were administered appropriate dose

of the reconstituted toxin solution corresponding to the body weight by oesophageal intubation twice weekly till the end of the experiment.

The control birds (Group A) were given the vehicle at appropriate concentration on the days when the birds of Group B and Group C were fed aflatoxin B_1.

3.2 Experimental parameters

Weekly body weights, haemogram values (Erythrocyte sedimentation rate, packed cell volume, total erythrocyte count, total leucocyte count and differential count) serum total protein levels, serum albumin concentration, serum globulin concentration, albumin-globulin ratios, organ weights of liver, spleen, bursa and haemagglutination inhibition titres were determined at fortnightly intervals during the experimental period. The birds were observed daily for any clinical symptoms.

3.3 Techniques

3.3.1 Body weight

All the birds were weighed individually at weekly intervals.

3.3.2 Collection of blood samples for laboratory examination

Seven birds from each group were sacrificed at fortnightly intervals (0 day, 15th day, 30th day, 45th day, 60th day) by decapitation for collection of blood. Approximately two ml of blood was collected for haematological studies using dipotassium salt of ethylene diamine tetra acetic acid (EDTA) at the rate of 1 mg/ml of blood as the anticoagulant. One millilitre of blood was collected separately in a sterile test tube without adding anticoagulant for serum separation. Peripheral blood smears were made from the wing vein prior to sacrificing the birds for differential count.

3.3.3 Separation of serum

The blood collected from the birds individually was left in the test tubes for one hour at room temperature, then kept at 4°C for six hours for contraction of the clot and the separated serum was collected in sterile vials and stored at -20°C until used.

3.3.4 Haemogram

Erythrocyte sedimentation rate and packed cell volume were estimated on 0, 15th, 30th, 45th and 60th day of the experiment using the method described by Wintrobe (1981).

Haemoglobin level was estimated on 0 day, 15th day, 30th day, 45th and 60th day employing the cyanmethoglobin method described by Miale (1967) and the final readings were taken in an Erma photometer with Haemochek(R) solution by Agappe Diagnostics Pvt. Ltd.

Total erythrocyte count (TLC) and total leucocyte count (TLC) were determined as per the method described by Sastry (1976). The differential leukocyte count (DLC) was done with copper peroxidase method of Sato and Sekiya (1965).

3.4 Serum chemistry

3.4.1 Total protein, albumin, globulin and albumin-globulin ratio

The estimation of total serum protein (TSP) and serum albumin (SAL) was done by using Qualigens commercial total protein and albumin estimation kits (Glaxo, India) and Chemetrics analyser. By deducting SAL from TSP, serum globulin (SGL) was determined. Serum albumin-globulin ratios were calculated using the above values.

3.4.2 Haemagglutination inhibition titre

The HI titres of the individual samples of serum collected from different groups at 30th, 45th, 60th, 75th and 90th day age of the birds (i.e., 0, 15, 30, 45 and 60 day of

experiment respectively) were estimated using microhaemagglutination trays with well (Laxbro) by beta method of evaluation of titres.

3.5 Organ weights

The weights of the liver, spleen and bursa were determined in all the sacrificed birds. The weight of liver comprised of the gall bladder with its contents, spleen-with its capsule and bursa trimmed of adnexa.

3.6 Histopathology

Birds which were sacrificed at different intervals or those found dead during the course of the experiment were subjected to detailed autopsy examination. Gross lesions in different organs were recorded. Liver, spleen and bursa were collected for determination of organ weights and representative samples of tissue of liver, spleen, and bursa were collected for histopathological studies. Tissues were fixed in 10 per cent neutral buffered formalin and were processed by the routine method. Paraffin sections cut at 4-5 μm thickness were stained with Harris haematoxylin and eosin as described by Sheehan and Hrapchak (1980).

3.7 Statistical analysis

The data obtained from various clinical parameters were subjected to analysis of variance (ANOVA) as explained by Snedecor and Cochran (1967).

Results

RESULTS

4.1 Body weight

All the birds belonging to the experimental as well as the control groups showed increase in body weights throughout the experimental period. Although the aflatoxin fed birds (Groups B and C) had depressed mean body weights from that of controls, there was no significant difference between the control and experimental groups in their weekly body weights. The values are shown in Table 1.

4.2 Organ weights

4.2.1 Liver weight

There was significant increase ($P<0.01$) in liver weights of aflatoxin treated birds (Group B and C) at both 45th and 60th day of the experiment from that of the control birds. The values are tabulated in Table 2 and shown in Fig.1.

4.2.2 Spleen weights

Birds of group B and Group C showed a relative increase in spleen weights from that of controls throughout the experimental period with a significant increase ($P<0.05$) on the 60th day of the experiment as shown in Table 2 and Fig.2.

Table 1. Average (Mean±S.E.) weekly body weight

Sl. No.	Experimental period	Group A (g)	Group B (g)	Group C (g)
1.	7th day	96.321± 3.137	95.179± 3.076	92.286± 2.975
2.	14th day	107.857± 3.594	105.429± 3.065	105.036± 3.420
3.	21st day	118.857± 3.976	113.952± 2.808	112.333± 3.613
4.	28th day	127.66± 2.978	124.952± 2.506	124.143± 3.334
5.	35th day	140.786± 3.356	135.714± 3.310	134.286± 2.987
6.	42nd day	151.643± 3.643	144.786± 3.216	145.143± 3.835
7.	48th day	155.571± 4.184	150.143± 3.019	148.714± 3.832
8.	60th day	162.00± 3.885	154.714± 2.842	155.143± 3.983

Table 2. Average (Mean±S.E.) organ weights

Sl. No.	Experimental period (in days)	Liver (g)			Spleen (g)			Bursa (g)		
		Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
1.	0	2.817 ± 0.0601	2.780 ± 0.0712	2.847 ± 0.0834	0.0523± 0.0037	0.0561± 0.0048	0.0592± 0.0052	0.0458± 0.0037	0.0472± 0.0042	0.0463± 0.0061
2.	15	3.074 ± 0.1137	3.137 ± 0.1248	3.211 ± 0.1209	0.0661± 0.0075	0.0722± 0.0037	0.0737± 0.0094	0.0467± 0.0037	0.0501± 0.0059	0.0540± 0.0042
3.	30	3.2402± 0.1360	3.6137± 0.1285	3.6533± 0.1474	0.0636± 0.0075	0.0860± 0.0056	0.08637± 0.0113	0.0862± 0.0056	0.0764± 0.0078 *	0.0701± 0.0151 *
4.	45	3.4992± 0.0793	3.9226± 0.0926 **	4.0494± 0.1133 **	0.1102± 0.0415	0.1227± 0.0461	0.1354± 0.0521	0.0874± 0.0037	0.0721± 0.0042 **	0.0735± 0.0064 **
5.	60	3.8102± 0.0756	4.3852± 0.1285 **	4.4701± 0.1928 **	0.1202± 0.0038	0.1623± 0.0078 *	0.1674± 0.0056 *	0.0732± 0.0038	0.0591± 0.0075 *	0.0602± 0.0567 *

* Significant at 5% level (P<0.05)
** Significant at 1% level (P<0.01)

Fig.1 AVERAGE LIVER WEIGHT (g)

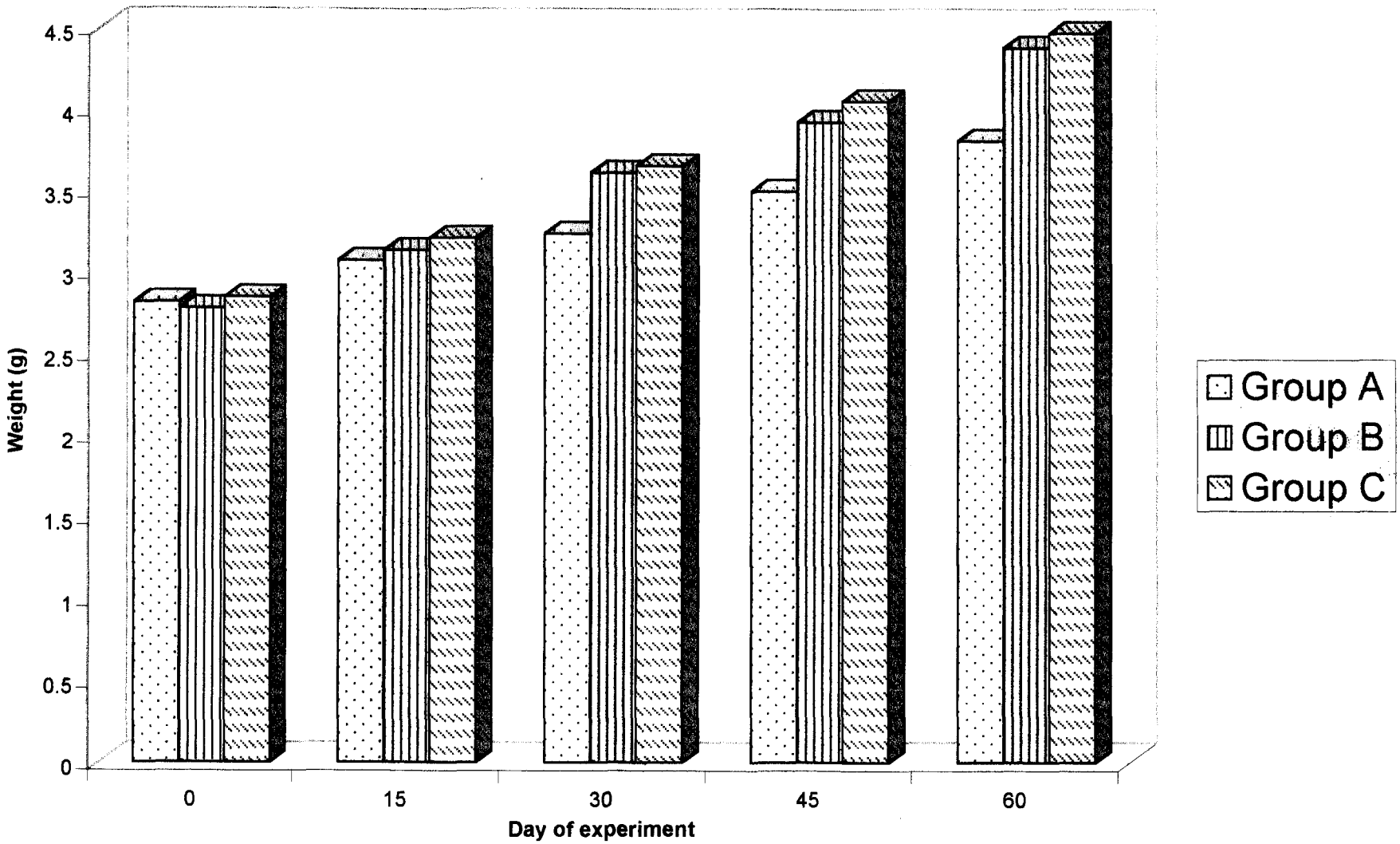


Fig.2 : AVERAGE SPLEEN WEIGHT (g)

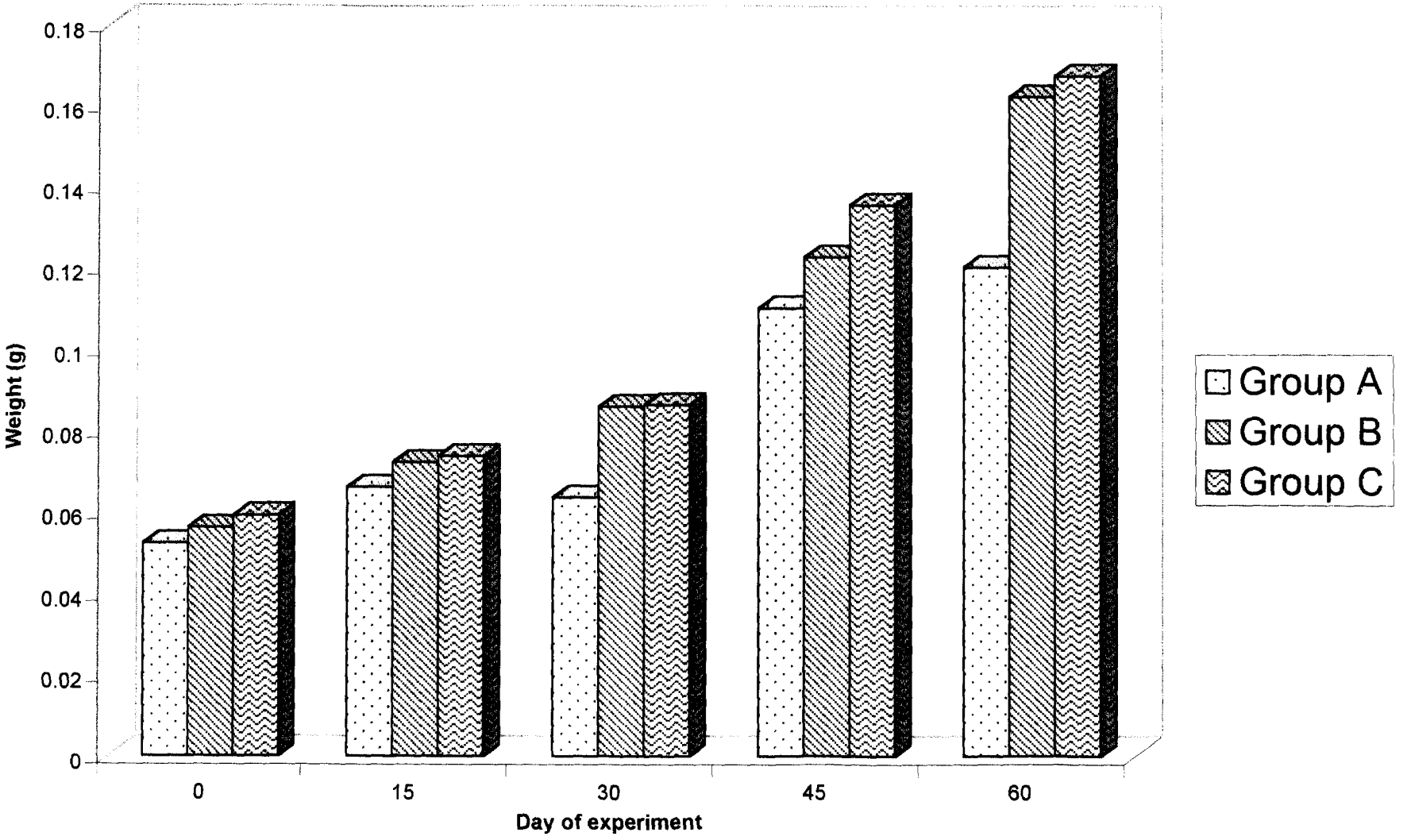
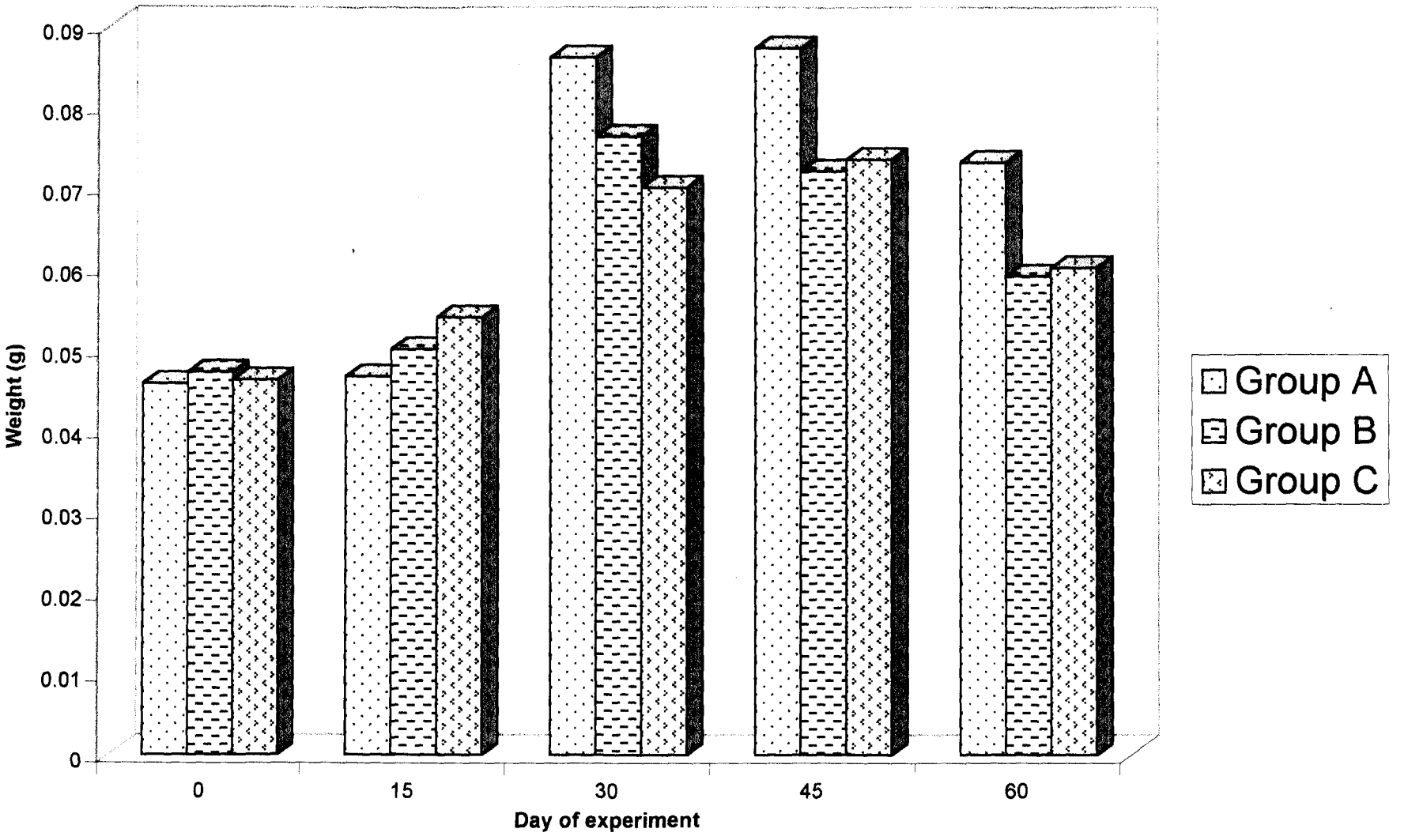


Fig.3 AVERAGE BURSA WEIGHT (g)



4.2.3 Bursal weights

The aflatoxin fed birds (Group B and C) showed significantly depressed bursal weights on the 30th day ($P<0.05$), 45th day ($P<0.01$) and 60th day ($P<0.05$) of the experimental period from that of controls. The values are tabulated in Table 2 and shown in Fig.3.

4.3 Haemogram

4.3.1 Haemoglobin

There was no significant difference in the haemoglobin values between the aflatoxin treated groups (Group B and C) and the control group (Group A) throughout the experimental period. The values are tabulated in Table 3a.

4.3.2 Packed cell volumes

A significant decrease in the packed cell volume ($P<0.05$) of the experimental groups (Group B and C) was noticed from that of controls (Group A) on the 60th day of the experiment. The results are detailed in Table 3a and shown in Fig.4.

4.3.3 Erythrocyte sedimentation rate

There was a significant increase ($P<0.05$) in the erythrocyte sedimentation rate of the aflatoxin treated groups

Table 3a. Average (Mean±S.E.) haemogram values

Experimental period (in days)	Hb (g/dl)			PCV (%)			ESR (mm/hr)			RBC ($10^6/\mu l$)			WBC ($10^3/\mu l$)		
	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
0	10.129± 0.3779	10.000± 0.3817	9.914± 0.3212	33.286± 0.6047	32.286± 0.7241	33.571± 0.8428	0.929± 0.170	0.929± 0.170	1.214± 0.1436	3.961± 0.1285	3.980± 0.158	3.870± 0.1474	32253.286± 360.956	32175.714± 337.900	31904.000± 453.55
15	11.00 ± 0.2381	10.629± 0.2532	10.557± 0.3488	34.857± 1.2624	33.571± 1.3267	34.429± 0.9486	1.214± 0.2154	1.286± 0.1814	1.357± 0.0907	4.227± 0.2118	4.159± 0.1474	4.256± 0.1133	32127.571± 577.124	31347.286± 621.751	31288.857± 600.207
30	11.957± 0.3893	11.643± 0.4164	11.581± 0.3124	36.000± 0.8995	35.571± 0.7823	34.178± 1.149	1.357± 0.0907	1.714± 0.1020 *	1.643± 0.1171 *	4.721± 0.1247	4.424± 0.0944	4.384± 0.1360	33047.571± 538.92	31682.286± 503.072	32603.000± 464.140
45	12.271± 0.1587	11.649± 0.4384	11.587± 0.4082	36.000± 1.572	33.857± 1.247 *	34.714± 1.852 *	1.071± 0.2003	1.714± 0.2418 *	1.929± 0.2683 *	4.807± 0.1549	4.580± 0.1247 *	4.417± 0.1663 *	34190.429± 234.33	33387.143± 367.381 *	32852.714± 394.562 *
60	12.743± 0.5215	12.229± 0.4006	12.571± 0.6228	39.857± 1.2435	35.429± 1.1112	34.868± 0.9978	1.071± 0.1700	1.786± 0.1889	1.714± 0.1020	4.819± 0.1360	4.283± 0.1096	4.179± 0.0642	34443.714± 298.59	33132.643± 337.90	33045.236± 541.67

* Significant at 5% level ($P<0.05$)
** Significant at 1% level ($P<0.01$)

Fig.4 : AVERAGE PACKED CELL VOLUME (%)

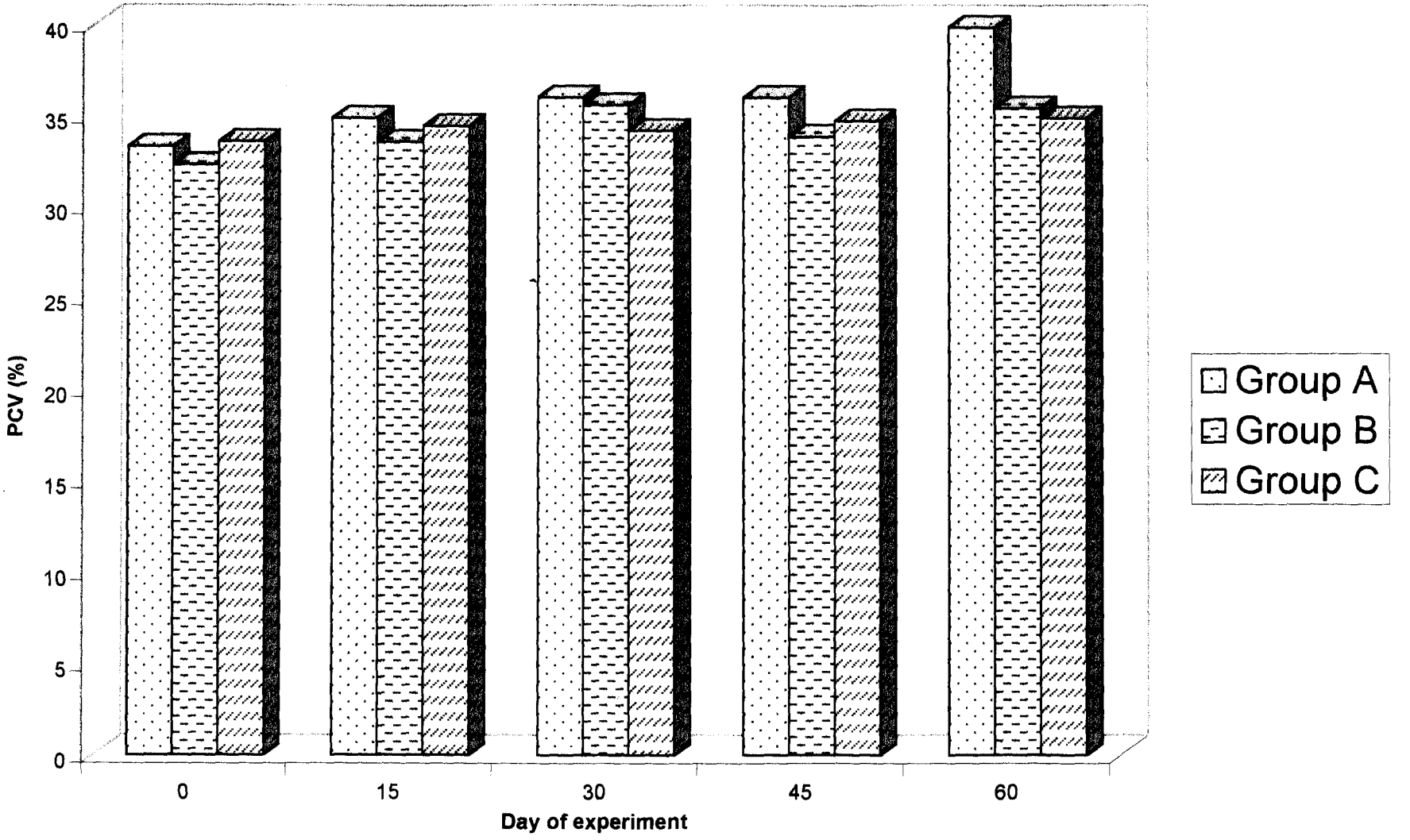


Fig. 5: AVERAGE ERYTHROCYTE SEDIMENTATION RATE (mm/hr)

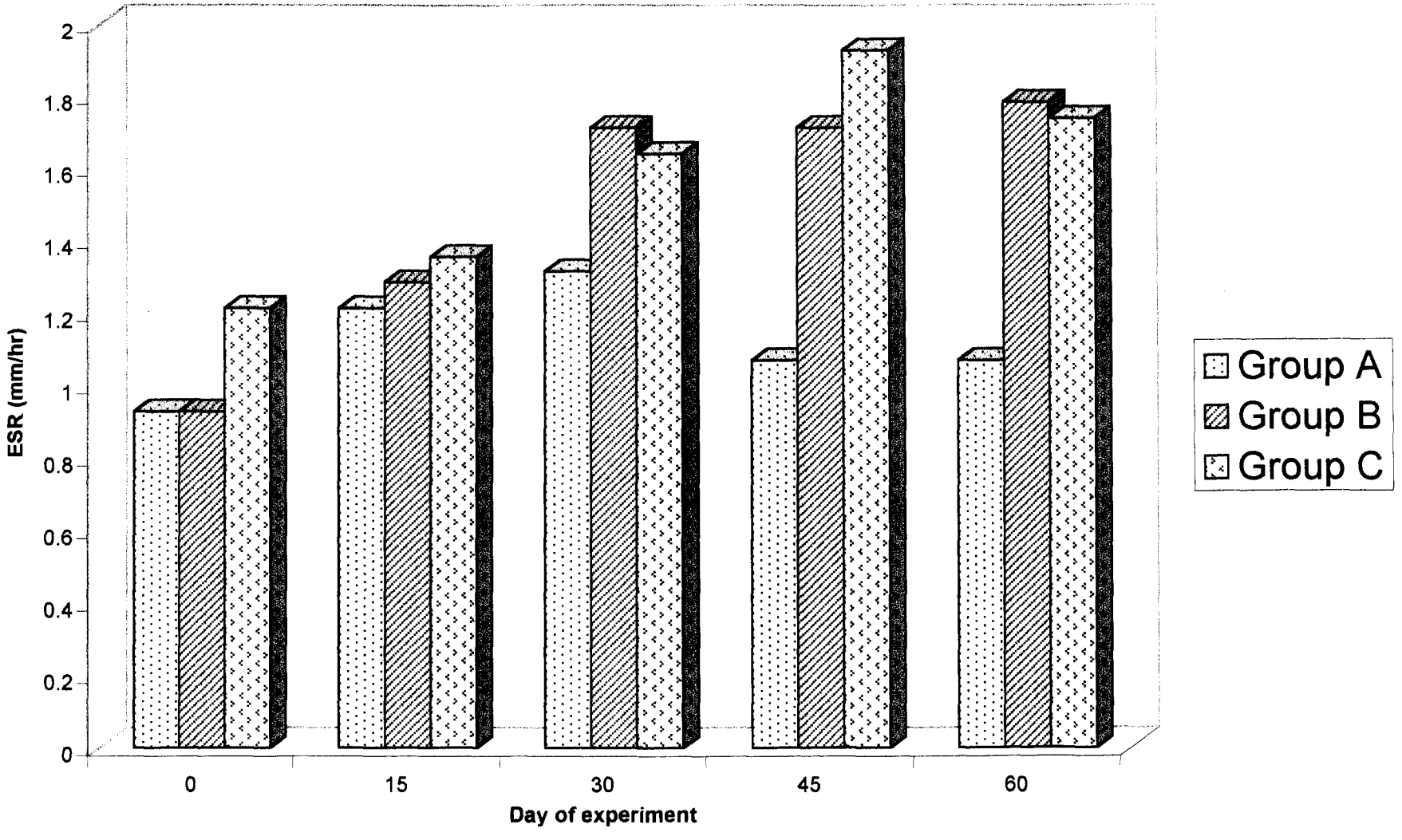


Fig.6: AVERAGE TOTAL ERYTHROCYTE COUNT (10^6/ul)

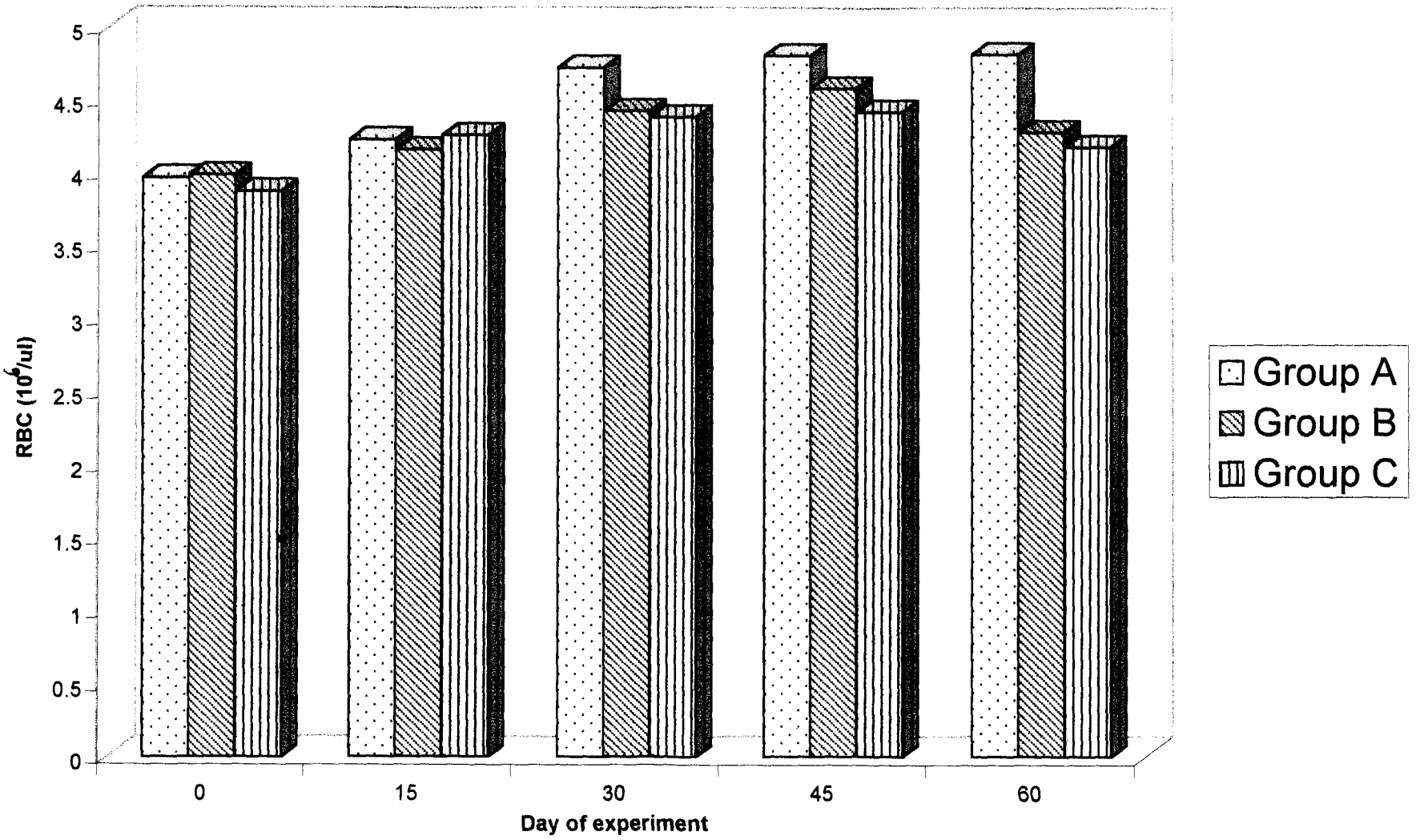
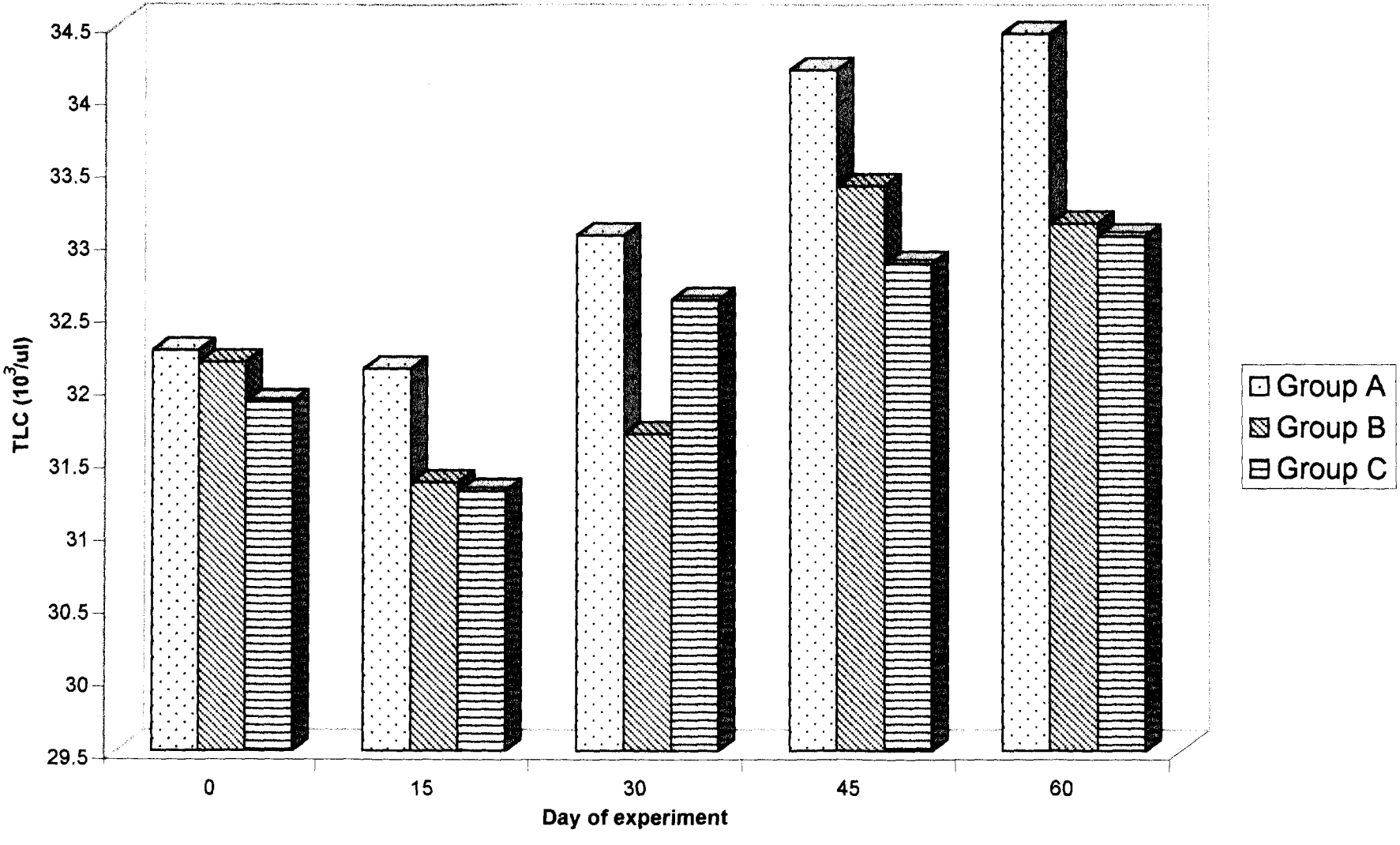


Fig.7: AVERAGE TOTAL LEUCOCYTE COUNT (10^3/ul)



(Groups B and C) from that of controls on the 45th and 60th day of the experiment. These values are tabulated in Table 3a and shown in Fig.5.

4.3.4 Erythrocyte count (RBC)

The values are detailed in Table 3a and shown in Fig.6. A significant decrease ($P<0.05$) in the RBC values of the aflatoxin treated birds (Group B and C) from that of controls was evident on the 60th day of the experiment.

4.3.5 Leucocyte count (WBC)

A significant decrease ($P<0.05$) in the leucocyte counts was noticed in the toxin treated birds (Group B and C) from that of controls only on the 60th day of the experiment. The values are tabulated in Table 3a and shown in Fig.7.

4.3.6 Differential leucocyte count (DLC)

There was no significant variation in the percentage of heterophils, lymphocytes, eosinophils, monocytes and basophils between the control and experimental groups throughout the experimental period. The toxin treated birds showed a relative increase in heterophil count and a relative decrease in lymphocyte count from that of the controls throughout the experimental period. The values are detailed in Table 3b.

Table 3(b). Average (Mean±S.E.) haemogram values

Experimental period (in days)	Heterophils (%)			Lymphocyte (%)			Eosinophils (%)			Monocyte (%)			Basophils (%)		
	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
0	26.714± 1.474	26.857± 1.383	27.000± 1.674	67.714± 1.583	67.571± 1.141	66.594± 1.043	1.857± 0.086	1.714± 0.200	2.428± 0.117	3.428± 0.102	3.285± 0.207	3.571± 0.105	0.286± 0.094	0.428± 0.105	0.286± 0.094
15	28.571± 1.043	28.143± 1.957	27.285± 1.424	66.000± 1.799	66.571± 1.515	68.008± 1.428	1.714± 0.086	1.857± 0.064	1.571± 0.098	3.143± 0.181	3.428± 0.170	2.857± 0.090	0.286± 0.035	0.286± 0.035	0.286± 0.035
30	29.142± 1.587	32.142± 0.910	32.428± 0.748	65.571± 1.228	63.857± 1.039	62.714± 1.039	1.571± 0.071	1.286± 0.075	1.714± 0.143	3.286± 0.117	2.286± 0.102	2.857± 0.136	0.429± 0.094	0.429± 0.094	0.286± 0.105
45	28.000± 1.602	30.285± 1.492	29.857± 1.920	66.571± 1.281	65.857± 1.504	64.285± 1.568	1.571± 0.071	1.714± 0.094	1.714± 0.056	3.285± 0.117	3.714± 0.014	3.429± 0.102	0.571± 0.105	0.429± 0.094	0.571± 0.105
60	28.857± 0.827	32.857± 2.158	33.429± 1.700	65.714± 0.703	62.286± 1.659	61.286± 2.233	1.714± 0.200	1.428± 0.143	1.857± 0.098	3.142± 0.050	3.000± 0.128	2.714± 0.090	0.571± 0.105	0.429± 0.094	0.714± 0.105

Fig.9 : AVERAGE SERUM TOTAL PROTEIN (g/dl)

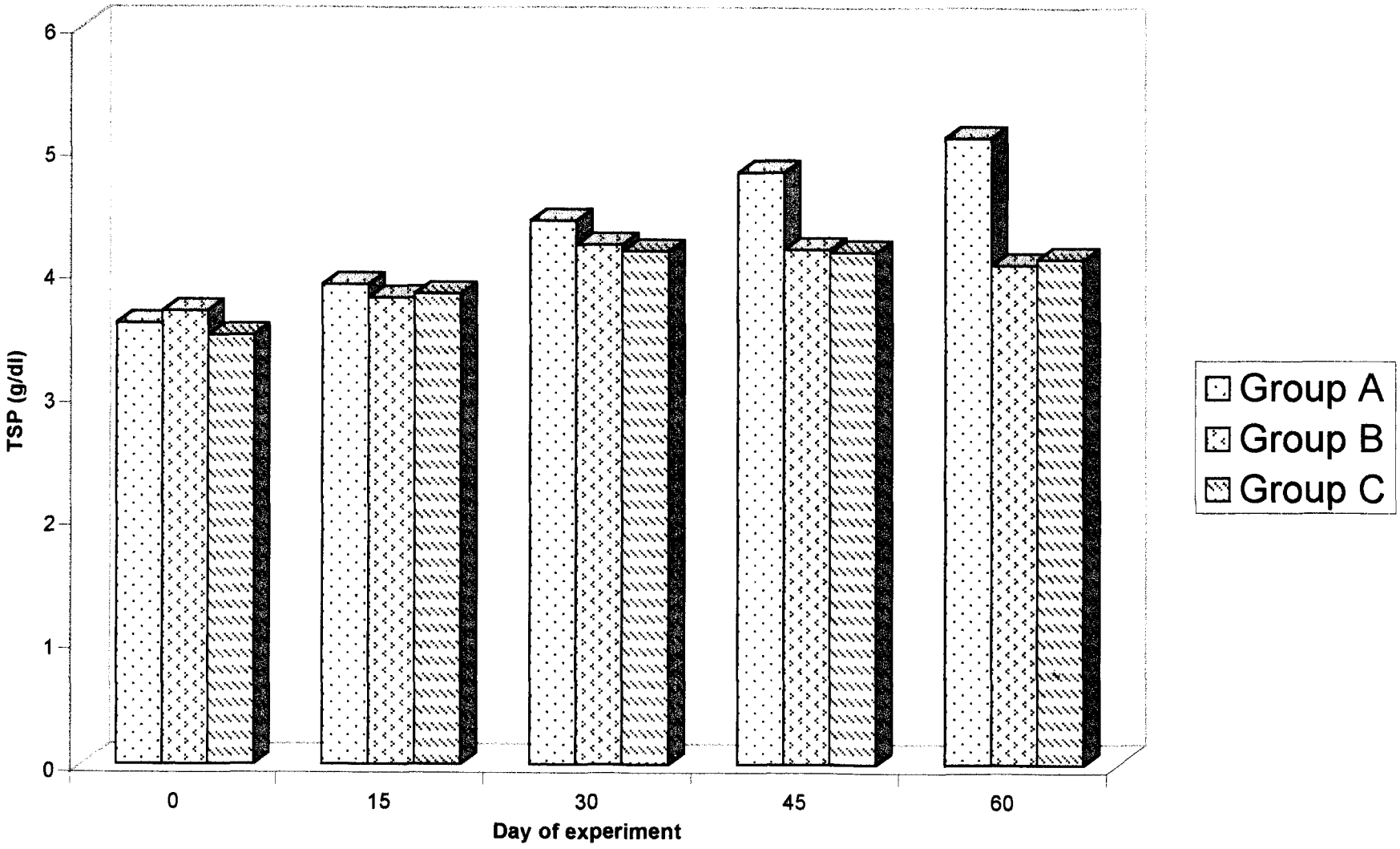


Fig.9 : AVERAGE SERUM ALBUMIN (g/dl)

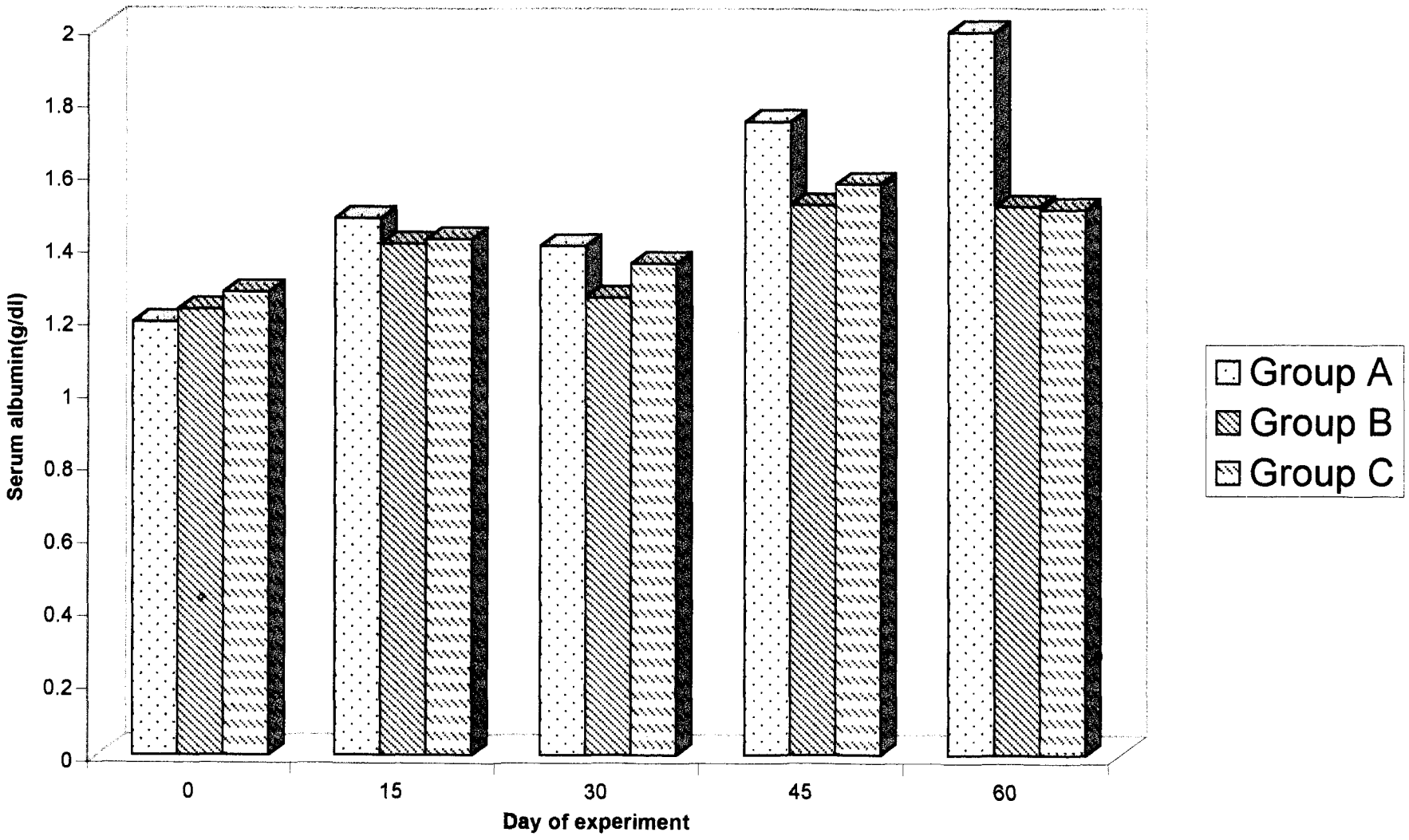
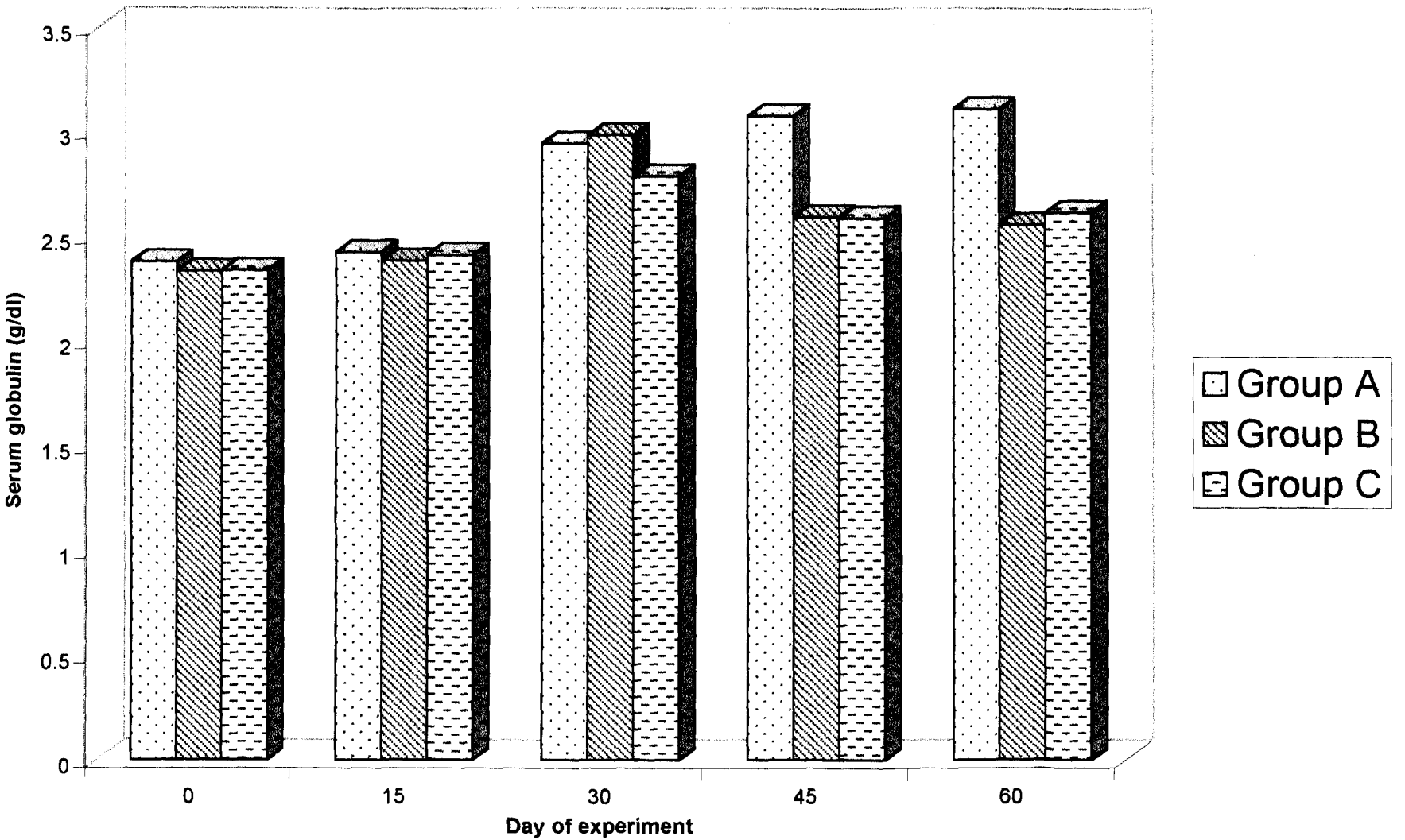


Fig.10 : AVERAGE SERUM GLOBULIN (g/dl)



4.4 Serum protein assay

4.4.1 Total serum protein

A significant decrease ($P<0.01$) in the serum protein levels of the aflatoxin treated birds (Group B and C) from that of controls was evident on the 45th and 60th day of the experiment. The values are shown in Table 4 and Fig.8.

4.4.2 Serum albumin

A significant decrease in the albumin values of the aflatoxin treated birds (Groups B andC) from that of controls was observed on the 45th day ($P<0.05$) and on the 60th day ($P<0.01$) of the experiment. The values are tabulated in Table 4 and represented in Fig.9.

4.4.3 Serum globulin

A significant depression ($P<0.01$) in the serum globulin levels of the aflatoxin treated groups (Group B and C) was noticed on the 45th and 60th day of the experiment. The values are shown in Table 4 and Fig.10.

4.4.4 A/G ratio

There was no significant difference in the albumin: globulin ratio between the toxin treated birds and the control birds throughout the experiment. The data are shown in Table 4.

Table 4. Average (Mean±S.E.) of TSP, SAL, SGL and albumin-globulin ratio

Experimental period (in days)	Total serum protein (g/dl)			Serum albumin (g/dl)			Serum globulin (g/dl)			A/G ratio		
	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
0	3.578± 0.139	3.679± 0.188	3.483± 0.090	1.187± 0.037	1.222± 0.045	1.270± 0.026	2.377± 0.120	2.334± 0.158	2.339± 0.124	0.508± 0.094	0.524± 0.154	0.543± 0.102
15	3.900± 0.049	3.791± 0.109	3.827± 0.120	1.474± 0.068	1.404± 0.034	1.416± 0.045	2.426± 0.071	2.385± 0.098	2.412± 0.086	0.608± 0.162	0.589± 0.090	0.594± 0.113
30	4.422± 0.071	4.227± 0.102 **	4.175± 0.120 **	1.399± 0.083	1.257± 0.064 *	1.351± 0.037 *	2.949± 0.105	2.987± 0.944 **	2.790± 0.139 **	0.470± 0.162	0.418± 0.124	0.486± 0.132
45	4.818± 0.132	4.193± 0.120 **	4.166± 0.102 **	1.741± 0.084	1.515± 0.086 **	1.571± 0.052 **	3.078± 0.072	2.595± 0.076 **	2.589± 0.126 **	0.566± 0.094	0.566± 0.109	0.607± 0.068
60	5.100± 0.094	4.066± 0.107	4.114± 0.094	1.988± 0.056	1.508± 0.068	1.499± 0.094	3.112± 0.068	2.558± 0.056	2.615± 0.147	0.651± 0.071	0.591± 0.120	0.576± 0.124

* Significant at 5% level ($P<0.05$)
** Significant at 1% level ($P<0.01$)

4.5 Haemagglutination inhibition titre values

The values are tabulated in Table 5 and shown in Fig.14.

There was no significant difference between the mean HI titre values of aflatoxin treated birds (Group B) and the control birds (Group A). However, a relative decrease in the mean titre values of the toxin treated birds was noticed from that of controls throughout the experiment.

4.6 Clinical symptoms

In general, the birds of the toxin fed groups were dull in appearance, particularly during the first half of the experimental period. During the second and third week of the experiment, the toxin treated birds showed signs of enteritis with fluid droppings.

The control birds were healthy and active throughout the experiment.

4.7 Gross pathology

4.7.1 1st fortnight (15th day)

Grossly, the livers of the toxin treated birds were brown to tan coloured with a few pin point haemorrhages in focal

Table 5. Average (Mean±S.E.) haemagglutination inhibition titre values

Sl. No.	Experimental period (in days)	Group A	Group B	Group C
1.	0	548.571± 58.962	525.714± 75.808	502.857± 88.526
2.	15	297.142± 29.507	251.429± 32.323	274.286± 29.507
3.	30	1174.280± 153.901	68.571± 7.377	914.286± 129.297
4.	45	960.000± 156.144	12.857± 2.857	777.143± 137.144
5.	60	434.286± 75.808	0.000	297.143± 64.650

Group A - Lasota vaccine + R_2 B vaccine
Group B - Lasota vaccine + Toxin
Group C - Lasota vaccine + Toxin + R_2 B vaccine

areas. The spleen, bursa and other organs were apparently normal in the toxin treated birds.

4.7.2 2nd fortnight (30th day)

The livers of the toxin treated birds were slightly swollen in appearance, pale to tan in colour with a few pin point haemorrhages in focal areas. The bursa of the toxin treated birds were slightly swollen and the spleen was congested. A few of the birds of experimental group also showed petechial haemorrhages in the muscles of the breast and thigh region (Fig.11). The lungs and kidneys were congested. Mild catarrhal enteritis was observed in a few birds.

The control birds did not reveal any gross pathological changes.

4.7.3 3rd fortnight (45th day)

The livers of the toxin treated birds were slightly enlarged in size, pale yellow in colour with rounded edges, a few of them showed pin point haemorrhages in focal areas on different lobes. The bursa was enlarged and the cut surface revealed the presence of increased colloid. The lungs and spleen were congested.

4.7.4 4th fortnight (60th day)

The livers of the toxin treated birds in general were grossly enlarged, pale yellow in colour, smooth and glistening with rounded edges (Fig.12). A few petechiae could be seen in focal areas on the capsular surface. The gross enlargement and intensity of yellowness of livers were more pronounced in the females than the males. The spleen was congested and some of the toxin treated birds exhibited splenomegaly. The lungs and kidney were congested and dark in appearance. A few birds of the toxin group also revealed petechial haemorrahges in the muscles of the thigh and breast region.

The control birds did not reveal any significant gross pathological lesions on examination.

4.8 Histopathology

4.8.1 Liver

4.8.1a 1st fortnight

The changes noticed in the livers of the toxin treated birds were focal haemorrhages, diffuse degeneration and necrosis of hepatocytes, focal mononuclear infiltration (Fig.13) dilatation of central vein, venous congestion, diffuse kupffer cell reaction and mild bile duct hyperplasia. The livers of the control birds were apparently normal and did not reveal any histopathological alterations.

4.8.1b 2nd fortnight

The liver of the toxin treated birds exhibited mild vacuolation of hepatocytes, all lobes were more or less uniformly involved. Besides, central venous congestion, focal infiltration of inflammatory cells, hepatocytomegaly, bile duct hyperplasia, and perivasular collections of inflammatory cells in the form of nodules (Fig.14 and 15) were the other significant changes observed.

4.8.1c 3rd fortnight

The pathological alterations were more or less similar in nature to the preceeding fortnight but were of a higher intensity. There was central venous congestion, bile duct hyperplasia, paracentral coagulative necrosis of hepatocytes, diffuse vacuolation, the vacuoles were large in some and smaller in others with diffuse involvement of all the lobules (Fig.16).

4.8.1d 4th fortnight

The liver of the toxin treated birds exhibited intense sinusoidal congestion, focal hepatocyte necrosis, infiltration of few inflammatory cells around the necrotic cells, extensive fatty changes (Fig.17), formation of very large vacuoles in many hepatocytes, presence of fatty cysts in some liver and bile duct hyperplasia were the prominent lesions observed.

The fatty changes were more pronounced in the liver of toxin treated females than that of males.

4.8.2 Spleen

4.8.2a 1st fortnight

The changes noticed in the spleen of birds treated with aflatoxin were necrosis of lymphocytes in focal areas of the cortex and the necrosed areas were occupied by macrophages with two to three nuclei.

4.8.2b 2nd fortnight

The spleen of the toxin groups showed mild cortical lymphoid depletion vascuar sclerosis and diffuse degeneration and necrosis of lymphocytes in the medullary areas (Fig.18).

4.8.2c 3rd fortnight

The changes observed in the toxin treated birds were cortical and paracortical lymphoid depletion (Fig.19), degeneration and necrosis of lymphocytes of higher intensity when compared to the previous fortnight. The spleens of the control birds were apparently normal on microscopic examination.

4.8.2d 4th fortnight

The pathological changes observed in the spleen of toxin treated birds were of higher intensity as compared to the previous fortnights. They were diffuse lymphoid depletion of the cortical and paracortical areas, degeneration and necrosis of lymphocytes, congestion and vascular sclerosis (Fig.20). The spleen of the control birds did not reveal any changes.

4.8.3 Bursa

4.8.3a 1st fortnight

The bursa of the toxin treated birds showed diffuse depletion and necrosis of lymphocytes from different follicles, (Fig.21) widening of the interfollicular space and focal interfollicular and intra follicular edema. The bursae of control birds were apparently normal on examination.

4.9.3b 2nd fortnight

The changes noticed in the bursa of the toxin treated birds were proliferation of interfollicular connective tissue and loss of lymphocytes in such follicles, depletion of lymphocytes in the follicles, extensive edema and intrafollicular cyst formation (Fig.22 and 23).

4.9.3c 3rd fortnight

The bursae of the toxin treated birds revealed follicular edema, complete loss of lymphocytes and epithelial components, proliferation of interfollicular connective tissue with cyst formation and lymphoid depletion of higher degree than the previous fortnight. The bursae of the control birds did not reveal any changes.

4.9.3d 4th fortnight

In general, the bursae of the toxin treated birds showed increased intensity of changes as compared to the previous fortnights. The changes observed were depletion of groups of follicles with cyst formation and interfollicular edema, increased fibrous tissue proliferation and degeneration and necrosis of lymphocytes in many follicles (Fig.24).

The bursae of the control birds were normal in appearance on examination.

Fig.11. **Diffuse haemorrhage in the breast region aflatoxin B_1 fed quail - 30th day**

Fig.12. **Liver - aflatoxin B_1 - hepatomegaly, yellowish discolouration - 60th day**

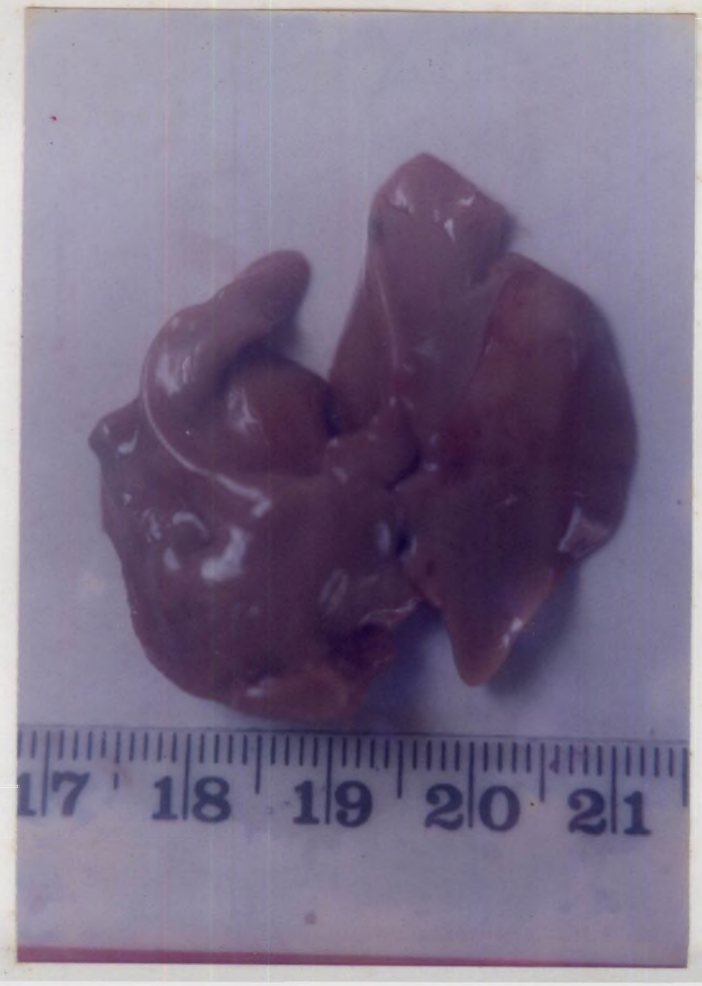

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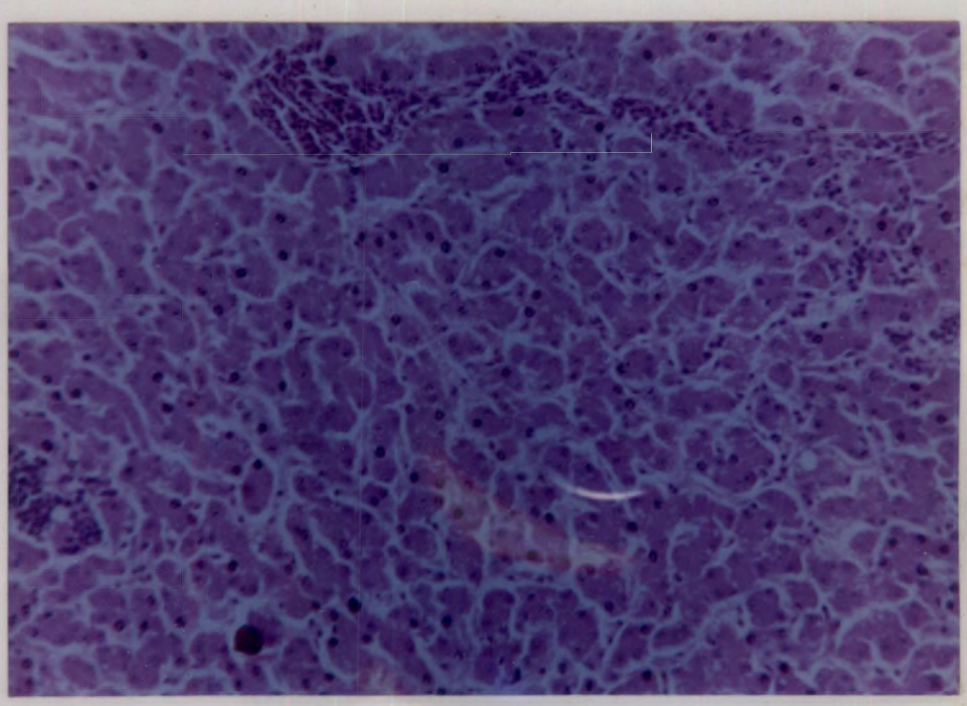
Fig.13. **Liver - haemorrhage, diffuse hepatic degeneration and focal mononuclear infiltration - aflatoxin B_1 - 15th day - H&E x400**

Fig.14. **Liver - Central venous dilatation, necrosis of hepatic cells and Kupffer cell reaction - aflatoxin B_1 - 30th day - H&E x 250**



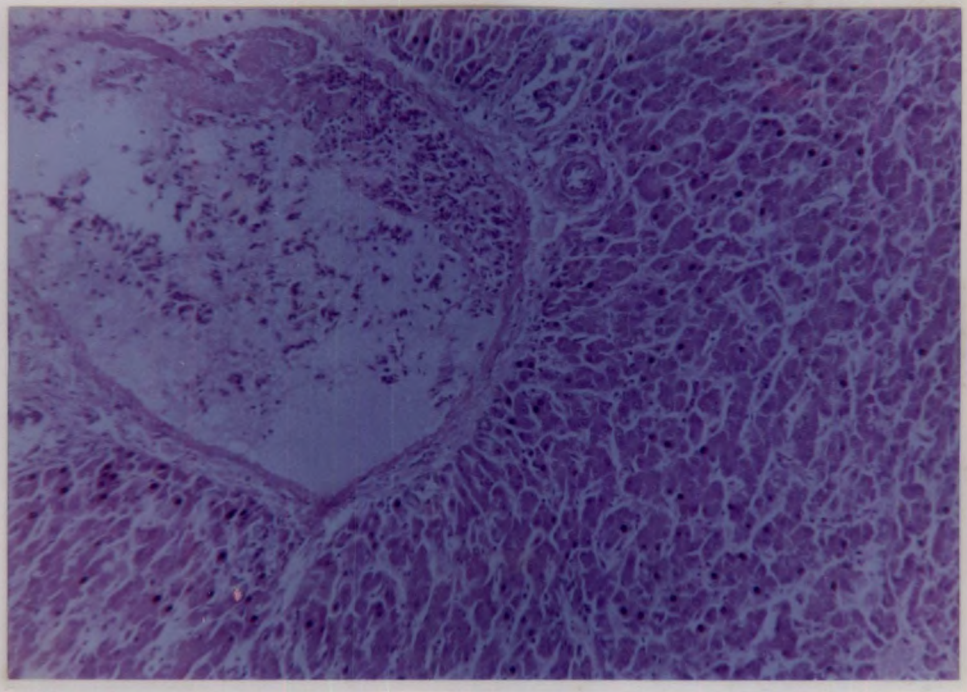
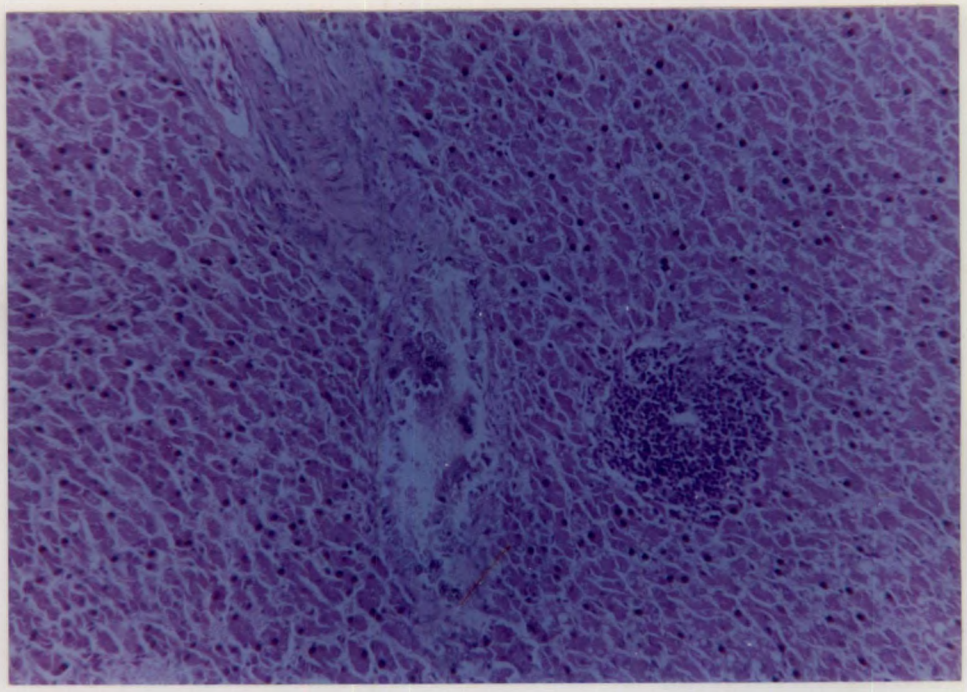


Fig.15. **Liver - bile duct hyperplasia, necrosis of hepatic cells and mononuclear infiltration - aflatoxin B_1 - 30th day - H&E x 250**

Fig.16. **Liver - fatty degeneration, necrosis of hepatocytes and mononuclear cell infiltration - aflatoxin B_1 - 45th day - H&E x 250**



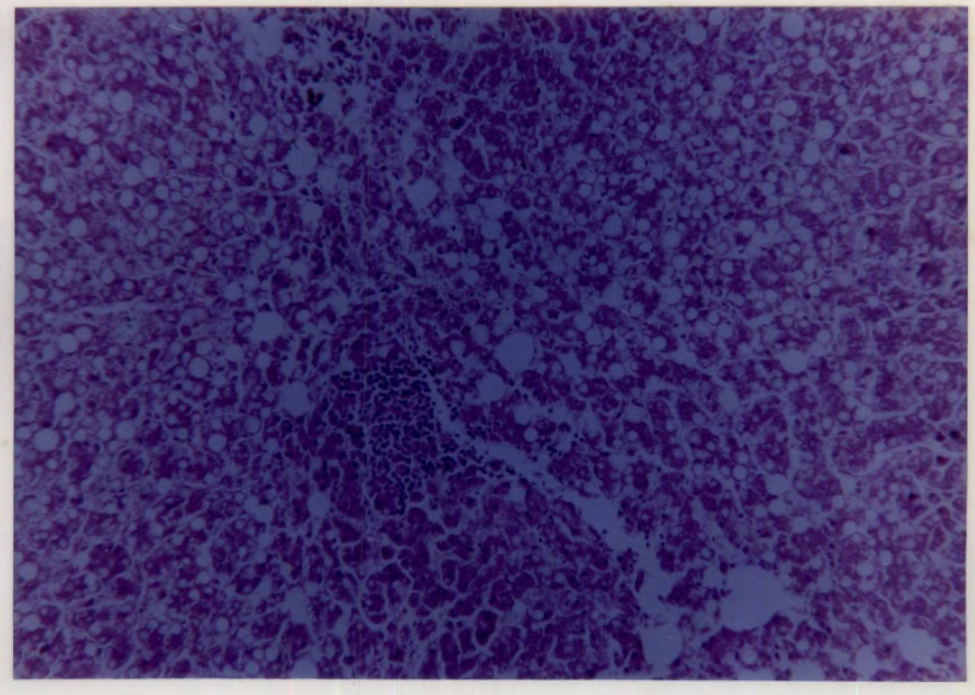
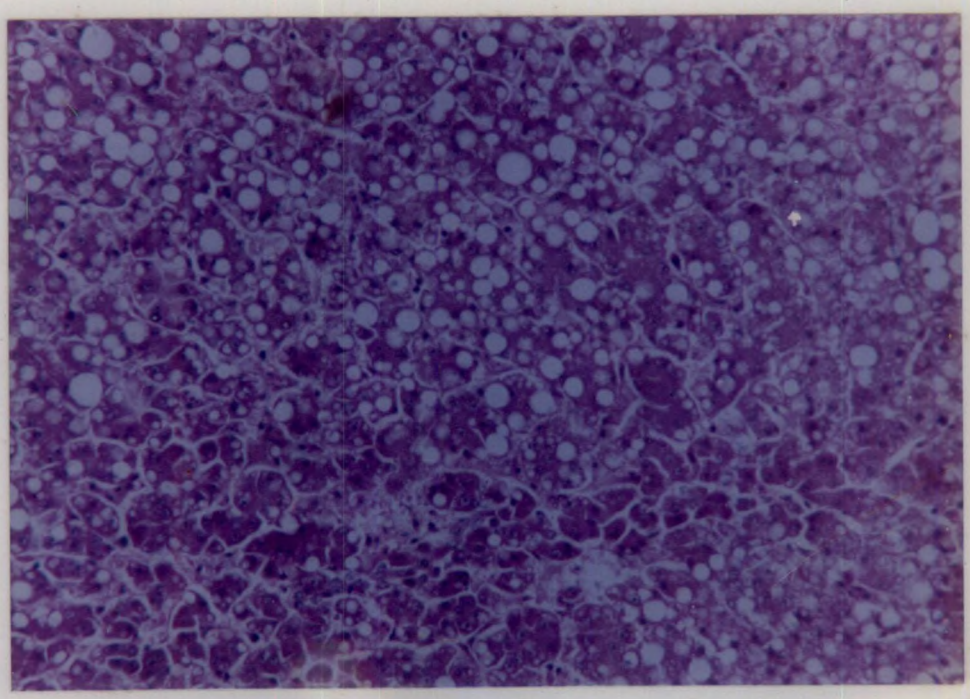


Fig.17. **Liver - diffuse Kupffer cell reaction, extensive fatty degenerative and necrotic changes - aflatoxin B_1 - 60th day - H&E x 400**

Fig.18. **Spleen - focal necrosis of lymphocytes and vascular sclerosis - aflatoxin B_1 - 30th day - H&E x 250**



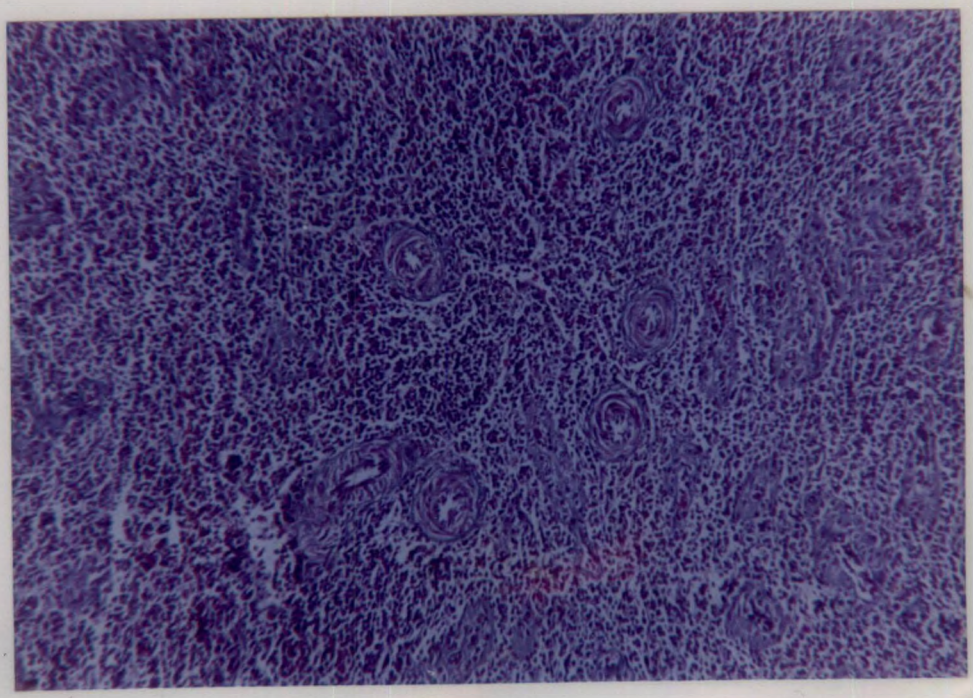
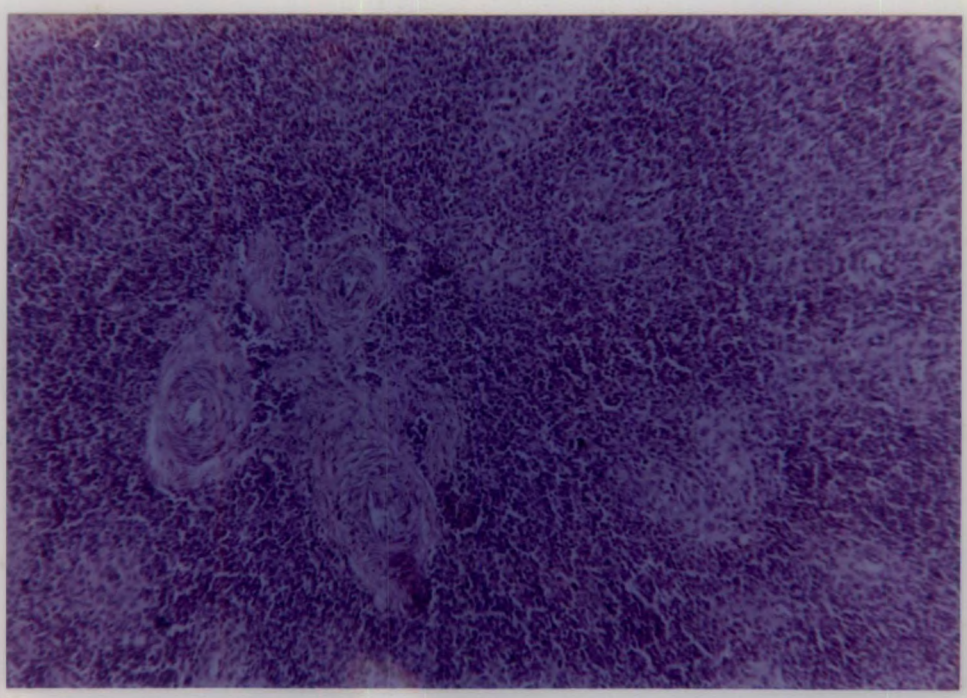


Fig.19. **Spleen - diffuse lymphoid depletion, necrosis of lymphoid cells and vascular sclerosis - aflatoxin B_1 - 45th day - H&E x 250**

Fig.20. **Spleen - lymphoid depletion - aflatoxin B_1 - 60th day**

H&E x 250



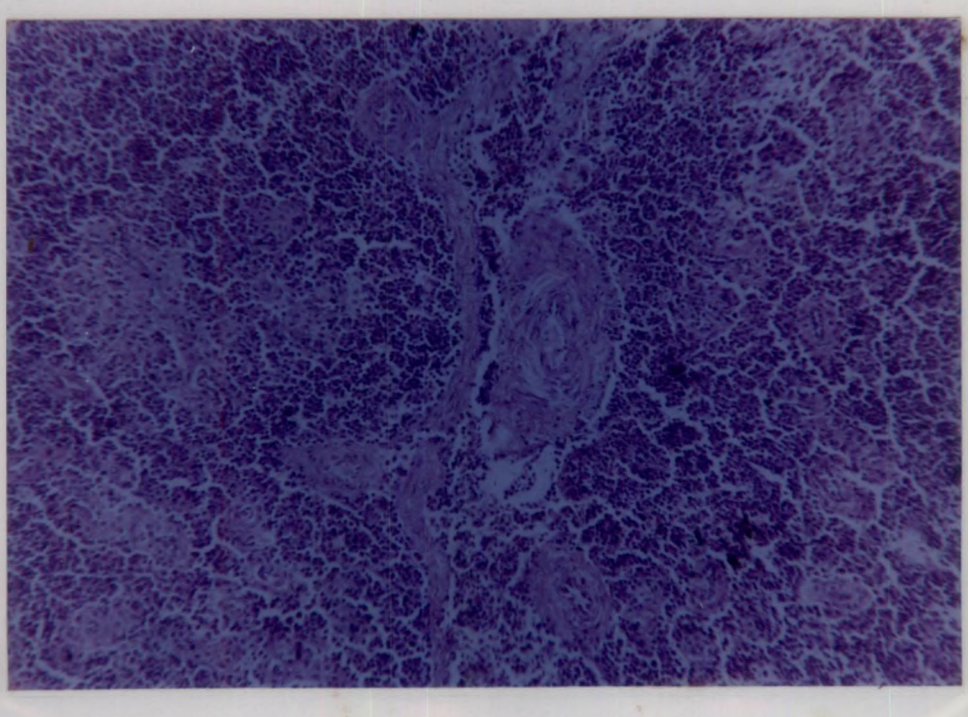
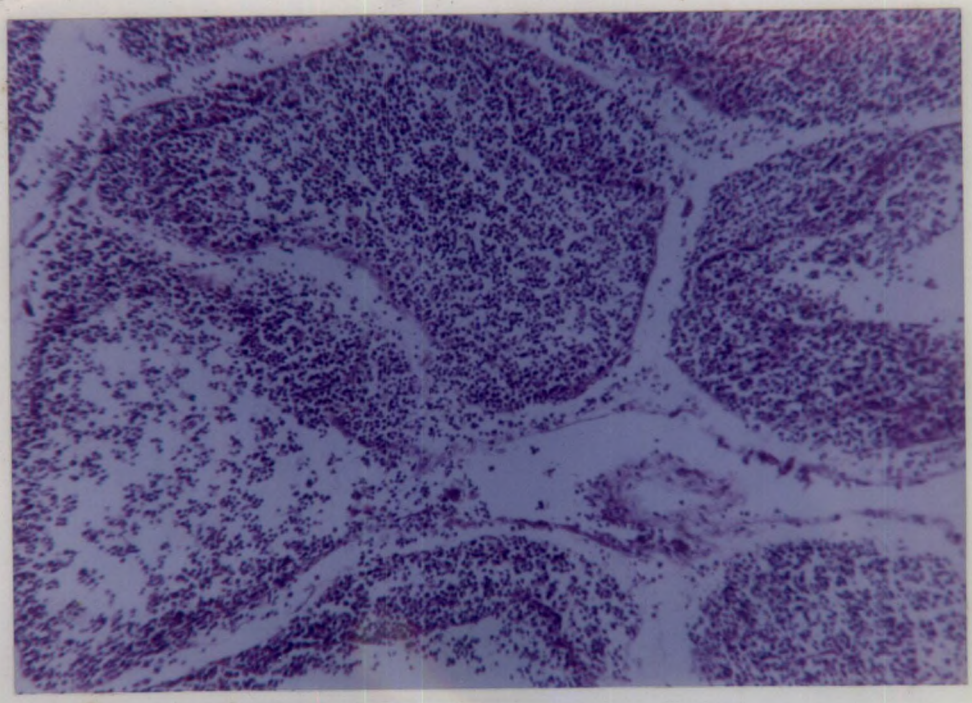


Fig.21. **Bursa - Lymphoid depletion and necrosis of follicular cells - aflatoxin B_1 - 15th day - H&E x 250**

Fig.22. **Bursa - lymphoid depletion and proliferation of interfollicular connective tissue - aflatoxin B_1 - 30th day - H&E x 250**



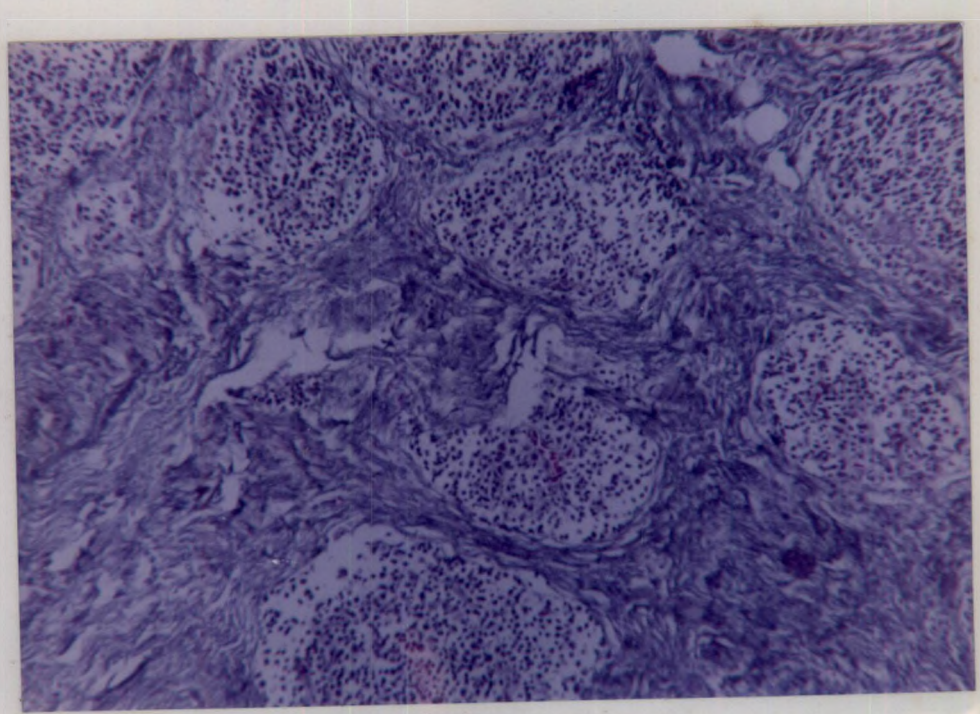
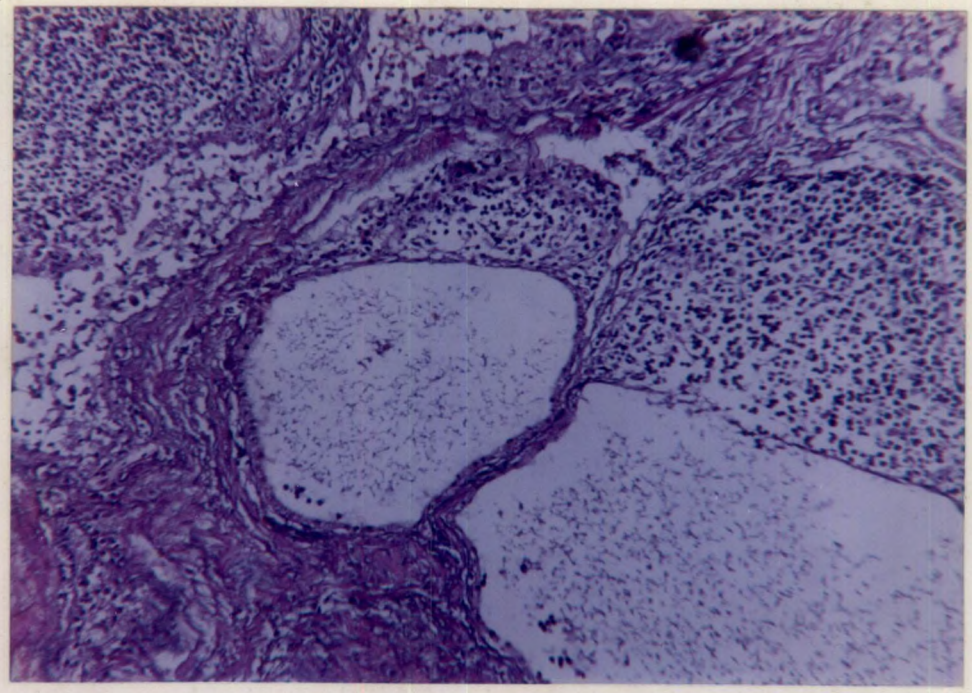
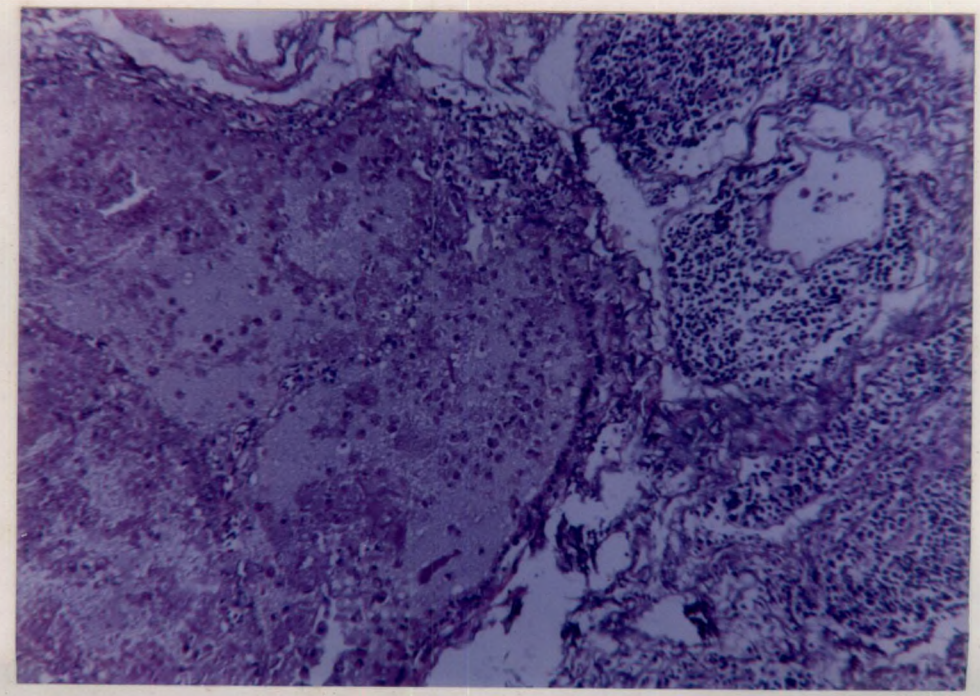


Fig.23. **Bursa - Lymphoid depletion, cyst formation, interfollicular connective tissue proliferation - aflatoxin B_1 - 30th day - H&E x 250**

Fig.24. **Bursa - Inter and intra follicular cyst formation, necrosis of lymphoid cells and lymphoid depletion - aflatoxin B_1 - 60th day - H&E x 250**





Discussion

DISCUSSION

The study was undertaken primarily to assess the pathological effects of aflatoxin B_1 in Japanese quails. A low dose of 0.5 ppm body weight was used twice weekly and the following parameters were analysed to evaluate its effect at such a low dose rate. The parameters were body weight, organ weights, haemogram, serum protein profiles, gross and histopathology of liver, spleen and bursa and the haemagglutination inhibition titres to Ranikhet disease vaccination.

The influence of aflatoxin B_1 on the body weight was studied and it was observed that the treatment did not cause any significant difference in the weekly body weight gain from that of controls. Extensive information in this aspect has been documented by Gumbmann *et al.* (1970), Sawhney *et al.* (1973), Doerr and Ottinger (1979), Pegram *et al.* (1985), Panda *et al.* (1987), Kumar *et al.* (1993) in Japanese quails and by Butler (1964), Gumbmann *et al.* (1970), Smith and Hamilton (1970) in other avian species. Most of them had observed a reduction of bodyweight gain in aflatoxicosis. This inhibitory effect may be attributed to the reduced intestinal absorption of nutrients and impaired liver function and synthisis of plasma proteins leading to decrease in feed efficiency. The finding in the present study is not in

confirmity with the earlier reports. This may be because of the low dose of aflatoxin used in the study which is below the minimal growth inhibitory dose of 5 μg/g in Japanese quails (Chang and Hamilton, 1982).

The effect of aflatoxin on liver weights has been studied by many workers and both increase and decrease in liver weights have been documented (Gumbmann *et al.*, 1970; Doerr and Ottinger, 1979; Chang and Hamilton, 1982; Carnaghan, 1964; Campbell *et al.*, 1983; Shiang, 1994). In this study, a significant increase in liver weights of the toxin treated birds was observed on the 45th day and 60th day of the experiment and this is in line with the findings of Doerr and Ottinger (1979) and Carnaghan (1964). The increase in liver weights may be due to direct toxic injury to the liver. This in turn would lead to defective phosphorylation of fat resulting in its accumulation in the hepatocytes. This is well evident from the number of lobules involved and presence of vacuoles of varying sizes in the hepatocytes.

A significant increase in spleenic weights was observed in the aflatoxin treated birds from that of controls and this correlates with similar findings of Rao *et al.* (1988) and Mani *et al.* (1993) in chicken. This could be due to the increase in the amount of reticular tissue as a response to the stimulation of the reticuloendothelial system by the aflatoxin

B_1 metabolites. However, Chang and Hamilton (1982) did not find any significant effect of aflatoxin on spleenic weights in Japanese quails.

Aflatoxin has been known to cause a depression in bursal weights as reported by Chang and Hamilton (1982) in Japanese quails and by Campbell *et al.* (1983) and Rao *et al.* (1988) in chicken. A significant depression in the bursal weights of the toxin treated birds from that of controls was evident in this study and is in agreement with the earlier findings. The reduction in bursal weights may be attributed to the depletion of lymphocytes and necrosis of the epithelium covering the gland and loss of cellular elements as a result of the toxic effects of aflatoxin.

Elevated erythrocyte sedimentation rate has been recorded in aflatoxicosis in different species of animals and birds (Yaman *et al.*, 1989; Fernandez *et al.*, 1995; Murthy *et al.*, 1984; Anbiah, 1996). The present study has revealed a significant increase in the erythrocyte sedimentation rate in the toxin treated birds from that of controls. This could be due to the altered serum protein levels, particularly albumin which is important for the stability of erythrocytes and hence loss of stability in the phase of reduced albumin level leads to increased erythrocyte clumping and rapid sedimentation.

The packed cell volume was found to be significantly decreased in the toxin fed birds (Group B and C) from that of controls at the end of the experiment. Similar supportive results in aflatoxicosis have been recorded by many workers in other species (Tung *et al.*, 1975; Reddy, 1981; Mohiuddin *et al.*, 1986; Anjaneyulu and Rao, 1993; Mani *et al.*, 1993). This decrease may be attributed to the depressing effect of aflatoxin on the haemopoietic tissues.

Lowering of hemoglobin at various dose levels of has been well established by many workers in different species (Brown and Abrams, 1965; Tung *et al.*, 1975; Reddy *et al.*, 1980; Reddy, 1981; Mohiuddin *et al.*, 1986; Panda *et al.*, 1987; Balachandran and Ramakrishnan, 1987; Anjaneyulu and Rao, 1993; Anbiah, 1996). However, in this study no significant reduction in hemoglobin was observed in the toxin treated birds and this may be accounted for the very low dose of aflatoxin used in the study which was not sufficient to cause considerable damage to the hemopoietic system.

A significant reduction in the erythrocyte counts of the toxin treated birds was observed at the end of the experiment. The findings of Wannop, 1961; Tung *et al.*, 1975; Reddy *et al.*, 1980; Reddy, 1981; Mohiuddin *et al.*, 1986; Anbiah *et al.*, 1996 in different species lend credence to the observation in the present study. The depressing effect of aflatoxin on the

hemopoietic tissues and the concurrent impairement of protein synthesis in the liver may have resulted in the reduction of erythrocyte count and the low dose used in the study accounts for the delayed appearance of the reduction in erythrocyte counts.

Leucocytosis has been observed in aflatoxicosis in many species of poultry as reported by Wannop (1961), Tung *et al.* (1975), Mohiuddin *et al.* (1986) and Anbiah (1996). This was attributed primarily to the elicitation of an inflammatory response primarily consisting of heterophils in aflatoxicosis. However, in this study there was a significant decrease in the total leucocyte count of the toxin treated groups (B&C) from that of controls on the 60th day. This may be due to the decrease in the number of circulating lymphocytes in levels countering the expected heterophilia in aflatoxicosis.

Heterophilia in aflatoxicosis in many species of poultry has been reported by Wannop (1961), Tung *et al.* (1975), Mohiuddin *et al.* (1986), Sova *et al.* (1991) and Anbiah (1996). Wannop (1961), Mohiuddin *et al.* (1986), Sova *et al.* (1991) had also reported lymphocytopenia in aflatoxicosis. Basophilia (Tung *et al.*, 1975), monocytosis (Wannop, 1961) and thrombocytopenia (Mohiuddin *et al.*, 1986) have also been reported as important haematological alterations encountered in aflatoxicosis in chicken and other species of poultry. In

the present study though a relative increase in heterophils and a decrease in the circulating lymphocytes were evident in the aflatoxin treated birds from that of controls, the findings were not of any statistical significance. Probably, the dose of the toxin was not sufficient to produce any significant variation.

Depression in serum total protein level in aflatoxicosis has been well documented (Chang and Hamilton, 1982, Pegram *et al*. 1986, Panda *et al*., 1987 and Kumar *et al*., 1993) in Japanese quails and by Reddy *et al*., 1982, Campbell *et al*., 1982, Harvey *et al*., 1989, Jassar *et al*., 1993, Shukla and Pachauri (1995) in other species of poultry. In the present study, a highly significant reduction in the total serum protein levels of the toxin treated birds was observed on the 45th and 60th day of the experiment and is in agreement with similar earlier findings. This indicates the effect of aflatoxin on protein synthesis and it is also worthy to note that a dose below the growth inhibitory level could considerably reduce the protein synthesis leading to a general depression of various physiological functional mechanisms.

A significant reduction in the serum albumin and serum globulin levels in the toxin treated birds was observed on the 45th and 60th day of the experimental period and is supported by similar findings of earlier workers (Pegram *et al*., 1986 in

Japanese quails and Harvey *et al.*, 1989, Ghosh *et al.*, 1990,, Shukla and Pachauri, 1995, Anbiah *et al.*, 1996 in other species of poultry). An interesting feature in this study is the lack of any significant variation in the albumin-globulin ratios in the experimental birds in contrast to the findings of Shukla and Pachauri (1995) in chicken who had reported otherwise. However, Anbiah (1996) had reported lack of any significant variation in the albumin-globulin ratios of the aflatoxin treated ducks from that of controls.

The gross changes of tan to yellow discolouration, hepatomegaly, fatty changes observed in the experimental birds indicated a primary liver injury. Sawhney *et al.* (1973), Doerr and Ottinger (1979), Panda *et al.* (1987), Sadana *et al.* (1992), Kumar *et al.* (1993) and Sridevi and Sriraman (1996) had observed similar pathological changes in Japanese quails in aflatoxicosis. The intensity of changes observed in the present study were in a time dependent manner. The histopathological changes of central venous congestion, focal degeneration and necrosis of hepatocytes, periportal aggregation of inflammatory cells, kupffer cell hyperplasia, bile duct hyperplasia, fatty vacuolation of hepatocytes in increasing intensities coincided with the duration of aflatoxin treatment. This is in conformity with the findings of earlier workers in Japanese quails.

The gross changes in the spleen, a secondary lymphoid organ, was primarily spleenomegaly and was observed in the third and fourth fortnights of the aflatoxin treatment. Similar findings have been reported by Balachandran and Ramakrishnan (1987) in chicken. An increase in the reticular tissue and vascular congestion may be attributed to this finding. Microscopically, the findings in the spleen were focal lymphoid depletion in the cortex and paracortex and degeneration and necrosis of lymphocytes in the paracortical areas in increasing intensities depending upon the duration of treatment and congestion. Balachandran and Ramakrishnan (1987), Ghosh *et al.* (1987) Rao *et al.* (1988) and Sridevi and Sriraman (1996) had reported similar findings in aflatoxicosis in chicken an Japanese quails. The lesions observed suggest a general depletion of the immune responsive cells.

The gross changes in the bursa, primarily consisted of increased colloid in the lymphoid organ and an initial mild enlargement followed by atrophy of the organ in the toxin treated birds. Sadana *et al.* (1992), Kumar *et al.* (1993) and Sridevi and Sriraman (1996) had reported similar findings in Japanese quails. The microscopic findings of diffuse lymphoid depletion, focal inter follicular and intrafollicular oedema, proliferation of inter follicular connective tissue, cyst formation in intensities varying with the duration of

treatment is in confirmation with the findings of Kumar *et al.* (1993), Sridevi and Sriraman (1996) in Japanese quails and with that of Rao *et al.* (1988) and Ghosh *et al.* (1987) in chicken. This clearly indicated a direct toxic injury and subsequent immunosuppression.

The other gross findings of congestion of lungs, kidneys and petechial haemorrhages in the muscles of the thigh and breast region have been recorded by many workers in aflatoxicosis (Balachandran and Ramakrishnan, 1987; Sadana *et al.*, 1992).

The effect of aflatoxin B_1 treatment on the immune response to Ranikhet disease vaccination (R_2B strain) at the age of 46 days was assessed by estimation of HI titres. In general, a relative decrease in the mean HI titre value was observed in the toxin treated birds to that of controls throughout the experiment, though it was not significant statistically. Iglaz (1983), Rao *et al.* (1988) and Mani *et al.* (1993) have reported suppression of immune responses to Ranikhet vaccination in comparable studies in chicken. The findings though not significant, show a trend towards immunosuppression. The low dose level of 0.5 ppm twice weekly and only brief exposure to the toxin prior to secondary vaccination on 46 days of age may be the reason for the nonsignificance of the results. In addition, Iglaz (1983) had

reported that aflatoxin administered three days after vaccination had no immunosuppressive effect.

To conclude, the findings of reduction in bursal weights, leukopenia, reduction of the total serum protein and serum globulin levels, histological picture of the bursa and spleen and a relative decrease in the HI titres to RD vaccination suggest the immunosuppressive effects of aflatoxin B_1 in Japanese quails even at low dose levels of 0.5 ppm.

Summary

SUMMARY

The study was undertaken to evaluate the immuno-pathological response of the Japanese quail in experimental aflatoxicosis. One month old Japanese quails were administered aflatoxin B_1 at the rate of 0.5ppm body weight twice weekly for two months and the immune response to Ranikhet disease vaccination was evaluated combined with body weights, organ weights, serum protein profiles and pathological studies of the liver, spleen and bursa at fortnightly intervals.

Clinically, all the experimental quails were healthy and revealed no signs of toxicity.

It was observed that the toxin did not cause any significant effect on the body weights of the treated birds. Liver weights were significantly increased in the toxin treated birds on the 45th and 60th day of the experiment. On day 60, a significant increase in the spleen weights of the toxin treated birds was noticed. On the contrary, a significant decrease in the bursal weights of the aflatoxin group was observed on the 30th, 45th and 60th day of the experiment.

Haematological studies indicated that the toxin did not produce any alteration in the haemoglobin levels of the

treated birds from that of the controls. The packed cell volume of the treated birds was significantly depressed from that of controls only at the end of the study. Elevated erythrocyte sedimentation rate of the toxin treated birds was a significant finding on the 45th and 60th day of the experiment.

Both the erythrocyte counts as well as the leucocyte counts of the toxin treated birds were significantly decreased on the 60th day of the study. Throughout the experiment, differential leucocyte counts were not of any significance. However, a relative leucocytosis and lymphopenia were observed in the toxin treated birds from that of controls.

Serum protein profiles revealed a significant depression of the total protein, albumin and globulin levels of the toxin groups from that of controls on the 45th and 60th day of the experiment. But the albumin-globulin ratios were unaltered.

Haemagglutination inhibition titre to Ranikhet disease vaccination did not show any significant variation in the toxin treated birds from that of controls. However, a relative decrease in the mean HI titres of the toxin treated birds was noticed throughout the experiment.

The quails which were administered the toxin revealed tan livers with a few pin point haemorrhages on day 30 followed by

yellow, enlarged, glistening livers on day 60 and the bursae revealed increased colloid. On day 45 and 60, the spleen was congested and slightly enlarged. Some of the toxin treated birds revealed petechial haemorrhages in the muscles of the thigh and breast region.

Histopathologically, the lesions in the liver of the toxin treated birds varied from central venous dilation, diffuse hepatic degeneration, focal haemorrhages, Kupffer cell reaction, mild bile duct hyperplasia and aggregation of mononuclear infiltrating cells on day 15 and 30 to intense venous congestion, diffuse Kupffer cell reaction, diffuse necrosis of the hepatocytes, severe fatty changes with vacuoles of varying sizes and numbers in the hepatocytes on day 45 and 60.

The histopathological changes in the spleen of toxin treated birds were paracortical and cortical lymphoid depletion, necrosis of lymphocytes in focal areas, vascular sclerosis in increasing intensities depending upon the duration of treatment.

The bursae of the toxin treated birds showed mild to severe lymphoid depletion, necrosis of lymphocytes, proliferation of interfollicular connective tissue, extensive oedema and intrafollicular and inter follicular cyst formation in higher degrees relative to the duration of toxin treatment.

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IMMUNOPATHOLOGICAL RESPONSE OF THE JAPANESE QUAIL *(Coturnix coturnix japonica)* IN EXPERIMENTAL AFLATOXICOSIS

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ABSTRACT OF A THESIS

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ABSTRACT

One month old Japanese quails vaccinated with Ranikhet disease vaccine (Lasota strain) on 7th day, were divided into three groups viz., A, B and C of 35 birds each for assessing the immunopathological responses in experimental aflatoxicosis. Birds of group B and C were administered aflatoxin B_1 by oral intubation at the rate of 0.5 ppm twice weekly for two months and group A were vaccinated with R_2B strain vaccine at the age of 46 days. Seven birds from each group were sacrificed at fortnightly intervals for the evaluation of various organ weights, haematological, biochemical and haemagglutination inhibition titre values.

Liver weights and spleen weights were significantly increased in the toxin treated birds while bursal weights were significantly depressed from that of controls. Packed cell volume, erythrocyte count and leucocyte counts were significantly increased in the toxin treated birds at the end of the experiment with a significant increase in erythrocyte sedimentation rate at the 45th and 60th day of the experiment. A relative heterophilia and lymphopenia were observed in the toxin treated birds.

Serum total protein, albumin and globulin levels were significantly depressed in the toxin treated birds but the albumin-globulin ratio was unaltered. A relative decrease in

the haemagglutination inhibition titre values of the toxin treated birds was recorded.

The gross and histopathological lesions in the liver of the toxin treated birds were suggestive of progressive hepatic degeneration, necrosis and fatty changes. There was lymphoid depletion and vascular sclerosis in the spleen while the bursae of the treated birds showed lymphoid depletion, necrosis of lymphocytes, intra and interfollicular cyst formation in increasing intensities in a time dependant manner.

The pathomorphological alterations and a relative depression of HI titres in the aflatoxin treated quails indicates a moderate depression of the immune status by aflatoxin at such a low dose level.