COPPER SULPHATE, ZINC SULPHATE AND MANCOZEB TOXICITY IN DUCKS

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Copper sulphate, Zinc sulphate and Mancozeb toxicity in ducks"** 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 this thesis entitled **"Copper sulphate, Zinc sulphate and Mancozeb toxicity in ducks"** is a record of research work done independently by **Mr. A.M.Chandrasekharan Nair** , 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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Chapter 1

INTRODUCTION

Rural India plays a significant role in animal production and allied activities. It is estimated that livestock including poultry contribute to 15-20 % of the income of 73 % of the rural households in the country. The livestock and poultry are concentrated with the poor section of the society. They provide economic security and social status to the family. Among animal production practices, poultry plays an important role in improving the family income. It also meets the food requirement (Tanuja, 1998). Compared to other livestock, poultry can be intensely reared by small and marginal farmers and even by land less. Duck and poultry provide the most economic, quality animal protein for common man. These factors contribute to the growth of poultry industry in India.

Poultry including duck rearing is reasonably well developed in Kerala. According to the Livestock Census of 1996, Kerala has 11.87 lakhs of ducks (showing an increase of 4.4 % over the previous 1987 Census). This accounts for about 7% of the total duck population of the country (Anon, 1999). The climatic condition, the topographic features, abundance of backwaters, presence of low- lying marshy lands and kole lands make Kerala ideal for duck rearing, in spite of its high density of population and limited land area. Alleppey, Ernakulam and Kottayam are the main duck rearing districts of Kerala.

Ducks are hardy in nature, comparatively more resistant to common avian diseases and can withstand extremes of climatic condition. They can easily be brooded and reared in marshy riverside, wet lands, which are not conducive for other poultry. In Kerala ducks are usually raised by small farmers as a means of livelihood. However,

large scale duck farming is not common. Ducks are let loose during day time in back waters and canals and they feed on various aquatic fauna and flora . Thus the average farmers spend very little money towards feeding of ducks. A general practice followed in Kerala, is to let the birds in to post harvested paddy fields. Hence the flocks are in continuous move from field to field in districts of Kottayam, Alleppey, Ernakulam and Thrissur.

Duck plague with high mortality was reported from Kuttanad area of Alleppey district from 1976 onwards. Inspite of regular vaccination out break occurred in different parts of the district. It was suspected that some factors which induce immunity break down may contribute to the disease out break. One of the suspect for the immunity break down in ducks in Kuttanad is the exposure of ducks to highly polluted soil, water in rivers, canals and ponds consequent to indiscriminate use of insecticides, fungicides and weedicides as part of agronomic practices.

Among fungicides, copper and zinc compounds are widely employed in agriculture and veterinary practice. The popular copper compounds in use include Cheshnut compound, Bordeaux mixture, copper carbonate, copper sulphate, copper naphthenate and copper oxychloride. Among the best known compounds, Bordeaux mixture is widely used against fungal diseases of crop plants especially rubber, coconut and pepper and also against snails.It contains 1-3% copper. These compounds drain into the rivers during rain and settle in stagnated back waters. Successive nontoxic doses of copper have cumulative effect in animals and is eliminated from the body very slowly. Even though it is an essential mineral element in animals and birds, excess copper is toxic, as it binds with SH group of enzymes inhibiting their action.

Zinc compounds are also widely used in agriculture in the organic form as fungicides in paddy cultivation. Like copper compounds these also pollute the soil and water by its residue. It is an essential trace element for normal growth and

development in mammals and birds. It accumulates in the body due to its long biological half life. Even though it is an essential trace element, excessive intake leads to toxicity in mammals and birds. When present in excess amounts it inactivates the enzymes by binding with SH groups.

Different locations in Alapuzha were prospected as a part of the investigation programme on duck mortality in Kuttanad area. Discussions were made with the local veterinary surgeons and Animal husbandary officers. Various areas frequented by flocks of ducks in and around Alapuzha were located and from them five sites, Kalarcode, Pallathuruthy, Kidangara, Chempumpuram and Pulimkunnu were selected for further investigation.

These places were visited for a period of one year, at four months intervals starting from April 1995 .Samples of water, sludge, small plants usually eaten by ducks, small fishes and mussels were collected during the visit observing sampling rules. Samples were analysed as per the procedure described by Anderson (1972) and Capar (1977). The heavy metal (copper and zinc) contamination in these areas are shown in Table 1 (under results).

The highest level of copper, 67.83 ppm was observed in the mud sediment collected from Kidangara . However, the highest concentration of zinc,198 ppm, was noted in fish from Pulimkunnu .

In the light of the above observations the present project was undertaken to investigate the toxicity of these two heavy metals *viz.* copper and zinc which form the important ingredient of fungicides widely used in Kuttanad, on the health status of ducks and their role on immune system in ducks.

REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

2.1 COPPER

2. 1. 1 Role of high dietary copper on growth and reproduction in birds.

Goldberg *et al.* (1956) recorded progressive loss of body weight in adult hens when they were fed with 50, 75 and 100 mg copper as copper acetate daily for first, second and third week respectively. Mayo *et al.* (1956) also noted an inhibition of growth at fourth week of age when diet was supplemented with 520 ppm copper as copper sulphate. At eighth week when copper was included as copper bound casein instead of copper sulphate, significant increase in growth was noted. The experiment conducted by Mehring *et al.* (1960) revealed that addition of copper to the feed up to the toxic level improved the feed efficiency of chicks up to the age of tenth week. The effect of various levels of copper and iron on growth, body weight and survival of chicks was studied by Lin *et al.* (1965). From their experiment they noticed that copper at 80 ppm or above depressed growth rate when iron level in the feed was maintained at a level of 40 ppm or above.

The depression of growth in birds due to 910-1000 ppm copper in the ration was found to be reduced after the addition of EDTA at 308 m.mol./kg of diet (Vohra and Kratzer, 1968).They could improve the survival and weight gain, but could not get any improvement with EDTA on 1620 ppm of copper. They also found that 3240 ppm of copper is lethal. Growth depression in birds fed on 500 ppm dietary copper was noticed by Hill (1974) and also by Jensen and Maurice (1976). Jensen and Maurice (1976) could prevent the growth depression caused by 500 ppm copper by addition of 0.4 % dl methionine to the diet. Poupoulis and Jensen (1976) noticed an improvement in body weight gain and feed efficiency with 250 ppm copper in the feed. But the growth rate and feed efficiency was supressed by 500-1000 ppm copper.

Jackson (1977) studied the influence of feed, supplemented with 120,240,480,960 and 1920 ppm copper for a period of 35 days in adult fowl.They noticed a suppression of feed intake and egg production with two highest levels of copper .As per the observation of Griminger (1977) 500 or 1000 ppm copper sulphate supplemented diet had no influence on body weight, egg production and feed intake in adult hen. But copper sulphate at 0.5 % of the ration reduced the feed intake to less than 1/3 of the normal and stopped egg laying completely. He noticed that more than 0.2 % copper sulphate in the feed caused loss of body weight and egg production. In turkey poults also copper supplementation reduced the growth (Christmas and Harms,1978) .Turkey poults were fed with 500 and 750 copper supplemented diet with or without 0.4% methionine in sulphur amino acid deficient diet. Methionine stimulated the growth, and addition of copper reduced the body weight. Copper at 120 ppm in the ration of turkey stimulated the growth rate (Guenthner *et al.*,1978)

A depression of body weight by supplementing the diet of hens with 600-800 ppm copper was observed by Jackson *et al.* (1979). In turkey poults feed was supplemented with 0, 500, and 700 ppm copper sulphate with and without 0.4% methionine and fed for 24 days of age (Christmas and Harms, 1979) . The results showed that addition of methionine increased growth. Addition of copper at either level greately reduced 21 day bodyweight in proportion to the level fed It also reduced feed consumption. Jackson *et al.* (1979) reported that lower levels (100 and 200 ppm) of copper enhanced feed and water intake and higher levels (600and 800 ppm) of copper depressed the body weight.

Jensen and Maurice (1979) reported a significant reduction in growth rate and feed efficiency by feeding chicks with 500 or 700 ppm copper . Stevenson and Jackson (1980) also noticed a significant reduction in body weight, feed intake and egg production in laying birds fed with a diet containing 500, 1000, and 2000 mg

added copper per kg feed and fed for 8 weeks. They noticed a reversal of this on withdrawal of copper supplemented diet and on feeding the control diet. An adverse effect on feed intake, egg production and body weight was also noted in hens by Jackson and Stevenson (1981) when they fed 0.2 and 1.0 g copper sulphate per kg feed for 280 days. Atlavin and Apsite (1982) observed a decreased growth rate in white leghorn chicken when they were fed with 1000 ppm copper sulphate supplemented diet.

In the experiment conducted by Southern and Baker (1982) in coccidia infected chicks, a decrease in weight gain and feed efficiency was recorded when infected chicks were fed with copper supplemented ration (500- 750 ppm). A decrease in feed intake and weight gain was also recorded by Andrews *et al.* (1988) when they fed 500 ppm copper supplemented diet to chicks.In turkey also a reduction in body weight was noted by Potter and Koru (1989) when turkey was maintained on a copper supplemented diet (200, 400, 600, 800, and 1000 ppm) .They also noticed an increase in mortality rate in 1000 ppm group. Stahl *et al.* (1989) fed laying hens with 250 μ g copper / g of feed with or without the addition of 10 μg zinc / g Egg production, feed conversion, feed intake, body weight, egg weight, fertility and hatchability were observed and no significant difference was noticed in the treated group.

Day old broiler chicks were fed with 480 ppm copper and different levels of calcium per kg diet (Leech, 1990) A substantial reduction in body weight was noted . The differences were least with 1% dietary calcium . Rosenblum *et al.* (1990) observed that, when the diet of chicks was deficient in calcium, the sensitivity to copper toxicity is increased They fed different combinations of calcium carbonate and copper sulphate to day old male broiler chicks . The addition of 480 mg copper per kg diet resulted in a reduction in body weight at the deficient and marginal level of

calcium. The differences were least with 1% dietary calcium. They concluded that the influence is mediated via intestinal absorption rather than through influence of copper excretion.

Anjum *et al.* (1992) observed a reduced feed intake and an increased water intake, but no effect on body weight, when broiler chicks were fed with diet containing 500 –1000 ppm copper sulphate. Ward *et al.* (1994) studied the influence of 204 ppm dietary copper for 5 days on growth, water intake and haemoglobin in poultry. They observed that feed and water intake were not affected, but supplementation of copper to water at this level reduced the growth and feed consumption. Gilbert *et al.* (1996) also noted a reduction in feed intake and egg production when 51 week old adult leghorn hens were fed with ration containing 1477 mg copper per kg.

2 . 1 . 2 Influence of sulphur containing aminoacids on copper toxicosis.

Jensen and Maurice (1976) noticed severe gizzard erosion and growth depression with 500 ppm copper in the ration of chicks . They could prevent growth depression, observed with 500 ppm copper by adding methionine (0.4%). Neither methionine nor cysteine significantly reduced the severity of gizzard erosion. Christmas and Harms (1978) studied the inter relationship of copper sulphate, methionine and potassium magnesium sulphate in turkey poults and broiler chicks. Turkey poults were fed with 500 – 700 ppm copper sulphate with or without 0.4% supplemented methionine in sulphur aminoacid deficient diets. In another trial, broiler chicks were fed graduated levels of supplemented methionine or one or two levels of potassium magnesium sulphate with or without 750 ppm copper for three weeks. In turkey addition of copper reduced body weight and feed consumption in proportion to the level of copper fed . Increased gizzard erosion and bleaching of gizzard were observed. Methionine stimulated the growth and partially reversed the effect of copper supplementation. In broiler also methionine increased the growth rate.

Copper supplementation at 750 ppm level decreased the performance of chicks. Both 0.4 and 0.8 % of methionine partially offset this effect. Feeding of potassium magnesium sulphate did not improve chick performance .

When chicks were fed a diet supplemented with copper at a level of 250,500 and 750 ppm in the ration, enlarged proventriculus and erosion of gizzard lining was noticed (Jensen and Maurice, 1978). They also noticed that addition of 0.4% methionine to the diet was unable to prevent gizzard erosion and abnormality of proventriculus But growth depression and reduced feed efficiency caused by 500 ppm copper was completely counteracted . Addition of 0.4 % methionine to the diet with 750 ppm copper did not significantly counteract the growth depression and reduction in feed efficiency.

Turkey poults were fed with a basal corn-soya diet which contained 26% protein and 0.765 % sulphur aminoacid (SAA). It was supplemented with 0, 500, and 700 ppm of copper as copper sulphate, with or without 0.4% supplemental methionine (Christmas and Harms,1979). The addition of copper at both the levels reduced the feed consumption. Supplementation of methionine to the copper treatment partially offset the effect of copper resulting in high weight and feed efficiency. Jensen and Maurice (1979) observed that addition of copper at 500,or 750 mg/kg to a diet deficient in SAA for chicks caused a reduction in growth rate and feed efficiency. Addition of dl methionine to the diet (0.4%) prevented the reduction in feed efficiency with 500 ppm copper but not with 750 ppm copper. The increase in hepatic copper level by 500-750 ppm is reduced by 0.4% added methionine.

Robbin and Baker (1980) revealed that copper addition to the ration at a level of 250 and 500 ppm caused erosion of gizzard and deposition of copper in gizzard lining of chicks . Copper concentration in the liver of chicks fed 250 mg/kg was reduced by one half when the SAA level exceeded the requirement.

Excess copper reduced the availability of sulphur compounds containing free SH group which resulted in growth depression .From their experiment they recommended a level of 0.64% SAA for 250 ppm copper, 0. 77 % for 500 ppm copper and 0.59 % for zero copper supplementation in diet to prevent bad effect.

Growth retardation in chicks were also noted by Ekperigin and Vohra (1981) when the diet was supplemented with 0, 500, and 1000 ppm copper along with 0, 0.4, 0.8 and 1.5 % of l methionine for 1-4 weeks. Growth was retarded after one week by all levels of supplementary methionine and by 500 and 1000 ppm excess copper. The growth retardation caused by 500 ppm excess copper was alleviated by 0.4 % supplemented methionine. Baker *et al.* (1982) demonstrated the interaction of copper snd sulphur aminoacids. They noticed that at upper level of copper ingestion (250 mg/kg and higher) it binds with sulfhydryl compounds such as cysteine and reduces glutathion. Dietary SAA requirement was increased in chicks fed at a copper level of 250 – 500 mg /kg . Hepatic copper deposition is enhanced by copper feeding.

Experiments were conducted in chicks by Southern and Baker (1982) to find out the effect of dietary methionine on *Eimeria acervulina* infection in chicks fed excess copper. Duodenal coccidiosis and supplementation of copper (500 –750 mg/kg) depressed weight gain and feed efficiency. Supplementation of copper increased concentration of copper in the liver and gall bladder. Coccidiosis resulted in two to four fold increase in copper in these tissues. Excess supplementation of methionine to feed (0.5 %) had little effect on toxicity in either healthy or infected chicks.

Kashani *et al.* (1986) studied the effect of added copper or methionine to diets of turkeys. Copper was added 0 or 120 ppm along with diet providing 75, 85 and 100 % requirement of SSA as supplemented by dl methionine. In another experiment four levels of copper 0, 60, 120 and 240, ppm added to diets containing

SAA 75, 100, or 125 % . Sixty ppm of copper improved 8 week body weights , but 120- 240 ppm was growth depressing up to 8 weeks of age. Increasing the methionine content of the diet to 100 % level consistently improved the weight gain of younger birds. The improved body weight from methionine addition for the older turkeys (16-24 weeks), however, was significant only in the first experiment. Copper did not influence the methionine requirement in this experiment.

2 . 1 . 3 Pathological changes due to excess copper in birds.

Goldberg *et al.* (1956) fed adult hens with copper 50, 75 and 100 mg daily for first, second and third week respectively. Hundred mg was continued until the birds developed anaemia or toxicity or death. They noticed a progressive loss of weight and anaemia concomitant with clinical signs of toxicity like reticulocytosis, erythrophagocytosis and haemosiderosis with Kupffer cells.Mayo *et al.* (1956) conducted experiment in chicks with purified diet and the same supplemented with 324, 520, and 1270 ppm copper up to fifth weeks of age . They noticed a faster growth in chicks fed with unpurified diet than chicks fed with purified diet with supplemented copper at all levels . Chicks fed 324 ppm copper caused muscular dystrophy. When copper was included as copper bound caseine instead of copper sulphate an increase in growth rate was noted but the incidence of muscular dystrophy remained the same . In chicks fed with 1270 ppm copper a high incidence of mortality was noticed.

Chicks were fed with a diet containing 0-1180 ppm copper (in the form of copper oxide)up to tenth week of age to find out the toxic level of copper in growing chicks by Mehring *et al.* (1960) . Body weight, feed efficiency and mortality were noted . The results showed that the minimum toxic level of copper is 500 ppm . In the case of turkey poults experimental diet with 50 ppm copper fed from 1-4 th week of

age was proved to be toxic (Waibel *et al.*, 1964). The effect of various levels of copper and iron on growth and survival of chicks were studied by Lin *et al.* (1965). From their studies it was noticed that up to 4 th week of age neither copper (5 – 160 ppm) nor iron (40 –1600 ppm) was toxic. Wiederanders (1968) suggested that the metabolic and excretory pathways for copper in the turkey and other fowls were different from those of the mammals as evidenced from the studies in heavy copper loading in turkeys.

Bubien *et al.* (1971) studied the acute and chronic copper poisoning in chicken. They administered a single large dose (0.8 g) of copper sulphate or a series of small dose (0.01 – 0.04 or 0.08 g) daily for 60 days or 0.16 g daily for 30 days . Death was noticed in most of the birds, given 0.8 g, in 48 hours. In chronic poisoning 0.8 g daily was tolerated for 60 days with out any apparent symptoms except a slight reduction in growth rate with 0.16 g daily. Icterus and hepatic lesions were absent even with the highest dosage when hepatic copper exceeded 1000 ppm.

Toxic effect could not be observed by Kamel *et al.* (1971), when they injected 0.0713 g/kg copper sulphate (1/10 of LD 50) into crop of fowls for 40 days. The relationship between copper and selenium was revealed by Jensen (1975). He observed a high mortality, muscular dystrophy and exudative diathesis in chicks fed a diet supplemented with 800 or 1600 ppm copper. Adding 0.5 ppm selenium to the basal diet containing 0.2 ppm, prevented the mortality and selenium deficient signs. Results suggested that higher copper induced selenium deficiency in chicks, when a diet relatively low in this element was fed .

Rangachar and Hegde (1975) conducted experiments in chicks to find out the effect of 100 and 200 ppm copper sulphate fed for 120 days on prothrombin time . At these levels there was no influence on prothrombin time. Experiments were

conducted in day old chicks by Poupoulis and Jensen (1976) by feeding 250 – 500 ppm copper supplemented diet for 4 weeks. They analyzed the fatty acid composition and found that it was not affected significantly. Studies were conducted by Guenthner *et al.* (1978) in turkey poults by feeding them with 60, 120 and 240 ppm copper supplemented diet . They observed no effect with 60 ppm copper, 10% increase in growth with 120 ppm , and no toxic action with 240 ppm .

Jackson *et al.* (1979) observed a depression of body weight in hens while feeding a diet with 600 and 800 ppm copper . Gross and microscopical examination of specific tissues revealed no pathological effect although gizzard and intestinal weight were increased and caecal weight was decreased . The liver copper conc. was found to be raised sharply by adding higher levels of copper. Rangachar and Jayaprakash (1979) noted an increase in haemoglobin synthesis when 10 weeks old chicks were reared on a diet supplemented with 100 ppm copper and zinc for 75 days.

Laying hens were fed for 48 hours with 250, 500, 1000 and 2000 ppm copper supplemented diet (Stevenson and Jackson, 1980). They noticed a reduction in Liver, Kidney, oviduct and ovarian weight per unit body weight by copper in the diet and the effect increased with period of time on the diet . The gizzard weight per unit body weight was increased by dietary added copper. The copper conc. in the liver were increased by dietary level of added copper . They also observed a negative linear relationship between liver lipid concentration and the level of added dietary copper . On the highest treated group the liver copper level continued to increase for 48 days but with 500 and 1000 mg added copper per kg a maximum level was reached after 12-24 days .

Latymer and Coates (1981) studied the role of copper supplementation on pantothenic acid in chicks by supplementing 250 mg copper per kg of feed .The results showed that high dietary supplementation of copper sulphate induced pantothenic acid

deficiency through interference in the biosynthesis of Co.A. Radzanowska (1989) noted an adverse effect on haemopoietic system,liver and kidney when adult chicks were fed with a diet containing 1000 mg/kg copper for 6 weeks. Ward *et al.* (1994) investigated the effect of 204 ppm copper supplemented diet on haemoglobin level. The result showed no significant changes between treated and control group

2 . 1 . 4 Gross and histopathological lesion in copper toxicosis.

Kamel *et al.* (1971) administered copper sulphate at a dose of 0.5-1 g per kg body weight in chicks. On post mortem they noticed lesions were mostly confined to kidney and intestine in those died in 24 hour The presence of acute tubular nephrosis and necrotic changes were noted . In those birds which died in 2 – 7 days the lesions were mainly in the liver which showed atypical acute toxic dystrophy and kidney showed advanced degenerative changes. Those birds which were sacrificed at 20 – 40 day had acute and chronic hepatitis. Poupoulis and Jensen (1976) observed that 500–1000 ppm copper in the feed caused gizzard erosion in all treated birds. The addition of 0.35 % cholic acid to the diet significantly decreased the gizzard erosion with 250 – 500 ppm supplemented copper. Jensen and Maurice (1976) also noted gizzard erosion in chicks fed with 500 ppm copper in the feed.

Addition of 120-250 mg copper per kg of feed in broiler ration resulted in the distension of caeca (Jensen and Maurice, 1978).The contents of caeca were dark and pasty. Adding monensin, sod. gentian violet or ferrous sulphate alone or in combination with copper to the ration did not significantly change the appearance of caeca. Copper concentration in the caecal content reached a level above 5000 mg/kg, suggesting that the effect of copper on the caeca may be related to an inhibition of normal fermentation. Gross appearance of gizzard lining and proventriculus was also changed by adding 240 mg/kg copper to the diet. Gizzard erosion was reported in

turkey poults also by Christmas and Harms (1979) when they fed a ration containing 500 – 700 ppm copper sulphate for 24 days .

Stevenson and Jackson (1980) could not observe any significant change in the haemoglobin level or pcv and serum copper of birds fed with 500, 1000 and 2000 ppm copper supplemented diet for 8 weeks and then fed with normal diet. Shivanandappa *et al.* (1983) studied the acute oral toxicity of copper sulphate and copper oxychloride in cocks. The median lethal dose (LD 50) for copper sulphate and copper oxychloride was found to be 693 and 1263 mg/kg body weight respectively. Severe diarrhoea, delayed mortality (3-6 days) and increased liver weight were characterestic of copper oxychloride. In both the cases a dose dependent testicular atrophy was noticed. Histochemically the interstitial (Leydig) cells and seminiferous tubule showed intense accumulation of cholesterol positive lipids.

Gizzard lesions were noticed by Wight *et al.* (1986) when they incorporated 2000 to 4000 ppm copper sulphate in the diet of fowl. Copper sulphate 0.05 to 0.2 % was added to the ration of broiler chicks and fed for 3 weeks to find out the influence on oral lesion (Jensen *et al.*, 1991) They noticed a significant increase in gizzard erosion and incidence and severity of oral lesion. A linear increase was noticed with increasing copper concentration. They suggested that copper sulphate supplementation should be considered as one of the possible causes of oral lesion seen during post mortem examination of avian species. Anjum *et al.* (1992) fed week old broiler chicks with diet containing 500 – 1000 ppm copper sulphate per kg . They could not observe any change in clinical appearance, weight of liver, or in gizzard and heart . The liver was pale and showed mild degenerative changes . The gizzard showed erosion of varying degrees. Erythrocyte count showed an increased trend. They concluded that dietary copper sulphate up to a concentration of 1000 ppm had no effect on performance of broilers.

The interaction between crude proventricular homogenate or filtered homogenate and copper sulphate (1g/kg) added diet was studied in female day old broilers by Bayyare *et al.* (1995). The result showed that there was no interaction between homogenate and copper.There was a reduction in feed conversion efficiency in broiler homogenate and copper sulphate . Natural exposure to low level of the infectious agent present in the homogenate may interact with excess dietary copper sulphate resulting in increase in proventricular size and decreased in body weight and feed conversion efficiency. Gilbert *et al.* (1996) noticed severe oral ulcers of pharynx in layer birds when the diet was supplemented with 1477 ppm copper and fed for one week.

2 . 1 . 5 Role of copper on the enzymes in birds.

Rucker *et al.* (1969) studied the role of copper on some of the enzyme systems of birds. They fed a copper deficient diet to chicks and noticed a marked reduction in cytochrome oxidase activity ,but the catalase activity was not changed . Stevenson and Jackson (1980) investigated the role of supplemented copper on aspartate amino transferase (AST) level in serum . Laying hens were fed with a diet containing 500, 1000 and 2000 mg added copper per kg feed for 8 weeks. The results showed that the level had no significant effect on AST

2 . 2 . 6 Role of copper on the immune system of birds.

Rangachar and Hegde (1974) studied the response of chicks to *Salmonella gallinarum* . The chicks were fed with 100 ppm copper supplemented diet. The results showed a greater primary response to *Salmonella gallinarum* in treated group than controls. Rangachar *et al* (1978) noticed no significant influence on the synthesis of immunoglobulin Ig G by feeding 10 weeks old cockerels with 100 ppm copper or zinc or both supplemented diet for 3 - 5 months. Stahl *et al.* (1989) studied the

antibody response of laying hen , feeding with a diet supplemented with 250 ppm copper, no difference was seen with the control birds

2 . 1 . 7 Role of dietary copper on the tissue copper accumulation.

The copper content of hepatic tissue of ducklings and chicks were assessed by Owood and Worden (1973) after feeding 200 ppm copper. The highest copper content recorded in chicks liver was 23 ppm after 28 days on diet containing 200 ppm copper. The maximum copper content recorded in the liver of ducklings on this diet was 140 ppm.

The mean plasma copper values for chicken were recorded at different age groups by Panic *et.al* (1974) after feeding a normal diet. The values for different age groups were as follows- 84 day old pullets – 10.0 μg/100 ml, 94 day old pullets –12.3 μg/100ml,at onset of laying –22.5 μg/100ml, 8 month old hens –27.6 μg/100ml, 12 month old hens – 26.5 μg/100ml, 24 month old hen stopped laying 13.6μg/100ml, cocks aged 84 days -9.6 μg/100ml,cocks aged 94 days –10.2 μg/100ml and cocks aged 210 days –12.2 μg/100ml.

Markarova (1978) reported that the plasma copper level of chicken will increase with age and that the peak concentration of copper was found at 60 days, during his study for a period of 15 – 210 days. Jackson *et al.* (1979) reported that by adding 600 – 800 ppm copper to diet caused a sharp rise in the liver copper concentration . An increase in liver copper level after feeding a high copper diet was also observed by Jensen and Maurice (1979) . They also noticed that the rise was significantly reduced on addition of 0.4% methionine to the copper supplemented diet . Martynyvk *et al.* (1980) estimated the copper content of poultry meat by atomic absorption spectrophotometry and colorimetry. The values were 0.61 – 4.2 and 0.59 – 4.5 mg/kg respectively.

Starvation of birds significantly reduced the serum level of copper (Richard *et al.,* 1980). However, the copper level in the liver, pancreas and duodenum was increased. Re-feeding resulted in a decline in copper level in liver and pancreas. A copper binding protein metallothionine was increased during fasting which was responsible for the increased concentration of copper. Metallothionine may function as a conservation mechanism to prevent the loss of copper during tissue catabolism. Robins and Baker (1980) noticed a reduction in the copper level of liver, when sulphur containing amino acid was added to copper supplemented diet of chicks. A relationship between dietary copper and the concentration of copper in the liver or plasma was also recorded by Ekpergin and Vohra (1981) .They also noted a lowered level of copper in the plasma and in the liver when excess methionine was included in the ration.

In coccidia infected birds a 2-4 fold increase in deposition of copper in liver was noticed by Southern and Baker (1982) ,when they fed 500 – 750 ppm copper supplemented diet in coccidia infected chicks. Southern and Baker (1983) observed an increase in liver copper concentration by excess feeding of dietary copper and also by coccidiosis , but a decrease is noticed by excess zinc addition to the ration. They have given 250 mg/kg copper in the diet. Andrews *et al* . (1988) found that addition of 500 ppm copper to corn -soya bean meal diet of chicks increased the liver and pancreas copper content.

Dressel *et al.* (1988) estimated the iron ,copper and zinc content of turkeys and geese liver . The liver copper content of slaughtered and naturally died Geese were 98.5 ± 94.8 and 318.5 ± 118.5 μg/g of dry matter respectively. The copper content in the liver of slaughtered turkeys was 36.3 ± 8.9. Copper content of liver, spleen, kidney, lung, heart, brain and breast muscles of Mallard and hybrid ducks were estimated by Proske *et al.* (1991). The age of the birds ranged from hatching to 22 weeks old. At hatching the liver copper level was low and increased from six weeks onwards.

Conversely in the spleen the copper concentration at the time of hatching was high which decreased rapidly during second week, and increased slightly when 16 weeks old . The concentration of copper was the highest in the liver of adult ones.

The liver and plasma copper concentration were investigated by Ward *et al.* (1994) after feeding poults with 204 ppm copper in the ration for 5 days It was found that liver copper concentration was increased in poults fed supplemented dietary copper, but plasma copper concentration was not affected by dietary copper.

2 . 2 ZINC

2 . 2 . 1 Role of high dietary zinc on body weight and reproduction in birds.

Roberson and Schaible (1960) conducted experiment to find out the upper limit of zinc which can be tolerated by the chicks . They added zinc oxide, zinc carbonate and zinc sulphate at a level ranging from 0-1500 ppm to the feed and fed for four weeks to, day -old chicks. The results showed that the carbonate and sulphate adversely affected growth and feed efficiency at 500 ppm and above . Mortality was not heavy except at 3000 ppm of zinc carbonate. In the studies conducted by Vohra and Kratzer (1968) the tolerance limit of zinc in poultry was found to be 2000 ppm. Zinc at 4000 ppm reduced weight gain which was counteracted by adding EDTA. Mortality could be observed only at 10000 ppm of supplemented zinc.

Gasaway and Buss (1972) fed diet supplemented with 3000 – 12000 ppm zinc to domestic Mallard ducks (*Anas platyrhynchos*).They noticed a reduction in feed

intake and body weight, which showed a proportional decrease as the level of zinc in the diet is increased .The ratio of adrenal and kidney weight to body weight was increased, but no change was observed in liver body weight ratio . Reduction in size of the gonads was also noticed. Hill (1974) observed that zinc at 200 ppm level in feed decreased the growth rate in chicks . Herland *et al.* (1975) did their work in day old chicks by giving diet supplemented with 25-30 mg/kg or 75 mg/kg of zinc for first week . In the second week the zinc level in the feed was reduced to deficient level. This showed a better growth rate than that of control group in which high level was not reduced in the second week. They concluded that bone stored the zinc consumed in excess of requirement, which may be available for utilization during subsequent period of deprivation in growing animals.

Kincaid *et al.* (1976) observed that the weight gain in broiler chicks was not reduced by 2400 ppm of added zinc in the diet. They have opined that the homeostatic mechanism of young chicks is effective up to 1200 ppm dietary zinc. A reduction in feed intake, egg production and body weight was noticed by Hermayer *et al.* (1977) when the feed was supplemented with zinc exceeding 794 ppm. A reduced egg production was also recorded by Palafox and Ho (1979) . In their experiments hens and pullets were fed with diet containing 200 ppm zinc from 0 –5 days and then control diet from 5 days to 12 weeks, they noticed a reduction in fertility and hatchability also.

Bessel and Lantzsch (1980) in their studies in white leghorn hens, given a diet to which 5000 – 20000 ppm zinc was added and fed for 2, 4, 6, and 8 days. They noticed a reduction in egg number and feed consumption with increased zinc content of the feed and also by increased duration of the treatment. Kibakin *et al.* (1980) concluded that 80 ppm zinc in the ration gave the highest values for egg yield and quality. Studies were conducted by Palafox and Ho (1980) in white leghorn laying pullets and hens to find out the effect of zinc toxicity in the performance in the birds.

Zinc, fed at 20 mg/g of diet for 5 days, showed that it significantly affected the fertility and hatchability of eggs. A reduction in body weight gain and number of eggs was also noticed.

A retardation of body weight gain was also reported by Paya (1980), when he fed day old Habbard chicks with diet supplemented by adding 1500 mg zinc per kg feed. He also noticed a reduction in the weight, length and width of femur. Southern and Baker (1983) found that zinc at 4000 mg/kg in ration reduced both feed efficiency and body weight gain. His experiment was aimed to find out the zinc toxicity, zinc deficiency and zinc copper inter relationship in *Eimeria acervulina* infected chicks. He found that coccidial infection had an ameliorative effect on zinc toxicity

Charles and Cunningham (1987) noticed a marked reduction in feed intake when they maintained white leghorn on diet supplemented with 20000 ppm zinc for 4 or 10 days. The egg production and body weight loss were not significantly affected . A reduction in feed intake by 4000 ppm zinc diet was noticed by Andrews *et al.* (1988). They got a significant reduction in body weight gain . Pimental *et al.* (1991) reported a suppression of body weight when chicks were fed with a diet containing less than 28 μg/g of diet.

2 . 2 . 2 Effect of high zinc on hormones and enzymes of birds.

The effect of zinc on testicular hormones was studied by Eltohamy *et al.* (1980).Zinc ammonium sulphate solution 100μg per kg body weight was injected once daily for four weeks intra muscularly in intact and caponised cockerels A decrease in weight of testis, an inhibition of spermatogenesis, disturbed testicular hormone production and increase in pituitary gonadotropic cell activity were observed. No discernible effect of zinc injection on pituitary and adrenal cells of caponised birds was observed. Paya (1980) noted an increase in alkaline phosphatase activity with increased zinc in day old chicks fed with 80 ppm zinc supplemented diet.

Schuster and Hindmursh (1980) suggested that measure of plasma alkaline phosphatase level can be used as a tool to assess the zinc status of birds. They showed a low plasma alkaline phosphatase activity in birds with low zinc status.

A reduction in amylase activity was recorded by Lu and Comb (1986) when they fed, chicks with 1000ppm zinc supplemented diet. They measured the amylase activity of duodenal luminal content after 8-15 days. The starch digestibility was reduced by 20-40% . In 1989 they also observed that even 500ppm zinc in the ration decreased the pancreatic amylase activity ,by reducing the synthesis . They have the opinion that zinc induced intracellular membrane damage might have reduced the amylase synthesis. Dean *et al.* (1991) in their investigation by feeding 5280 ppm zinc supplemented diet to, day -old chicks for 1-2 weeks, recorded an impairment in the post natal growth and multiple endocrinopathy. They concluded that depressed level of circulating thyroid hormones might be the cause for impaired growth in birds.

2 . 2 . 3 Zinc toxicity and inter relationship with other metals.

The growth in poults due to 4000 ppm zinc in the diet was overcome by 15.4 or 30.8 m.mol EDTA (Vohra and Kratzer, 1968),but the effect of 8000 ppm zinc could not be overcome by EDTA. They observed no mortality even at 10000 ppm zinc in the diet. Supplementing the diet with 2000 and 4000 ppm zinc Southern and Baker (1983) noted that the excess dietary zinc decreased tissue copper deposition, but excess copper did not affect tissue zinc deposition in chicks. Park and Kim (1985) observed that zinc supplementation could alleviate the toxicity caused due to nickel in the diet. It was also noticed that copper supplementation can alleviate the nickel toxicity. They could reverse the low zinc level in tibia and plasma by zinc supplementation. They concluded that nickel toxicity was by interference with zinc metabolism.

2 . 2 . 4 Role of zinc on the immune system of birds.

Rangachar *et al* .(1978) studied the immunoglobulin level in cockerels fed with copper and zinc at the rate of 100 mg/kg feed for 3-5 months. They could not observe any significant change in immunoglobulin IgG level. Fowls which were maintained on zinc deficient diet was immunized with bovine albumin I/p injection or by a combination of ocular drop and i/p injection (Burns, 1983). The birds showed no antibody response as judged by immuno electrophoresis and immuno fluorescence, where as, birds on zinc sufficient diet had serum antibody and anti bovine serum albumin cells in the Harderian glands, payers patches, spleen and thymus, which indicated that zinc is essential for the immune system.

Shoyinka and Daudu (1986) also reported that the antibody response to B1 vaccine and Komarov vaccine were higher in cockerels fed with 200 mg zinc per kg feed than either 50 mg zinc or 400 mg zinc in the feed. Pimentel and Cook (1988) observed that zinc deficiency had no effect on primary humoral immune response to sheep RBC. In chicks zinc supplementation at a level of 30, 40, 50, 60, 70 and 80 ppm did not influence primary and secondary immune response to sheep RBC or delayed hypersensitivity to phytohemagglutinin -P or human gamaglobulin. Decreasing the level of dietary zinc to 8 ppm did not alter immune parameters. In contrast chicks fed 58 ppm zinc had a marked reduction in primary and secondary humoral immune response.

In another experiment by Stahl *et al.* (1989) chicks were fed on diet containing zinc 28, 38, 48, 68, and 178 μg/g of feed and they were evaluated for zinc related feather frawing and immune status . It was noticed that 38 μg/g is necessary for maximal immune response. They have suggested that supplementation of hens diet with zinc 150 μg/g may be excessive and causes marginal immune suppression of young chicks. The results of the study by Pimentel *et al.* (1991) revealed that zinc

at a level of 8-88 μg/g of diet had no influence on the immune response. Zinc was fed to chicks at a level of 8-88 μg/g of diet for 4 – 7 weeks. The immune response was studied against sheep RBC and phytohemagglutinin-P by hemagglutination and delayed type skin hypersensitivity test . Zinc at 8 μg/g of diet had smaller bursa of fabricius and thymus .

Kidd *et al.* (1992) evaluated the immune status of chicks by feeding 72 and 152 ppm zinc oxide or organic zinc-methionine (ZM) supplemented diet to dams and to chicks for the first one month . The results indicated that supplementation of ZM in hens diet increased cellular immunity response in progeny and an increase in primary antibody titers to *Salmonella pullorum* antigen. Kidd et. al. (1993) recorded an increase in basal hypersensitivity to phytohemagglutinin-p in the progeny of hens fed with supplemental ZM in corn soyabean meal. It also increased the antibody titers in the progeny. Kidd *et al.* (1994) studied the influence of diet containing ZM on *in vitro* and *in vivo* uptake of *E. coli* by mononuclear phagocytic system in female turkeys .The turkeys were maintained on a ration supplemented with 40 μg ZM ,for 1 – 3 weeks of age so that the basal diet contained 130 μg zinc/g and ZM diet contained 165 μg zinc/g .The number of phagocytized *E.coli* per macrophage did not differ significantly. The clearance of *E.coli* from blood was significantly improved in ZM higher zinc group.

2 . 2 . 5 Gross and histo- pathological changes in the organs of birds in zinc toxicity.

It was reported by Gasaway and Buss (1972) that feeding of 3000 to 12000 ppm of zinc supplemented diet to domestic Mallard ducks (*Anas platyrhynchos*) caused yellowish red colouration of kidney.It was suggested that this can be used for

diagnosis of zinc toxicosis in Mallard ducks . A reduction in size of pancreas and gonads was also noticed. In an acute and chronic toxicity studies of zinc in birds conducted by Sofeietti and Bestettl (1975) , they found that high level of zinc as zinc sulphate or zinc oxide was moderately toxic and caused gastric ulcer ,pancreatic lesion and colloid goiter.

Dewar *et al.* (1983) fed chicks with diet containing 2000, 4000 and 6000 ppm of zinc. All of them grew poorly and showed gizzard erosion and lesion of the exocrine pancreas. Dissecting aneurysm occurred in a few chicks on 6000 ppm group . The gizzard lesion varied from excessive desquamation of epithelial cells, heterophils and erythrocytes into an abnormaly spongiform koilin to erosion of koilin glands . The pancreatic lesions were detected only microscopicaly and consisted of delution of acinar,luminocytoplasmic vaculation, cytoplasmic globule formation and necrosis of the exocrine cell with inter parenchymal fibrosis.

Lesions in pancreas and gizzard were also recorded by Wight *et al.* (1986) in hens fed with diet containing 20000 –25000 ppm zinc as zinc oxide . Breeding *et al.* (1992) could not observe any lesion in chicks fed with 2800 ppm zinc in the form of zinc sulphate.

2 . 2 . 6 Tissue zinc concentration.

Makarova (1978) assessed the zinc level in serum of normal white leghorn hens of 15 – 210 days of age. The values were increased with age. Peak concentration was reached when egg laying was in full swing. Relatively low serum zinc values were noted in hens with disturbed egg laying. Makarova and Chekalova (1979) stated that during the growth of ovarian follicle, the zinc content of the blood was closely correlated with the albumin fraction of blood serum. The zinc content increased from

162 – 445 µg/ 100 ml . Oh *et al.* (1979) studied concentration of zinc in liver, kidney, pancreas and intestine of chicks after feeding 500 – 4000 and 1000 – 16000 ppm zinc in the ration .The concentration of zinc was maximum in metallothionine. Results revealed the involvement of metallothionine in zinc homeostasis.

In turkey poults serum concentration of zinc was significantly reduced by starvation (Richard *et al.*, 1980) The level of zinc increased in liver,duodenum, pancreas and kidney on fasting. Refeeding resulted in a decline of zinc level in these tissues. Analysis of metal bound zinc in the cytoplasmic fraction of these tissues indicated that metallothionine, a zinc copper binding protein is increased during starvation and declined with refeeding. Metallothionine acted as a conservation mechanism to prevent the loss of zinc and copper during tissue catabolism.

Bettger *et al.* (1981) fed 1,5,10,20 and 100 ppm zinc supplemented diet to chicks. The plasma zinc concentration observed was 0.2, 0.4, 0.6, 1.0 and 1.3 µg/ml. The haematocrit values were 39, 36, 33, 30 and 29 respectively. The plasma zinc conc. dropped significantly within 12 hours after a zinc deficient diet. Dressel *et al.* (1988) studied the zinc level in various tissues of slaughtered ducks and those which have a natural death and of slaughtered turkeys. The zinc content in the liver of slaughtered ducks was 154.1 ± 27.4 compared to 91.1 ± 23.1 in slaughtered turkeys. The difference in values was attributed to the effect of inflammation and infection.

Iron, copper and zinc content of liver, spleen, kidney, lung, heart, brain and breast muscle was estimated in growing Mallard and hybrid ducks by Proske *et al.* (1991) . The zinc content was low at hatchability and increased from 6 weeks onwards. In the spleen there was higher concentration of zinc at hatching when the spleen was extremely small (0.03 g) . Concentration decreased rapidly during the

second week after hatching and increased slightly at 16 weeks onwards. Concentration of zinc was the highest in the liver of adult duck.

Improvement in the performance of the *Ascaridia gallinarum* infected chicks fed with basic salt of zinc at the rate of 30 μg zinc per kg body weight was noticed by Morros *et al.* (1995).

2 . 3 MANCOZEB

2 . 3 . 1 Toxic action of Mancozeb and related compounds in animals and birds.

Begliomini *et al.* (1967) noted a reduction in weight gain in broiler chicks when they were fed with 0.01 – 0.1 % Ziram in the feed. They noticed a delay in the onset of laying and reduction in egg production by 28 % in group fed with 0.1 % Ziram. On postmortem examination they could observe enteritis, renal and hepatotropy, bone fragility and extreme deficiency of calcium. Acute and chronic toxicity study of Zineb in fowls were made by Soffietti and Bestettle (1975) . Zineb was given by stomach tube as a single dose for acute test and as 1% of feed for 31 weeks in chronic test. Clinical and histo pathological studies were made and was found that Zineb was almost non toxic except for causing cloudy swelling of hepatocytes and kidney cells.

Soffietti (1978) fed day old chicks with a diet containing 2% Zineb for 24 months and noticed that after one month of feeding birds developed goiter which grew progressively. After ten months one thyroid weighed over 43 g. Histological examination revealed desquamation in the alveoli and hyperplasia of the follicular epithelia, assuming a papillomatous appearance. Intestinal haemorrhage and sclerosis

of intra follicular walls were also noted. Carlonebbia *et al.* (1991) studied the toxicity of Zineb in calves with immature rumen function. Zineb was administered at a dose of 200 mg/kg body weight daily for 80 days. The treated calves had unthrifty appearance and reduction in body weight gain, remarkable impairment in thyroid function was also recorded .

The influence of Mancozeb on human erythrocyte carbonic anhydrase isoenzyme and bovine erythrocyte carbonic anhydrase were determind *in vitro* by Celik *et al.* (1996). They observed no effect of Mancozeb on these enzymes. The toxicity of Mancozeb was tested in rats by Hore *et al.* (1997) . The fungicide was given at a rate of 400 mg/kg body weight orally every morning for 49 days. The treated rats showed reduced body weight, reduced appetite and had an abnormal huddled posture. The PCV, TEC, HB and TLC values were low.The animals developed a normocytic normochromic anaemia. The pathological examination of the 2 rats that died during the experiment at 22 and 35 days showed congestion, haemorrhage on heart and spleen , sinusoidal congestion of the liver and haemorrhages in the kidney.

Kara and Celik (1997) studied the in vitro effect of Mancozeb on human serum enzymes, aspartate amino transferase, serum glutamyl pyruvic transferase, alkaline phosphatase, delta glutamyl transferase,lactate dehydrogenase, amylase and myocardial creatinkinase. They found that it strongly inhibited these enzymes except lactate dehydrogenase.

MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS.

Based on the results of the studies conducted in Kuttanad area, a controlled study in ducks was taken up in the College of Veterinary and Animal sciences Mannuthy, to assess the chronic toxicity of these two heavy metals and one organic fungicide Mancozeb (a combination of Maneb and Zineb).

One hundred and fifty six, one month old clinically healthy White Peckin ducks (*Anas platyrhyncos domesticus*) obtained from the Kerala Agricultural University poultry and duck farm were used for the study. The study was conducted in three phases with copper, zinc and Mancozeb supplemented diet

3 . 1 Lay out of the experiment.

Phase one – After wing banding and recording the initial body weight, sixty ducklings were devided into four groups (I, II, III and IV) of fifteen each in such a way that difference among the groups were fairly similar. The birds were reared under same management condition in deep litter system with ration having the composition given below

Composition of the ration.

Maize	410 kg
Gingly oil cake	130 kg
Fish	190 kg
Rice polish	200 kg
Salt	2.5 kg

Mineral mixture	17.5	kg
Shell grit	50	kg
Total	1000	kg

Group I, II and III served as the treatment groups and they were fed on a ration supplemented with 100, 200 and 300 ppm of elemental copper respectively (as copper sulphate) dissolved in water. The feed was moistened with the copper sulphate solution, so that to get a uniform distribution of copper sulphate in the feed .Group IV served as control and fed on the same diet of treatment group without copper supplementation.

Phase two- In the second phase forty eight ducklings of one month age were used. They were divided into four groups of 12 each in such a way that the difference among the groups were similar. Group I, II and III were served as treatment groups and they were fed on diet supplemented with 100, 200 and 300 ppm elemental zinc respectively (as zinc sulphate) .The feed was moistened with the zinc sulphate solution. Group IV served as control and fed on the same diet of treatment groups with out supplementation of zinc.

Phase three- In the third phase also forty eight ducklings of one month age were use. They were divided into four groups of twelve each. First three groups were fed on ration added with 1000, 1500 and 2000 ppm Mancozeb respectively as Dithane M 45. (zinc manganese ethylene bis dithiocarbamate). A suspension was made in water and moistened the feed with the suspension. Group four served as controls fed with the same diet of treatment group without the addition of Mancozeb.

3. 2 Details of the study.

3 . 2 . 1 Body weight: Body weight of the ducks was recorded at fifteen days intervals until they were sacrificed after six months.

3. 2. 2 Haemogram : Erythrocyte count, Leucocyte count , Haemoglobin , Erythrocyte sedimentation rate, Packed cell volume and Differential count were recorded at monthly intervals.

Blood samples were collected from the jugular vein, with anti coagulant Na EDTA for the routine haematological tests and without anticoagulant for collecting serum . A fresh smear was also prepared without adding anticoagulant for the differential count.

3 . 2 . 2 . 1 Erythrocyte count : The method described by Sastry , G.A. (1989) was followed. The diluent medium consisting of sodium citrate 2%,gentian violet 0.1% in Ringer sol., brilliant crysyl blue 0.1% in Ringer sol. and neutral formalin mixed in the ratio 5:10:5:1.

Ringer solution-Sodium chloride,0.7 g,Sodium bicarbonate 0.03 g, Pot. Chloride 0.026 g and Calcium chloride 0.003 g in 100 ml distilled water.

Neutral formalin- Disodium hydrogen phosphate (Na_2 H PO_4) 7.83 g, Sodium dihydrogen phosphate (NaH_2 PO_4 $2H_2O$) 5.673 g in 100 ml formalin and make up the volume to 1000 ml with distilled water.

For both erythrocyte and leucocyte count , same diluting pipette and same diluting solution were used.

3 . 2 . 2 . 2 Erythrocyte sedimentation rate: (ESR) The ESR was estimated as per the method described by Wintrobe (1981).

3 . 2 . 2 . 3 Packed cell volume: (PCV) The PCV was estimated as per the method described by Wintrobe (1981).

3 . 2 . 2 .4 Haemoglobin: For estimation of haemoglobin cyan methaemoglobin method described by Miale (1972) was followed.

3 .3 .2 .5 Leucocyte count : The method described by Sastry, G.A. (1989) was followed.

3 .2 . 2 6 Differential count : Modified copper peroxide method of Valsala (1968) was followed.

Reagent 'A' Copper sulphate solution 0.5 % in water.

Reagent 'B' Benzidin reagent –Rubbed 0.2 g benzidin with few drops of water in a mortar and to this 200 ml of distilled water was added . The solution was filtered and added 4 drops of 3% hydrogen peroxide.

Technique-Solution 'A'was applied to fresh dry smears and allowed to act for ½-1min, poured it off and applied sol. 'B' allowed to act for ½-1 min poured it off. Flushed with distilled water drained and allowed to dry. Counter stain with Wright's stain for 10 min. Washed dried and examined under oil immersion.

3 . 2 . 3 Enzyme profile.

Collection of serum –Blood was collected from jugular vein, into a clean dry glass tube of 15 mm diameter. It was allowed to stand in a slanting position undisturbed for one hour at room temperature for clot formation. Then the clot was carefully detached from the wall of the tubes with the help of a glass rod and allowed to

stand at room temperature for one more hour for serum separation .Then the tubes were centrifuged at 2000 rpm for 5 min to get the serum separated. The serum was aspirated with an aspiration needle and preserved in deep freezer for enzyme study.

3 . 2 . 3 . 1 Aspartate amino transferase (AST / SGOT) : The method of Reitman's and Frankel's (1957) was followed. AST kit supplied by Glaxo was used for the study. The procedure laid down in the literature provided along with the kit was followed . The concentration of the enzyme was calculated from the calibration curve prepared earlier.

3 .2 . 3 . 2 Alanine amino transferase (ALT / SGPT) : The method of Reitman's and Frankel's (1957) was followed. ALT kit supplied by Glaxo was used for the study. The procedure laid down in the literature provided along with the kit was followed . The concentration of the enzyme was calculated from the calibration curve prepared earlier.

3 .2 . 3 . 3 Alkaline phosphatase : Alkaline phosphatase kit provided by Glaxo was used. The concentration of enzyme was calculated by comparing with phenol standard provided with the kit.

3 . 2 . 4 Immune status of birds.

3 .2 . 4 . 1 Antibody titre: All the birds, used for the experiment, were vaccinated with Egg Drop syndrome-76 (ED-76) virus vaccine produced in the Department of Microbiology. Two vaccinations were done on 15^{th} day and on 90^{th} day from the beginning of the experiment. All the birds, on one phase of study, were vaccinated on the same day. The serum samples from six birds each from the groups were collected fifteen days after the vaccination and there after at every fifteen days intervals to asses

the antibody titre. The non haemolysed serum samples collected in serum vials and stored in deep freezer until use (within 3-4 days after collection) The antibody titer was assessed by haemagglutination and Heamagglutination inhibition test. Haemagglutination test was first performed with washed chicken RBC to find out the virus titer, and then haemagglutination inhibition was performed to find out antibody titre in the serum of experimental birds as per the methods described by Poultry biologics (1963).

3 . 2 . 4 . 2 Cell mediated immunity (Phytohaemagglutinin-P test): PHA-P from Sigma chemicals was used for the study. Five microgram of PHA-P (in 0.1 ml distilled water) was administered intradermally(0.05 ml) at two sites on the wing web . All the birds were injected and the area was marked. Injection was repeated at half months intervels . The skin thickness was measured using a Venier calipers at 0,24,48 and 72 hours after injection, the readings were recorded (Rajan , *et. al,* 1986).

3 . 2 . 5 The element content in the serum: The copper, zinc and manganese content of the serum from six birds each, from all batches were noted at monthly intervals. The element content was estimated using Atomic absorption spectrophotometer,after diluting appropriately with distilled water (Anderson,1972).

3 . 2 . 6 Gross and histo-pathological lesion: All the birds were sacrificed after six months of the experiment. After sacrificing by cutting the jugular vein the carcass was opened and observed for any gross lesion. Internal organs like liver, kidney, muscle, bursa and available thymus were collected and examined for gross lesion. Gross weight of the liver, and spleen were recorded. Samples from liver, kidney, spleen, bursa and thymus were taken and preserved in formalin. Tissues were processed by routine paraffin embedding technique. (Armed Force Institute of Pathology , 1968). Paraffin sections were cut at 5-6 microns thickness and were stained

routinely with haematoxyline and eosin method of Harris as described by Disbery and Rack (1970).

3 . 2 . 7 Element content in the tissues : Samples from liver, kidney and muscle were taken for estimating the element (copper, zinc and manganese)content. The samples were dried in a hot air oven and powdered in a mortar and pestle. Five g each of the tissue samples were digested with triacid digestion and the element was estimated with atomic absorption spectrophotometer (Anderson, 1972 and Capar, 1977).

RESULTS

Chapter 4

RESULTS

4.1 COPPER

4.1.1 Body weight.

The mean body weight of ducks (g) fed on diet supplemented with 100, 200, 300 and 0 ppm copper is shown in table 2 .The body weight was recorded at 15 days intervals. All the birds fed on copper supplemented diet showed a mean body weight higher than that of the controls, even though all are not significant, of the corresponding period. The group I showed a significantly (significantly) higher body weight than that of the controls on 60^{th}, 105^{th}, 120^{th} and 135^{th} day (vide table 2). While the group II showed significantly higher body weight only on 135^{th} and 165^{th} day. The mean values on 105^{th}, 120^{th} and 165^{th} day of the group III also were significantly higher than the corresponding values of controls. In group I the values ranged from 1206.67± 38.38 to 1836.67 ± 33.97,group II from 1006.67 ± 81.34 to 1743.33 ± 44.15,group III from 1013.33± 63.89 to 1826.67 ± 46.77 and group IV from 1106.67 ± 55.61 to 1710.00 ± 35.92.

4.1.2 Erythrocyte count .

The mean erythrocyte count (x 10000 per cmm) of four groups of ducks at monthly intervals is shown in table 3 .The mean value of group I in second and fifth month were significantly higher than the corresponding values of the control birds. In group II significantly higher value was noticed in the fifth month and lower value on the sixth month . Group III showed significantly lower values on fourth, fifth and sixth month. The mean erythrocyte count ranged from 281.20 ± 7.23 to 349.13 ± 11.36 in group I, 292.27 ± 10.37 to 328.00 ± 8.89 in group II, 255.67 ± 6.69 to 314.8 ± 5.17 in group III and 275.87 ± 7.44 to 343.67 ± 11.74 in group IV.

Table 1 Copper and zinc content (ppm on dry matter basis) in biological samples collected from Kuttanad area.

Location	Copper				Zinc			
	Water	Mussels & Fishes	Plants	Sedi-ments	Water	Mussels & fishes	Plants	Sedi-ments
Kalarcode	0.00	12.8	30.00	55.00	0.02	141.00	153.66	165.50
Pallathuruthy	0.00	12.66	20.33	31.80	0.13	160.80	141.66	91.00
Chempumpuram	0.01	15.66	19.00	49.16	0.00	185.66	86.00	126.00
Kidangara	0.00	14.33	21.66	67.83	0.03	157.80	100.66	155.30
Pulimkunnu	0.02	14.5	32.30	42.66	0.34	198.00	155.30	111.66

Table 2 Mean body weight of Ducks (g) at 15 days intervals, fed on diet supplemented with copper.

Group	Days											
	15	30	45	60	75	90	105	120	135	150	165	180
I	1206.67 ±38.38	1546.67 ±36.34	1760.00 ±34.91	1820.00* ±41.40	1826.67 ±30.03	1836.67 ±33.97	1810.00* ±36.25	1783.33* ±40.73	1766.67* ±35.41	1713.33 ±33.62	1563.33 ±26.93	1556.67 ±22.29
II	1006.67 ±81.34	1373.33 ±70.69	1580.00 ±55.38	1733.33 ±50.40	1723.33 ±40.79	1743.33 ±44.15	1726.67 ±42.22	1700.00 ±39.94	1720.00* ±35.79	1673.33 ±31.57	1606.67* ±28.40	1556.67 ±34.80
III	1013.33 ±63.89	1373.33 ±72.68	1653.33 ±104.59	1733.33 ±54.92	1760.00 ±45.04	1826.67 ±46.47	1786.67* ±42.67	1713.33* ±42.39	1696.67 ±33.97	1633.33 ±31.87	1580.00* ±29.19	1500.00 ±41.40
# IV	1106.67 ±55.61	1440.00 ±63.84	1586.67 ±43.22	1686.67 ±36.34	1690.00 ±37.86	1710.00 ±35.92	1653.33 ±32.90	1600.00 ±30.47	1603.33 ±32.90	1586.67 ±33.62	1493.33 ±20.62	1446.67 ±25.57
CD(0.05)				131.34			109.76	109.57	97.88		75.11	

N=15

control

* Sig.at 5% level (P< 0.05)

** Sig. at 1% level (p< 0.01)

Table 2.1 ANOVA table (M.S.S),influence of dietary level of copper on the body weight of Ducks at 15 days intervals.

Source of variation	df	Days											
		15	30	45	60	75	90	105	120	135	150	165	180
Group	3	132666.667	100444.444	104611.111	131777.778	51444.444	58152.778	73486.117	85597.222	70777.778	44166.667	35152.778	41833.333
Error	56	57238.095	58785.714	64101.190	32214.286	22601.190	24678.571	22500.000	22422.619	17892.857	16023.810	10535.714	15285.714
F value		2.318	1.709	1.632	4.091*	2.276	2.356	3.266*	3.817*	3.956*	2.756	3.337*	2.737

Fig.1 Mean body weight (g) of Ducks at 15 days intervals, fed on diet supplemented with copper.

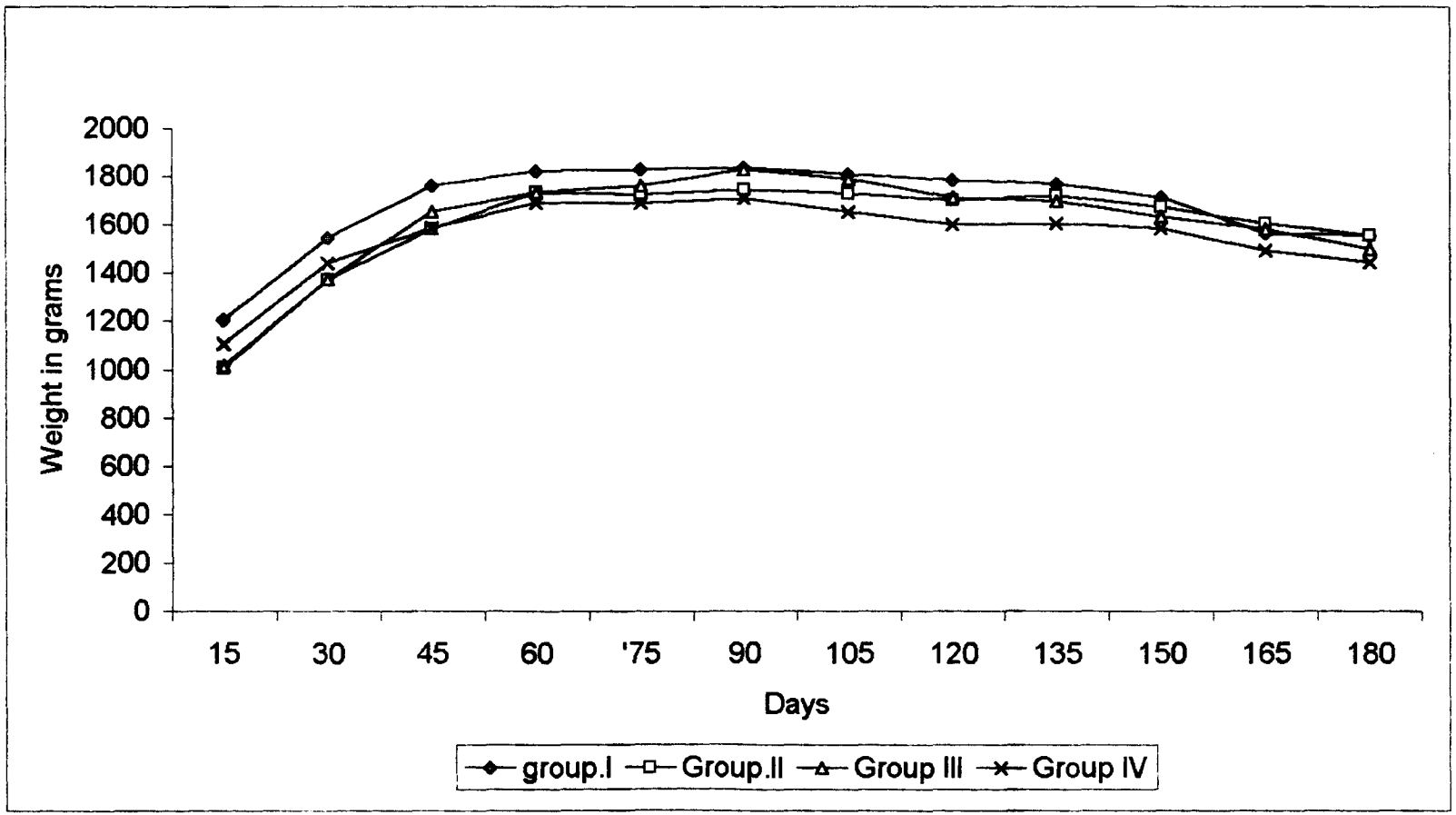


Table 3 Mean Erythrocyte count (x 10000 per cmm) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	322.40 ±8.81	349.13** ±11.36	289.20 ±7.23	308.13 ±5.21	344.67** ±10.18	347.47 ±6.36
II	292.27 ±10.37	296.73 ±6.69	293.20 ±5.65	301.40 ±4.80	328.00** ±8.89	303.73** ±10.53
III	314.80 ±5.17	300.20 ±6.12	293.53 ±6.43	269.20** ±5.73	255.67** ±6.69	301.87** ±4.35
# IV	308.93 ±5.40	293.47 ±5.03	275.87 ±7.44	305.07 ±6.80	289.53 ±7.99	343.67 ±11.74
CD (0.05) N = 15 # control		21.965		16.108	24.178	24.878

Table 3.1 ANOVA table (M.S.S), influence of dietary level of copper on the Erythrocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	2458.844	10383.794	1171.528	4884.061	8086.511	6541.000
Error	56	903.783	901.086	679.969	484.619	1091.757	1155.888
F value		2.720	11.524**	1.723	10.078**	7.407**	5.659**

4.1.3 Haemoglobin.

The mean haemoglobin value (g percentage) of four groups of ducks at monthly intervals is shown in table 4. All the mean values of the treated groups were lower (not significant) than the corresponding values of the controls. The mean values of the group I in the first, second and fourth months were significantly lower than that of the corresponding control values. In group II values in the first and fourth month and in the group III values in the first, second, fourth and fifth month were significantly lower than that of the controls. The mean haemoglobin values of group I ranged from 10.71 ± 0.16 to 13.13 ± 0.09, Group II from 10.65 ± 0.16 to 12.90 ± 0.14, Group III from 9.94 ± 0.12 to 12.67 ± 0.40 and group IV from 11.21 ± 0.12 to 13.07 ± 0.10.

4.1.4 Erythrocyte sedimentation rate.

The mean erythrocyte sedimentation rate (mm/hr)of four groups of ducks at monthly intervals is shown in table 5. The mean values were not significantly different except for the group I and II in second month in which the values were significantly lower . The mean values of the group one ranges from 0.11± 0.01 to 0.19 ± 0.01, group II from 0.12 ± 0.01 to 0.13 ± 0.01, group III from 0.11 ± 0.01 to 0.19 ± 0.01 and group IV 0.11 ± 0.01 to 0.2 ± 0.04.

4.1.5 Packed cell volume.

The mean packed cell volume (percentage) of four groups of ducks at monthly intervals is shown in table 6. Even though all the values were less than the corresponding values of the controls, none of the value was significantly lower. The values of group I ranged from 39.93 ± 1.03 to 41.13 ± 0.43, group II from 39.40 ± 0.87 to 41.00 ± 0.57, group III from 39.93 ± 0.95 to 41.13 ± 1.12 and group IV from 40.67 ± 0.89 to 42.80 ± 0.93.

Table 4 Mean Haemoglobin level (g percentage) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	10.71** ±0.16	11.80** ±0.15	12.12 ±0.19	11.95** ±0.17	13.13 ±0.09	12.55 ±0.14
II	10.65** ±0.16	12.41 ±0.12	12.50 ±0.14	11.82** ±0.12	12.86 ±0.11	12.90 ±0.14
III	9.94** ±0.12	11.26** ±0.17	12.51 ±0.17	11.77** ±0.11	11.82** ±0.17	12.67 ±0.40
# IV	11.21 ±0.12	12.75 ±0.17	12.69 ±0.36	12.50 ±0.21	13.01 0.19	13.07 ±0.10
CD (0.05) N = 15 # control	0.401	0.439	0.654	0.446	0.706	

Table 4.1 ANOVA table (M.S.S), influence of dietary level of copper on the haemoglobin level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	4.068	6.563	2.265	1.698	5.408	0.816
Error	56	0.301	0.361	0.799	0.373	0.931	0.771
F value	13.530**	18.167**	2.834*	4.546**	5.807**	1.057	

Table 5 Mean Erythrocyte sedimentation rate (c m /hr.) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	0.19 ±0.01	0.11** ±0.01	0.12 ±0.01	0.12 ±0.01	0.14 ±0.01	0.12 ±0.01
II	0.21 ±0.01	0.13** ±0.01	0.13 ±0.01	0.12 ±0.01	0.12 ±0.01	0.13 ±0.01
III	0.19 ±0.01	0.16 ±0.01	0.13 ±0.01	0.12 ±0.01	0.11 ±0.01	0.12 ±0.01
# IV	0.2 ± 0.04	0.18 ±0.01	0.13 ±0.01	0.11 ±0.01	0.12 ±0.01	0.13 ±0.01
CD (0.05)		0.032				

N = 15
# control

Table 5.1 ANOVA table (M.S.S), influence of dietary level of copper on the Erythrocyte sedimentation rate of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.002	0.014	0.000	0.000	0.002	0.000
Error	56	0.002	0.002	0.002	0.002	0.002	0.002
F value		1.047	7.321**	0.083	0.104	1.105	0.117

Table 6 Mean packed cell volume (percentage) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	40.53 ±0.87	40.27 ±0.69	43.13 ±0.43	40.13 ±0.92	40.87 ±1.02	39.93 ±1.03
II	39.80 ±0.64	40.53 ±0.84	41.00 ±0.57	40.80 ±1.02	39.40 ±0.87	40.53 ±1.31
III	40.27 ±0.51	39.93 ±0.95	40.67 ±0.72	39.93 ±1.00	40.80 ±1.09	41.13 ±1.12
# IV	41.13 ±0.42	40.67 ±0.89	42.80 ±0.93	42.27 ±0.67	42.20 ±0.96	41.27 ±1.10

CD (0.05)
N =15
# control

Table 6.1 ANOVA table (M.S.S), influence of dietary level of copper on the packed cell volume of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	4.644	7.156	13.644	16.728	19.617	13.533
Error	56	6.014	10.838	7.169	12.536	14.645	19.631
F value		0.772	0.660	1.903	1.334	1.339	0.689

4.1.6 Leucocyte count.

The mean leucocyte count (x 100 per cmm) of four groups of ducks at monthly intervals are shown in table 7. The mean values of the group I in the first, second and fifth month were significantly lower than the corresponding values of the control birds. The mean values of other groups were not significantly different from the control values. The mean leucocyte count ranged from 233.87 ± 6.17 to 308.07 ± 6.78 in group I, 271.80 ± 5.23 to 351.80 ± 14.86 in group II, 271.93 ± 6.45 to 330.93 ± 11.38 in group III, and 280.40 ± 4.90 to 336.07 ± 12.95 in group IV.

4.1.7 Differential count

4.1.7.1 Lymphocyte.

The mean counts of lymphocyte (percentage) of four groups of ducks at monthly intervals are shown in table 8 . Significantly higher values were noticed in the third month for group one and two .In the sixth month all the treated groups showed a significantly lower value In fifth month also the mean count of all the treated groups are less but not significant. The lymphocyte count of group I ranged from 58.53 ± 0.83 to 69.33 ± 0.93, group II from 59.47 ± 1.06 to 70.07 ±1.33, group III from 57.20 ± 1.07 to 66.73 ± 1.02 and group IV from 61.20 ± 1.61 to 67.67 ± 1.00.

4.1.7.2 Heterophil .

The mean heterophil counts (percentage) of four groups of ducks at monthly intervals are shown in table 9. None of the value except in the third month of group I and II was significantly lower from those of controls. All the values in fifth and sixth month were more than the corresponding control values. The values of group I ranged from 22.00 ± 0.73 to 31.20 ± 1.05, group II from 21.87 ± 1.07 to 29.87 ± 0.94, group III from 23.80 ± 0.98 to 31.27 ± 1.05 and group IV from 23.40 ± 0.62 to 29.80 ± 0.85.

Table 7 Mean Leucocyte count (x 100 / c m m) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	295.33** ±9.74	233.87** ±6.17	282.87 ±7.23	262.80 ±9.19	272.13** ±7.84	308.07 ±6.78
II	338.27 ±7.87	271.80 ±5.23	304.13 ±4.62	181.67 ±12.27	351.80 ±14.86	335.53 ±10.36
III	300.33 ±8.22	290.07 ±6.19	282.87 ±5.83	271.93 ±6.45	330.93 ±11.38	319.47 ±7.70
# IV	336.07 ±12.95	280.40 ±4.90	299.40 ±5.52	281.93 ±5.21	326.73 ±8.21	316.67 ±6.38
CD (0.05)	28.06	16.012			31.022	

N = 15
# control

Tyable 7.1 ANOVA table (M.S.S), influence of dietary level of copper on the Leucocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	7810.156	11413.089	1842.061	1250.328	17333.467	1678.333
Error	56	1470.617	478.833	517.479	1139.279	1797.286	949.960
F value		5.311**	23.835**	3.560	1.097	9.644**	1.767

Table 8 Mean Lymphocyte count (percent of total leucocytes) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	58.53 ±0.83	61.73 ±0.97	67.73** ±0.95	69.33 ±0.93	65.73 ±1.60	62.20** ±1.22
II	62.53 ±1.56	59.47 ±1.06	67.13** ±1.14	70.07 ±1.33	64.13 ±1.50	66.40** ±1.12
III	58.93 ±1.14	57.20 ±1.07	62.73 ±1.00	66.73 ±1.02	65.53 ±1.28	63.20** ±0.66
# IV	61.27 ±1.20	61.20 ±1.61	63.47 ±1.10	67.67 ±1.00	67.00 ±0.76	67.27 ±0.88
CD (0.05)Transforme values.		2.014	1.987			1.696

N = 15
# control

Table 8.1 ANOVA table (M.S.S, Transformed values), influence of dietary level of copper on the Lymphocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	19.497	21.723	35.429	13.626	7.258	32.419
Error	56	7.814	7.577	7.379	6.707	9.384	5.377
F value		2.495	2.888*	4.801**	2.032	0.774	6.028**

Table 9 Mean Heterophil count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	31.20 ±1.05	29.67 ±1.21	22.13** ±0.77	22.00 ±0.73	25.27 ±1.53	26.73** ±1.09
II	28.00 ±1.07	29.87 ±0.94	23.47** ±1.07	21.87 ±1.07	25.47 ±1.20	26.40** ±0.99
III	30.73 ±1.06	31.27 ±1.05	25.80 ±0.79	23.80 ±0.98	25.00 **±0.98**	26.67** **±0.73**
# IV	29.80 ±0.85	28.40 ±1.48	26.60 ±0.95	23.40 ±1.01	23.67 ±0.74	23.40 ±0.62
CD (0.05)Transformed values			1.705			1.653

N = 15
# control

Table 9.1 ANOVA table (M.S.S, transformed values) influence of dietary level of copper on the Heterophil count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	12.132	8.831	28.632	6.663	3.958	23.814
Error	56	5.937	8.515	5.433	6.256	8.495	5.107
F value		2.043	1.037	5.270**	1.065	0.466	4.663**

4.1.7. 3 Eosinophil.

The mean eosinophil counts (percentage) of four groups of ducks at monthly intervals are shown in table 10. No significant difference in eosinophil count was observed. The values of group I ranged from 4.27 ± 0.35 to 5.80 ± 0.28, group II from 4.47 ± 0.27 to 5.67 ± 0.31,group III from 4.87 ± 0.29 to 5.47 ± 0.46 and group IV varies from 4.47 ± 0.39 to 5.27 ± 0.65.

4.1.7. 4 Monocyte.

The mean monocyte counts (percentage) of four groups of ducks at monthly intervals are shown in table 11. None of the values were significantly different from that of the controls. All the values were higher than controls except second month of group I. The values of group I ranged from 3.00 ± 0.04 to 5.33 ± 0.06, group II from 3.27 ± 0.35 to 4.53 ± 0.41, group III from 3.40 ± 0.39 to 5.40 ± 0.19 and group IV from 2.87 ± 0.40 to 4.27 ± 0.48.

4.1.7.5 Basophil.

The mean basophil counts (percentage) of four groups of ducks at monthly intervals are shown in table 12. None of the values were significantly different from that of the controls. All the values in fifth and sixth month were lesser than that of the corresponding control values. In group I the values ranged from 0.67 ± 0.19 to 0.87 ± 0.29, in group II from 0.27 ± 0.11 to 0.93 ± 0.23, in group III from 0.73 ± 0.28 to 1.20 ± 0.22 and in group IV from 0.67± 0.23 to 0.93 ± 0.21.

4.1.8 Enzyme content

4.1.8.1 Aspartate Amino Transferase (AST).

The mean serum AST values (i.u/ml) of four groups of ducks at monthly intervals are shown in table 13. In group I significantly lower value was noticed in the third and the fifth month. In group II significantly Lower value was noticed in the

Table 10 Mean Eosinophil count (percent of total leucocytes) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	4.27 ±0.35	4.80 ±0.36	4.93 ±0.25	4.93 ±0.32	4.60 ±0.29	5.80 ±0.28
II	4.53 ±0.34	5.60 ±0.31	5.07 ±0.27	4.47 ±0.27	5.67 ±0.39	5.33 ±0.32
III	5.33 ±0.51	5.47 ±0.46	5.27 ±0.37	4.87 ±0.29	5.13 ±0.31	5.07 ±0.51
# IV	4.47 ±0.39	5.27 ±0.65	4.93 ±0.44	5.27 ±0.30	5.00 ±0.35	5.00 ±0.43

CD (0.05)
N = 15
# control

Table 10.1 ANOVA table (M.S.S, transformed values), influence of dietary level of copper on the Eosinophil count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	5.274	3.411	6.726	2.919	4.746	4.177
Error	56	4.877	5.823	3.230	2.393	2.919	3.916
F value		1.082	0.586	0.225	1.220	1.626	1.067

Table 11 Mean monocyte count (percent of total leucocytes) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	5.33 ±0.06	3.00 ±0.04	4.27 ±0.27	3.27 ±0.28	3.73 ±0.37	4.20 ±0.20
II	4.00 ±0.34	4.33 ±0.39	4.27 ±0.45	3.27 ±0.35	4.53 ±0.41	4.00 ±0.22
III	4.13 ±0.52	5.40 ±0.19	5.00 ±0.45	3.40 ±0.39	3.73 ±0.23	4.13 ±0.24
# IV	3.73 ±0.27	4.27 ±0.48	4.00 ±0.28	2.87 ±0.40	3.67 ±0.40	3.53 ±0.34
CD(0.05)transformed value		1.831				

N = 15
# control

Table 11.1 ANOVA table (M.S.S, transformed value), influence of dietary level of copper on the Monocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	12.703	38.172	4.946	2.956	5.269	4.036
Error	56	5.929	6.261	3.878	7.002	4.969	2.433
F value		2.142	6.097**	1.275	0.422	1.180	1.659

Table 12 Mean Basophil count (percent of total leucocytes) of Ducks at monthly intervals, fed on diet supplemented with copper.

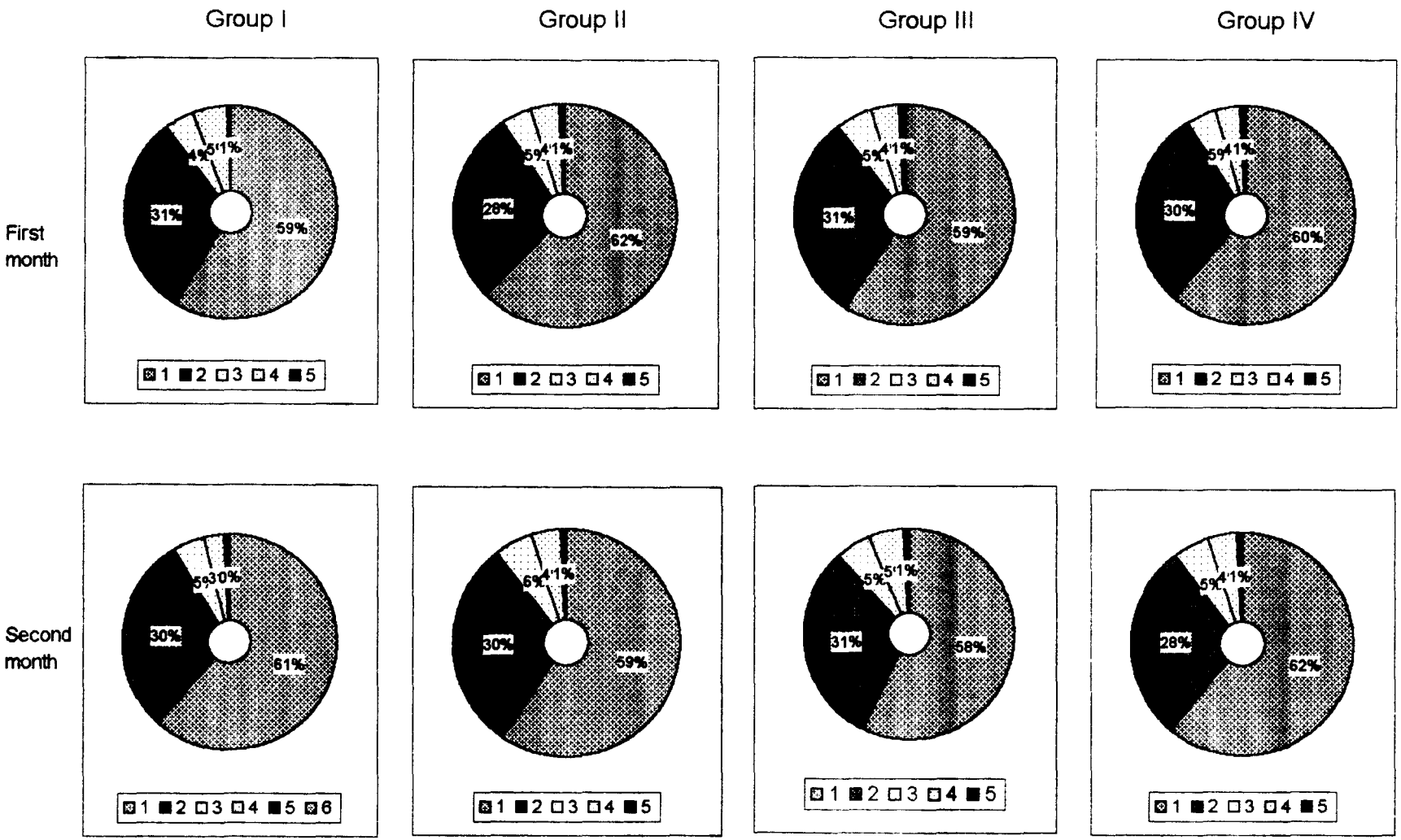
Group	Months					
	1	2	3	4	5	6
I	0.67 ±0.19	0.87 ±0.19	0.73 ±0.18	0.80 ±0.22	0.87 ±0.29	0.80 ±0.26
II	0.93 ±0.23	0.93 ±0.23	0.47 ±0.22	0.27 ±0.11	0.40 ±0.16	0.80 ±0.20
III	0.93 ±0.25	0.73 ±0.28	1.20 ±0.22	0.93 ±0.25	0.80 ±0.22	0.87 ±0.29
# IV	0.73 ±0.21	0.80 ±0.3	0.67 ±0.23	0.73 ±0.23	0.93 ±0.21	0.93 ±0.21

N =15
# control

Table 12.1 ANOVA table (M.S.S, transformed values), influence of dietary level of copper on the Basophol count of Ducks at monthly intervals.

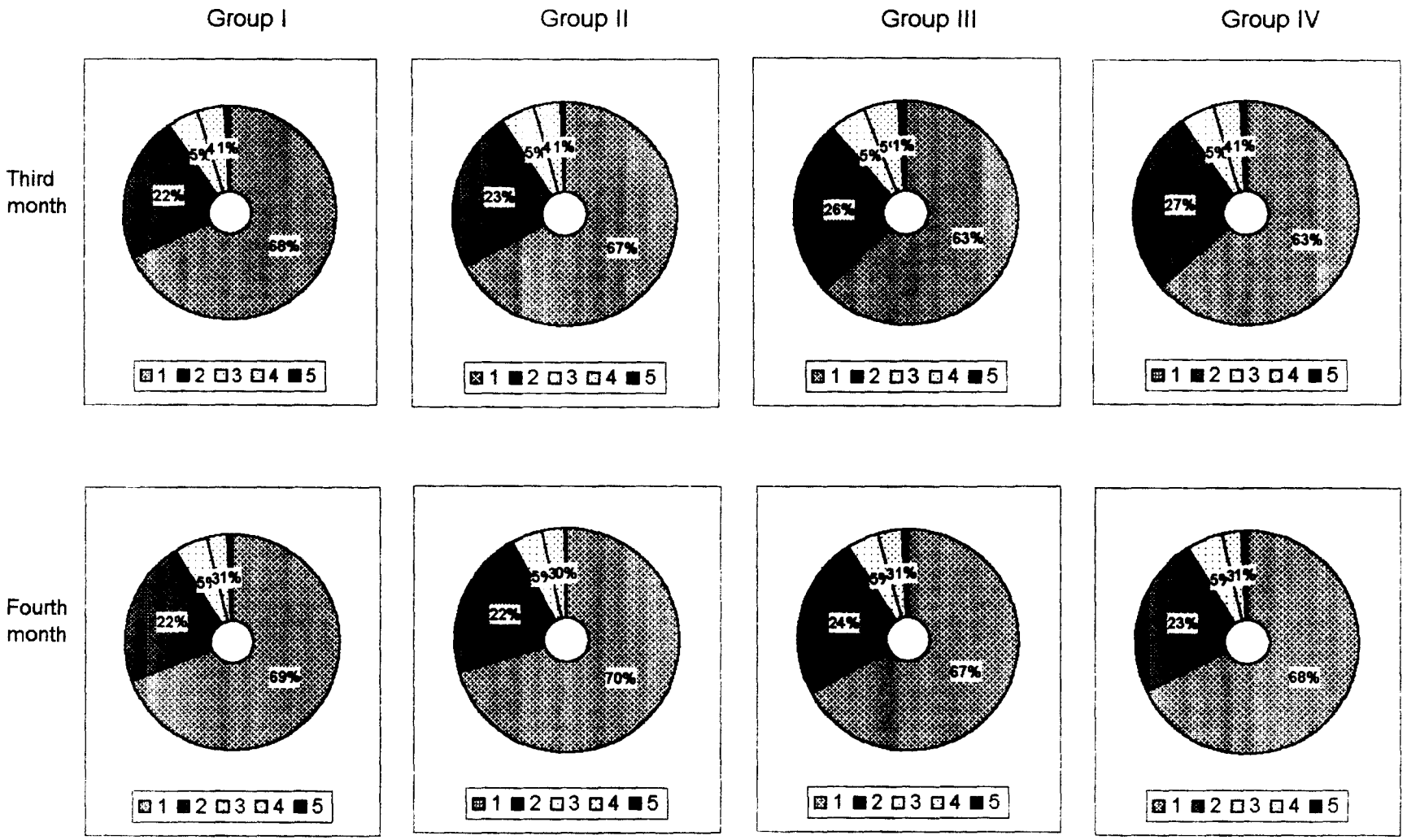
Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	1.692	1.808	10.431	7.402	5.817	0.577
Error	56	4.798	6.004	4.543	4.551	4.957	5.751
F value		0.353	0.301	2.294	1.627	1.173	0.100

Fig.2 Mean differential count (percentage) of Ducks at monthly intervals, fed on diet supplemented with copper.



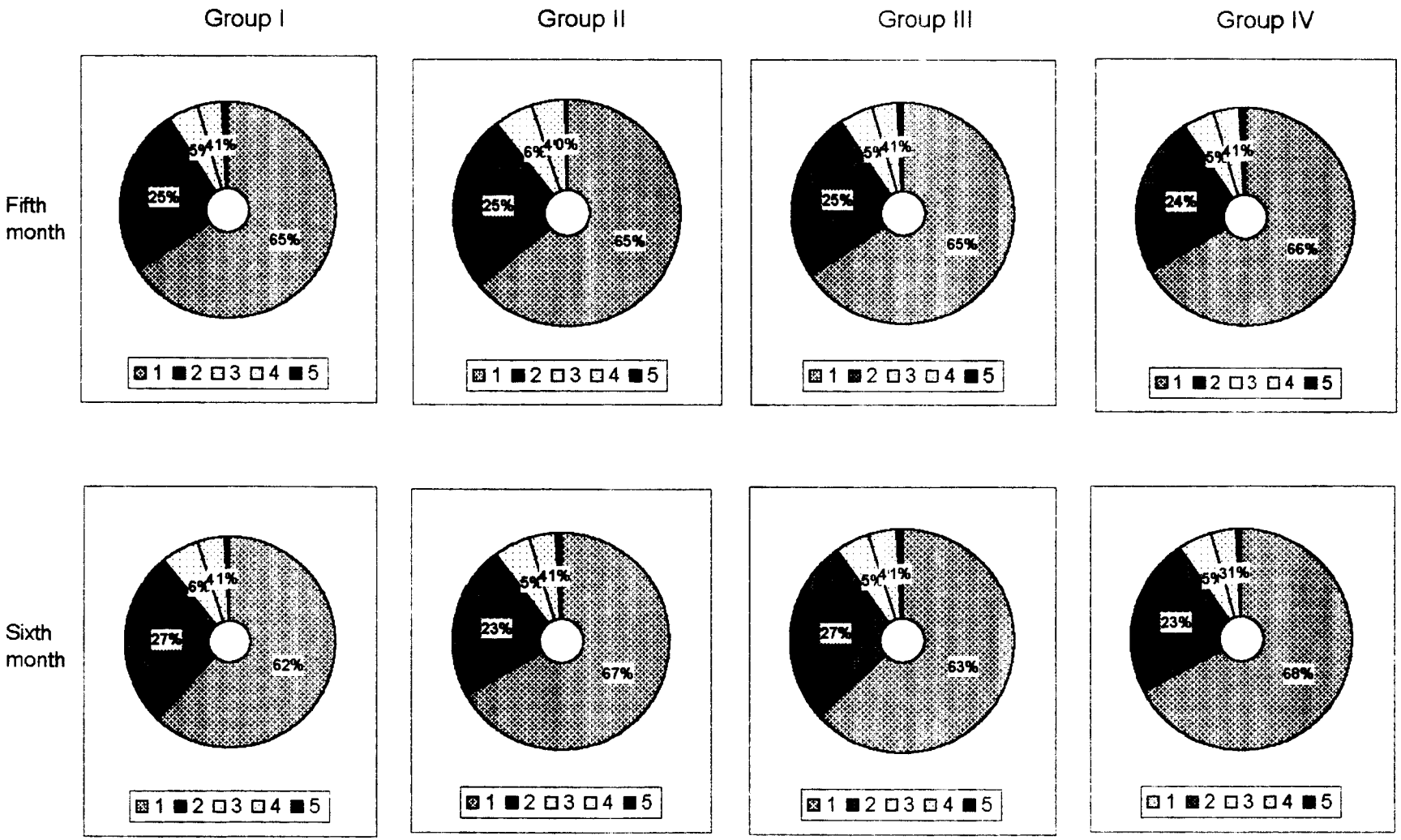
1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils.

Fig.2 Mean differential count (percentage) of Ducks at monthly intervals, fed on diet supplemented with copper.



1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils

Fig.2 Mean differential count (percentage) of Ducks at monthly intervals, fed on diet supplemented with copper.



1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils

Table 13 Mean serum Aspartate Amino Transferase level (I.units/ml) of Ducks at monthly intervals, fed on diet supplemented with copper.

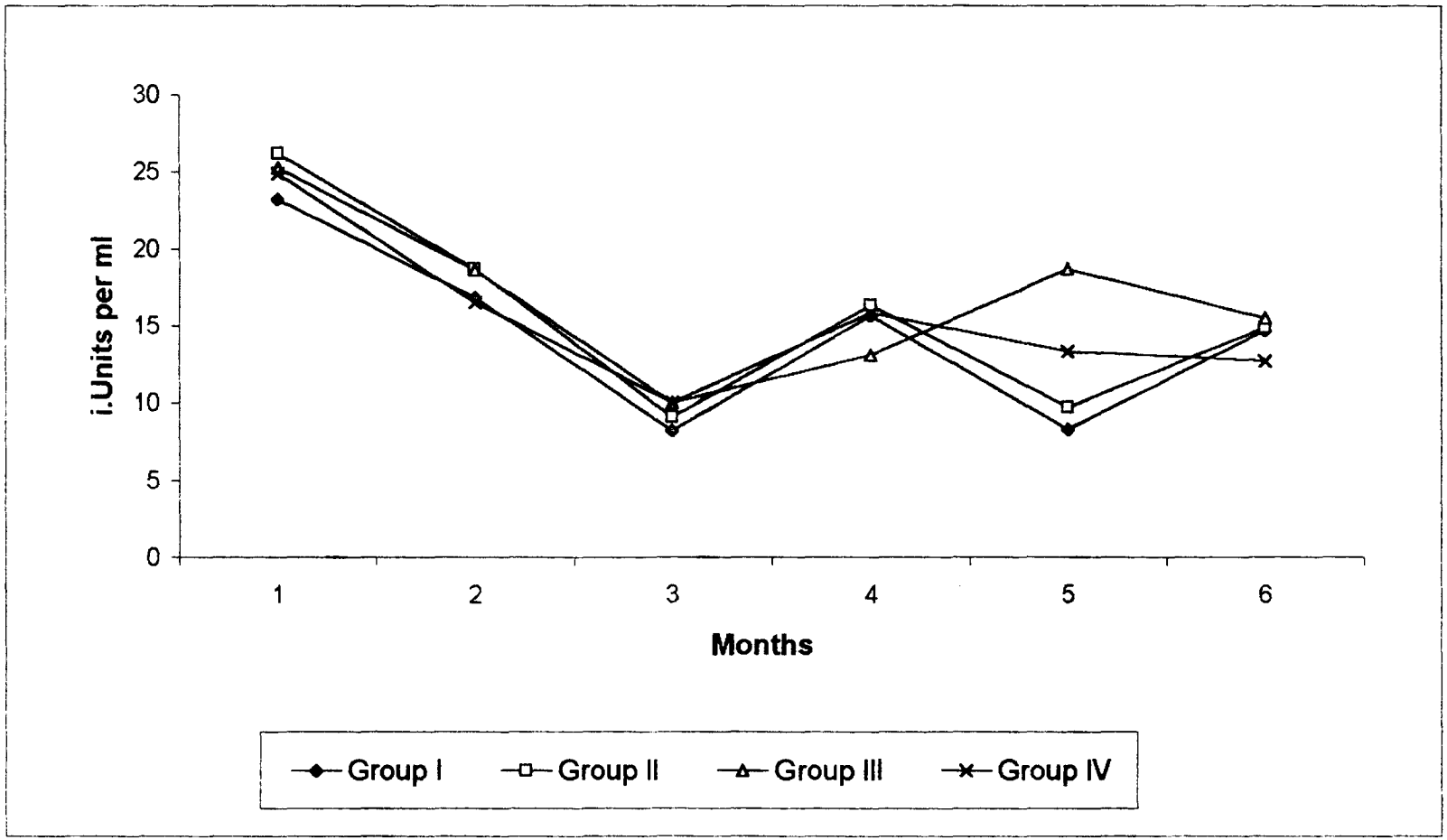
Groups	Months					
	1	2	3	4	5	6
I	23.20 ±1.26	16.80 ±1.01	8.20** ±0.41	15.60 ±0.65	8.27** ±0.46	14.27 ±1.00
II	26.20 ±1.13	18.67 ±0.89	9.13 ±0.41	16.33 ±0.93	9.73** ±0.75	14.87 ±0.82
III	25.27 ±1.73	18.60 ±1.01	10.07 ±0.43	13.13 ±1.00	18.73 ±1.62	15.53 ±0.94
# IV	24.87 ±1.51	16.53 ±0.53	10.07 ±0.27	15.80 ±0.85	13.33 ±0.49	12.67 ±1.19
CD (0.05)			1.088		2.696	

N = 15
# control

Table 13.1 ANOVA table(M.S.S),influence of dietary level of copper on the serum Aspartate Amino Transferase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	23.572	19.528	11.978	30.372	325.617	22.533
Error	56	30.419	11.698	2.214	11.305	13.574	14.960
F value		0.775	1.669	5.409**	2.687	23.989**	1.506

Fig.3 Mean serum Aspartate Amino Transferase level of Ducks at monthly intervals, fed on diet supplemented with copper.



fifth month .In group III the values were significantly higher in the fifth month . The values of group I ranges from 8.20 ± 0.41 to 23.20 ± 1.26, group II from 9.13 ± 0.41 to 26.20 ± 1.13, group III from 10.07 ± 0.43 to 25.27 ± 1.73 and group IV from 10.07 ± 0.27 to 24.87 ± 1.51.

4.1.8.2 Alanine Amino Transferase (ALT).

The mean serum ALT values (i.u/ml) of four groups of ducks at monthly intervals are shown in table 14. In group I for the first three months all the three groups showed a higher values than the controls even though all are not significant. In the fourth month groups I & II and in the sixth month all the treated groups showed significantly lower values than that of controls. The values of four groups ranged from 5.53 ± 0.71 to 20.33 ± 0.51, 5.07 ± 0.44 to 19.80 ± 0.55, 9.80 ± 0.94 to 22.40 ± 1.52 and 6.93 ± 0.37 to 17.33 ± 0.72 respectively.

4.1.8.3 Alkaline phosphatase.

The mean levels of alkaline phosphatase (KA units) of four groups of ducks at monthly intervals are shown in table 15. All the values of treated birds were higher than the corresponding values of the controls. In group I mean values of the first ,the second the third and the sixth month were significantly higher . In group II all the values were significantly higher except in the fourth month. In group III significantly higher values were noticed in the first, the third, the fifth and the sixth month. The mean values of group I ranged from 5.29 ± 0.44 to 34.13 ± 1.41, group II from 5.91 ± 0.85 to 29.99 ± 1.30, group III from 6.59 ± 0.29 to 28.47 ± 1.27 and group IV from 3.13 ± 0.43to 20.93 ± 1.96.

Table 14 Mean serum Alanine Amino Transferase level (I.units/ml) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	20.33** ±0.51	12.93 ±0.61	10.93** ±0.57	9.93** ±0.64	9.27 ±0.42	5.53** ±0.71
II	19.80** ±0.55	13.67 ±0.42	10.93** ±0.89	10.20** ±0.69	9.27 ±0.76	5.07** ±0.44
III	20.73** ±0.99	18.80** ±0.72	11.00** ±0.68	22.40** ±1.52	11.00 ±0.94	9.80** ±0.94
# IV	17.33 ±0.72	12.53 ±0.65	6.93 ±0.37	15.13 ±1.29	8.80 ±0.59	13.6 ±0.77
CD (0.05) N = 15 # control	2.042	1.734	1.847	3.124		2.085

Table 14.1 ANOVA table (M.S.S), influence of dietary level of copper on the serum Alanine Amino Transferase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Group	3	34.950	127.528	60.683	510.639	14.106	241.444
Error	56	7.786	56.140	6.371	18.226	7.798	8.119
F value		4.489**	22.715**	9.524**	28.017**	1.907	29.738**

Fig. 4 Mean serum Alanine Amino Transferase level of Ducks at monthly intervals, fed on diet supplemented with copper.

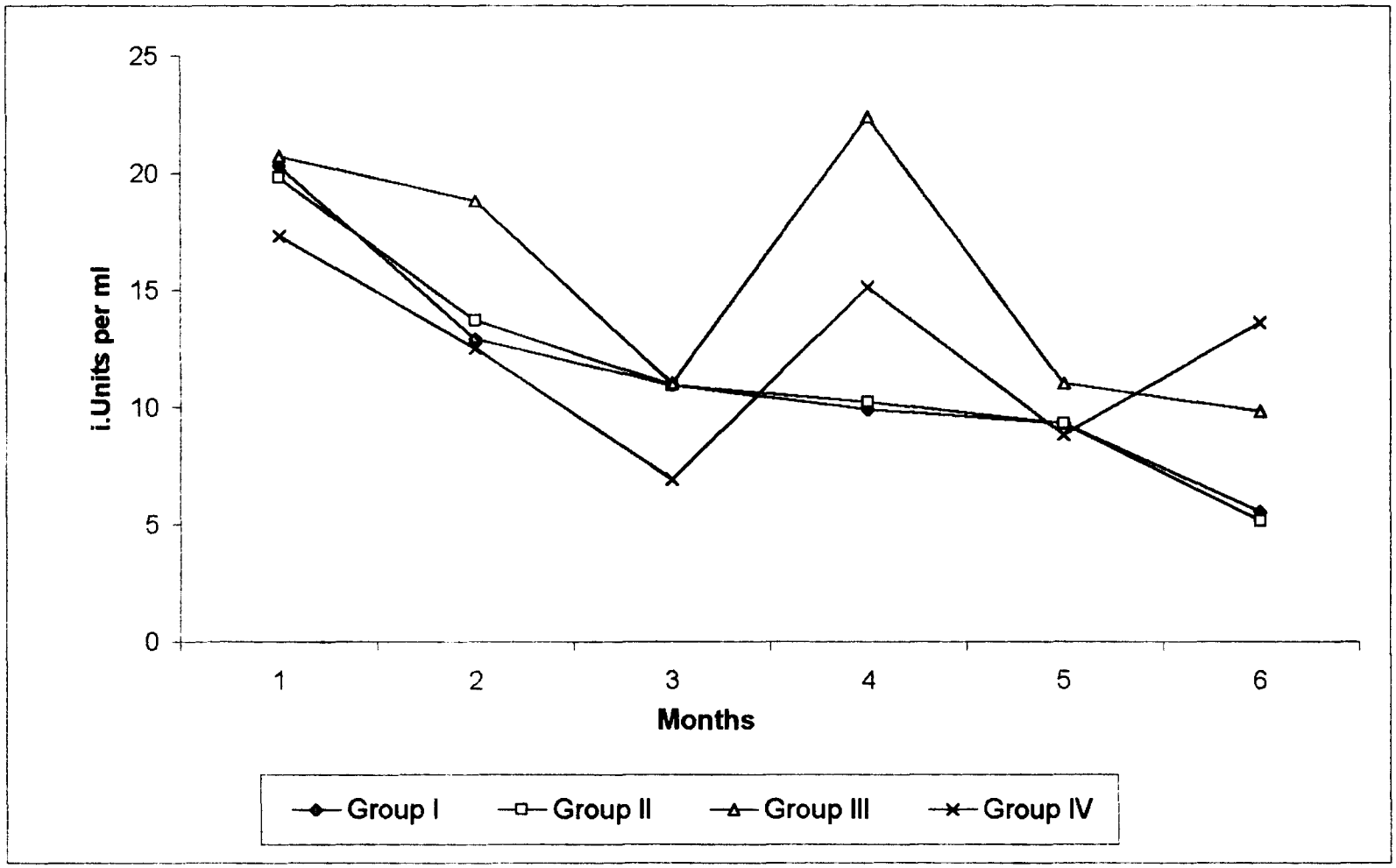


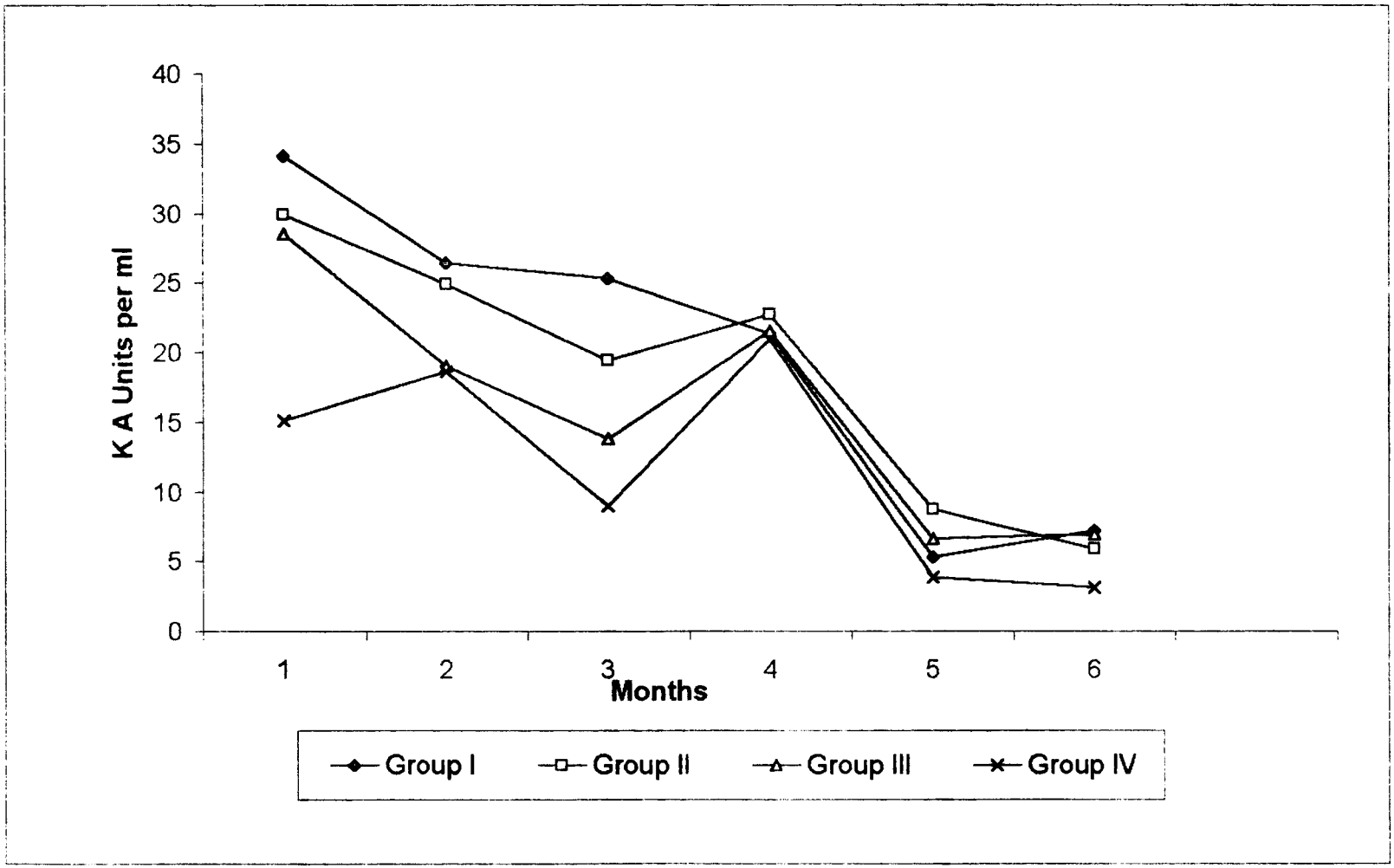
Table 15 Mean serum Alkaline Phosphatase level (KA units) of Ducks at monthly intervals, fed on diet supplemented with copper.

Group	Months					
	1	2	3	4	5	6
I	34.13** ±1.41	26.45** ±0.95	25.31** ±2.25	21.37 ±0.91	5.29 ±0.44	7.19** ±0.42
II	29.99** ±1.30	24.99** ±0.81	19.36** ±0.82	22.72 ±2.31	8.73** ±1.34	5.91** ±0.85
III	28.47** ±1.27	19.09 ±0.89	13.88** ±1.22	21.55 ±1.60	6.59** ±0.29	6.91** ±0.49
# IV	15.15 ±1.03	18.63 ±1.02	9.03 ±0.34	20.93 ±1.96	3.80 ±0.31	3.13 ±0.43
CD (0.05) N = 15 # control	3.572	2.606	3.853		1.656	1.656

Table 15.1 ANOVA table (M.S.S),influence of dietary level of copper on the serum Alkaline Phosphatase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	1011.72	55.067	692.822	102.122	64.524	51.438
Error	56	23.87	12.689	27.736	47.046	8.104	5.125
F value		42.385**	4.340**	24.979**	2.171	7.962**	10.037**

Fig . 5 Mean serum Alkaline Phosphatase level of Ducks at monthly intervals, fed on diet supplemented with copper.



4.1. 9 . Phytohaemaglutinin –P (PHA-P) skin sensitivity test.

The mean wing web thickness (mm) of ducks subjected to PHA-P skin sensitivity test at one and a half months intervals are shown in table 16 . None of the values were significantly different from that of controls. All the values at 24 hours were higher than the corresponding values of the controls. The skin thickness of group I ranged from 0.44 ± 0.02 to 1.43 ± 0.05, group II from 0.43 ± 0.01 to 1.36 ± 0.03. group III from 0.45 ± 0.01 to 1.41 ± 0.03 and group IV from 0.44 ± 0.01 to 1.29 ± 0.03.

4.1.10 . Antibody titre.

The mean antibody titre of four groups of ducks at fifteen days intervals are shown in table 17. None of the values were significantly different from that of the corresponding control values. The values of group I ranged from 384.000 ± 57.243 to 188406.667 ± 82005.796, group II from 384.000 ± 57.243 to 104448.000 ± 50780.489, group III from 256.000 ± 57.248 to 51882. 667 ± 8890. 407 and for group IV from 597.333 ± 142. 790 to 49152.000 ± 7327.148.

4.1.11 . Copper content in the serum .

The mean values of copper content in the serum (ppm) of four groups of ducks at monthly intervals are given in the table 18. The mean values increased from first to sixth month in all the groups. In group III from fourth month onwards the mean values were significantly higher than the corresponding control values. In group I the values ranged from 0.213 ± 0.03 to 0.593 ± 0.03, in group II from 0.180 ± 0.01 to 0.682 ± 0.05, in group III from 0.163 ± 0.02 to 1.057 ± 0.18 and in group IV it ranges from 0.153 ± 0.02 to 0.398 ± 0.03.

Table 16 Mean wing web thickness (mm) of Ducks, in PHA-P skin sensitivity test at one and a half months intervals, fed on diet supplemented with copper.

Group	One and a half month				Three month				Four and a half month				Six month			
	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
I	0.48 ±0.01	1.36 ±0.05	0.88 ±0.03	0.49 ±0.02	0.44 ±0.02	1.43 ±0.05	0.95 ±0.04	0.47 ±0.02	0.48 ±0.02	1.43 ±0.05	1.06 ±0.04	0.51 ±0.01	0.47 ±0.01	1.41 ±0.05	0.97 ±0.04	0.49 ±0.02
II	0.47 ±0.01	1.36 ±0.03	0.92 ±0.03	0.51 ±0.01	0.45 ±0.02	1.35 ±0.04	0.85 ±0.04	0.57 ±0.03	0.43 ±0.01	1.33 ±0.05	1.09 ±0.05	0.49 ±0.02	0.47 ±0.01	1.31 ±0.05	0.83 ±0.05	0.49 ±0.02
III	0.46 ±0.01	1.41 ±0.03	0.96 ±0.03	0.49 ±0.02	0.46 ±0.02	1.36 ±0.06	0.87 ±0.03	0.51 ±0.02	0.45 ±0.01	1.37 ±0.04	1.05 ±0.04	0.51 ±0.03	0.47 ±0.01	1.29 ±0.06	0.85 ±0.03	0.48 ±0.02
# IV	0.46 ±0.01	1.27 ±0.05	0.97 ±0.03	0.47 ±0.02	0.45 ±0.02	1.29 ±0.03	0.87 ±0.02	0.51 ±0.02	0.46 ±0.02	1.24 ±0.05	1.06 ±0.04	0.47 ±0.01	0.44 ±0.01	1.19 ±0.05	0.93 ±0.04	0.49 ±0.02
CD(0.05)								0.065	0.033						0.11	

N = 15
# control

Table 16.1 ANOVA table (M.S.S), influence of dietary level of copper on the PHA-P sensitivity test on Ducks at 45 days intervals.

Source of variation	df	One and a half month				Tthree month				Four and a half month				Six month			
		0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
Group	3	0.001	0.045	0.024	0.004	0.001	0. 050	0.029	0.025	0.007	0.091	0.035	0.006	0.004	0.122	0.061	0.000
Error	56	0.002	0.024	0.011	0.004	0.004	0.033	0.014	0.008	0.002	0.031	0.027	0.008	0.002	0.068	0.021	0.005
F value		0.670	1.926	2.123	1.000	0.301	1.512	2.013	3.150*	3.218*	2.491	1.304	0.797	1.653	1.788	2.978*	0.086

Table 17 Mean antibody titre in Duck serum at 15 days intervals,fed on diet supplemented with copper.

Group	Days										
	15	30	45	60	75	90	105	120	135	150	165
I	1792.000 ±524.644	1578.667 ±554.667	2474.667 ±1170.654	640.000 ±128.000	384.000 ±57.243	188406.667 ±82005.796	2218.667 ±615.347	1365.333 ±215.875	1962.667 ±501.946	2048.000 ±647.634	2133.333 ±653.232
II	1194.667 ±170.667	1280.000 ±256.000	2304.000 ±1195.276	640.000 ±128.000	384.000 ±57.243	104448.000 ±50780.489	1621.333 ±535.632	1365.333 ±215.878	1450.667 ±535.632	2048.000 ±457.947	1706.667 ±215.878
III	1536.000 ±228.973	853.333 ±107.937	597.333 ±85.333	426.667 ±53.970	256.000 ±57.248	51882.667 ±8890.407	3072.000 ±457.947	5461.333** ±2222.602	2560.000 ±512.000	3498.667 ±1093.466	5963.000 ±2416.327
# IV	2560.000 ±512.000	1706.667 ±215.878	938.667 ±244.357	981.333 ±250.973	597.333 ±142.790	49152.000 ±7327.148	1408.000 ±308.265	1536.000 ±228.973	1365.333 ±215.878	1194.667 ±170.667	2218.667 ±615.357
CD(0.05,log values)								0.258			

N= 6

control

table 17.1 ANOVA table (M.S.S), influence of dietary level of copper on the antibody titre of Ducks at 15 days intervals.

Source of variation	df	Days										
		15	30	45	60	75	90	105	120	135	150	165
Groups	3	0.096	0.091	0.272	0.091	0.125	0.256	0.195	0.356	0.106	0.126	0.173
Error	20	0.048	0.065	0.104	0.056	0.045	0.351	0.084	0.046	0.065	0.083	0.129
F value		1.979	1.395	2.609	1.622	2.797	0.732	2.327	7.732**	1.628	1.515	1.341

Fig. 6 Mean antibody titre in Duck serum at 15 days intervals, fed on diet supplemented with copper.

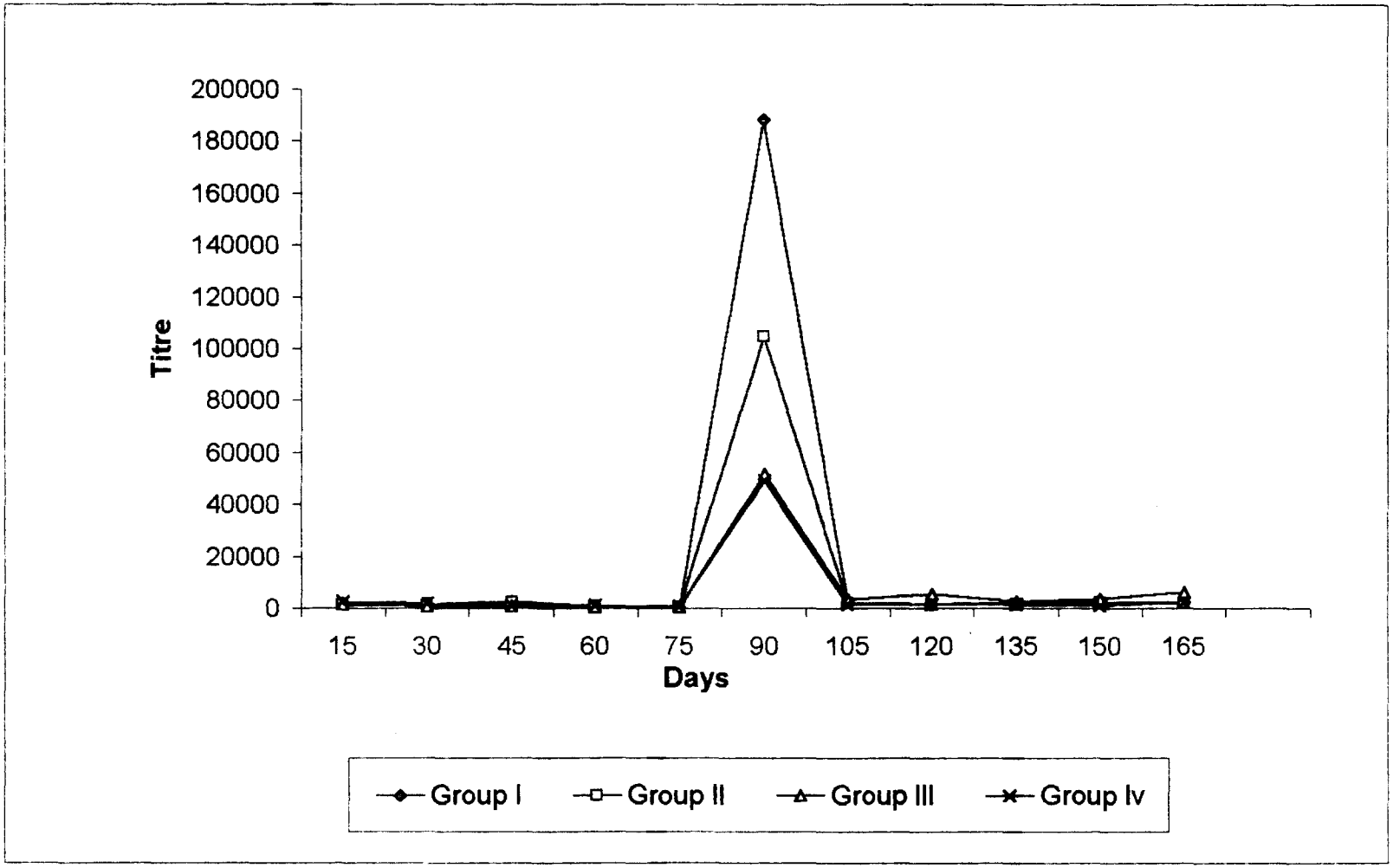


Table 18 Mean serum copper level (ppm) of Ducks at monthly intervals, fed on diet supplemented with copper.

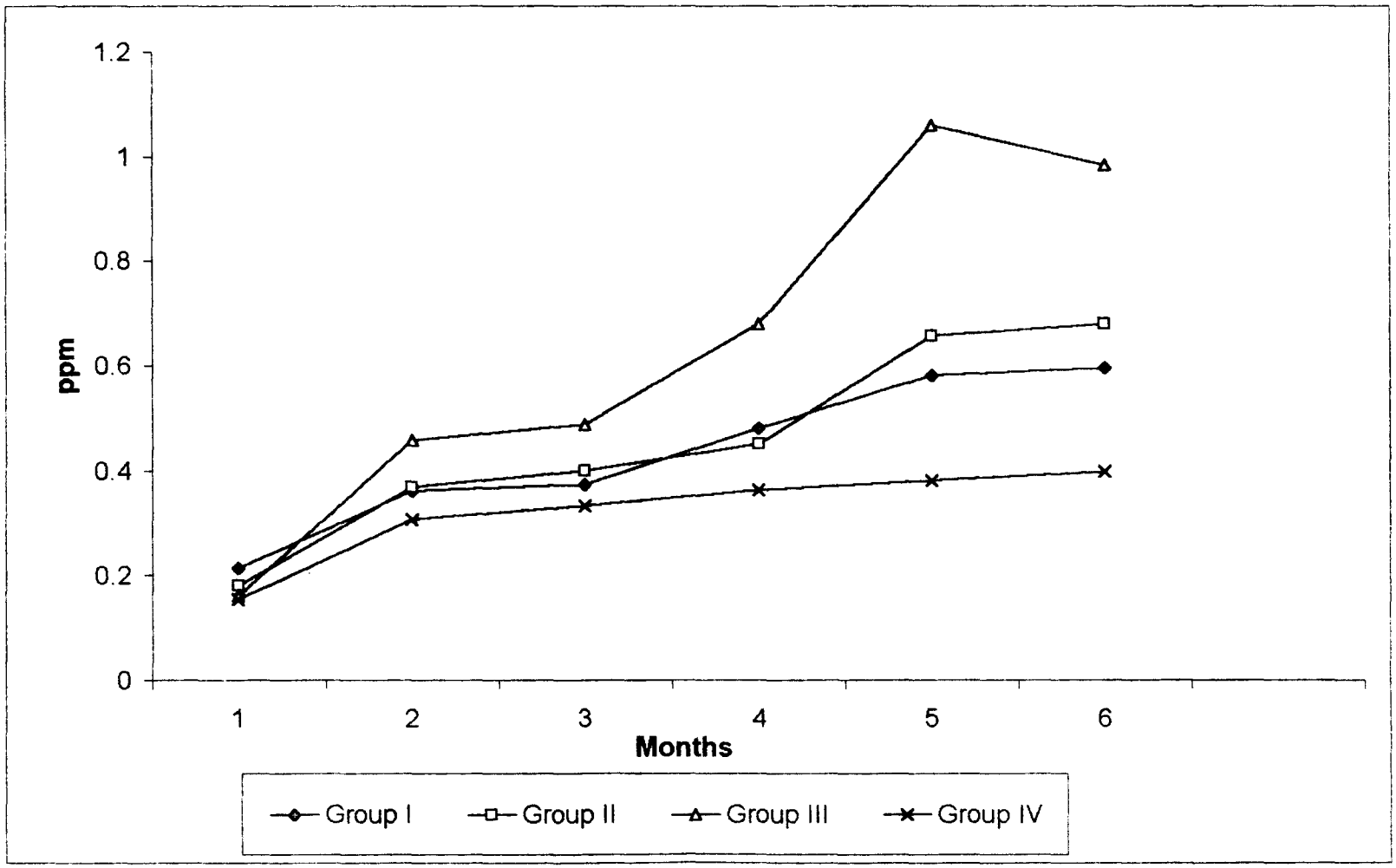
Group	Months					
	1	2	3	4	5	6
I	0.213 ±0.03	0.360 ±0.01	0.373 ±0.04	0.480 ±0.03	0.583 ±0.03	0.595 ±0.03
II	0.180 ±0.01	0.368 ±0.04	0.400 ±0.04	0.450 ±0.03	0.657 ±0.08	0.682 ±0.05
III	0.163 ±0.02	0.457 ±0.05	0.487 ±0.05	0.680** ±0.07	1.057** ±0.18	0.983** ±0.19
# IV	0.153 ±0.02	0.307 ±0.02	0.333 ±0.03	0.363 ±0.02	0.383 0.02	0.398 0.03
CD (0.05)				0.132	0.293	0.299

N = 6
# control

Table18.1 ANOVA table (M.S.S),influence of dietary level of copper on the serum copper level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.004	0.023	0.025	0.103	0.479	0.355
Error	20	0.003	0.008	0.009	0.012	0.059	0.062
F value		1.352	3.071	2.800	8.962**	8.071**	5.775**

Fig.7 Mean serum copper level of Ducks at monthly intervals, fed on diet supplemented with copper.



4.1.12 Copper content in tissues.

The mean copper levels (ppm on dry matter basis) in liver, kidney and muscle tissues of four groups of ducks are shown in table 19.The mean copper content of these tissues of all the treated groups were higher than that of the controls. But a significantly higher value was noticed only in the liver tissue of the group II and III and kidney tissue of all the treated groups.

4.1.13 Weight of liver and spleen.

The mean weight of liver (g) and spleen (mg) of four groups of ducks is shown in table 20. The mean weight of liver of treated group was more than that of the controls, but none of the value was significantly different from the controls.

4.1.14 Gross and histo-pathological changes.

4.1.14.1 Gross lesions.

Gross lesions could not be seen in the internal organs of any of the birds.

4.1.14. 2 Hist- pathology.

The histo-pathological lesions are shown in Fig. 8a to 8d. In the liver tissue no significant lesion could be detected . Mild depletion of lymphoid cells in the peri arteriolar aggregations and mild reduction in the number of Malphegion corpuscles were noted in the spleen of birds in group III. In bursa of fabricius moderate to severe depletion of lymphoid follicle were detected in all groups. In group III extensive fibrosis was found around cystic follicles. Other lymphoid organs did not reveal any lesion. Focal necrosis of tubular epithelium and desquamation of cells were noted in the kidney of birds in group II and III..Thickening of blood vessels, vacuolation of tubular epithelium, diffuse calcification of endothelium and congestion of small blood vessels were also seen in the kidney.

Table 19 Mean copper content (ppm on dry matter basis) of Liver, Kidney and Muscle of Ducks after six months of feeding on diet supplemented with copper.

Group	Liver	Kidney	Muscle
I	658.667 ±58.800	43.833* ±1.600	22.000 ±2.634
II	822.333** ±72.164	40.167* ±2.809	30.667 ±5.484
III	1021.333** ±52.829	44.000** ±3.466	25.000 ±1.984
# IV	523.333 ±36.243	29.833 ±4.765	20.833 ±2.287
CD (0.05)	166.600	9.911	

N = 6
# control

Table 19.1 ANOVA table (M.S.S), influence of dietary level of copper On the tissue copper content of Ducks after six months of feeding.

Source of variation	df	Liver	Kidney	Muscle
Groups	3	276817.500	265.819	115.819
Error	20	19147.467	67.725	69.208
F value		14.457**	3.925*	1.673

Fig. 8 Mean copper content (ppm on dry matter basis) of liver,kidney and muscle of Ducks after six months of feeding on diet supplemented with copper.

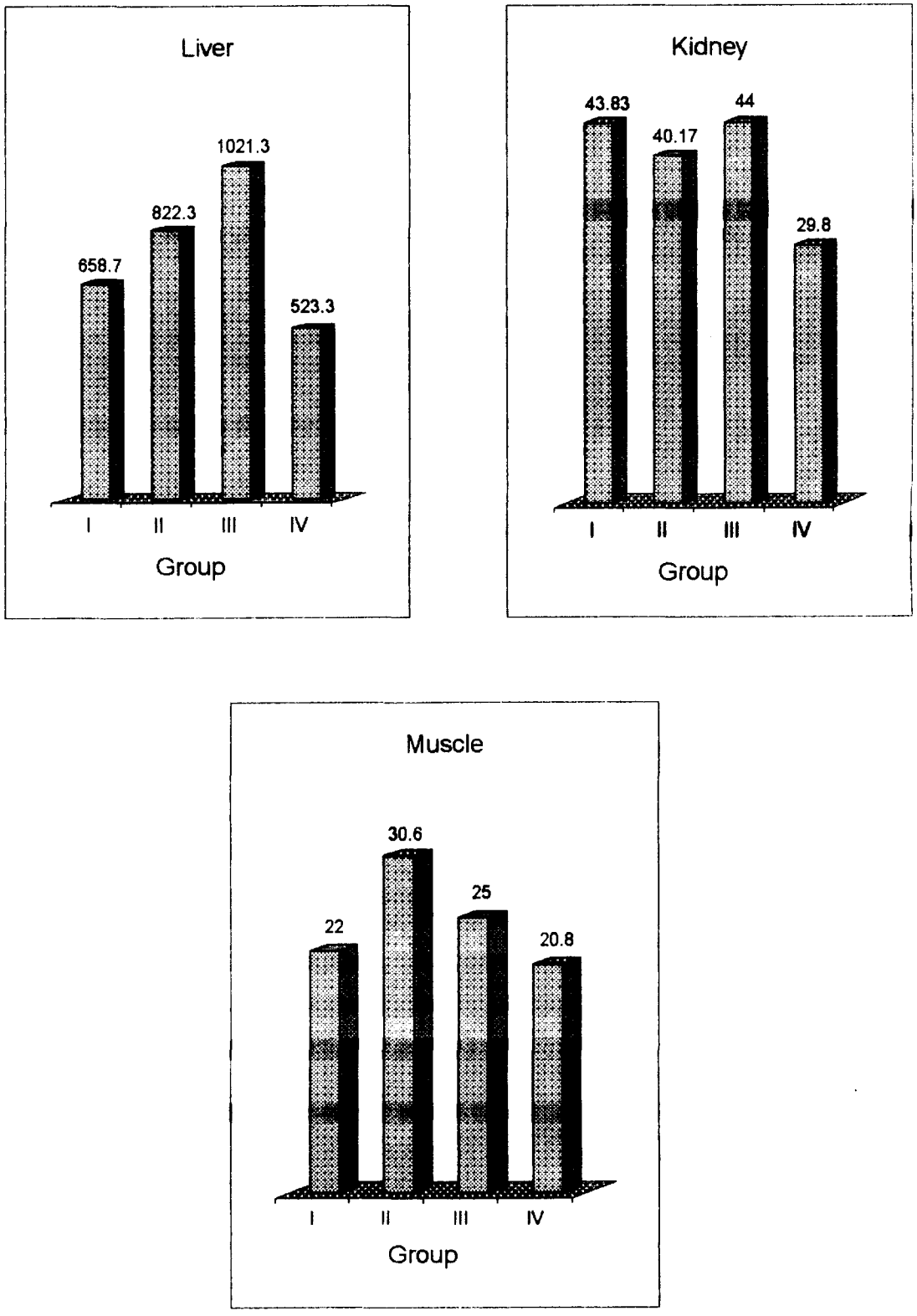


Table 20 The mean weight of Liver (g) and Spleen (mg) of sacrificed Ducks after six months of feeding on diet supplemented with copper.

Group	Liver	Spleen
I	46.000 ±2.832	568.000 ±38.345
II	49.000 ±2.845	683.333 ±52.479
III	47.200 ±8.480	613.333 ±55.735
# IV	45.867 ±3.429	656.667 ±42.781

N =15
# control

Table 20.1 ANOVA table (M.S.S), influence of dietary level of copper on the weight of Liver and Spleen of Ducks after six months of feeding.

Source of variation	df	Liver	Spleen
Group	3	31.617	38384.444
Error	56	134.967	34353.393
F value		0.234	1.117

Fig. 8a Duck kidney - group III –treated with copper, destruction of endothelium and Vascular sclerosis. H&E X 160

Fig. 8b Duck bursa – group I, II, III & IV - treated with copper, fibrous tissue proliferation H&E X 160

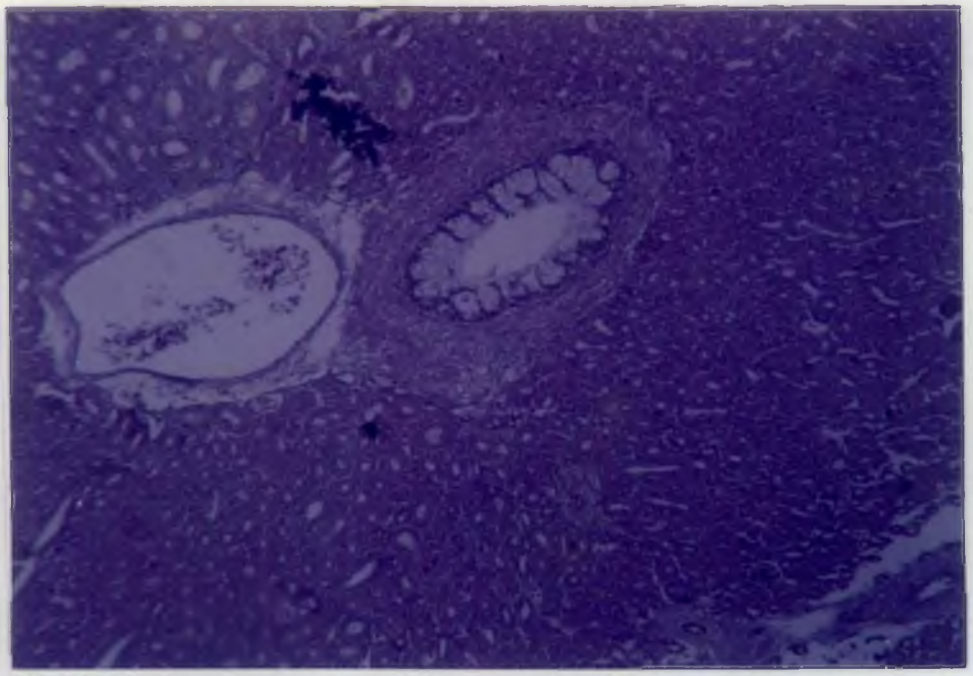


Fig. 8a

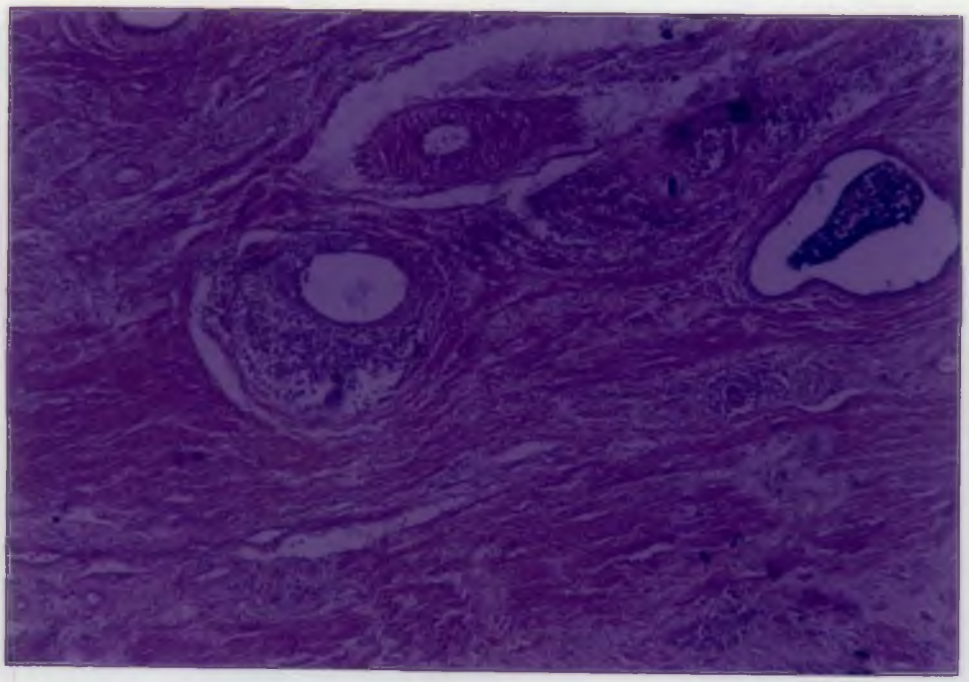


Fig. 8b

Fig. 8c Duck thymus - group I, II, III, & IV- treated with copper, depletion of lymphoid element H&E X 160

Fig. 8d Duck bursa- group I, II, III & IV –treated with copper, depletion of lymphoid element and moderate fibrous tissue proliferation H&E X 160

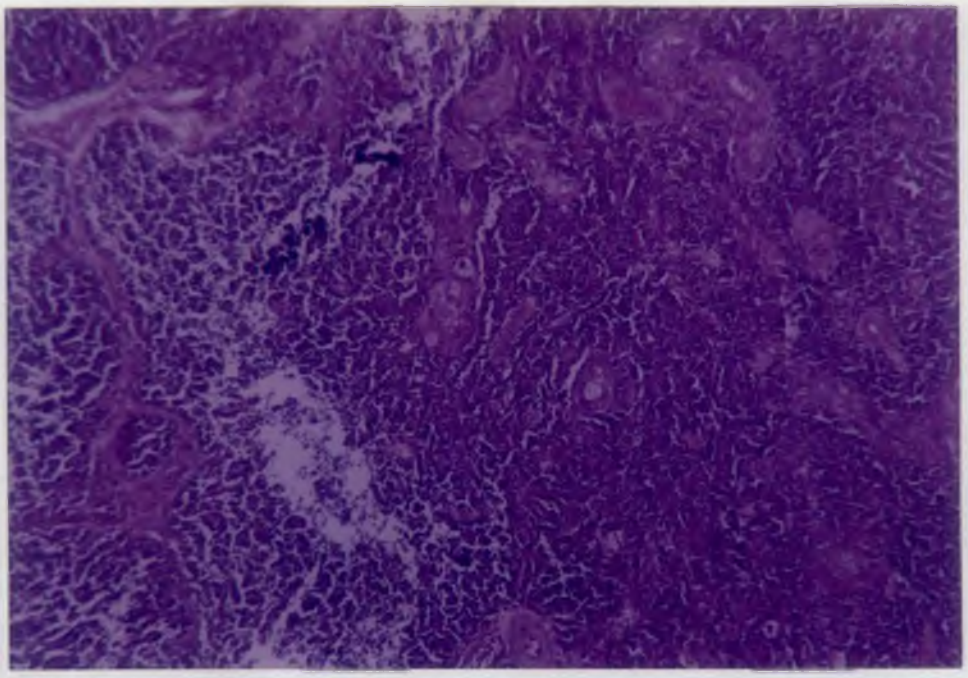


Fig. 8c

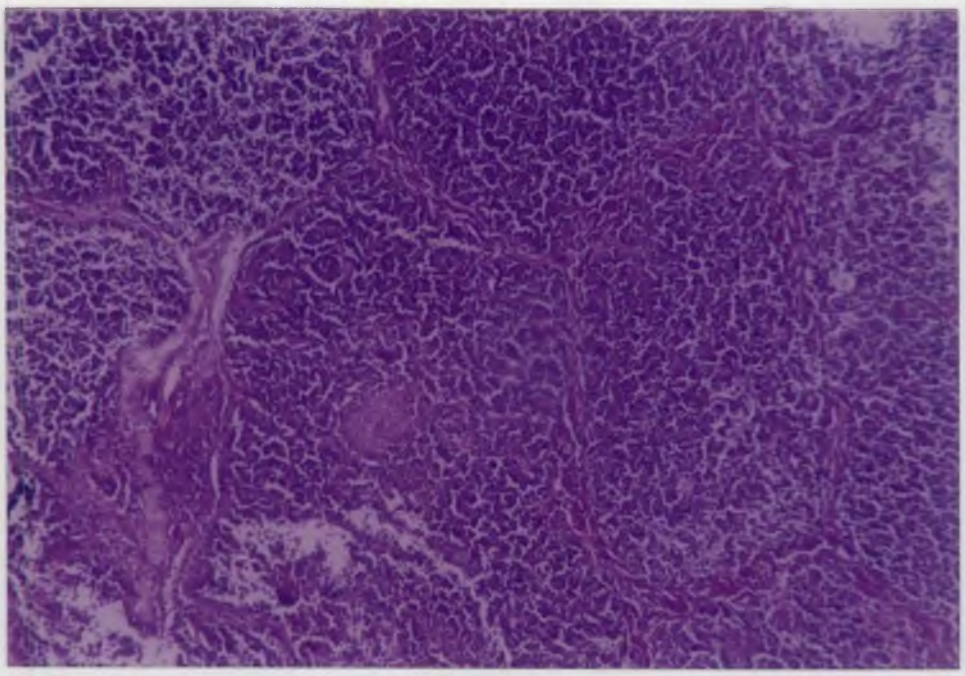


Fig. 8d

4.2. ZINC

4.2.1 Body weight..

The mean body weight of ducks fed on diet supplemented with 100,200,300 and 0 ppm zinc is shown in table 21and Fig.9. The body weight was recorded at 15 days intervals. Even though not significantly different, the mean body weight of group I on 30,45 and 60th day is more than that of the controls and all other values were lesser. All mean values of group II except on 15th day were higher than the control values. In group III all values were lower than the control values. In group I the values ranged from 983.33 ± 72.65 to 1812.50 ± 69.12, in group II from 1008.33 ± 85.69 to 1925.00 ± 49.43,in group III from 966.67 ± 68.90 to 1779.17±34.52 and group IV from 1025.00 ± 92.22 to 1858.33 ± 46.81

4.2.2 Erythrocyte count.

The mean erythrocyte count of four groups of ducks at monthly intervals is shown in table 22. None of the values were significantly different from those of controls. In group III all the values except in the second month were lower than the control values. In group I the values ranged from 297.42 ± 8.87 to 337.92 ± 10.37, group II 303.00 ± 7.37 to 325.25 ± 10.74, group III 275.67 ± 6.65 to 316.50 ± 8.43 and group IV from 297.17 ± 6.25 to 319.67 ± 7.47.

4.2.3 Haemoglobin.

The mean haemoglobin value of four group of ducks at monthly intervals is shown in table 23 . In group I the values in the month of first second and fourth were significantly higher than the controls. In the other two groups the values are not consistent. In group I the values ranged from 11.5 ± 0.22 to 12.65 ± 0.18 group II from

Table21 Mean body weight of Ducks (g) at 15 days intervals, fed on diet supplemented with zinc.

	Days											
Group	15	30	45	60	75	90	105	120	135	150	165	180
I	983.33 ±72.65	1408.33 ±78.29	1616.67 ±66.10	1800.00 ±63.96	1808.33 ±65.67	1812.50 ±69.12	1779.17 ±53.81	1750.00 ±54.35	1679.17 ±47.85	1650.00 ±54.35	1637.5 ±44.86	1625.00** ±53.83
II	1008.33 ±85.69	1366.67 ±61.96	1625.00 ±59.19	1816.67 ±47.41	1870.83 ±43.72	1925.00 ±49.43	1908.33 ±43.00	1900.00 ±47.67	1845.83 ±43.72	1791.67 ±37.86	1808.33 ±43.38	1791.67 ±31.28
III	966.67 ±68.90	1358.33 ±52.89	1475.00 ±60.77	1583.33 ±72.65	1675.00 ±62.00	1750.00 ±50.00	1779.17 ±33.41	1779.17 ±34.52	1679.17 ±34.52	1625.00 ±25.00	1608.33 ±28.09	1591.67** ±37.86
# IV	1025.00 ±92.22	1341.67 ±57.02	1575.00 ±55.22	1741.67 ±54.30	1808.38 ±51.04	1858.33 ±46.81	1858.33 ±38.84	1816.67 ±44.09	1745.83 ±43.28	1745.83 ±37.16	1729.17 ±38.66	1750.00 ±46.87
CD(0.05)				171.876					121.396	113.872	105.632	123.384

N=12

control

Fig. 9 Mean body weight (g) of Ducks at 15 Days intervals, fed on diet supplemented with zinc.

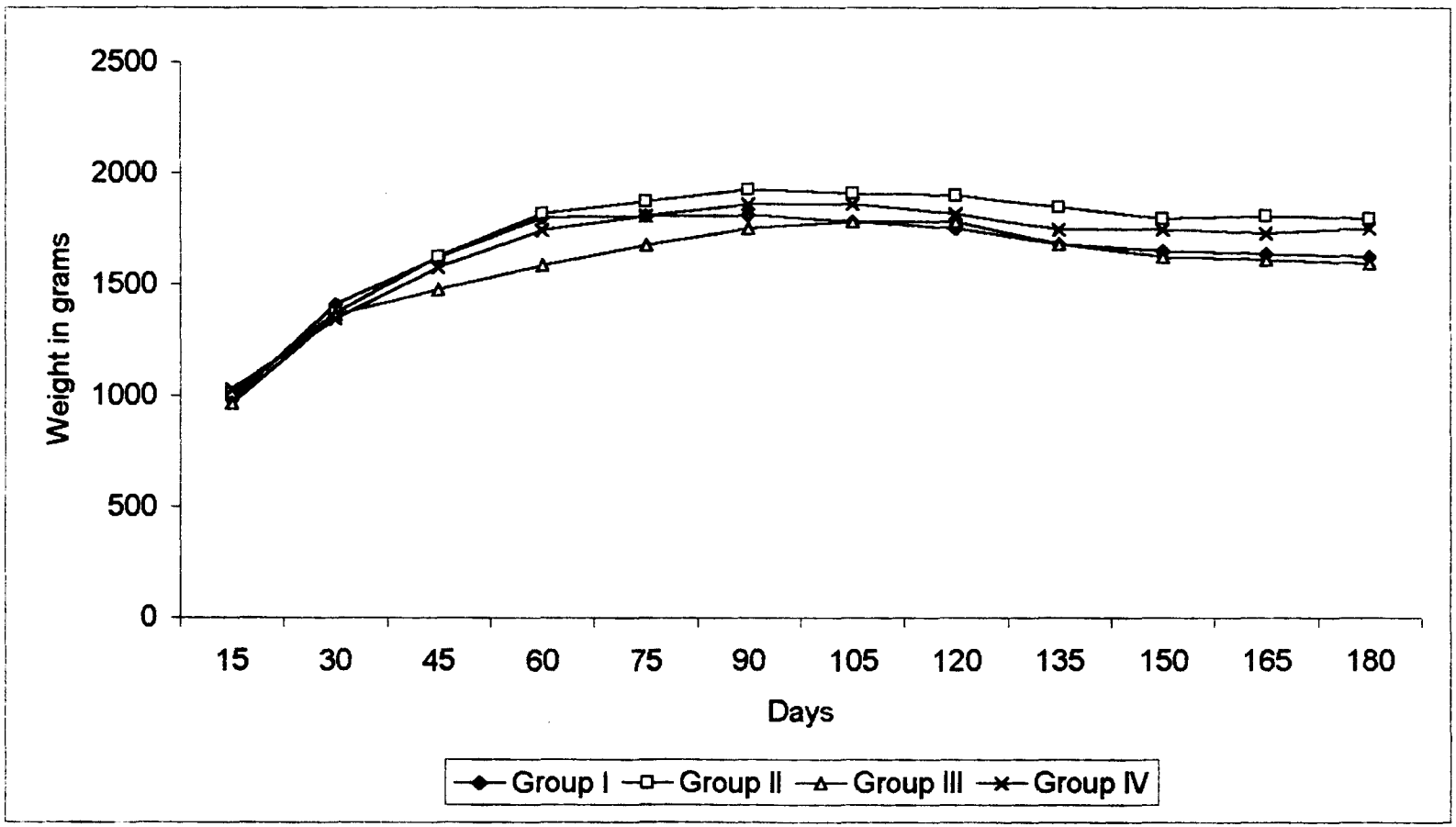


Table 21.1 ANOVA table (M.S.S), influence of dietary level of zinc on the body weight of Ducks at 15 days intervals.

Source of variation	df	Days											
		15	30	45	60	75	90	105	120	135	150	165	180
Group	3	8055.556	9652.778	56875.000	135763.889	817118.750	65468.750	48402.778	50746.528	74444.444	74357.639	99305.556	14319.444
Error	44	77613.636	48049.242	43844.697	43693.182	38025.568	35733.902	22111.742	25089.962	21799.242	19180.871	16505.682	22518.939
F value		0.104	0.201	1.297	3.107*	2.149	1.832	2.189	2.023	3.415*	3.877*	6.016**	4.943**

Table 22 Mean Erythrocyte count (x10000 per c m m) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	297.42 ±8.87	318.33 ±6.64	320.92 ±6.24	312.50 ±7.36	337.92 ±10.37	308.17 ±9.85
II	305.00 ±12.06	303.00 ±7.37	306.58 ±9.53	315.50 ±6.83	325.25 ±10.74	303.50 ±12.29
III	296.25 ±5.83	316.50 ±8.43	311.58 ±4.02	309.92 ±9.60	316.08 ±4.84	275.67 ±6.65
# IV	297.17 ±6.25	312.00 ±9.60	318.33 ±4.17	309.83 ±6.97	319.67 ±7.47	305.17 ±5.72

N = 12
# control

Table 22.1 ANOVA table (M.S.S), influence of dietary level of zinc on the Erythrocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	379.694	1852.083	507.854	86.076	1098.243	2734.750
Error	44	890.837	785.174	488.850	724.650	905.563	975.205
F value		0.426	2.359	1.039	0.119	1.213	2.804

Table 23 Mean Haemoglobin level (g percentage) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	11.50** ±o.22	12.24** ±0.20	11.91 ±0.21	12.65* ±0.18	12.26 ±0.16	12.21 ±0.27
II	11.25** ±0.18	11.14 ±0.18	11.42 ±0.08	11.60 ±0.24	12.42 ±0.18	12.30 ±0.28
III	11.05 ±0.13	11.62 ±0.20	11.97 ±0.14	12.24 ±0.24	12.06 ±0.19	11.45 ±0.27
# IV	10.55 ±0.18	11.17 ±0.20	11.99 ±0.11	11.82 ±0.24	12.07 ±0.31	11.67 ±0.26
CD(0.05)	0.507	0.556		0.643		

N = 12
# control

Table 23.1 ANOVA table (M.S.S), influence of dietary level of zinc on the Haemoglobin levels of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	1.948	3.620	1.989	2.599	0.271	1.942
Error	44	0.380	0.458	0.835	0.612	0.571	0.864
F value		5.127**	7.911**	2.382	4.245*	0.476	2.247

11.14 ± 0.18 to 12.30 ± 0.28, group III from 11.05 ± 0.13 to 12.24 ± 0.24 and group IV from 10.55 ± 0.18 to 12.07 ± 0.31.

4.2.4 Erythrocyte sedimentation rate.

The mean erythrocyte sedimentation rate of four group of ducks at monthly intervals is shown in table 24. None of the values were significantly different from that of controls. The values of group I ranged from 0.10 ± 0.01 to 0.12 ±0.01, group II from 0.11 ± 0.01 to 0.12 ±0.01, group III from o.10 ± 0.01 to 0.13 ± 0.01 and group IV from 0.11 ± 0.01 to 0.12 ± 0.01.

4.2.5 Packed cell volume

The mean packed cell volume of four groups of ducks at monthly intervals is shown in table 25. In the sixth month mean values of all the groups were significantly higher than control values. In the fourth month all the values were significantly lower than that of the controls. The values of group I ranged from 39.82 ± 0.99 to 43.08 ± 0.62, group II from 40.25 ± 0.88 to 43.75 ± 0.79, group III from 39.75 ± 0.65 to 42.33 ± 0.72 and group IV from 39.67 ± 0.72 to 40.75 ± 0.59.

4.2. 6 Leucocyte count.

The mean leucocyte count of four groups of ducks at monthly intervals is shown in table 26 . In group I the mean values in the third and fifth months were significantly higher than the controls . In groups II and III all values except those in the month of first and second were higher than the controls . Significant difference was noticed only in the third, fifth and sixth months of group III.In group I the mean values ranged from 294.42 ± 9.13 to 344.08 ± 18.14, in group II from 285.08 ± 9.50 to 340.25 ± 8.87, in group III from 283.33 ± 6.56 to 380. 53 ± 22.29 and group IV from 270.75 ± 14.46 to 329.50.± 6.65.

Table 24 Mean Erythrocyte sedimentation rate (mm / hr) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	0.12 ±0.01	0.11 ±0.01	0.11 ±0.01	0.10 ±0.01	0.11 ±0.01	0.11 ±0.01
II	0.12 ±0.01	0.12 ±0.01	0.11 ±0.01	0.11 ±0.01	0.12 ±0.01	0.11 ±0.01
III	0.13 ±0.01	0.11 ±0.01	0.11 ±0.01	0.12 ±0.01	0.10 ±0.01	0.11 ±0.01
# IV	0.11 ±0.01	0.12 ±0.01	0.11 ±0.01	0.12 ±0.01	0.12 ±0.01	0.11 ±0.01

N = 12
# control

Table 24.1 ANOVA table (M.S.S), influence of dietary level of zinc on the Erythrocyte sedimentation rate of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.001	0.000	0.001	0.001	0.001	0.000
Error	44	0.001	0.001	0.000	0.001	0.001	0.001
F value		0.376	0.237	0.000	0.791	0.791	0.000

Table 25 Mean packed cell volume (percentage) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	39.82 ±0.99	40.42 ±0.72	43.08 ±0.62	43.75** ±0.58	41.50 ±0.73	42.67** ±0.33
II	42.58 ±0.47	40.25 ±0.88	42.08 ±0.89	41.75 ±0.70	40.75 ±0.84	43.75** ±0.79
III	41.25 ±1.07	40.67 ±0.51	41.67 ±0.55	39.75 ±0.65	42.17 ±0.56	42.33** ±0.72
# IV	39.92 ±0.63	40.75 ±0.59	40.08 ±0.57	39.67 ±0.72	41.5 ±0.67	40.33 ±0.51
CD(0.05) # control				2.189		1.754

Table 25.1 ANOVA table (M.S.S), influence of dietary level of zinc on the packed cell volume of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	111.556	0.632	14.021	65.806	4.021	24.410
Error	44	8.250	5.729	5.441	7.087	5.998	4.551
F value		13.552**	0.110	2.577	9.285**	0.670	5.363**

Table 26 Mean Leucocyte count (x 100 per c m m) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	249.92 ±5.44	310.83 ±10.14	374.92** ±16.45	294.42 ±9.13	344.08** ±18.14	368.33** ±11.52
II	302.33 ±8.46	309.75 ±8.72	336.58 ±5.09	285.08 ±9.50	326.67** ±20.10	340.25 ±8.87
III	283.33 ±6.56	299.25 ±9.38	367.92** ±9.13	292.25 ±12.56	322.17** ±15.94	380.53** ±22.29
# IV	303.33 7.05	328.25 ±6.93	321.75 ±9.50	275.42 ±11.26	270.75 ±14.46	329.50 ±6.65
CD(0.05)			30.881		49.251	39.071

N = 12
# control

Table 26.1 ANOVA table (M.S.S), influence of dietary level of zinc on the Leucocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	1022.021	1732.187	7678.31	3990.97	63645.389	11038.611
Error	44	581.248	944.509	1410.61	1873.48	3588.125	2258.110
F value		1.758	1.834	5.443**	2.130	17.738**	4.888**

4.2.7 Differential count.

4.2.7.1 Lymphocyte.

The mean counts of Lymphocyte of four groups of ducks at monthly intervals are shown in table 27 and Fig.10.The mean values in the first month of groups I & II and in the second month of group II & III were significantly higher than the control values. The mean values of group I ranged from 59.03 ± 1.58 to 65.17 ± 1.90, group II from 60.83 ± 0.67 to 68.75 ±0.69, group III from 57.00 ± 1.34 to 65.75 ± 1.57 and group IV from 56.58 ± 1.55 to 63.50 ± 1.83.

4.2.7.2 Heterophils.

The mean Heterophil counts of four groups of ducks at monthly intervals are shown in table 28and Fig.10. In the first month the mean values of group I & II and in the second month the mean values of group I,II & III showed significantly lower values than that of controls. There was no significant difference for any other values. The mean values of group I ranged from 23.42 ± 1.02 to 30.00 ± 1.75, group II from 21.08 ± 0.72 to 28.42 ± 0.92, group III from 23.58 ± 1.46 to 32.50 ± 1.36 and group IV from 26.42 ± 1.61 to 33.17 ± 1.66.

4.2.7.3 Eosinophils

The mean Eosinophil counts of four groups of ducks at monthly intervals are shown in table 29and Fig.10. None of the values were significantly different from that of controls. The mean values of group I ranged from 5.33 ± 0.56 to 7.03 ±0.38, group II from 4.83 ± 0.29 to 6.00 ± 0.52,group III from 5.00 ± 0.35 to 6.92 ± 1.10 and group IV from 5.00 ± 0.30 to 6.33 ± 0.54.

4.2.7.4 Monocytes

The mean Monocyte counts of four groups of ducks at monthly intervals are

Table 27 Mean Lymphocyte count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	61.33** ±1.04	64.75 ±1.21	62.50 ±1.40	59.03 ±1.58	64.58 ±1.93	65.17 ±1.90
II	60.83** ±0.67	68.75** ±0.69	61.92 ±1.70	61.83 ±2.41	62.83 ±1.36	64.51 ±3.02
III	57.00 ±1.34	65.75** ±1.57	61.00 ±1.71	65.33 ±1.64	61.58 ±1.72	57.33 ±2.52
# IV	56.58 ±1.55	61.51 ±1.60	63.50 ±1.83	62.17 ±1.55	63.08 ±1.55	63.33 ±1.48
CD(0.05) (Transformed values)	1.984	2.249				

N = 12
# control

Table 27.1 ANOVA table (M.S.S,transformed values), influence of dietary level of zinc on the Lymphocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	25.240	38.433	4.654	27.836	6.788	54.257
Error	44	5.824	7.483	11.899	14.401	11.915	22.547
F value		4.334**	5.136**	0.391	1.933	0.570	2.406

Table 28 Mean Heterophil count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Month					
	1	2	3	4	5	6
I	26.83** ±0.55	23.42** ±1.02	25.83 ±1.02	30.00 ±1.75	25.33 ±1.42	25.58 ±1.91
II	28.42** ±0.92	21.08** ±0.72	26.83 ±1.49	28.17 ±2.36	26.67 ±1.31	26.83 ±2.91
III	32.50 ±1.36	23.58** ±1.46	28.67 ±1.66	25.42 ±1.54	26.42 ±1.21	22.58 ±2.45
# IV	33.17 ±1.66	27.25 ±1.43	26.50 ±1.32	28.17 ±1.16	26.42 ±1.61	27.08 ±1.46
CD(0.05) (Transformed values)	2.099	2.243				

N = 12
# control

Table28.1 ANOVA table (M.S.S, transformed values), influence of dietary level of zinc on the Heterophil count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	43.759	34.139	6.988	17.606	1.962	46.490
Error	44	6.518	7.441	9.867	14.426	9.998	23.677
F value		6.714**	4.588**	0.708	1.220	0.196	1.964

Table 29 Mean Eosinophil count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	6.67 ±0.48	7.03 ±0.38	6.67 ±0.67	6.50 ±0.45	6.21 ±0.62	5.33 ±0.56
II	5.67 ±0.47	6.00 ±0.52	5.83 ±0.53	5.92 ±0.50	5.58 ±0.36	4.83 ±0.29
III	6.08 ±0.48	6.92 ±1.10	6.33 ±0.47	5.00 ±0.35	6.67 ±0.80	6.00 ±0.28
# IV	5.75 ±0.43	6.33 ±0.54	5.83 ±0.44	5.00 ±0.30	6.08 ±0.38	5.58 ±0.29
CD(0.05,transformed values)				1.440		

N = 12
# control

Table 29.1 ANOVA table (M.S.S,transformed values), influence of dietary level of zinc on the Eosinophil count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	3.551	3.722	2.504	9.703	49.403	4.921
Error	44	4.062	7.587	5.224	3.066	55.758	2.677
F value		0.874	0.491	0.479	3.165*	0.886	1.838

Table 30 Mean Monocyte count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	4.33 ±0.31	3.67 ±0.28	4.33 ±0.45	3.58 ±0.34	3.50 ±0.38	3.33 ±0.33
II	4.25 ±0.65	3.33 ±0.43	4.67 ±0.36	3.58 ±0.47	4.17 ±0.32	3.00 ±0.35
III	4.00 ±0.41	3.08 ±0.45	3.33 ±0.31	3.50 ±0.50	4.42 ±0.36	2.83 ±0.47
# IV	3.83 ±0.32	4.25 ±0.37	3.58 ±0.38	3.50 ±0.36	3.75 ±0.35	3.42 ±0.38

N = 12
# control

Table 30.1 ANOVA table (M.S.S, transformed values), influence of dietary level of zinc on the Monocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	1.271	8.870	10.232	0.331	4.687	3.397
Error	44	4.881	4.790	4.015	6.171	3.332	5.562
F value		0.260	1.852	2.548	0.054	1.407	0.611

shown in table 30 and Fig . 10. None of the values were significantly different from that of the controls. The mean values of group I ranged from 3.33 ± 0.33 to 4.33 ± 0.45, group II from 3.00 ± 0.35 to 4.67 ± 0.36, group III from 2.83 ± 0.47 to 4.42 ± 0.36 and group IV from 3.42 ± 0.38 to 4.25 ± 0.37.

4.2.7.5 basophils

The mean Basophil counts of four groups of ducks at monthly intervals are shown in table 31 and Fig.10. None of the values were significantly different from that of the controls. The mean values of group I ranged from 0.63 ± 0.26 to 1.23 ± 0.38 ,group II from 0.81 ± 0.22 to 0.98 ± 0.31, group III from 0.65 ± 0.16 to 1.29 ± 0.20 and group IV from 0.63 ± 0.11 to 0.88 ± 0.22.

4.2.8 Enzymes

4.2.8.1 Aspartate Amino Transferase(AST).

The mean serum AST values of four groups of ducks at monthly intervals are shown in table 32 and Fig.11. Group I showed a significantly lower value in the first, second and fourth months. In the fifth and the sixth months all the groups showed a higher value than that of the controls, but significant difference was noticed only in the sixth month. A significantly lower value was noticed in the second and the fourth month of group II and the first and the second month of group III. The values of group I ranged from 11.17 ± 0.37 to 14.83 ± 0.72, group II from 13.42 ± 0.73 to 16.92 ± 0.70, group III from 12.03 ± 0.72 to 16.25 ± 0.59 and group IV from 9.67 ± 0.26 to 20.25 ± 0.58.

4.2.8.2 Alanine Amino Transferase (ALT).

The mean serum ALT levels of four group of ducks at monthly intervals are shown in table 33 and Fig. 12.The group I showed a significantly reduced value than

Table 31 Mean Basophil count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet supplemented with zinc.

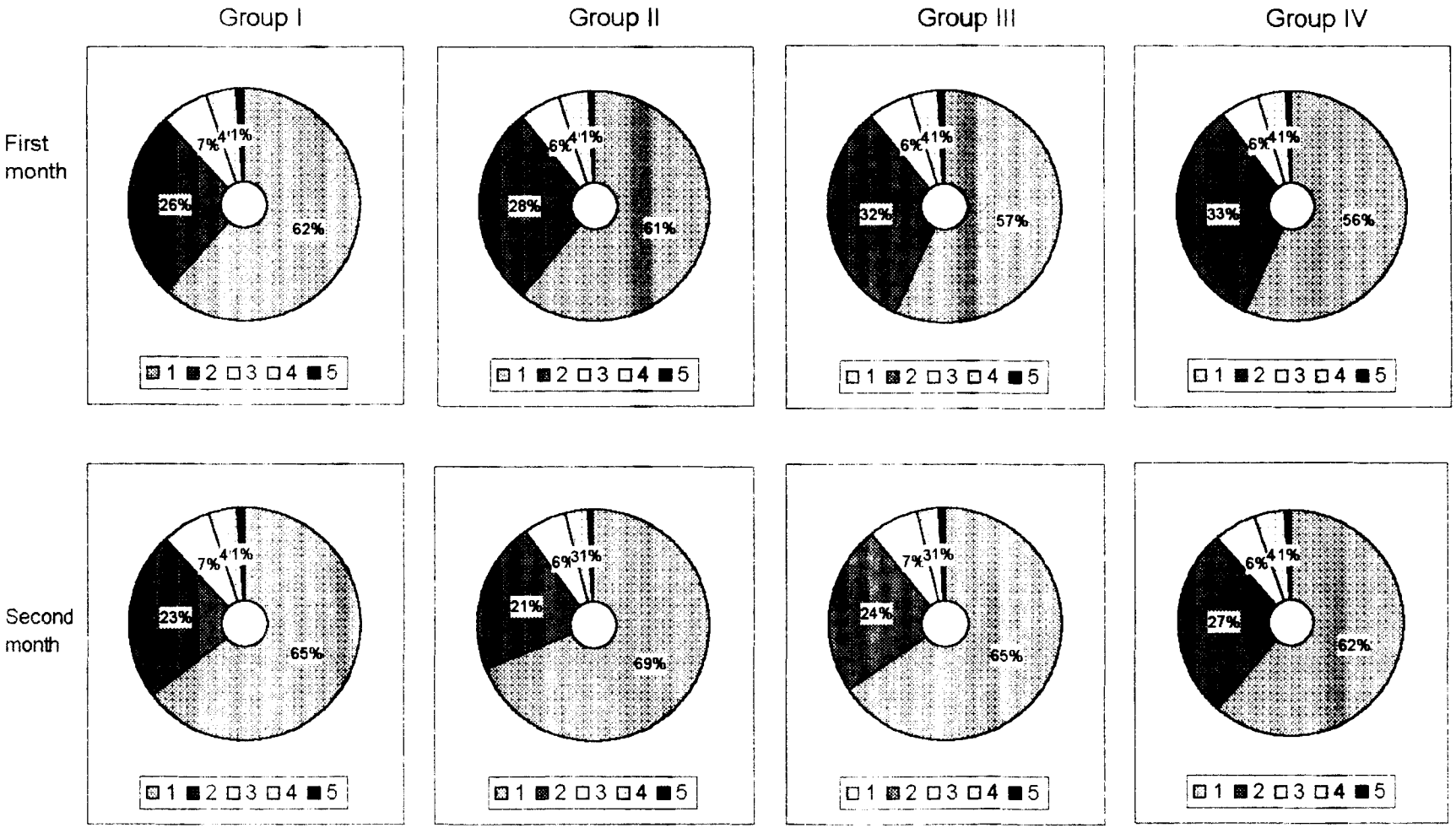
Group	Months					
	1	2	3	4	5	6
I	0.98 ±0.26	1.23 ±0.38	0.63 ±0.26	0.92 ±0.18	0.65 ±0.16	0.71 ±0.16
II	0.94 ±0.21	0.98 ±0.31	0.92 ±0.32	0.83 ±0.15	0.81 ±0.22	0.85 ±0.19
III	0.83 ±0.28	0.81 ±0.22	0.65 ±0.16	0.75 ±0.11	0.88 ±0.22	1.29 ±0.20
# IV	0.79 ±0.19	0.88 ±0.22	0.63 ±0.11	0.85 ±0.19	0.71 ±0.16	0.71 ±0.16

N = 12
# control

Table 31.1 ANOVA table (M.S.S, transformed values), influence of dietary level of zinc on the Basophil count of Ducks at monthly intervals.

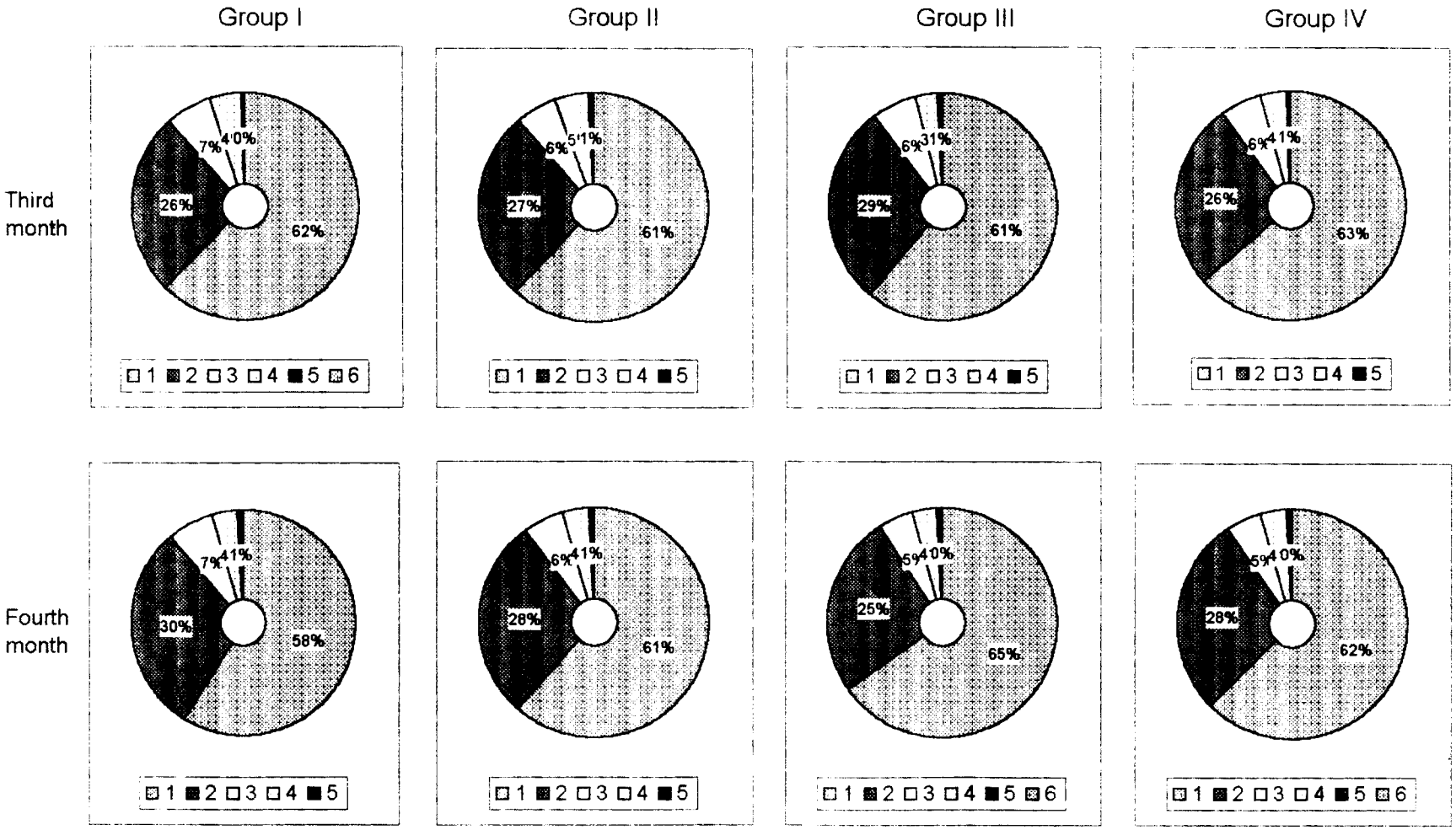
Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.897	1.492	1.392	0.323	0.865	8.283
Error	44	5.963	7.672	5.072	3.176	4.401	3.656
F value		0.150	0.195	0.274	0.102	0.197	2.266

Fig. 10 Mean differential count (percentage) of Ducks at monthly intervals, fed on diet supplemented with zinc.



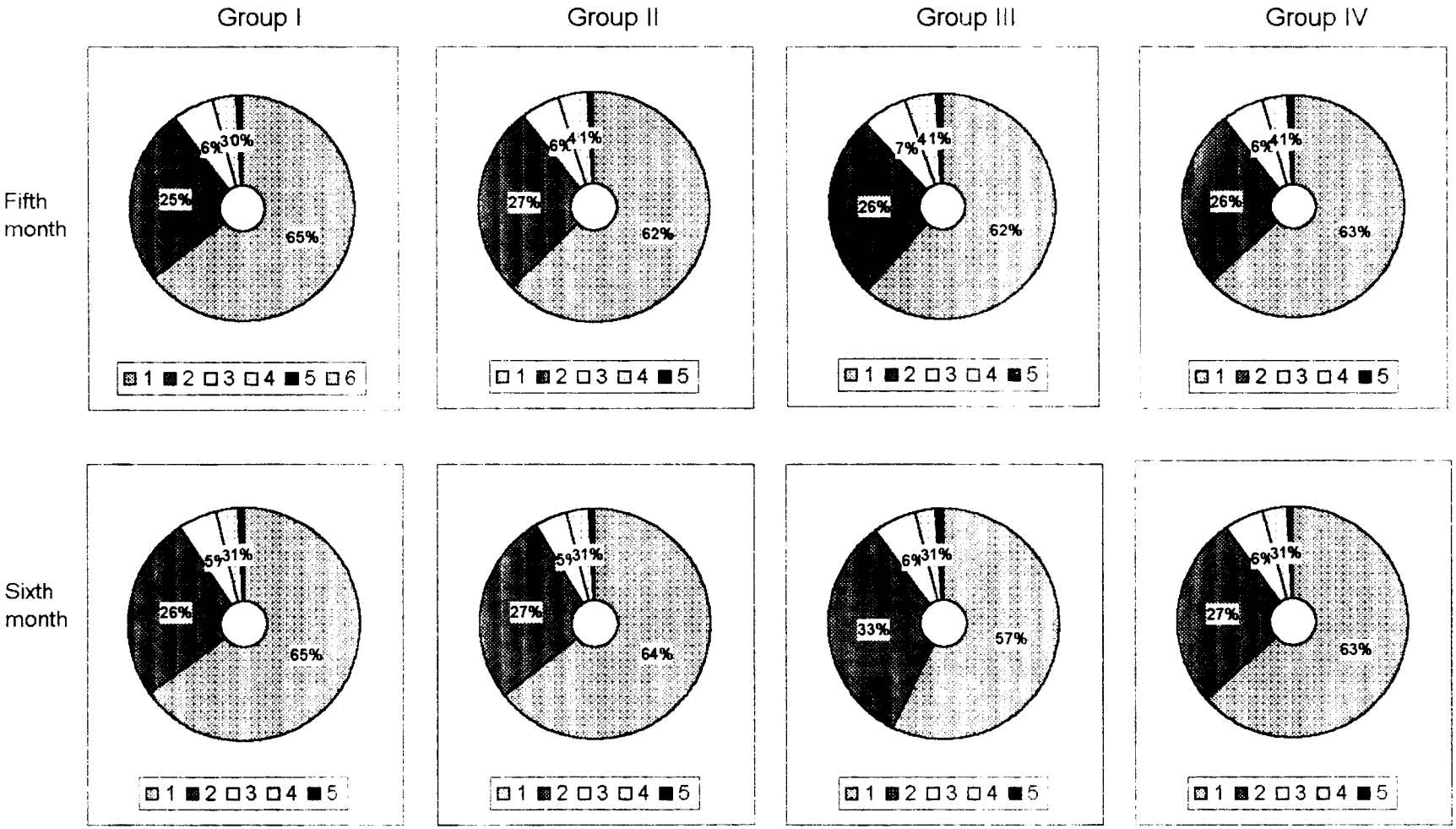
1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils

Fig .10 Mean differential count (percentage) of Ducks at monthly intervals, fed on diet supplemented with zinc.



1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils

Fig .10 Mean differential count (percentage) of Ducks at monthly intervals, fed on diet supplemented with zinc.



1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils

Table 32 Mean serum Aspartate Amino Transferase level (I.units per ml) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	11.17** ±0.37	14.83** ±0.72	12.00 ±0.39	12.92** ±0.53	13.58 ±0.69	11.78** ±0.39
II	11.58** ±0.95	13.42** ±0.73	13.92 ±0.63	13.67** ±1.08	15.50 ±0,75	16.92** ±0.70
III	12.03** ±0.72	15.25** ±0.08	13.33 ±0.70	16.25 ±0.59	14.17 ±1.07	12.58** ±0.66
# IV	14.25 ±0.73	20.25 ±0.58	13.00 ±0.48	16.92 ±0.83	12.58 ±0.51	9.67 ±0.26
CD(0.05) N = 12 # control	2.055	2.048		2.248		1.522

Table 32.1 ANOVA table (M.S.S), influence of dietary level of zinc on the serum Aspartate Amino Transferase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	76.389	106.576	7.743	45.354	17.806	111.576
Error	44	6.250	6.206	3.809	7.472	7.330	3.426
F value		12.222**	17.172**	2.033	6.070**	2.429	32.566**

Fig. 11 Mean serum Aspartate Amino Transferase level of Ducks at monthly intervals, fed on diet supplemented with zinc.

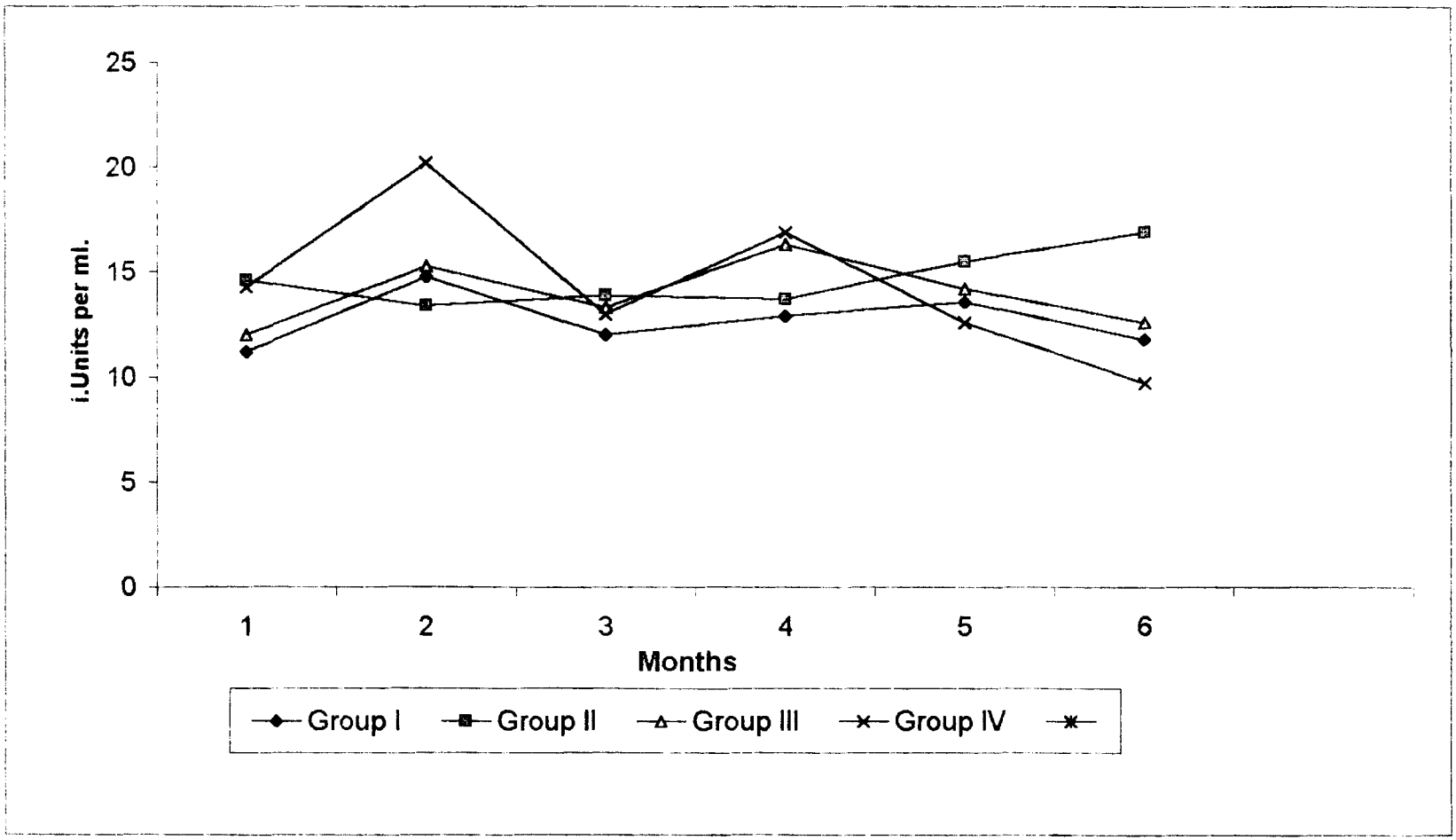


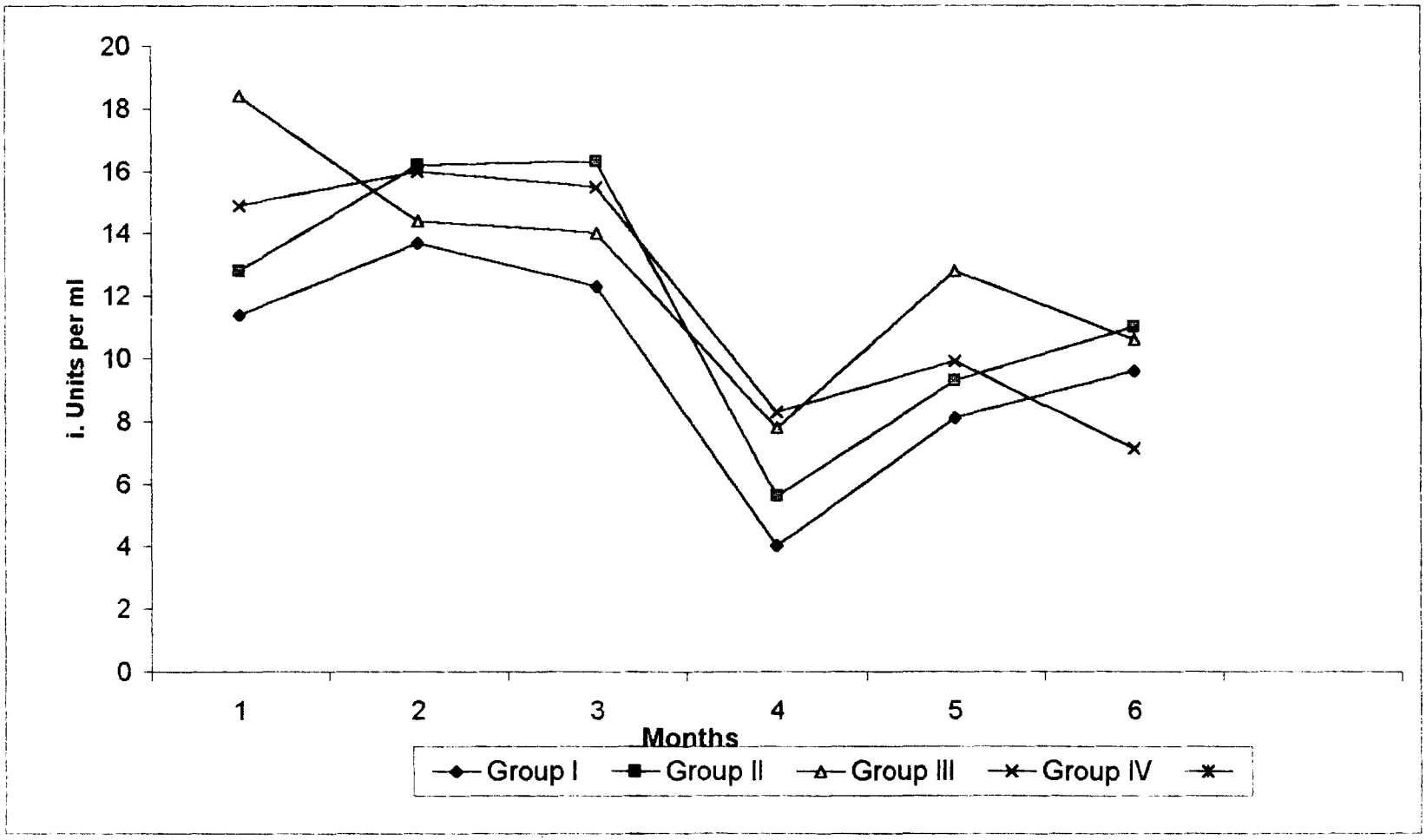
Table 33 Mean serum Alanine Amino Transferase level (I.units per ml) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	11.42 ±0.38	13.05** ±0.77	12.25** ±1.35	4.00** ±0.46	8.17 ±0.47	9.58** ±0.60
II	12.75** ±1.24	16.17 ±0.75	16.25 ±0.85	6.58 ±0.86	9.25 ±0.75	11.05** ±0.51
III	18.42** ±0.70	16.42 ±0.71	15.00 ±0.75	8.83 ±0.63	12.83** ±0.66	10.58** ±0.57
# IV	14.92 ±2.49	16.08 ±1.08	15.50 ±1.06	7.33 ±0.40	9.92 ±0.79	7.17 ±0.57
CD(0.05) N = 12 # control	2.113	2.389	2.927	1.741	1.938	1.609

Table 33.1 ANOVA table (M.S.S), influence of dietary level of zinc on the serum Alanine Amino Transferase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	455.417	51.243	37.500	36.688	47.806	36.354
Error	44	25.025	8.449	12.670	4.483	5.557	3.828
F value		18.200**	6.065**	2.960*	8.184**	8.603**	9.498**

Fig. 12 Mean serum Alanine Amino Transferase level of Ducks at monthly intervals, fed on diet supplemented with zinc.



controls in the first to the fourth months. In group II significantly Reduced value was noticed only in the first month. In the sixth month all the treated groups showed a significantly higher value than the controls. In group III in addition to the sixth the first and the fifth month also showed a significantly higher values. In group I the values ranged from 4.00 ± 0.46 to 13.05 ± 0.77, in group II from 6.58 ± 0.86 to 16.25 ± 0.85, in group III from 8.83 ± 0.63 to 18.42 ± 0.70 and in group IV from 7.17 ± 0.57 to 16.08 ± 1.08.

4.2.8.3 Serum alkaline phosphatase.

The mean level of serum alkaline phosphatase of four groups of ducks at monthly intervals are shown in table 34 and Fig.13.Group I showed significantly higher values in the second, the third and the fifth month and a lesser value in the sixth month. Group II also showed significantly higher values in all the observations except in the sixth month which showed significantly lesser value . The group III showed significantly higher value in the second, the fourth, the fifth and the sixth month. The values of group I ranged from 12.66 ± 0.26 to 26.06 ± 0.92, group II from 14.69 ± 1.41 to 33.23 ± 1.30, group III from 17.60 ± 2.54 to 26.88 ± 2.09 and group IV from 8.92 ± 1.24 to 25.16 ± 2.17.

4.2. 9 Phytohaemaglutinin-p skin sensitivity test.

The mean wing web thickness of four groups of ducks subjected to PHA-P skin sensitivity test at 1½ months intervals are shown in table 35 and Fig.14. None of the values except 72 hour value of group I & II at 1½ month and 48 hour reading of group I at third month were significantly different. The values of the group I ranged from 0.33 ± 0.01 to 1.15 ± 0.10,group II from 0.34 ± 0.01 to 1.14 ± 0.07, group III from 0.33 ± 0.01 to 1.07 ± 0.03 and group IV from 0.33 ± 0.01 to 1.13 ± 0.05.

Table 34 Mean serum Alkaline phosphatase level (KA units per ml) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	26.06 ±0.92	20.58* ±1.39	21.59** ±2.01	12.66 ±0.26	19.26** ±1.49	18.68** ±0.53
II	33.23* ±1.30	27.45* ±0.62	26.17** ±1.71	14.69** ±1.41	21.21** ±1.83	16.19** ±0.64
III	26.88 ±2.09	25.17* ±0.38	18.47 ±1.03	17.60** ±2.54	22.88** ±1.23	19.74** ±1.65
# IV	25.16 ±2.17	17.21 ±1.01	16.86 ±1.16	8.92 ±1.24	12.72 ±1,35	11.44 ±0.98
CD(0.05) N = 12 # control	4.863	2.663	4.366	4.534	4.247	2.978

Table 34.1 ANOVA table (M.S.S), influence of dietary level of zinc on the serum alkaline phosphatase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	123.957	59.989	201.570	255.940	237.743	416.347
Error	44	34.975	10.488	28.201	30.402	26.686	13.120
F value		3.544*	5.720**	7.148**	8.419**	8.909**	31.734**

Fig. 13 Mean serum Alkaline Phosphatase level of Ducks at monthly intervals, fed on diet supplemented with zinc.

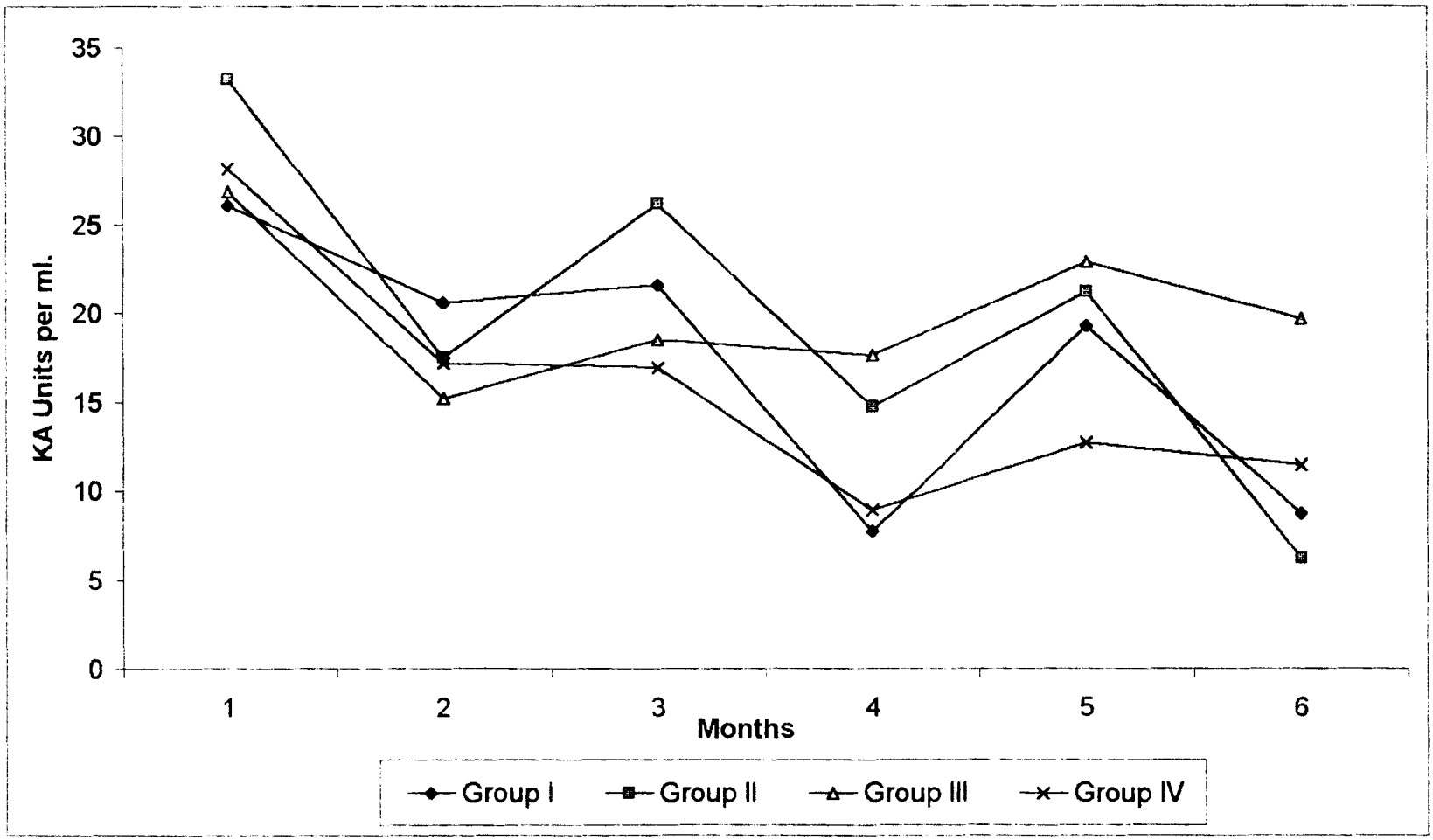


FIG 61

Table 35 Mean wing web thickness of Ducks, in PHA-P skin sensitivity test at one and a half months intervals, fed on diet supplemented with zinc.

Group	One and a half month				Three month				Four and a half month				Six month			
	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
I	0.48 ±0.01	1.07 ±0.07	0.70 ±0.04	0.51** ±0.02	0.40 ±0.02	0.88 ±0.04	0.63* ±0.05	0.48 ±0.03	0.34 ±0.02	1.15 ±0.01	0.72 ±0.05	0.50 ±0.04	0.33 ±0.01	1.13 ±0.09	0.77 ±0.06	0.36 ±0.02
II	0.48 ±0.01	1.14 ±0.07	0.82 ±0.05	0.52** ±0.03	0.38 ±0.03	0.97 ±0.03	0.77 ±0.03	0.44 ±0.02	0.34 ±0.01	1.05 ±0.10	0.61 ±0.06	0.48 ±0.04	0.35 ±0.02	1.08 ±0.04	0.77 ±0.04	0.40 ±0.03
III	0.47 ±0.01	1.01 ±0.06	0.84 ±0.06	0.65 ±0.04	0.43 ±0.02	0.94 ±0.04	0.78 ±0.04	0.50 ±0.03	0.33 ±0.01	0.89 ±0.05	0.64 ±0.07	0.45 ±0.03	0.35 ±0.02	1.07 ±0.03	0.75 ±0.03	0.38 ±0.02
# IV	0.48 ±0.01	1.11 ±0.06	0.88 ±0.03	0.70 ±0.05	0.40 ±0.02	0.99 ±0.05	0.75 ±0.03	0.46 ±0.01	0.33 ±0.01	0.92 ±0.02	0.60 ±0.06	0.40 ±0.03	0.34 ±0.01	1.13 ±0.05	0.78 ±0.03	0.41 ±0.03
CD(0.05)				0.101			0.107									

N = 12

control

Table 35.1 ANOVA table (MSS), influence of dietary level of zinc on the PHA-P sensitivity test on Ducks at 45 days intervals.

Source of variation	df	One and a half month				Three month				Four and a half month				Six month			
		0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
Group	3	0.000	0.040	0.074	0.111	0.005	0.030	0.056	0.007	0.001	0.175	0.034	0.022	0.001	0.012	0.002	0.006
Error	44	0.002	0.059	0.029	0.015	0.006	0.021	0.017	0.006	0.003	0.067	0.042	0.017	0.004	0.040	0.024	0.008
F value		0.163	0.678	2.711	7.211**	0.882	1.433	3.305*	1.164	0.396	2.609	0.813	1.314	0.216	0.295	0.094	0.766

4.2.10 Antibody titre.

The mean antibody titre of four groups of ducks at 15 days intervals are shown in table 36 and Fig.14. The mean values of group II on 105^{th} day and values of group III on 105^{th}, 135^{th}, 150^{th} and 165^{th} day were significantly higher than the control values. Other values are not significantly different. The mean values of group I ranged from 138.667 ± 25.689 to 9557.333 ± 2284.640, group II from 202.667 ± 66.954 to 12629.333 ± 4800.556,group III from 154.667± 73.166 to 105130.667 ± 37505.684 and group IV from 106.667 ± 31.642 to 15018.33 3± 3909.696..

4.2.11 Zinc content in the serum.

The mean zinc content in the serum of four group of ducks at monthly intervals are shown in table 37 and Fig.15. The mean values of group I.II & III in the fourth .the fifth and the sixth months showed significantly higher values than that of controls. Other values showed no significant difference The mean values of group I ranged from 2.77 ±0.14 to 8.07 ± 1.05, group II from 2.78 ± 0.08 to 9.55 ± 1.12, group III from 2.60 ± 0.23 to 17.54 ± 1.51 and group IV from 2.54 ± 0.31 to 3.99 ± 0.40.

4.2.12 Zinc content in tissues.

The mean zinc values in liver, kidney and muscle tissues of four groups of ducks are shown in table 38 and Fig.16. None of the values are significantly different from the controls except group I which showed higher values .The zinc content of liver, kidney and muscle tissues of group I showed higher values than that of the controls.

Table 36 Mean antibody titre in Duck serum at 15 days intervals, fed on diet supplemented with zinc.

Group	Days										
	15	30	45	60	75	90	105	120	135	150	165
I	640.000 ±128.000	362.667 ±69.456	202.667 ±66.954	138.667 ±25.689	181.333 ±34.728	9557.333 ±2284.640	8730.667 ±431.756	3754.667 ±1079.391	1450.667 ±277.825	3669.333 ±2551.167	3840.000 ±2521.308
II	618.667 ±182.272	362.667 ±69.456	256.000 ±57.245	245.333 ±62.743	202.667 ±66.954	8874.667 ±1644.079	12629.333** ±4800.559	5632.000 ±2390.552	1058.333 ±5021.198	7680.000 ±2941.216	8192.000 ±2747.680
III	448.000 ±131.161	256.000 ±57.243	202.667 ±66.945	213.333 ±69.456	154.667 ±73.166	11605.333 ±2222.602	23893.333** ±5637.686	30720.000 ±8444.120	105130.667** ±37505.684	45056.000** ±9864.474	68266.667** ±20905.427
# IV	256.000 ±57.243	384.000 ±140.217	245.333 ±84.932	128.000 ±28.622	106.667 ±31.642	13653.333 ±4050.233	13072.000 ±1121.736	15018.333 ±3909.696	3840.000 ±1397.488	9556.667 ±4778.819	12458.667 ±6435.288
CD(0.05)(log value)								0.452	0.585	0.565	0.601

N = 6
# control

Fig.14 Mean antibody titre in Duck serum at 15 days intervals, fed on diet supplemented with zinc.

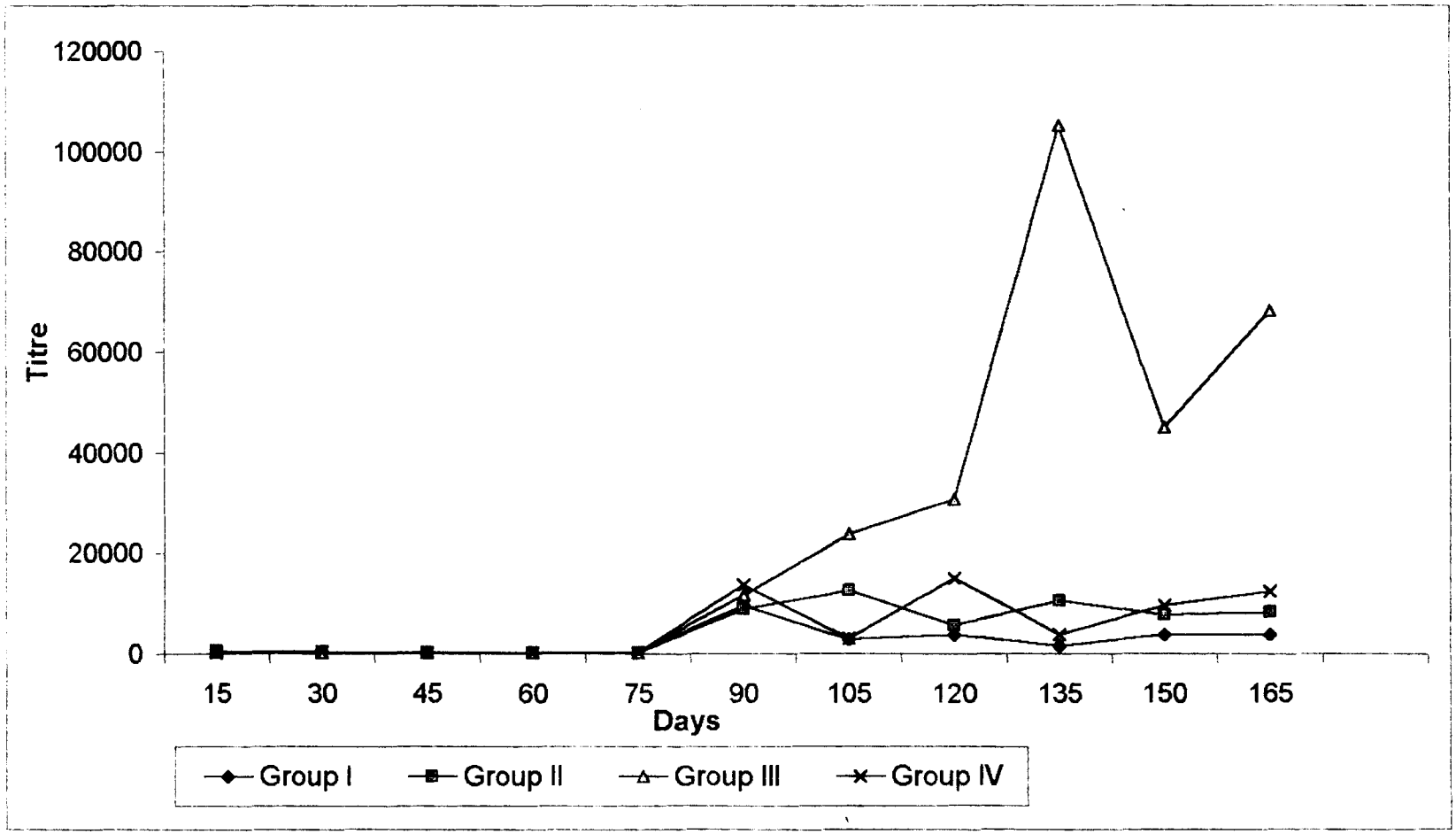


Table 36.1 ANOVA table (M.S.S), influence of dietary level of zinc on the antibody titre of Ducks at 15 days intervals.

Source of variation	df	Days										
		15	30	45	60	75	90	105	120	135	150	165
Group	3	0.176	0.030	0.030	0.074	0.104	0.034	0.413	1.131	3.323	1.907	2.245.
Error	20	0.091	0.074	0.095	0.079	0.097	0.058	0.131	0.141	0.236	0.220	0.249
F value		1.944	0.408	0.317	0.937	1.072	0.584	0.314	8.012**	14.103**	8.677**	9.010**

Table 37 Mean serum zinc level (ppm) of Ducks at monthly intervals, fed on diet supplemented with zinc.

Group	Months					
	1	2	3	4	5	6
I	2.77 ±0.14	2.95 ±0.08	3.53 ±0.06	7.02** ±0.83	7.15** ±1.08	8.07** ±1.05
II	2.78 ±0.08	3.12 ±0.22	3.66 ±0.27	8.70** ±1.50	9.13** ±1.70	9.55** ±1.12
III	2.60 ±0.23	3.42 ±0.19	3.46 ±0.09	9.05** ±0.67	12.18** ±1.52	17.54** ±1.51
# IV	2.54 ±0.31	2.96 ±0.20	3.37 ±0.16	3.44 ±0.16	3.58 ±0.14	3.99 ±0.40
CD(0.05)				2.72	3.526	3.248

N = 6
# control

Table 37.1 ANNOVA table (M.S.S), influence of dietary level of zinc on the serum zinc level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.086	0.317	0.545	42.436	77.918	193.192
Error	20	0.264	0.202	0.741	5.102	9.570	7.275
F value		0.328	1.569	0.735	8.318**	8.142**	26.557**

Fig .15 Mean serum zinc level of Ducks at monthly intervals, fed on diet supplemented with zinc.

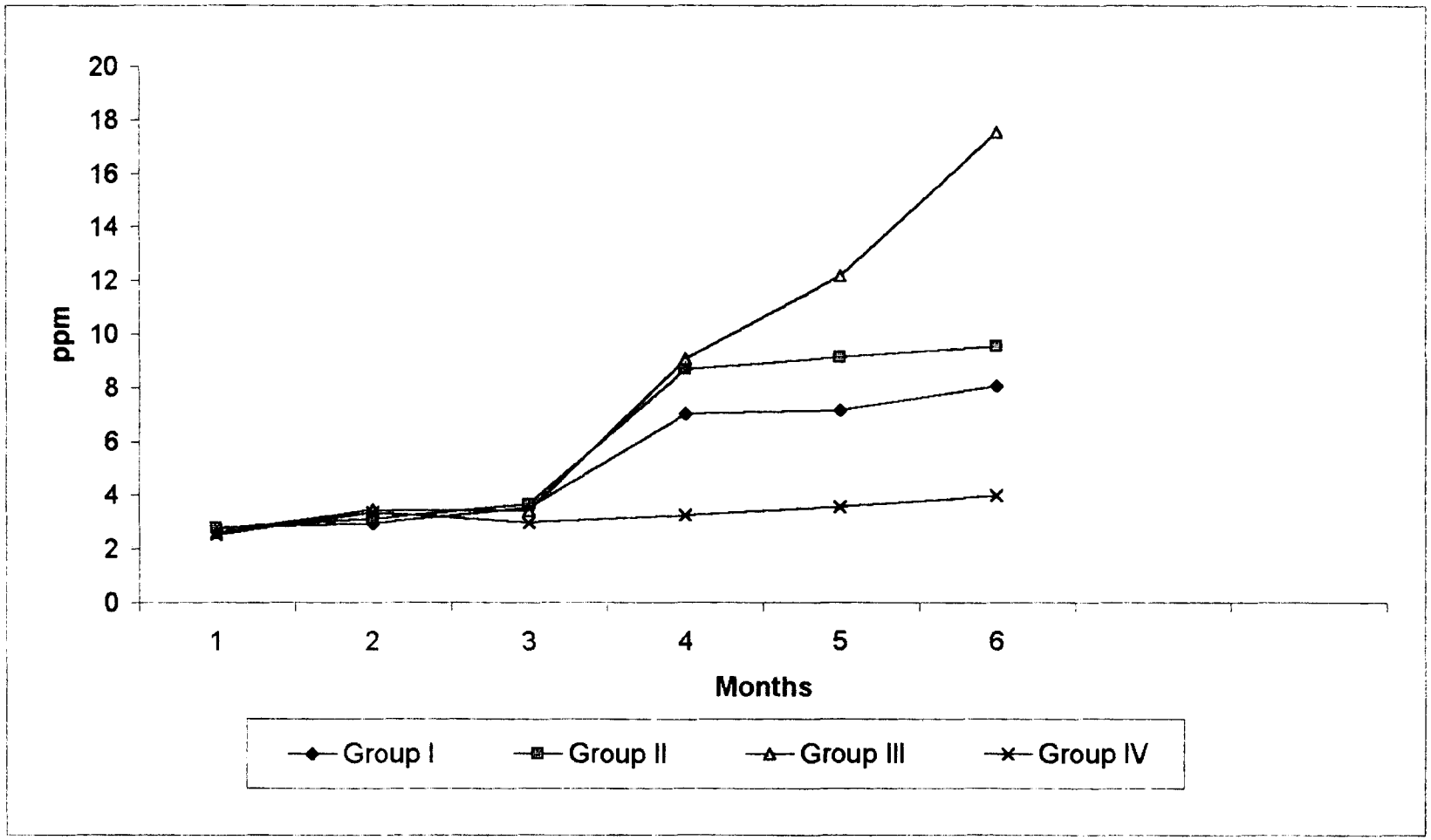


Table 38 Mean zinc content (ppm on dry matter basis) of Liver,Kidney and Muscle of Ducks after six months of feeding on diet supplemented with zinc.

Group	Liver	Kidney	Muscle
I	148.167** ±7.247	170.667 ±19.073	150.167 ±8.693
II	107.833 ±6.586	131.833 ±9.245	142.667 ±6.582
III	116.167 ±5.884	128.667 ±5.626	174.333 ±15.790
# IV	110.833 ±4.945	152.333 ±6.778	149.667 ±10.862
CD(0.05) # control	18.361		

Table 38.1 ANOVA table (M.S.S),influence of dietary level of zinc on the tissue zinc content of Ducks after six months of feeding.

Source of variation	df	Tissues		
		Liver	Kidney	Muscle
Groups	3	2075.722	2299.26	1150.375
Error	20	232.367	789.842	729.042
F value		8.933**	2.911	1.578

Fig. 16 Mean zinc content (ppm on dry matter basis) of liver, kidney and muscle of Ducks after six months of feeding on diet supplemented with zinc.

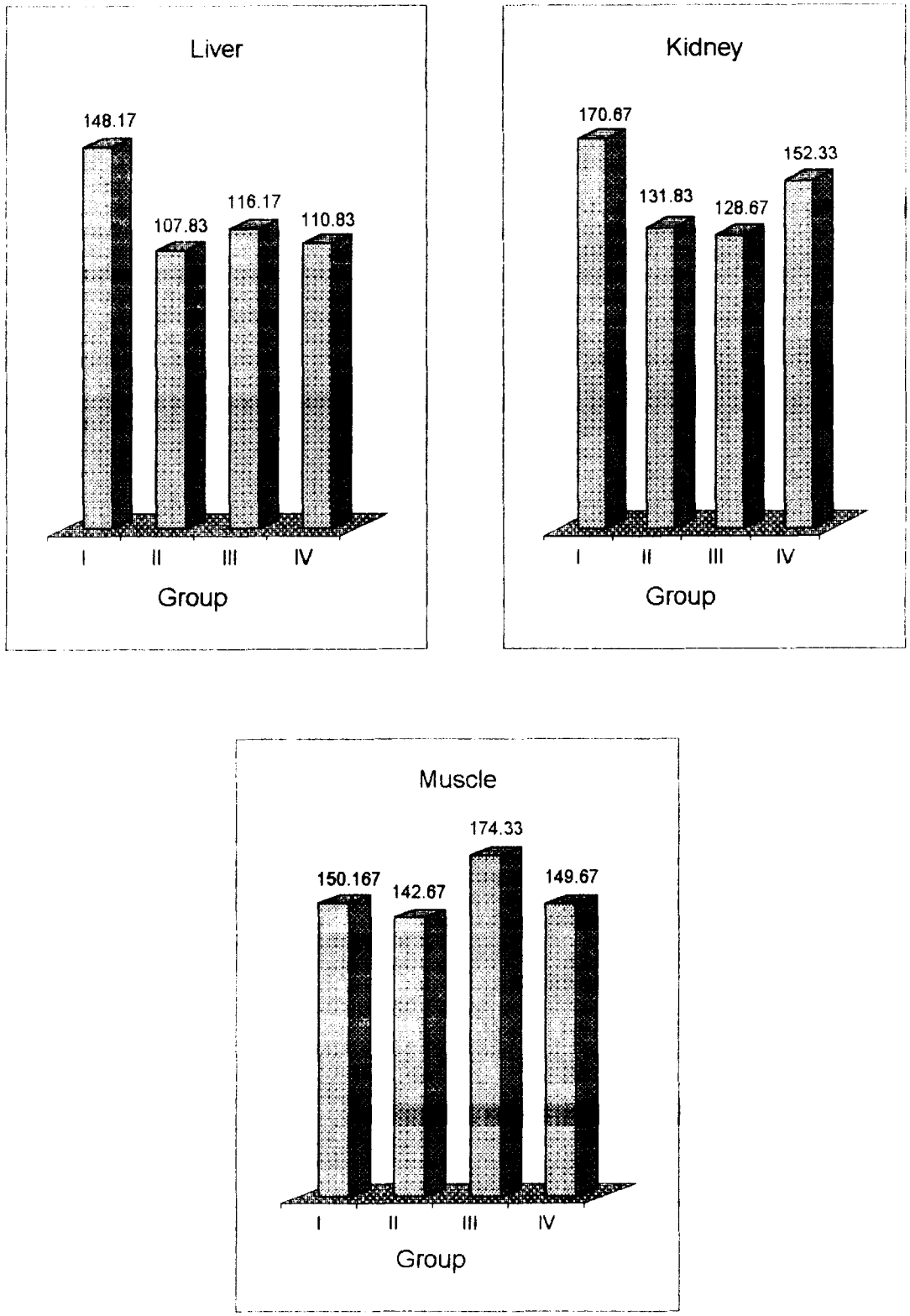


Table 39 Mean weight of Liver (g) and Spleen (mg) of sacrificed Ducks after six months of feeding on diet supplemented with zinc.

Group	Liver	Spleen
I	48.583 ±4.509	854.167 ±55.207
II	54.833 ±3.545	979.167 ±118.467
III	51.083 ±3.158	1085.833 ±124.244
# IV	50.333 ±4.532	875.000 76.005

N = 12
# control

Table 39.1 ANOVA table (M.S.S), influence of dietary level of zinc on the weight of Liver and spleen of Ducks after six months of feeding.

Source of variation	df	Liver	Spleen
Groups	3	83.25	136407.639
Error	44	190.277	114876.705
F value		0.438	1.187

4.2.13 Weight of liver and spleen.

The mean weight of liver and spleen of four groups of ducks is shown in table 39. The mean weight of liver and spleen of group I was lower and group II & III was higher than that of the control

4.2.14 Gross and histo-pathological changes.

4.2.14.1 Gross lesions.

Gross lesions could not be noted in any of the birds except for diffuse petechiae in the liver of one bird in the group III.

4.2.14.2 Histo-pathology.

The histo-pathological lesions are shown in Fig.16a to 16d. Mild lymphoid infiltration was observed in the liver tissue of the birds belonging to group III. However no microscopic lesions could be detected in the liver tissue of birds in the other groups Group III birds also exhibited mild depletion of lymphoid cells and moderate fibrous tissue replacement in the medullary area of the spleen. In all the groups including control, thymus tissue showed moderate fibrous tissue proliferation. Diffuse calcification of endothelium and congestion of the small blood vessels were noted in the kidney of birds in group II and III. Depletion of lymphoid follicle in the bursa was observed in all the groups.

Fig. 16a Duck kidney- group III – treated with zinc, tubular degeneration , necrosis and calcification H&E X 250

Fig. 16b Duck bursa-g roup I, II, III & IV-treated with zinc, extensive fibrous tissue proliferation and depletion of lymphoid element H&E X 160

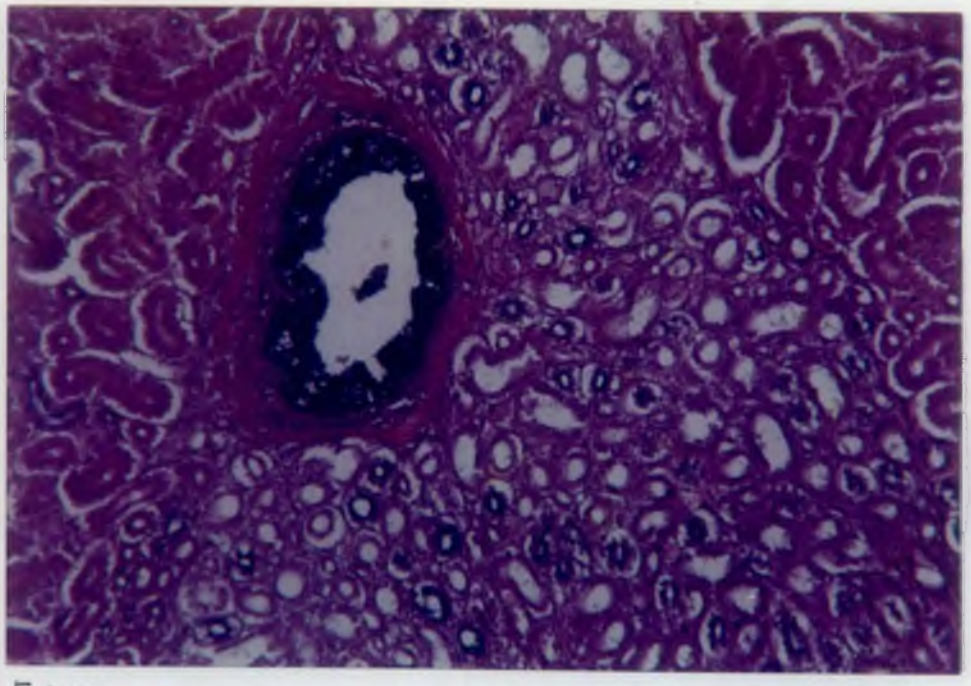


Fig. 16a

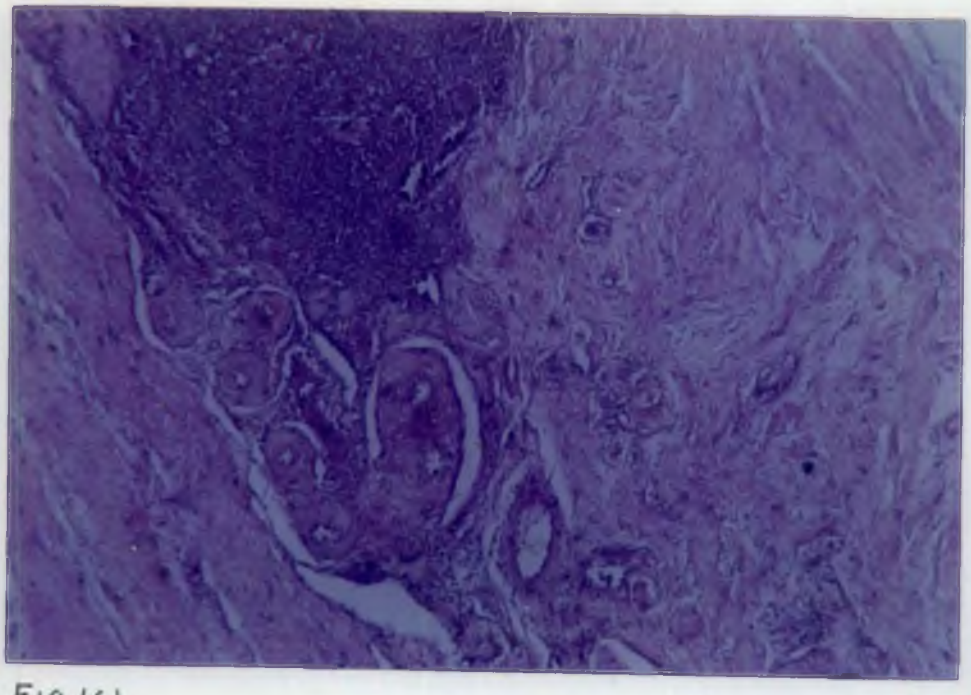


Fig 16b

Fig. 16c Duck caecal tonsil – group III – treated with zinc, depletion of lymphoid element H&E X 160

Fig. 16d Duck thymus - group I, II, III & IV - treated with zinc, moderate fibrous tissue proliferation and depletion of lymphoid element H&E X 160

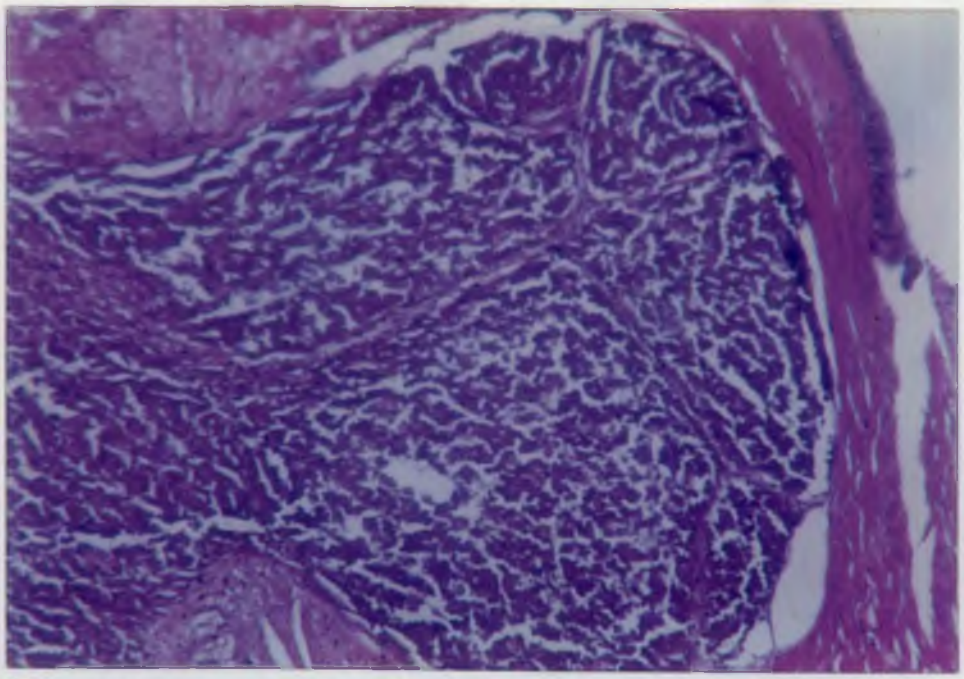


Fig. 16c

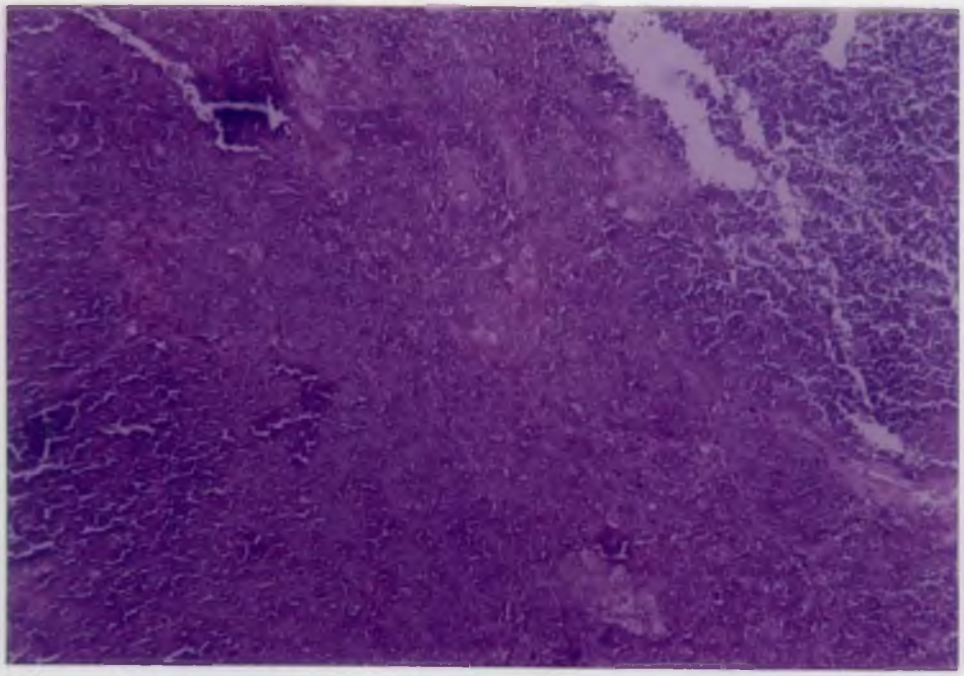


Fig. 16d

4.3 MANCOZEB

4.3.1 Body weight.

The mean body weight of ducks fed on diet to which Mancozeb was added at the rate of 1000, 1500, 2000 ppm are shown in Table 40 and Fig.17. The mean body weight of the group I showed no significant change from the control. All the mean values (except on 15^{th} day) of the group II and the group III was lower than that of the controls but significant reduction was noticed only on 90^{th}, 105^{th}, 120^{th}, 135^{th}, and 150^{th}, day. The mean value of group I ranged from 1200.00 ± 71.77 to 1750.00 ± 41.57, group II from 1183.33 ± 44.10 to 1620.83 ± 49.03 g, group III from 1191.67 ± 46.81 to 1700.00 ± 53.03g, and group IV (control) from 1158.33 ± 52.89 to 1800.00 ± 32.57g.

4.3.2 Erythrocyte count.

The mean erythrocyte counts of four groups of ducks at monthly intervals are shown in table 41. The mean value of the group I was significantly high in fourth and fifth month and low in third month. In the group II significantly higher value was noticed in the first month and low value in the third month. In the group III all the values except in sixth month was high even though significant increase was noticed only in the first and the second month. The mean value of group I ranged from 272.92 ± 8.82 to 326.75 ± 8.11, group II from 267.42 ± 10.43 to 301.92 ± 9.54, group III 284.00 ± 7.98 to 338.00 ± 5.68 and group IV from 258.33 ± 6.48 to 322.08 ± 9.14.

4.3.3 Haemoglobin.

The mean haemoglobin values of four groups of ducks at monthly intervals are shown in table 42. In the group I significantly higher value was noticed in the fifth month and significantly lower value was noticed in the third and the fourth month. In the group II also the same trend was seen. In the group III significant reduction was

Table 40 Mean body weight of Ducks (g) at 15 days intervals, fed on diet added with Mancozeb.

Group	Days											
	15	30	45	60	75	90	105	120	135	150	165	180
I	1200.00 ±71.77	1412.50 ±57.45	1541.67 ±51.43	1600.00 ±45.23	1658.33 ±41.67	1768.33 ±39.33	1750.00 ±41.57	1733.33 ±30.98	1683.33 ±40.52	1604.17 ±28.51	1566.67 ±35.53	1516.67 ±30.36
II	1183.33 ±44.10	1408.33 ±41.20	1566.67 ±33.33	1870.50 ±40.42	1620.83 ±49.03	1566.67* ±46.60	1516.67** ±50.50	1504.17** ±42.84	1483.33** ±40.52	1479.17* ±37.16	1487.50 ±35.95	1462.50 ±36.48
III	1191.67 ±46.81	1420.83 ±47.85	1600.00 ±47.67	1645.83 ±53.11	1700.00 ±53.03	1595.83* ±59.82	1516.67** ±50.50	1508.33** ±39.33	1491.67** ±37.35	1495.83 ±35.60	1529.17 ±112.84	1466.67 ±36.06
# IV	1158.33 ±52.89	1495.83 ±41.04	1687.50 ±40.88	1737.50 ±36.48	1800.00 ±32.57	1737.50 ±38.99	1658.33 ±48.40	1641.67 42.12	1608.33 ±43.45	1591.67 ±35.80	1600.00 ±38.92	1579.17 ±36.65
CD(0.05) N =12	14.000					133.720	136.489	111.371	115.394	98.080		

control

Table 40.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the body weight of Ducks at 15 days intervals.

Source of variation	df	Days											
		15	30	45	60	75	90	105	120	135	150	165	180
Group	3	13888.889	20468.750	48663.194	55468.750	71579.861	83680.556	157430.556	148246.53	111666.667	49635.417	28194.444	35625.000
Error	44	36250.000	26917.614	23101.326	23489.583	26055.871	26448.864	27556.818	18347.538	19696.970	14228.220	17717.803	14687.500
F value		0.383	0.760	2.107	2.361	2.747	3.164*	5.713**	8.080**	5.669**	3.489*	1.591	2.426

Fig. 17 Mean body weight (g) of Ducks at 15 days intervals, fed on diet added with Mancozeb.

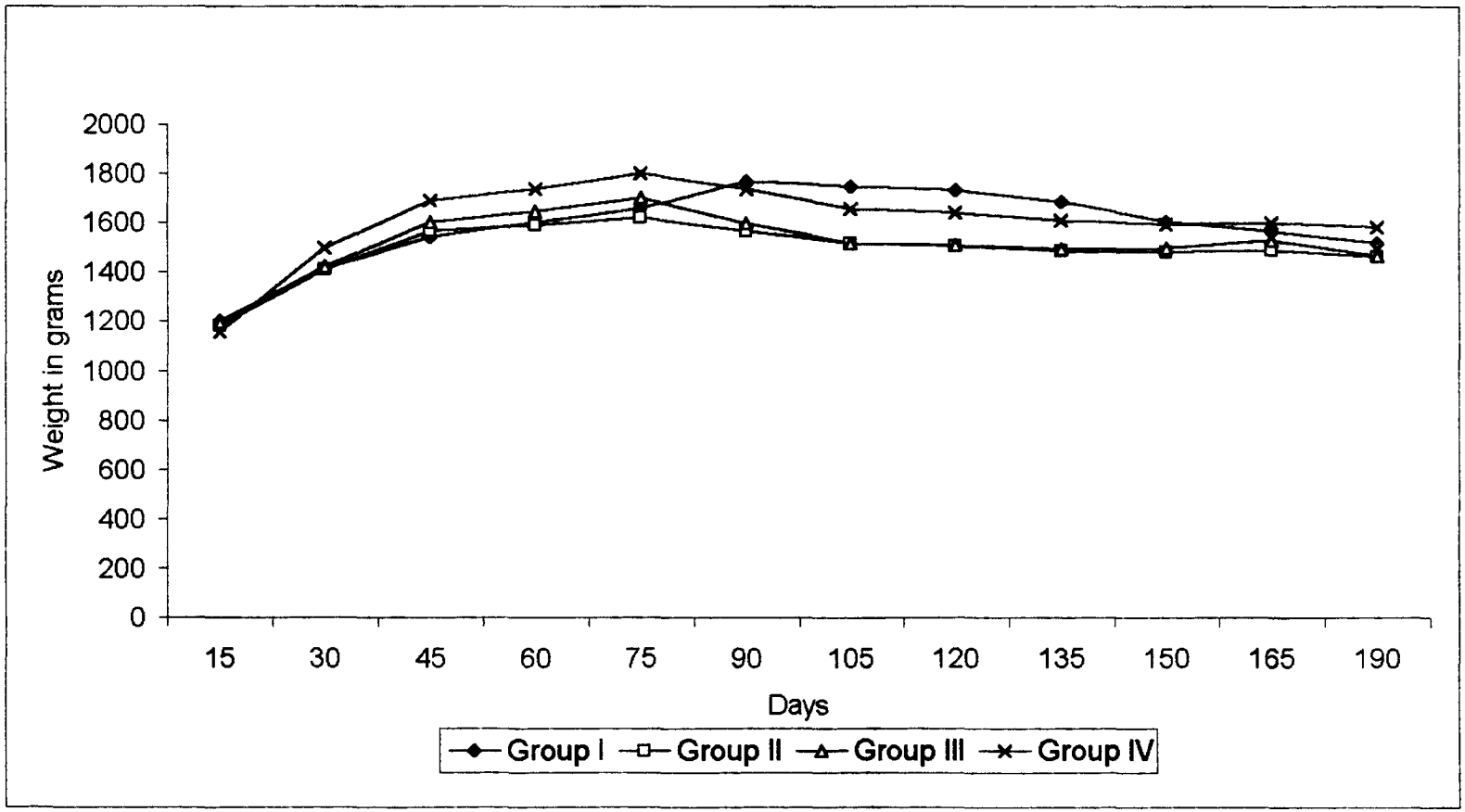


Table 41 Mean Erythrocyte count (x10000 per c m m) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	272.9 ±8.82	278.75 ±7.51	277.75** ±6.94	326.75** ±8.11	303.08* ±9.32	278.83 ±9.28
II	299.50*# ±6.40	301.92 ±9.54	267.42** ±10.43	295.83 ±5.37	280.33 ±5.07	296.42 ±4.86
III	284.00*# ±7.98	309.33* ±7.32	338.00 ±5.68	295.50 ±9.37	297.67 ±4.01	301.83 ±6.50
# IV	258.3 ±6.48	287.83 ±4.35	322.08 ±9.14	284 ±9.01	275.42 ±10.79	304.83 ±8.11
CD(0.05)	21.34	21.118	23.519	23.13	22.296	

N = 12
# control

Table 41.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the Erythrocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	3635.910	2270.139	13926.076	4032.354	2132.028	1623.354
Error	44	673.831	659.670	818.275	791.384	735.345	653.044
F value		5.396**	3.441*	17.019**	5.095**	2.899*	2.486

Table 42 Mean Haemoglobin level (g percentage) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	11.14 ±0.18	10.73 ±0.16	11.09** ±0.15	11.66** ±0.14	12.20** ±0.21	11.89 ±0.14
II	10.79 ±0.22	11.62 ±0.22	11.20** ±0.20	11.45** ±0.12	11.82** ±0.15	11.98 ±0.23
III	10.61 ±0.21	10.39** ±0.16	11.91 ±0.11	11.83** ±0.20	11.88** ±0.27	12.33 ±0.16
# IV	10.53 ±0.15	11.22 ±0.20	11.98 ±0.15	12.34 ±0.20	11.15 ±0.18	11.83 ±0.25
CD(0.05)		0.52	0.488	0.486	0.587	

N = 12
# control

Table 42.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the Haemoglobin level of Ducks at monthly intervals.

Source of variation	df	Month					
		1	2	3	4	5	6
Groups	3	0.848	3.504	2.578	1.74	4.852	0.606
Error	44	0.443	0.4	0.298	0.349	0.511	0.477
F value		1.961	8.764**	8.653**	4.980**	9.494**	1.269

noticed in the second and the fourth month and an increase in the fifth month . The mean value of the group I ranged from 10.73 ± 0.16 to 12.20 ± 0.21, group II from 10.79 ± 0.22 to 11.98 ± 0.23, group III from 10.39 ± 0.16 to 12.33 ± 0.16 and group IV from 10.53 ± 0.15 to 12.34 ± 0.20.

4.3.4 Erythrocyte sedimentation rate.

The mean erythrocyte sedimentation rates of four groups of ducks at monthly intervals are shown in table 43. The mean values were not significantly different from that of the controls. The mean values of group I ranged from 0.10 ± 0 to 0.12 ± 0.01, group II from 0.10 ± 0 to 0.13 ± 0.01, group III from 0.10 ± 0 to 0.13 ± 0.01 and group IV from 0.10 ± 0 to 0.14 ± 0.01.

4.3.5 Packed cell volume.

The mean packed cell volume of four groups of ducks at monthly intervals are shown in table 44. The mean value of group I in the third month is significantly low and in the fifth month is significantly high. In group II, significantly higher value was noticed in the third month and lower value in the fifth month. In group III, in the first month significantly higher value was noticed and in the third month significantly lower value was noticed. The mean value of group I ranged from 36.17 ± 0.51 to 45.92 ± 0.23, group II from 35.00 ± 0.48 to 47.67 ± 0.26, group III from 37.17 ± 0.47 to 45.50 ±0.31 and group IV from 35.00 ± 0.35 to 45.17 ± 0.42.

4.3.6 Leucocyte count .

The mean leucocyte count of four groups of ducks at monthly intervals are shown in table 45. The mean values of group I showed significant increase in the fourth month and significant decrease in the first month, other values showed an increase trend even though not significantly different . In the group II all the values

Table 43 Mean Erythrocyte sedimentation rate (cm / hr) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	0.12 ±0.01	0.11 ±0.01	0.12 ±0.01	0.11 ±0.01	0.11 ±0.01	0.10 ±0.00
II	0.12 ±0.01	0.11 ±0.01	0.11 ±0.01	0.11 ±0.01	0.13 ±0.01	0.10 ±0.00
III	0.11 ±0.01	0.12 ±0.01	0.11 ±0.01	0.10 ±0.00	0.13 ±0.01	0.10 ±0.00
# IV	0.11 ±0.01	0.14 ±0.01	0.11 ±0.01	0.10 ±0.01	0.12 ±0.01	0.10 ±0.00

N = 12
# control

Table 43.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the Erythrocyte sedimentation rate of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.000	0.003	0.000	0.000	0.001	0.000
Error	44	0.001	0.001	0.001	0.001	0.002	0.000
Fvalue		0.237	2.048	0.208	0.333	0.475	0.000

Table 44 Mean packed cell volume (percentage) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	36.17 ±0.51	39.50 ±0.40	41.08** ±0.23	44.17 ±0.21	45.92** ±0.23	44.58 ±0.40
II	35.00 ±0.48	39.50 ±0.66	47.67** ±0.26	44.75 ±0.13	41.83** ±0.32	45.50 ±0.34
III	37.17** ±0.47	40.83 ±0.67	43.33** ±0.36	44.67 ±0.14	44.50 ±0.29	45.50 ±0.31
# IV	35.00 ±0.35	38.75 ±0.48	45.17 ±0.42	44.50 ±0.26	44.00 ±0.58	44.50 ±0.36
CD(0.05)	1.296		0.926		1.091	1.000

N = 12
# control

Table 44.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the packed cell volume of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	13.111	3.021	34.132	0.799	34.410	6.187
Error	44	2.485	3.816	1.271	0.445	1.763	1.498
F value		5.276**	2.364	26.858**	1.794	19.515**	4.13*

Table 45 Mean Leucocyte count (x100 per c m m) of Ducks at monthly intervals, fed on diet added with Mancozeb .

Group	Months					
	1	2	3	4	5	6
I	301.00*# ±22.27	348.67 ±15.16	438.75 ±21.43	438.17** ±10.83	385.92 ±9.83	361.67 ±12.14
II	465.08*# ±26.08	463.17** ±16.90	462.33 ±10.14	431.33** ±11.92	365.50 ±9.90	366.25 ±5.26
III	491.00*# ±7.14	439.67** ±30.46	467.33 ±19.38	400.58** ±20.67	324.42** ±6.84	332.83 ±11.00
# IV	383.7 18.80	333.92 13.18	434.92 11.31	344.00 9.15	361.25 7.69	351.08 7.38
CD(0.05)	56.61	57.269		39.532	24.681	26.671

N = 12
# control

Table 45.1 ANNOVA table (M.S.S), influence of dietary level of Mancozeb on the Leucocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	162594.6	114699.7	3903.389	22100.910	7870.132	25677.47
Error	44	4741.263	4851.498	3195.830	2311.756	901.070	1052.261
F value		34.294**	23.642**	1.221	9.560**	8.734**	24.402**

showed an increase trend but significant increase was noticed only in the first second and fourth months . In group III significant Increase was noticed in the first, second and fourth month and decrease was noticed in the fifth month. The mean value of group I ranged from 301.00 ± 22.27 to 438.75 ± 21.43, group II from 365.50 ± 9.90 to 465.08 ± 26.08,group III from 324.42 ± 6.84 to 439.67 ± 30.46 and group IV from 333.92 ± 13.18 to 434.92 ± 11.31.

4.3.7 Differential counts

4.3.7.1 Lymphocytes.

The mean lymphocyte counts of four groups of ducks at monthly intervals are shown in table 46 and Fig.18. The mean value of group I did not differ significantly from group IV except in the third month. Group III also showed significantly lower value in the third month. All other values were not significantly different from that of controls . The mean values of group one ranged from 66.75 ± 1.21 to 75.42 ± 1.23 group II from 68.67 ± 1.00 to 73.58 ± 2.24, group III from 68.00 ± 1.68 to 74.83 ± 1.51 and group IV from 67.67 ± 1.33 to 74.83 ± 0.64.

4.3.7.2 Heterophils.

The mean heterophil counts of four groups of ducks at monthly intervals are shown in table 47 and Fig.18. None of the value was significantly different from that of the controls except the value of the group I in the first month, which is significantly higher . All the values in the fourth, the fifth and the sixth month showed a decreasing trend than control values. The mean values of group I ranged from 15.17 ± 0.92 to 23.83 ± 1.08, group II from 15.92 ± 0.84 to 21.00 ± 0.44, group III from 16.17 ± 1.70 to 22.08 ± 0.60 and group IV from 19.00 ± 0.72 to 22.08 ± 0.65.

Table 46 Mean Lymphocyte count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	66.75 ±1.21	71.67 ±1.56	68.17** ±1.25	70.33 ±1.00	75.42 ±1.23	74.58 ±1.04
II	68.67 ±1.00	69.08 ±0.85	71.75 ±0.94	69.33 ±1.05	72.83 ±1.59	73.58 ±2.24
III	68.00 ±1.68	71.58 ±1.36	68.33** ±0.72	71.83 ±0.66	74.83 ±1.51	72.00 ±0.98
# IV	71.83 ±0.75	67.67 ±1.33	71.58 ±0.73	68.58 ±1.15	74.83 ±0.64	69.92 ±1.77

CD(0.05,transformed values). 1.676
N = 12
# control

Table 46.1 ANOVA table (M.S.S, transformed values), influence of dietary level of Mancozeb on the Lymphocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	21.421	18.644	18.252	9.131	15.016	20.423
Error	44	7.63	7.99	4.159	4.537	8.446	12.178
F value		2.808	2.333	4.388**	2.013	1.778	1.677

Table 47 Mean Heterophil count (percent of total Leucocyte) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	23.83* ±1.08	19.33 ±1.33	21.75 ±0.99	18.92 ±0.54	15.17 ±0.92	18.67 ±1.15
II	21.00 ±0.44	19.75 ±0.66	18.67 ±0.54	20.08 ±0.70	15.92 ±0.84	18.50 ±1.18
III	21.75 ±1.43	19.50 ±1.25	22.08** ±0.60	19.33 ±0.63	16.17 ±1.70	18.67 ±0.88
# IV	19.58 ±0.81	22.08 ±0.65	19.75 ±0.57	21.42 ±1.12	19.00 ±0.72	20.50 ±0.04
CD(0.05, transformed values)	1.950		1.410			

control

Table 47.1 ANOVA table (M.S.S,transformed values), influence of dietary level of Mancozeb on the Heterophil count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	17.913	10.905	15.948	6.995	20.859	7.101
Error	44	5.626	6.494	2.941	3.584	8.281	7.105
F value		3.184*	1.679	5.422**	1.952	2.519	1.281

4.3.7.3 Eosinophils.

The mean eosinophil counts of four groups of ducks at monthly intervals are shown in table 48 and Fig.18. None of the value was significantly different from controls. The mean value of group I ranged from 3.33 ± 0.28 to 5.83 ± 0.41, group II from 5.33 ± 0.36 to 7.58 ± 1.14, group III from 4.92 ± 0.36 to 6.58 ± 0.58 and group IV from 4.92 ± 0.54 to 6.33 ± 0.61

4.3.7.4 Monocyte.

The mean monocyte counts of four groups of ducks at monthly intervals are shown in table 49 and Fig.18. No significant difference could be noticed between treated and control group. The mean values of group I ranged from 2.75 ± 0.25 to 4.58 ± 0.51, group II from 1.83 ± 0.21 to 3.75 ± 0.55, group III from 2.92 ± 0.23 to 3.75 ± 0.35 and group IV from 2.92 ± 0.23 to 3.83 ± 0.32.

4.3.7.5 Basophil.

The mean basophil counts of four groups of ducks at monthly intervals are shown in table 50 and Fig.18. No significant difference is noticed between the treated and the controls. The mean value of group I ranged from 0.56 ± 0.11 to 1.17 ± 0.23, group II from 0.63 ± 0.11 to 0.92 ± 0.18 , group III from 0.56 ± 0.11 to 1.17 ± 0.26 and group IV from 0.63 ± 0.11 to 0.92 ± 0.18.

4.3.8 Enzymes

4.3.8.1 Aspartate Amino Transferase(AST) .

The mean serum aspartate amino transferase level of four groups of ducks at monthly intervals are shown in table 51 and Fig.19. The mean values of all the groups showed a decreasing tendency than that of the controls from the third month onwards. Only significant reduction was noticed in the fourth month (all groups) and in the

Table 48 Mean Eosinophil count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	5.83 ±0.41	5.42 ±0.42	5.58 ±0.43	5.08 ±0.40	5.25 ±0.65	3.33 ±0.28
II	6.58 ±0.87	6.83 ±0.79	5.33 ±0.36	6.03 ±0.74	7.58 ±1.14	5.42 ±1.27
III	6.58 ±0.58	5.25 ±0.35	5.92 ±0.53	5.25 ±0.35	4.92 ±0.36	6.00 ±0.28
# IV	5.00 ±0.37	6.33 ±0.61	5.25 ±0.37	5.67 ±0.43	5.42 ±0.29	4.92 ±0.54
CD(0.05,transformed values)						2.42

N = 12
# control

Table 48.1 ANOVA table (M.S.S, transformed values), influence of dietary level of Mancozeb on the Eosinophil count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	8.785	8.648	1.454	2.932	20.129	28.437
Error	44	5.408	5.017	3.297	4.342	7.708	8.673
F value		1.624	1.724	0.441	0.675	2.611	3.279*

Table 49 Mean Monocyte count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	3.17 ±0.44	3.00 ±0.37	3.75 ±0.49	4.58 ±0.51	3.08 ±0.40	2.75 ±0.25
II	2.92 ±0.42	3.42 ±0.36	3.75 ±0.55	3.75 ±0.28	3.00 ±0.25	1.83 ±0.21
III	3.17 ±0.27	3.25 ±0.33	2.92 ±0.23	3.00 ±0.37	3.00 ±0.37	3.75 ±0.35
# IV	3.08 ±0.34	3.42 ±0.34	2.92 ±0.23	3.83 ±0.32	3.17 ±0.37	3.33 ±0.54
CD(0.05,transformed values)				0.674		1.796

N = 12
# control

Table 49.1 ANOVA table (M.S.S, transformed values), influence of dietary level of Mancozeb on the Monocyte count of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.551	1.340	5.082	11.815	0.173	24.514
Error	44	4.527	3.705	4.632	4.147	4.349	4.772
F value		0.122	0.362	1.097	2.849*	0.040	5.137**

Table 50 Mean Basophil count (percent of total leucocyte) of Ducks at monthly intervals, fed on diet added with Mancozeb.

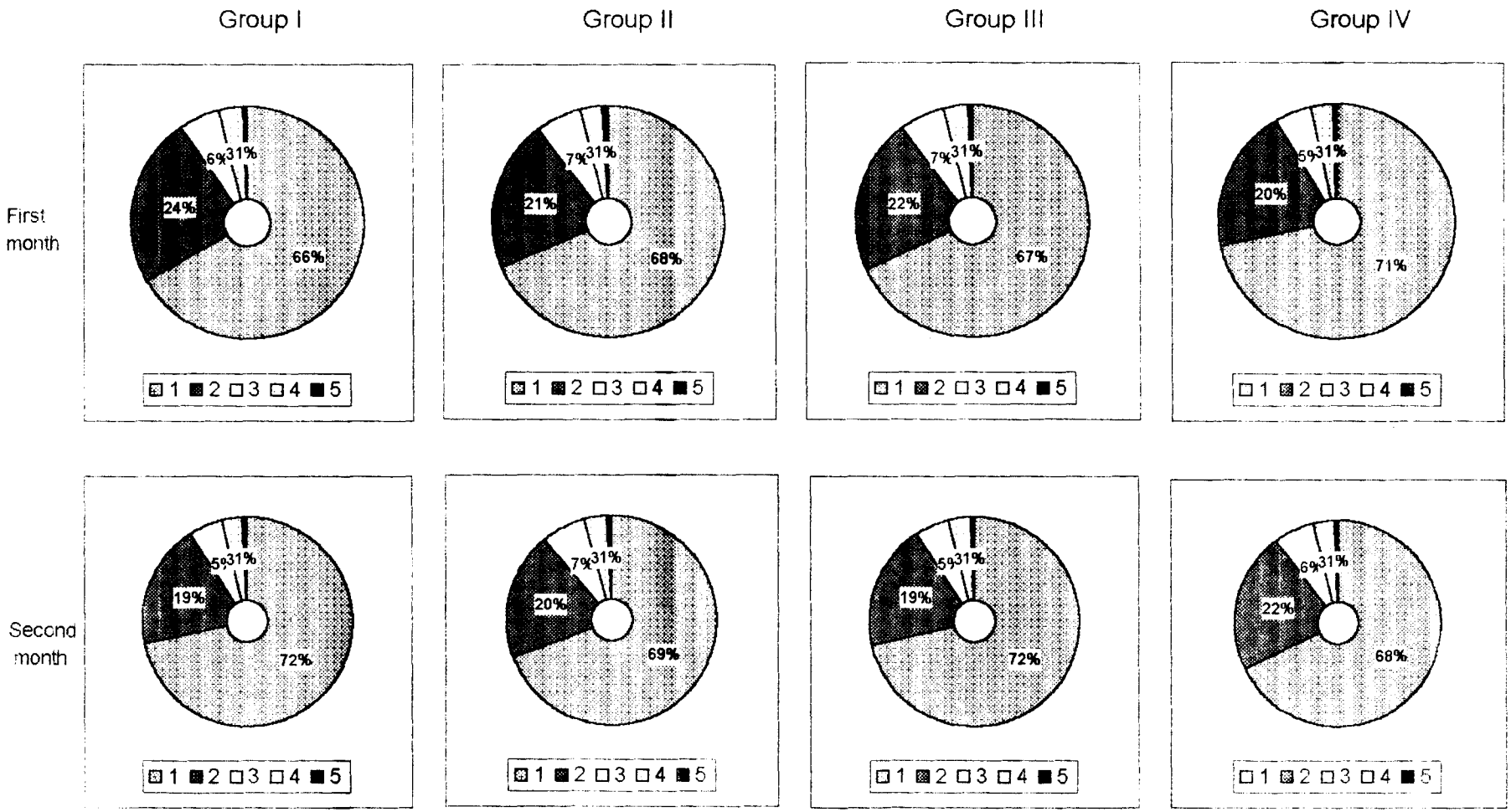
Group	Months					
	1	2	3	4	5	6
I	0.56 ±0.11	0.75 ±0.11	0.83 ±0.15	1.17 ±0.23	0.85 ±0.19	0.77 ±0.16
II	0.92 ±0.18	0.75 ±0.11	0.63 ±0.11	0.85 ±0.19	0.79 ±0.19	0.77 ±0.15
III	0.63 ±0.11	0.56 ±0.11	0.83 ±0.15	0.73 ±0.20	1.17 ±0.26	0.67 ±0.20
# IV	0.63 ±0.11	0.63 ±0.11	0.79 ±0.19	0.75 ±0.10	0.75 ±0.23	0.92 ±0.18

N = 12
# control

Table 50.1 ANOVA table (M.S.S, transformed values), influence of dietary level of Mancozeb on the Basophil count of Ducks at monthly intervals.

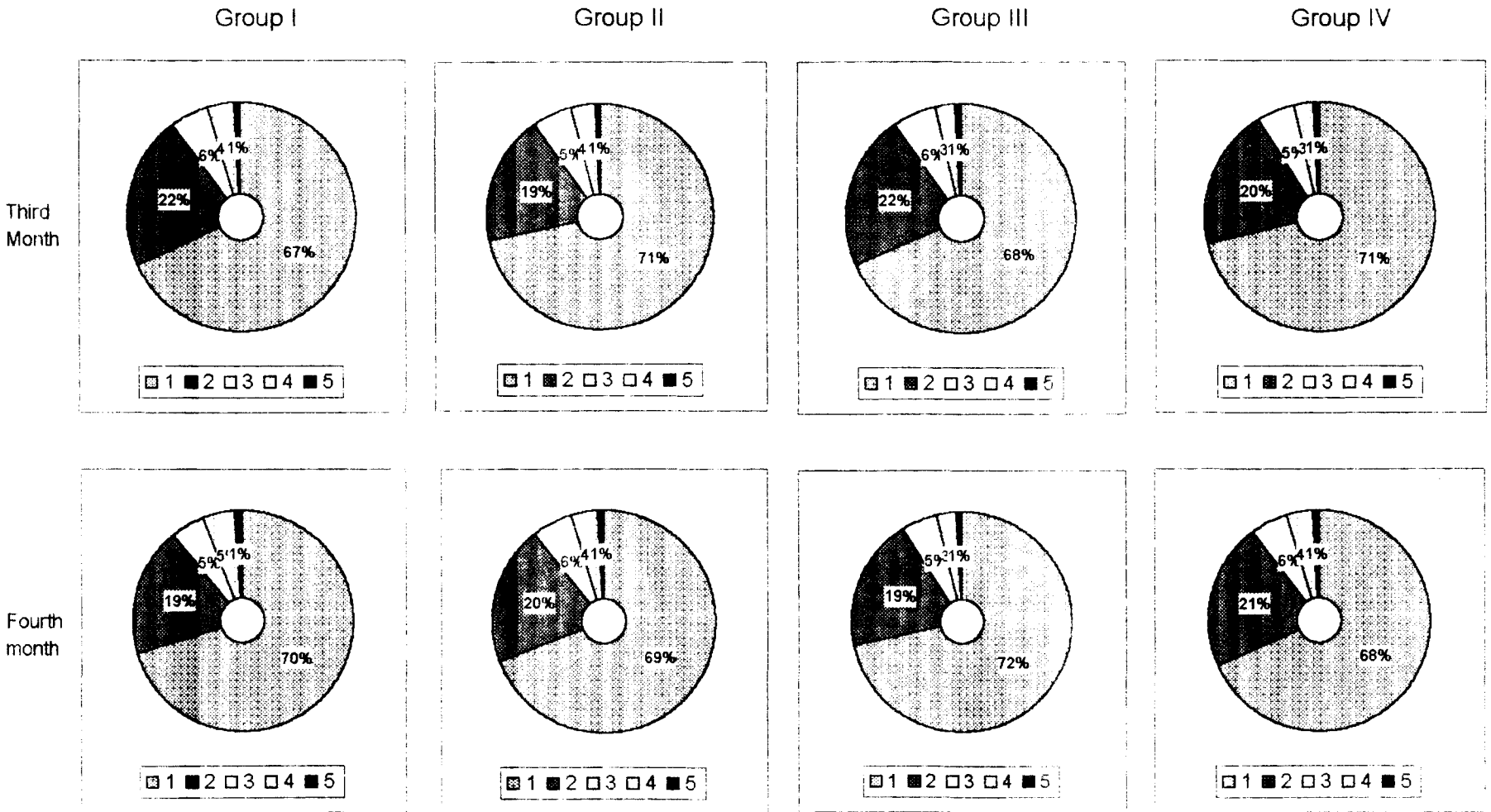
Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	2.904	1.549	1.236	3.786	3.816	1.843
Error	44	2.601	2.113	3.105	4.056	5.086	3.655
F value		1.117	0.733	0.398	0.933	0.750	0.504

Fig.18 Mean differential count (percent) of Ducks at monthly intervals, fed on diet added with mancozeb.



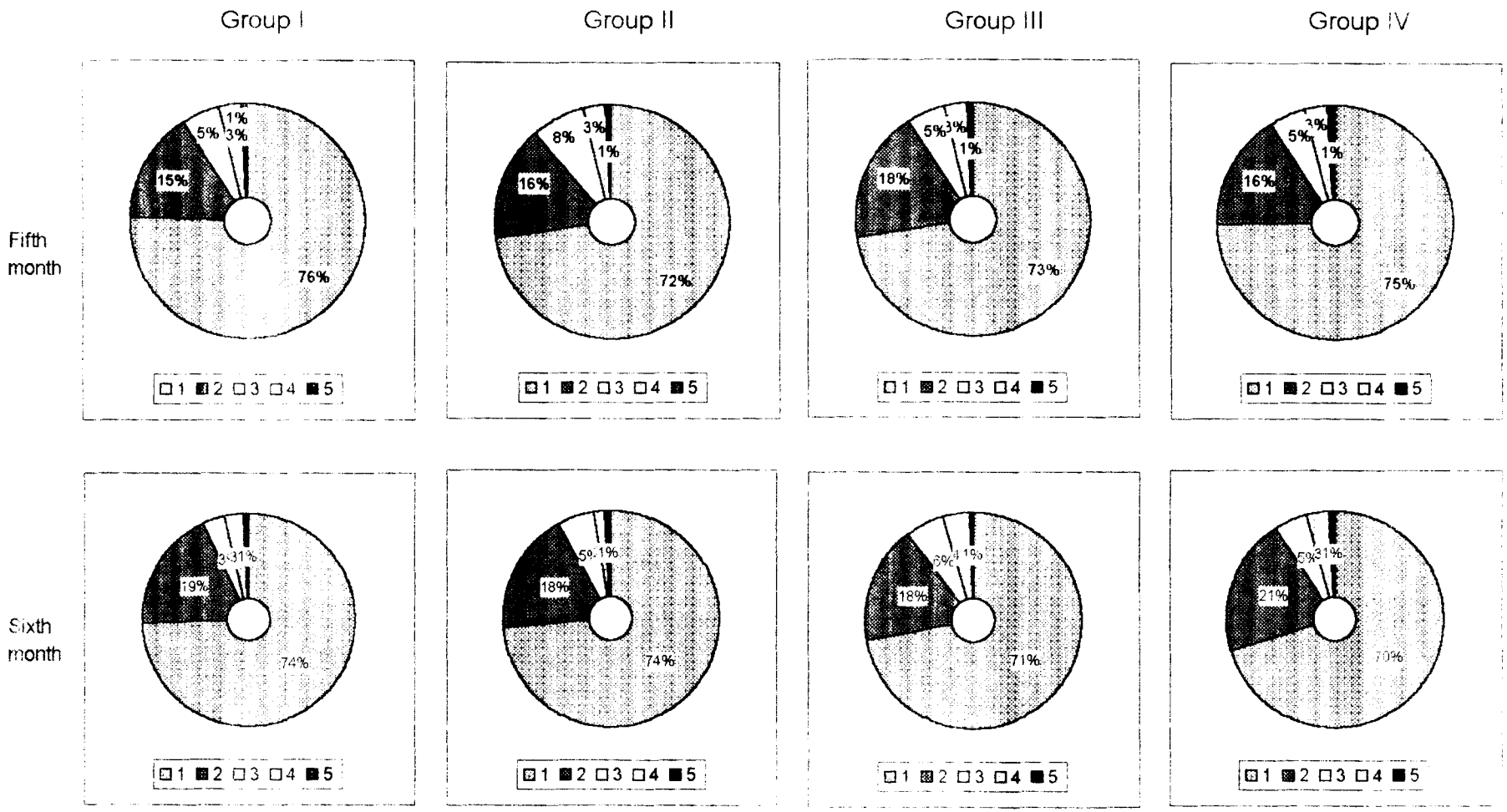
1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils.

Fig.18 Mean differential count (percent) of Ducks at monthly intervals, fed on diet added with mancozeb.



1. Lymphocytes, 2 Heterophils. 3 Eosinophils, 4 Monocytes, 5 Basophils.

Fig 18 Mean differential count (percent) of ducks at monthly intervals, fed on diet added with mancozeb.



1 Lymphocytes, 2 Heterophils, 3 Eosinophils, 4 Monocytes, 5 Basophils

Table 51 Mean serum Aspartate Amino Transferase level (I.units per ml) of Ducks at monthly intervals, fed on diet added with Mancozeb .

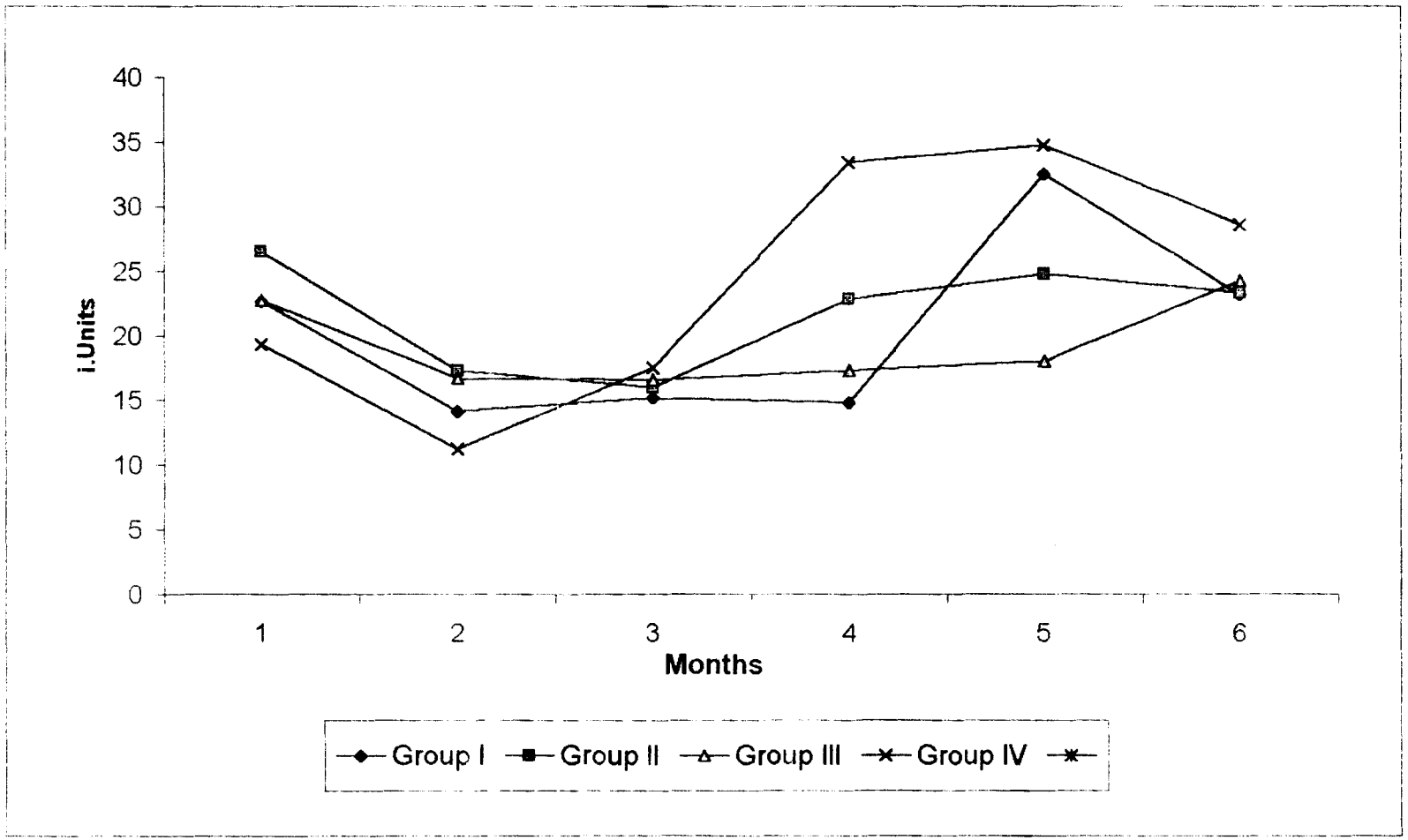
Group	Months					
	1	2	3	4	5	6
I	22.67 ±0.87	14.17** ±0.85	15.17 ±2.68	14.83** ±1.31	32.50 ±2.29	23.17 ±1.09
II	26.50* ±2.63	17.25** ±0.60	16.08 ±1.05	22.83** ±2.41	24.75** ±1.49	23.33 ±2.01
III	22.75 ±1.17	16.67** ±0.64	16.58 ±3.51	17.33** ±1.29	18.00** ±1.87	24.25 ±2.16
# IV	19.33 ±1.28	11.25 ±0.31	17.50 ±2.69	33.42 ±1.80	34.75 ±3.84	28.58 ±1.13
CD(0.05)	4.655	1.799		5.017	7.223	

N = 12
# control

Table 51.1 ANOVA table (M.S.S),influence of dietary level of Mancozeb on the serum Aspartate Amino Transferase levelof Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	102.910	243.278	203.056	816.521	701.500	77.722
Error	44	32.059	4.792	83.511	37.384	77.170	33.534
F value		3.210*	50.771**	2.431	21.841**	9.090**	2.318

Fig. 19 Mean serum Aspartate Amino Transferase level of Ducks at monthly intervals, fed on diet added with Mancozeb.



fifth month (group II and III). The mean values of all the groups were higher than the control values in the first and the second month. The mean values of group I ranged from 14.17 ± 0.85 to 32.50 ± 2.29, group II from 16.08 ± 1.05 to 26.50 ± 2.63, group III from 16.58 ± 3.51 to 24.25 ± 2.16 and group IV from 11.25 ± 0.31 to 34.75 ± 3.84.

4.3.8.2 Alanine Amino Transferase(ALT).

The mean serum alanine amino transferase level of four groups of ducks at monthly intervals are shown in table 52 and Fig.20. The mean values of all the groups in the fifth and the sixth month showed a significantly lesser value than the controls. All other values were higher than the values of the controls. Significant difference was noticed only in the third and the fourth month of group I ,first to fourth month of group II and first to third month of group III . The mean values of group I ranged from 7.83 ± 0.89 to 20.67 ± 1.53, group II from 7.00± o.51 to 22.17 ± 1.84, group III from 6.58 ± 0.57 to 27.42 ± 3.00 and group IV from 3.37 ± 0.31 to 8.52 ± 1.43.

4.3.8.3 Serum alkaline phosphatase.

The mean serum alkaline phosphatase level of four groups of ducks at monthly intervals are shown in table 53 and Fig. 21.The mean values of group I showed significantly lower value in the fourth and the sixth month and significantly higher value in the second, third and fifth months. Group II showed significantly higher value in the second month and lower value in the fourth and sixth month. Group III showed significantly higher value in the second month and lower value in the first and sixth month. The mean value of group one ranged from 0.75 ± 0.44 to 45.46 ± 1.93, group II from 0.77 ± 0.08 to 46.48 ± 1.05 ,group III from o.46 ± 0.05 to 36.83 ± 2.53 and group IV from 0.87 ± 0.06 to 42.88 ± 2.09.

Table 52 Mean serum Alanine Amino Transferase level (I.units per ml) of Ducks at monthly intervals, fed on diet added with Mancozeb .

Group	Months					
	1	2	3	4	5	6
I	15.33 ±1.13	12.00 ±0.88	7.83** ±0.89	20.67** ±1.53	15.00** ±1.91	9.17** ±0.68
II	18.42* ±1.37	22.17** ±1.84	8.03** ±0.69	21.58** ±1.53	13.17 ±1.70	7.00** ±0.51
III	16.75* ±1.81	27.42** ±3.00	7.25** ±0.64	14.17 ±1.70	9.75 ±1.14	6.58** ±0.57
# IV	12.25 ±1.4	6.92 ±1.01	3.42 ±0.31	10.58 ±1.39	8.52 ±1.43	3.37 ±0.87
CD(0.05)	4.13	5.37	2.073	4.896	6.305	1.915

N = 12
# control

Table 52.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the serum Alanine Amino Transferase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	82.076	6217.250	57.076	333.611	15837.63	2069.472
Error	44	25.229	42.670	5.358	35.458	58.809	5.428
F value		3.253*	145.704**	10.653**	9.409**	269.308**	381.257**

Fig. 20 Mean serum Alanine Amino Transferase level of Ducks at monthly intervals, fed on diet added with Mancozeb.

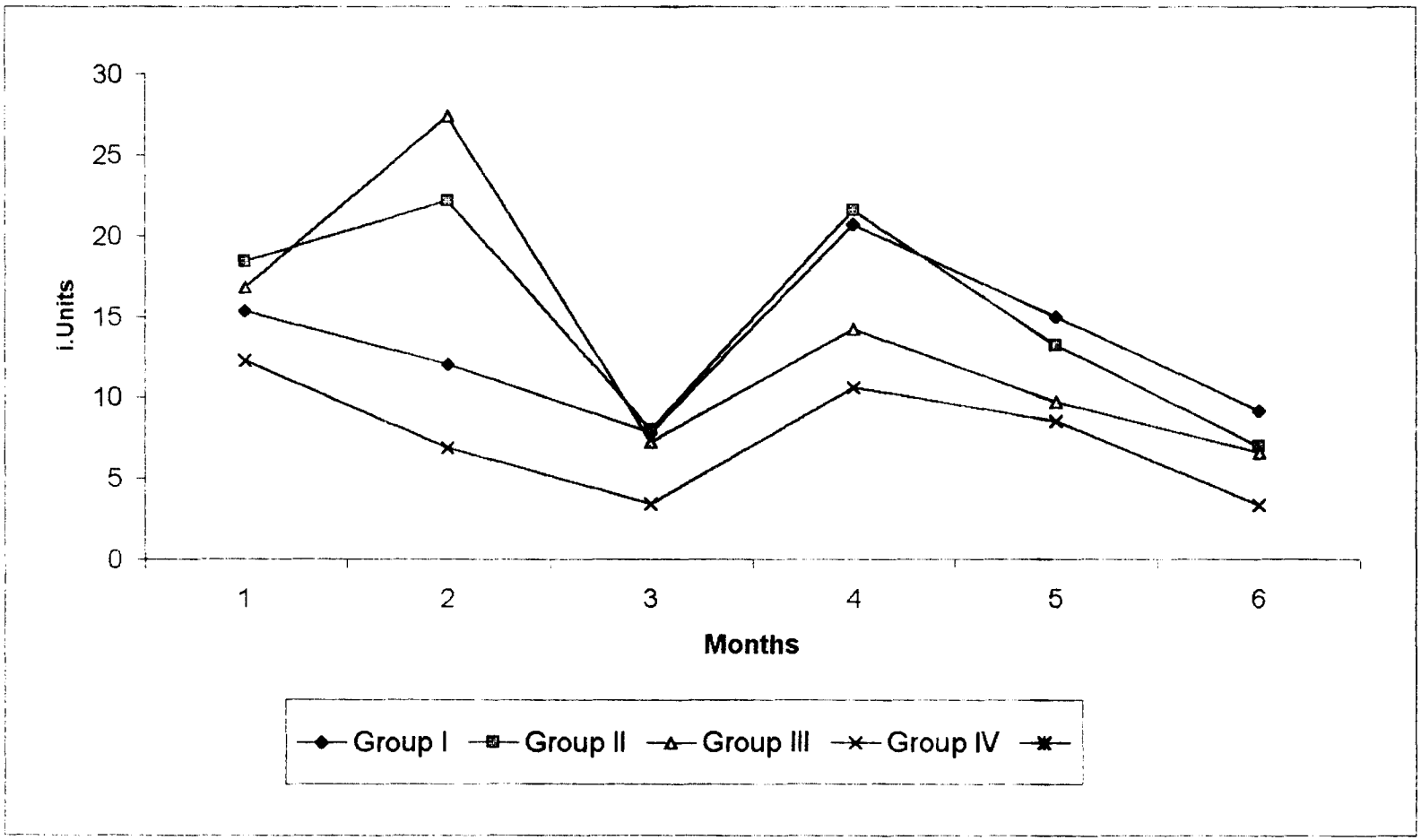


Table 53 Mean serum alkaline phosphatase level (KA units per ml),of Ducks at monthly intervals, fed on diet added with Mancozeb.

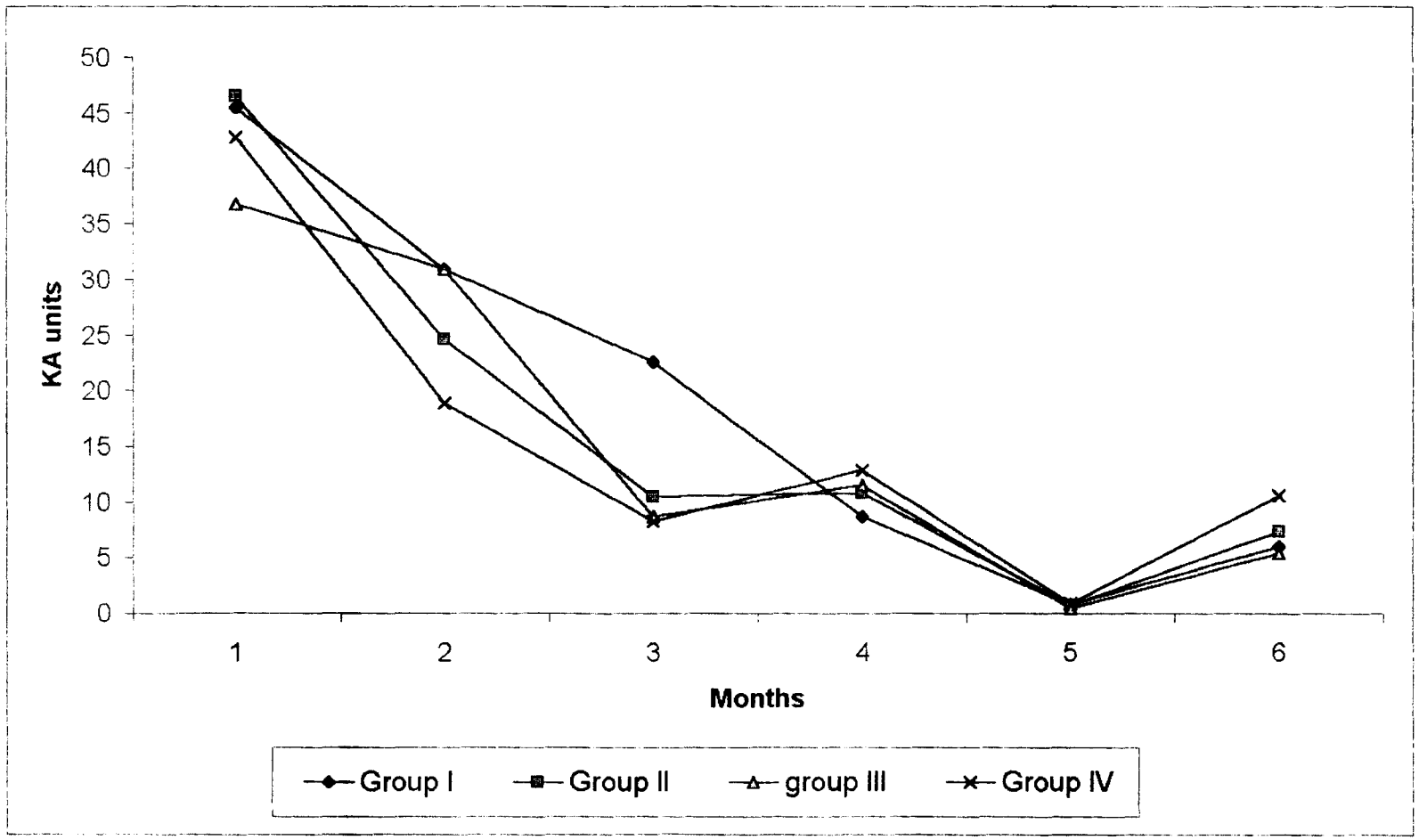
Group	Months					
	1	2	3	4	5	6
I	45.46 ±1.93	30.90** ±1.51	22.59** ±2.12	8.66** ±0.84	0.75 ±0.04	6.04** ±0.37
II	46.48 ±1.05	24.60** ±0.99	10.49 ±0.88	10.79** ±0.70	0.77 ±0.08	7.26** ±0.45
III	36.83** ±2.53	30.89** ±2.91	8.66 ±0.67	11.50 ±0.57	0.46 ±0.05	5.40** ±0.29
# IV	42.88 ±2.09	18.92 ±0.89	8.30 ±0.35	12.87 ±0.46	0.87 ±0.06	10.56 ±1.16
CD(0.05)	5.622	5.036	3.462	1.916	0.648	1.897

N = 12
# control

Table 53.1 ANOVA table (M.S.S),influence of dietary level of Mancozeb on the serum alkaline phosphatase level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	225.175	398.431	552.202	36.904	125.131	63.186
Error	44	46.766	37.516	17.732	5.433	0.622	5.328
F value		4.825**	10.620**	31.142**	6.793**	201.044**	11.860**

Fig. 21 Mean serum Alkaline Phosphatase level of Ducks at monthly intervals, fed on diet added with Mancozeb.



4.3.9 Phytohaemagglutinin-p (PHA-P) skin sensitivity test.

The mean wing web thickness of ducks subjected to PHA-P skin sensitivity test at 1½ months intervals are shown in table 54. Only very few values are significantly different from controls. The mean values of group I ranged from 0.32 ± 0.01 to 1.5 ± 0.08, group II from 0.33 ± 0.01 to 1.3 ± 0.04, group III from 0.32 ± 0.01 to 1.24 ± 0.04 and group IV from o.33 ± 0.01 to 1.43 ± 0.08.

4.3.10 Antibody titre.

The mean antibody titre of four groups of ducks at 15 days intervals are shown in table 55 and Fig.22. In the group I and II the mean values were not significantly different from that of the controls .In group III significantly higher value was noticed only on 45th, 60th, and 75th, day. Other values were not significantly different. The mean value of group I ranged from 69.333 ± 12.844 to 4266.667 ± 1036.464, group II from 96.000 ± 14.311 to 3754.667 ± 1415.608, group III from 80.00 ± 16.000 to 4736.000 ± 2623.844 and group IV from 61.333 ± 15.686 to 3328.00 ± 1150.577.

4.3.11 Zinc content in the serum.

The mean zinc levels in the serum of four groups of ducks at monthly intervals are shown in table 56 and Fig.23. The mean value of the group I and II showed no significant difference except in the fourth month in which significantly lower value was noticed. In the group III significantly higher value was noticed in 2nd, 3rd, 5th, and 6th month . In fifth and sixth month all the values are higher than the that of the controls. The mean value of the group I ranged from 2.80 ± 0.20 to 7.59 ± 0.84, group II from 2.89 ± 0.31 to 9.39 ± 0.93, group III from 4.10 ± 0.66 to 12.69 ± 1.03 and group IV from 2.59 ± 6.18 to 7.00 ± 0.52.

Table 54 Mean wing web thickness of Ducks, in PHA-P skin sensitivity test at one and a half months intervals, fed on diet added with Mancozeb.

Group	One and a half month				Three month				Four and a half month				Six month			
	0 hr	24 hr	48 hr	72 hr	0 hr	24hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
I	0.35 ±0.01	1.50 ±0.08	0.88 ±0.04	0.41 ±0.02	0.32 ±0.01	1.05* ±0.03	0.62 ±0.06	0.30 ±0.01	0.33 ±0.01	0.93* ±0.05	0.53 ±0.03	0.34 ±0.01	0.33 ±0.01	1.02 ±0.04	0.74* ±0.03	0.37 ±0.02
II	0.36 ±0.01	1.31 ±0.04	0.81 ±0.04	0.43 ±0.02	0.33 ±0.01	0.85 ±0.03	0.70 ±0.04	0.38 ±0.02	0.33 ±0.01	0.75 ±0.05	0.72* ±0.03	0.35 ±0.02	0.33 ±0.01	0.93 ±0.03	0.64 ±0.02	0.39 ±0.02
III	0.35 ±0.01	1.24* ±0.04	0.68 ±0.04	0.37 ±0.02	0.33 ±0.01	0.96 ±0.05	0.63 ±0.04	0.35 ±0.01	0.32 ±0.01	0.78 ±0.05	0.63* ±0.04	0.35 ±0.02	0.33 ±0.01	0.94 ±0.03	0.66 ±0.03	0.33 ±0.01
# IV	0.36 ±0.01	1.43 ±0.08	0.76 ±0.06	0.40 ±0.02	0.33 ±0.01	0.90 ±0.03	0.65 ±0.03	0.38 ±0.02	0.33 ±0.01	0.72 ±0.05	0.53 ±0.03	0.36 ±0.02	0.33 ±0.01	0.95 ±0.03	0.59 ±0.03	0.36 ±0.02
CD(0.05)		0.176	0.140			0.100				0.138	0.093				0.082	

N = 12
# control

Table 54.1 ANOVA table (MSS), influence of dietary level of mancozeb on PHA-P sensitivity test on Ducks at 45 days intervals.

Source of variation	df	One and a half month				Three month				Four and a half month				Six month			
		0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
Group	3	0.000	0.165	0.085	0.009	0.001	0.089	0.016	0.002	0.001	0.110	0.099	0.001	0.000	0.019	0.047	0.01
Error	44	0.003	0.046	0.029	0.005	0.002	0.015	0.022	0.004	0.002	0.028	0.013	0.004	0.002	0.019	0.010	0.01
F value		0.103	3.579*	2.970*	1.799	0.363	5.822**	0.703	0.667	0.363	3.925*	7.359**	0.137	0.124	1.430	4.496**	1.44

Table 55 Mean antibody titre in Duck serum at 15 days intervals,fed on diet added with Mancozeb.

Group	Days										
	15	30	45	60	75	90	105	120	135	150	165
I	554.667 ±102.755	256.000 ±57.245	149.333 ±35.697	106.667 ±13.492	80.000 ±13.492	69.333 ±12.844	1194.667 ±285.580	2133.333 ±653.232	4266.667 ±1036.464	3072.000 ±1121.736	2048.000 ±457.947
II	469.333 ±42.667	448.000 ±131.161	341.333 ±138.913	192.000 ±28.622	149.333 ±35.697	96.000 ±14.311	1877.333 ±707.804	3754.667 ±1415.608	2901.333 ±1165.042	2645.333 ±1140.406	2030.667 ±1223.574
III	1024.000 ±228.973	853.333* ±277.825	384.000* ±57.243	234.667** ±21.333	117.333 ±10.667	80.000 ±16.000	2218.667 ±411.020	4736.000 ±2623.844	2133.333 ±653.232	981.333 ±339.730	768.000 ±280.434
# IV	725.333 ±291.261	277.333 ±51.377	192.000 ±28.622	128.000 ±28.622	66.667 ±14.557	61.333 ±15.686	3328.000 ±1150.577	1536.000 ±560.868	1664.000 ±575.288	981.333 ±250.973	896.000 ±262.322
CD(0.05)(log value) N = 6		0.341	0.293	0.205							

control

Fig. 22 Mean antibody titre in Duck serum at 15 days intervals, fed on diet added with Mancozeb.

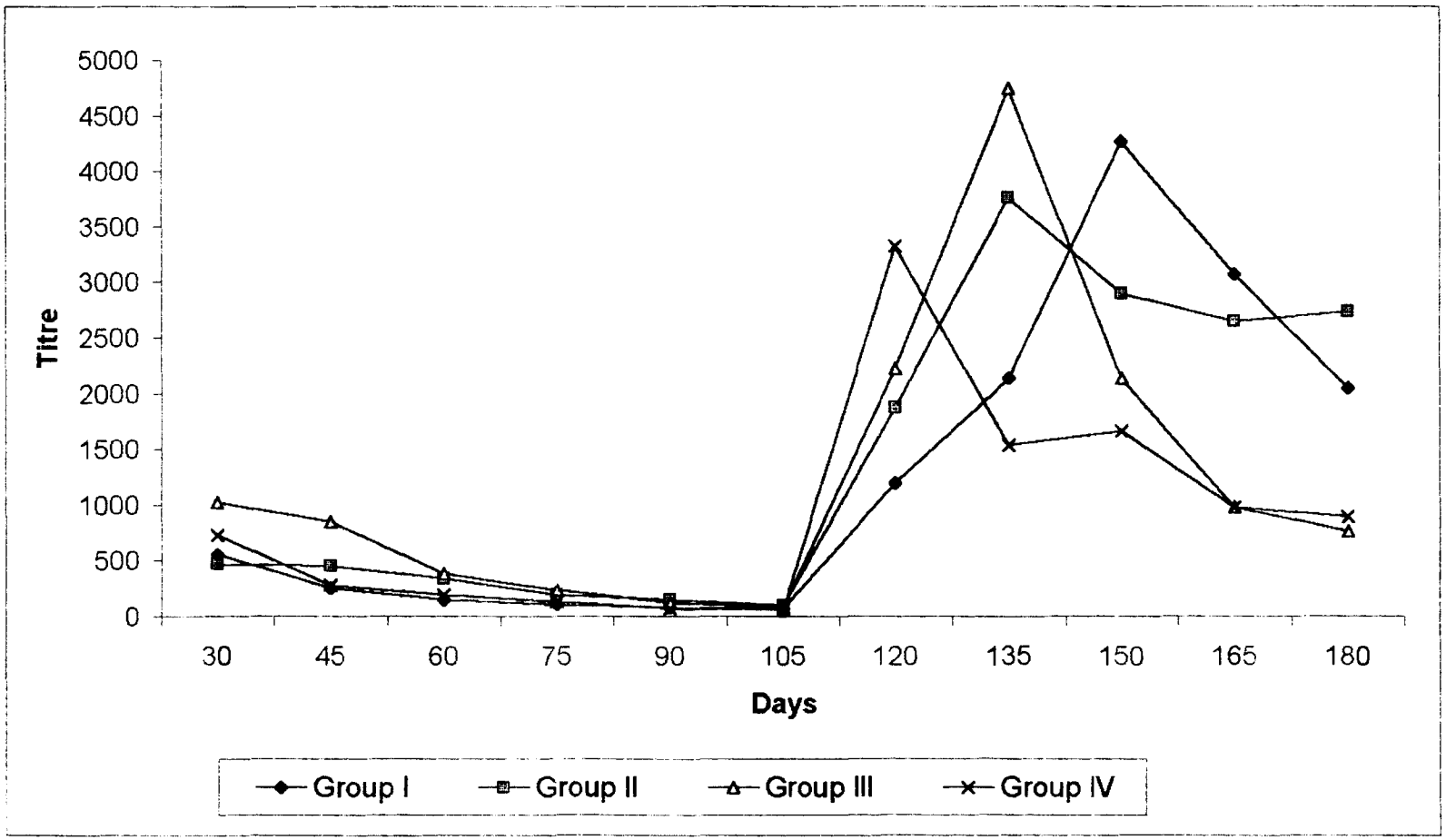


Table 55.1 ANOVA table(M.S.S), influence of dietary level of mancozeb on the antibody titre of Ducks at 15 days intervals.

Source of variation	df	Days										
		15	30	45	60	75	90	105	120	135	150	165
Group	3	0.111	0.247	0.227	0.165	0.165	0.065	0.165	0.126	0.215	0.376	0.376
Error	20	0.062	0.080	0.059	0.029	0.057	0.053	0.119	0.228	0.152	0.129	0.129
F value		1.789	3.082*	3.846*	5.598**	2.911	1.238	1.391	0.552	1.418	2.914	2.914

Table 56 Mean serum zinc level (ppm) of Ducks at monthly intervals, fed on diet added with Mancozeb.

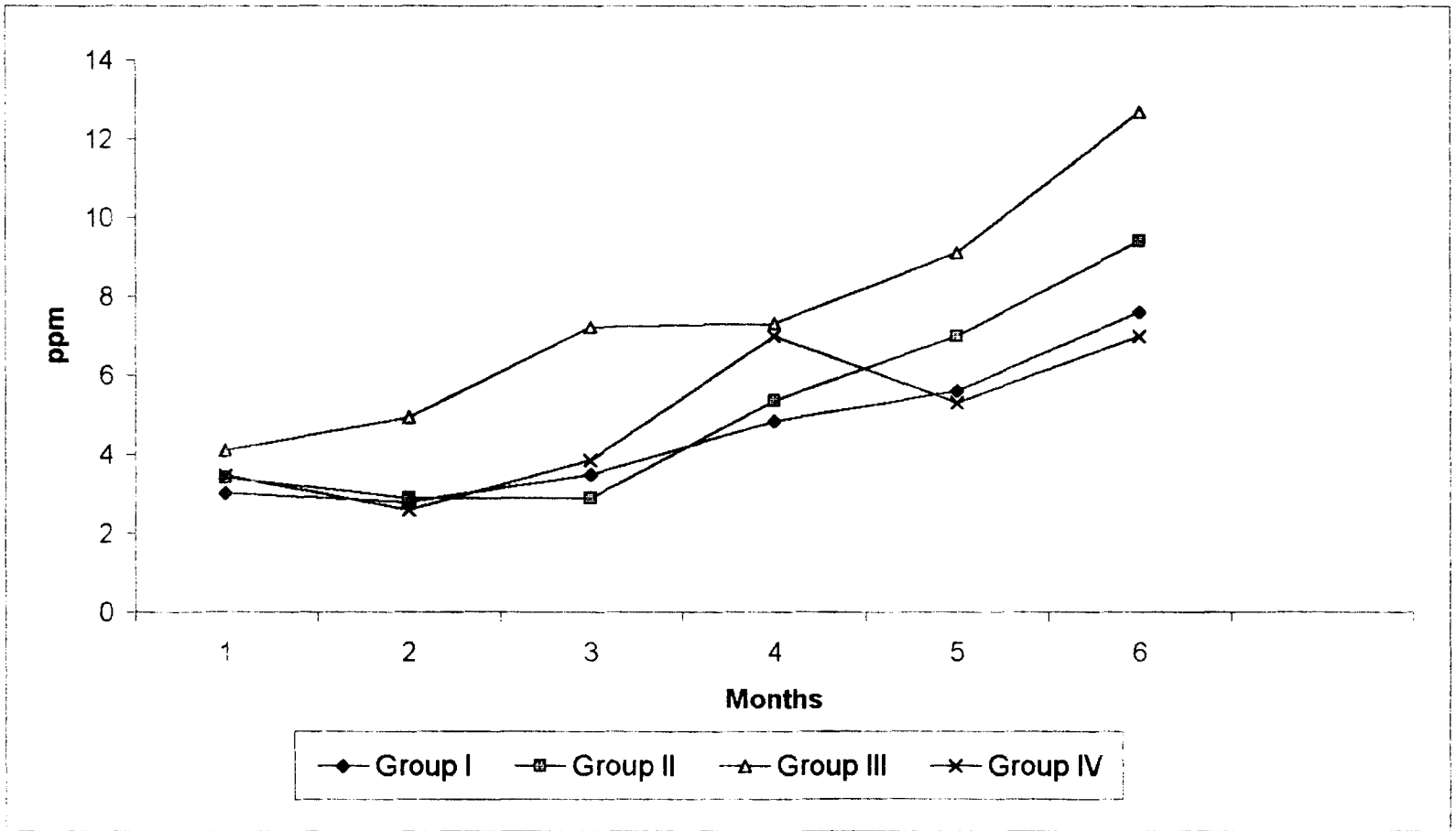
Group	Months					
	1	2	3	4	5	6
I	3.02 ±0.22	2.80 ±0.20	3.48 ±0.35	5.85** ±0.47	5.56 ±0.27	7.59 ±0.84
II	3.43 ±0.23	2.89 ±0.31	3.92 ±0.22	5.86** ±0.50	7.00 ±0.80	9.39 ±0.93
III	4.10 ±0.66	4.95** ±0.38	7.19** ±0.54	7.32** ±0.49	9.10** ±0.89	12.69** ±1.03
# IV	3.46 ±0.40	2.59 ±6.18	3.86 ±0.44	4.04 ±0.46	5.33 ±0.36	7.00 ±0.52
CD(0.05)		0.555	1.194	1.483	1.883	2.510

N = 6
# control

Table 56.1 ANOVA table (M.S.S) influence of dietary level of Mancozeb on the serum zinc levels of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	1.193	7.293	22.167	8.986	18.103	39.23
Error	20	1.048	0.456	0.983	1.516	2.445	4.346
F value		1.138	16.018**	22.556**	9.928**	7.403**	9.028**

Fig. 23 Mean serum zinc level of Ducks at monthly intervals, fed on diet added with Mancozeb.



4.3.12 Zinc content in the tissues.

The mean zinc levels in the liver, kidney, and muscle of four groups of ducks sacrificed after six month of feeding with mancozeb added diet are shown in table 58 and Fig.24..None of the value was significantly different from that of the controls. The group I showed more zinc in the liver tissue but group II and III more zinc in muscle.

4.3.13 Manganese content in the serum.

The mean manganese levels in the serum of four groups of ducks at monthly intervals are shown in table 57and Fig.25. The mean value of the group I showed no significant difference. In group II the values are significantly higher in 3rd, and 4th, month. Other values were not significantly different from that of the controls The mean value of group I ranged from 0.050 ± 0.01 to 0.160 ± 0.02 , group II from 0.120 ± 0.04 to 0.260 ± 0.03 ,group III from 0.130 ± 0.01 to 0.340 ± 0.03 and group IV from 0.090 ± 0.02 to 0.160 ±0.01.

4.3.14 Manganese content in the tissues.

The mean manganese levels in the liver, kidney and muscle of four groups of ducks sacrificed after six months of feeding with mancozeb added diet are shown in table 59 and Fig.26. None of the value was significantly different from that of the controls. In all the groups liver tissue showed more manganese than kidney or muscle.

4.3.15 Weight of liver and spleen.

The mean weight of liver and spleen of four groups of ducks sacrificed after six months of feeding with mancozeb added diet are shown in table 60. The mean weight of liver in group I was significantly higher than that of the controls. The mean weight of

Table 57 Mean zinc content (ppm on dry matter basis) of Liver,Kidney and Muscle of Ducks after six months of feeding on diet added with Mancozeb.

Group	Liver	Kidney	Muscle
I	163.333 ±11.584	127.167 ±14.747	153.833 ±9.505
II	198.000 ±16.459	116.500 ±4.994	302.667 ±56.292
III	188.000 ±4.932	135.500 ±7.076	328.667 ±86.794
# IV	128.833 ±24.283	119.000 ±3.985	178.000 ±14.981

N = 6
# control

Table 57.1 ANOVA table (M.S.S),influence of dietary level of Mancozeb on the tissue zinc content of Ducks after six months of feeding.

Source of variation	df	Tissues		
		Liver	Kidney	Muscle
Groups	3	4409.264	444,708	46110.15
Error	20	1528.208	462.792	16517.48
F value		2.885	0.961	2.792

Fig24 Mean zinc content (ppm on dry matter basis) of liver, kidney and muscle of Ducks after six months of feeding on diet added with Mancozeb.

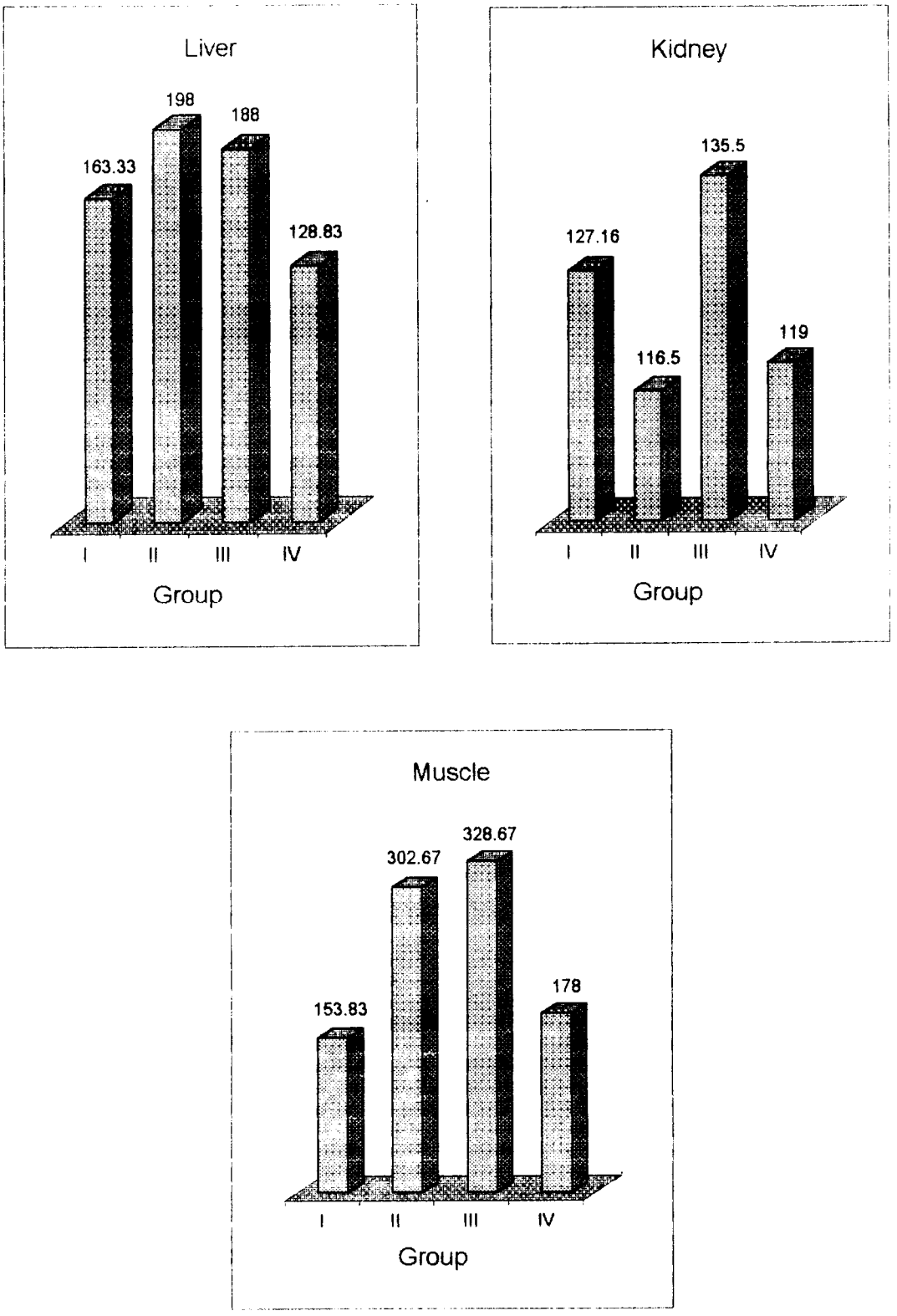


Table 57 Mean serum manganese level (ppm) of Ducks at monthly intervals, fed on diet added with Mancozeb.

Group	Months					
	1	2	3	4	5	6
I	0.110 ±0.02	0.050 ±0.01	0.080 ±0.02	0.160 ±0.02	0.133 ±0.01	0.130 ±0.02
II	0.120 ±0.04	0.120 ±0.04	0.213** ±0.04	0.260** ±0.03	0.236 ±0.06	0.190 ±0.02
III	0.130 ±0.01	0.160 ±0.03	0.253** ±0.03	0.340** ±0.03	0.154 ±0.02	0.160 ±0.02
# IV	0.090 ±0.02	0.120 ±0.02	0.108 ±0.02	0.160 ±0.01	0.131 ±0.02	0.130 ±0.02
CD (0.05)			0.093	0.076		

N = 6
# control

Table 58.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the serum manganese level of Ducks at monthly intervals.

Source of variation	df	Months					
		1	2	3	4	5	6
Groups	3	0.001	0.013	0.040	0.048	0.015	0.002
Error	20	0.004	0.005	0.006	0.004	0.007	0.002
F value		0.283	2.812	6.673**	12.752**	2.250	0.747

Fig. 25 Mean serum manganese level of Ducks at monthly intervals, fed on diet added with Mancozeb.

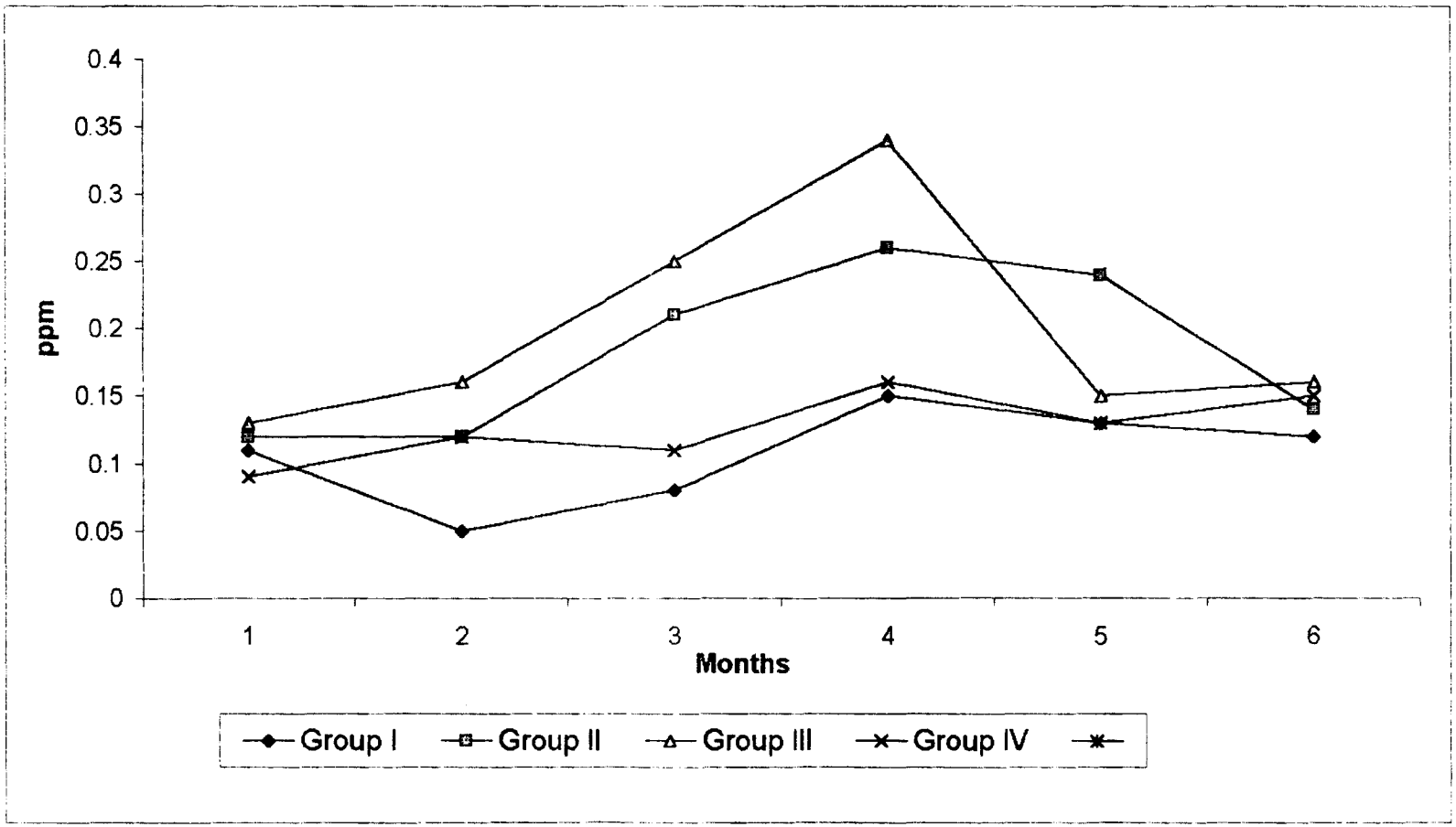


Table 59 Mean manganese content (ppm on dry matter basis) of Liver, Kidney and Muscles of Ducks after six months of feeding on diet added with Mancozeb.

Group	Liver	Kidney	Muscle
I	59.000 ±5.026	56.167 ±1.662	39.833 ±2.690
II	62.667 ±2.095	53.167 ±2.332	43.500 ±1.670
III	58.500 ±1.335	55.333 ±0.955	46.333 ±1.665
# IV	60.167 ±2.213	52.667 ±1.499	43.667 ±1.584

N = 6
# control

Table 59.1 ANOVA table (M.S.S),influence of dietary level of Mancozeb on the tissue manganese content of Ducks after six months of feeding.

Source of variation	df	Tissues		
		Liver	Kidney	Muscle
Groups	3	20.722	17.000	42.778
Error	20	54.483	17.017	22.950
F value		0.380	0.999	1.864

Fig.26 Mean manganese content (ppm on dry matter basis) of Liver, Kidney and Muscle of Ducks after six months of feeding on diet added with Mancozeb.

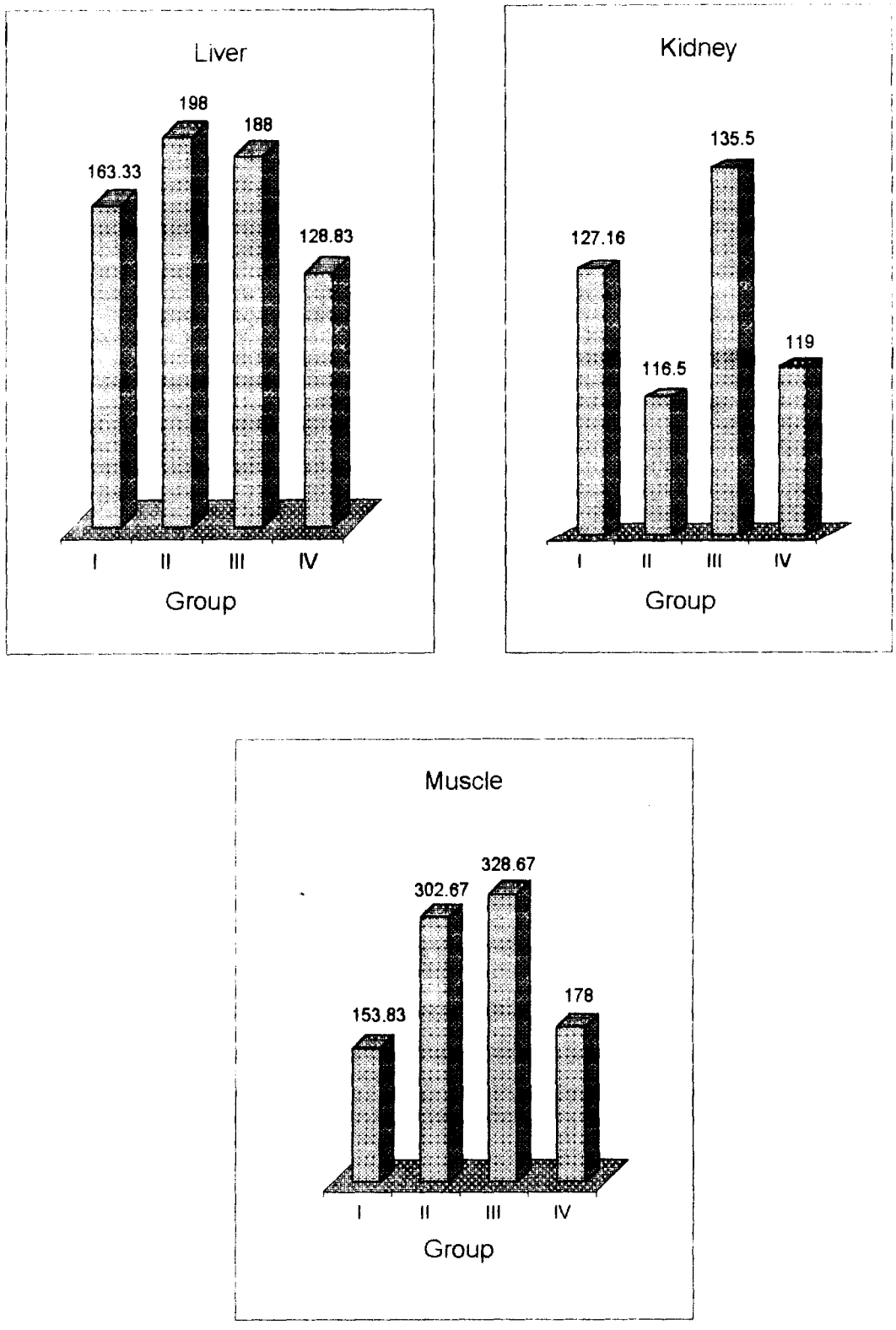


Table 60 Mean weight of Liver (g) and Spleen (mg) of sacrificed Ducks after six months of feeding on diet added with Mancozeb.

Group	Liver	Spleen
I	37.917** ±1.668	812.500 ±23.132
II	34.667 ±2.300	808.333 ±37.857
III	28.250 ±0.889	575.000** ±39.168
# IV	30.167 ±1.983	720.833 ±51.296
CD(0.05)	5.098	111.600

N = 12
# control

Table60.1 ANOVA table (M.S.S), influence of dietary level of Mancozeb on the weight of Liver and Spleen of Ducks after six months of feeding.

Source of variation	df	Tissues	
		Liver	Kidney
Groups	3	229.167	148194.444
Error	44	38.398	18399.62
F value		5.968**	8.054**

Fig. 26a Duck liver- group I, II & III-treated with Mancozeb, extensive vacuolation H&E X 160

Fig. 26b Duck liver - group I, II & III-treated with Mancozeb, vacuolation and leucocytic infiltration H&E X 160

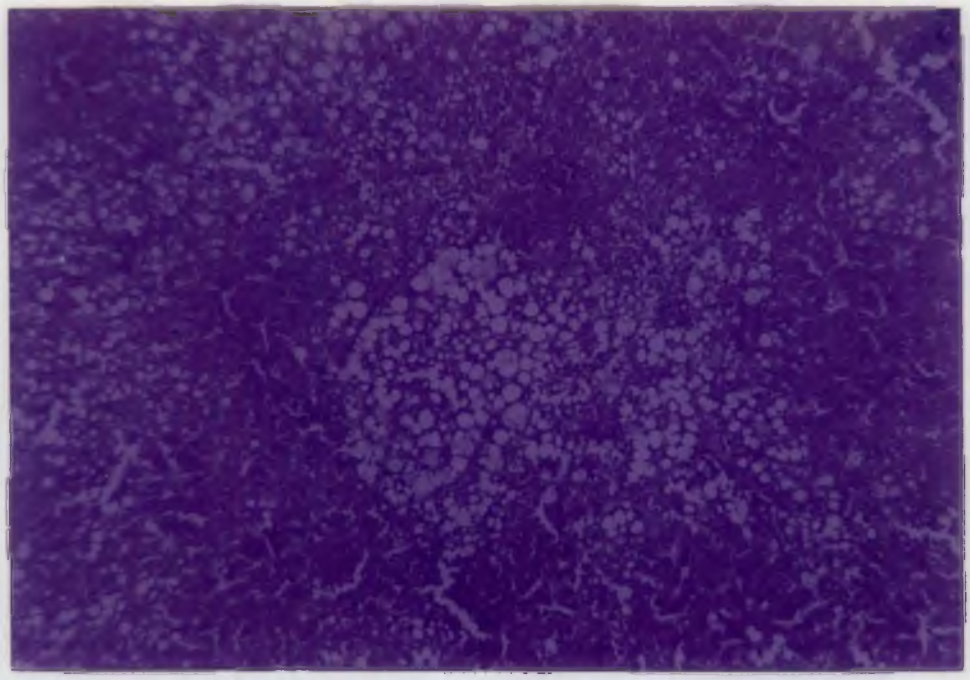


Fig. 26a

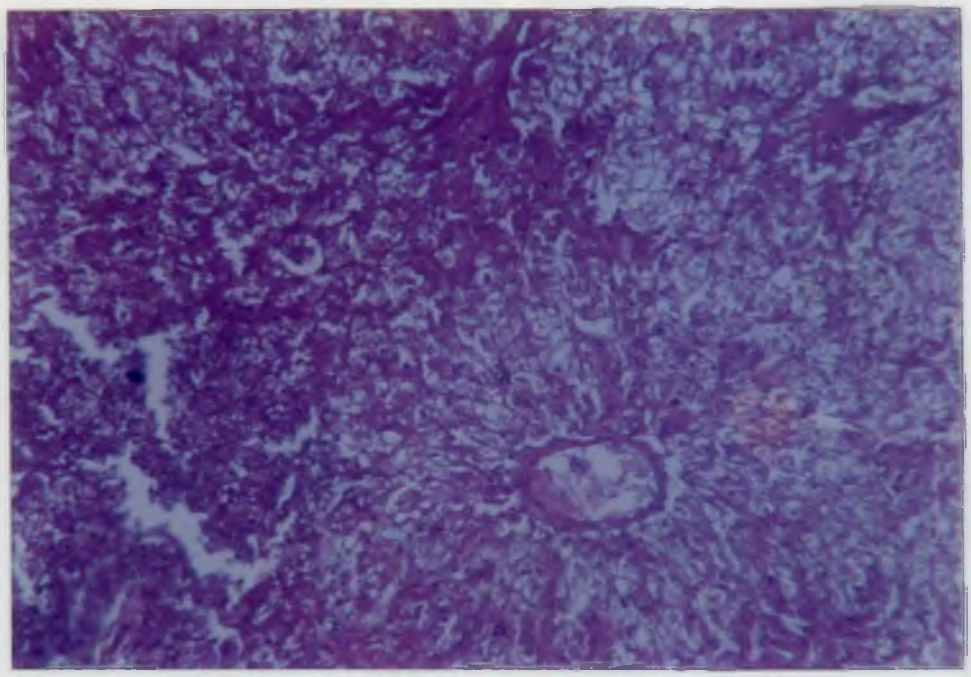


Fig. 26b

Fig. 26c Duck kidney-group I, II & III-treated with Mancozeb, hyalin degeneration and endothelial calcification H&E X 250

Fig. 26d Duck kidney-group I, II & III-treated with Mancozeb,degenerative necrosis, vascular sclerosis and infiltration with mononuclear cells H&E X 250

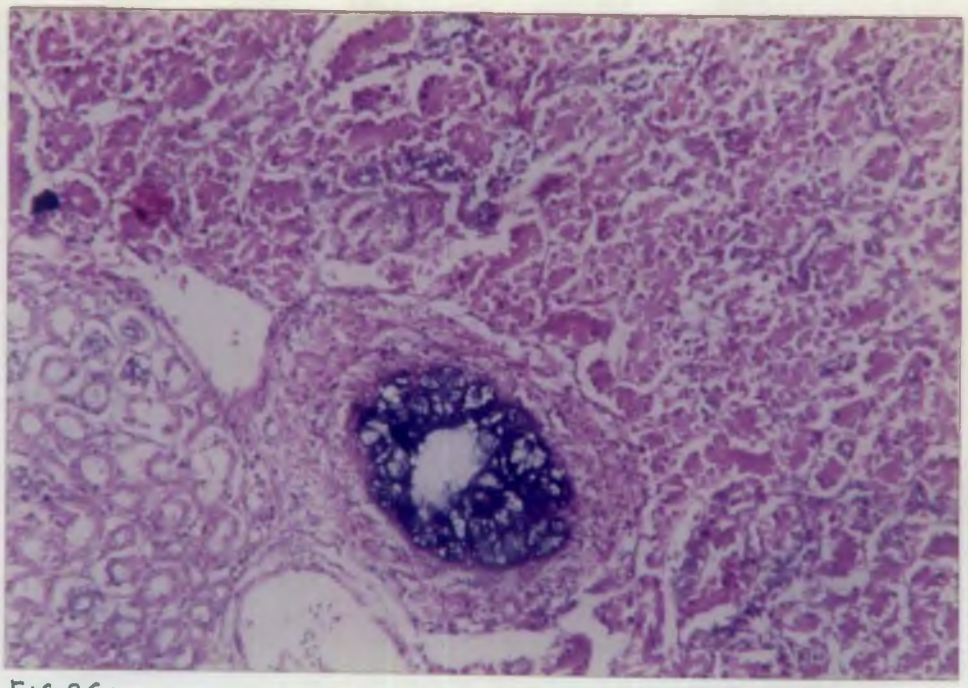


Fig. 26c

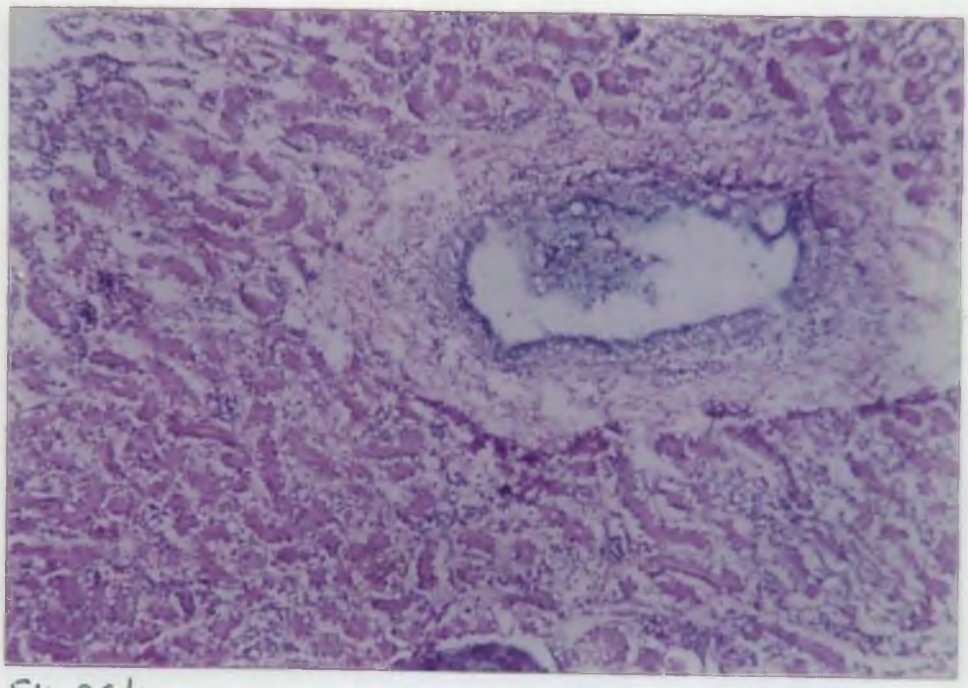


Fig. 26d

Fig. 26e Duck bursa – group I, II, III & IV – treated with Mancozeb, fibrous tissue Proliferation H&E X 160

Fig. 26f Duck spleen-group I, II & III-treated with Mancozeb, depletion of germinal Center and vascular sclerosis H&E X 160

Fig. 26e

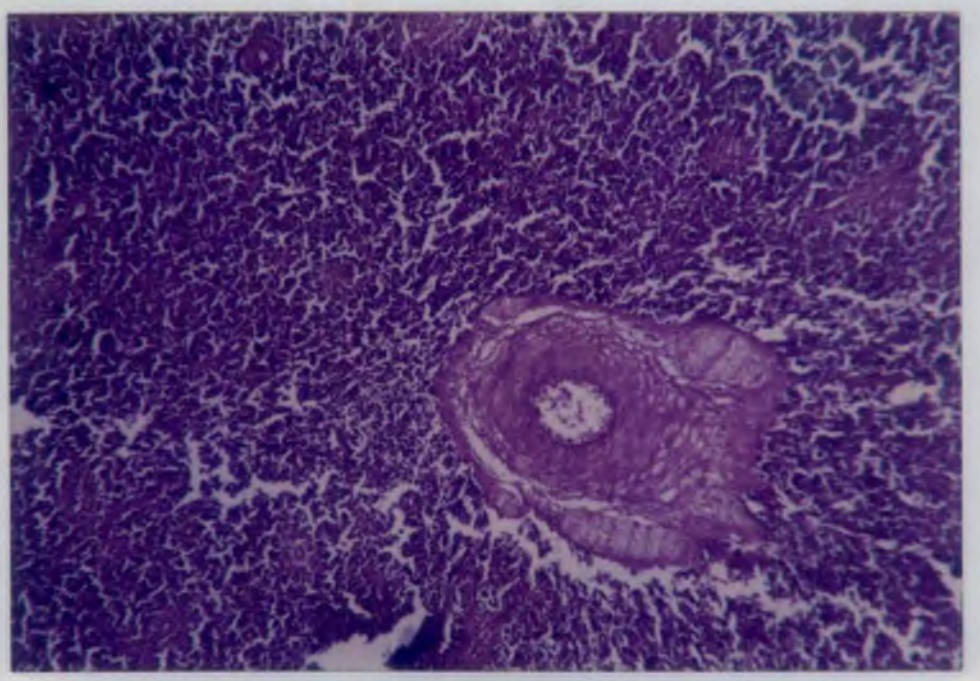


Fig. 26f

spleen in group III was significantly lower than that of the controls. In group III both liver and spleen showed decreased weight than other groups.

4.3.16 Gross and histo-pathological lesions.

4.3.16.1 Gross lesions.

In the group I and II liver, showed focal area of sub capsular haemorrhage and diffuse greyish streaks of necrosis in the borders. In group III liver showed diffuse whitish area and linear streaks and focal areas of petechiae. In two birds haemorrhage and haematoma were noticed. Out of twelve livers examined ten were affected . In the control group no lesion could be detected. In the spleen of birds in treated groups, petechiae and suffusion in the parenchyma were noticed. In the group I and II kidney showed no gross lesion. In the group III kidney showed focal areas of petechiae. Haemorrhagic streaks were found in the thigh muscles of the same group. In control animal no lesion could be detected

4.3.16.2 Histo-pathology.

Histo-pathological lesions are shown in Fig. 26a to 26f .Severe fatty changes, focal area of necrosis, infiltration of lymphoid and mononuclear cells and congestion of dilated blood vessels were seen in the liver tissue of all the treated groups. Granular degenerative changes of hepatic cells were evident in some sections of liver. Disruption of the hepatocytes and haemorrhage in between hepatic cods were also detected in group III. Dilated blood vessels, loosely arranged lymphoid cells, depletion of germinal center, thickening of the blood vessel wall and fibrous tissue hyperplasia were found in the spleen of all the treated groups. Moderate degree of fibrous tissue proliferation, depletion of lymphoid cells as evidenced by lack of lymphoblastic activity and tendency for formation of corpora amylacia were seen in the thymus of all the treated groups. Vacuolation of tubular epithelium, haemorrhage at interstitial tissue, calcification of endothelium of small arterioles and homogenous pink stained materials were noted in the tubular lumen in the kidney of all the treated groups. Depletion of lymphoid follicle was noticed in the bursa of all the treated groups.

DISCUSSION

Chapter 5

DISCUSSION

5 . 1 COPPER

5 . 1. 1 Body weight

The mean body weight of four groups of ducks reared on feed supplemented with copper is presented in Table 2 and Fig.1. The mean copper content of the control feed is 28 ppm. The group I, which is fed with 100 ppm supplemented copper showed an increase in growth rate up to 90 days of experiment *i.e.* four months of age (the experiment was started in ducklings of one month age). From 90^{th} day onwards a gradual decrease is seen up to 180^{th} day of the experiment. The same trend is seen in other groups also as evidenced from the table and chart. Hence it can be concluded that these levels of copper have no influence on the period of peak gain in body weight. Though it was not significant, all the treated birds showed higher body weight than the control except for the first month.

Literature on the effect of copper on body weight in ducks is scarce. In poultry Poupoulis and Jensen (1976) reported an increase in body weight by feeding 250 ppm copper for four weeks. Similar observation was also reported by Mehring *et al.* (1960) and Jackson *et al.* (1979) . But Lin *et al.* (1965) reported a reduction in growth rate in poultry when they fed copper at 80 ppm or above along with 40 ppm or above iron for four weeks. Ward *et al.* (1994) observed that 204 ppm copper for five days in feed did not produce any effect on growth. Jackson and Stevenson (1981) noticed a reduction in body weight when 200 ppm copper was added in the feed . Stahl *et al.* (1989) also reported a reduction in body weight gain with 250 ppm copper supplemented diet.

Higher levels of copper (above 500 ppm) reduced the feed intake and growth according to Jensen and Maurice (1976 , 1979) , Jackson (1977), Jackson *et al* (1979), Atlavin and Aspite (1982), Southern and Baker (1982), Andrews (1988), Leech *et al.* (1990) and Gilbert *et al.* (1996). But Griminger (1977)could not observe any reduction in body weight gain by 500 ppm copper in the ration Anjum *et al.* (1992) also made similar observations.

In turkey also an increase in growth rate by 120 ppm copper in the diet was observed by Guenthner *et al.* (1978). But more than 500 ppm reduced the weight gain, observed by Christmas and Harms (1978, 1979) and Potter and Koru (1989).

The present study in ducks fed with 100-300 ppm copper supplemented diet though not significant shows an increase in body weight gain. The body weight gain of group I,II,III and IV are 694, 737, 813, and 594 g respectively which indicate that copper 100-300 ppm has a stimulating trend on body weight gain in ducks.

5 . 1 . 2 Haemogram

5 . 1 . 2 . 1 Erythrocyte count

The mean erythrocyte count of four groups of ducks is presented in Table 3 . In group IEven though all are not significant, a higher erythrocyte count is seen throughout the experiment. It shows that 100 ppm copper supplemented diet stimulates the erythropoiesis with no other adverse effect, on feeding for six months. In group II eventhough a stimulation of erythropoiesis is seen up to fifth month and a reduction in the number of erythrocyte is seen towards the end of the treatment period. At this stage the serum level of copper is found to be more than 0.68 ppm (Table 18) In group III a reduction in erythrocyte count is seen from fourth month onwards (when the serum level of copper exceeds 0.68 ppm, Table 18). In the initial period this group

also showed an increasing tendancy, when copper is gradually accumulated in the system starts showing adverse effect on erythropoiesis. Stevenson and Jackson (1980) could not observe any change in haemogram with 1000 ppm copper in the feed for 2 months. But Radzanowsk (1989) noted an adverse effect on haemopoiesis with 1000 ppm copper fed for 1 ½ months . Anjum *et al.* (1992) observed a stimulation of erythropoiesis with 500-1000 ppm copper sulphate (copper is only 40%) in broiler chicks of one week age.

Several factors like age, sex, change in climate, muscular activity etc. influence the erythrocyte count. Sreeraman *et al.* (1979) reported a normal erythrocyte count of 3.02 m/cmm in ducks. Surendranathan (1966) recorded 2.96 and 2.42 m/cmm for adult male and female ducks respectively. In the present study the erythrocyte count of control group ranges from 2.75 to 3.43 m/cmm (on an average 3.02) The overall mean of the observations taken at monthly intervals for group I, II, and III are 3.25. 3.02 and 2.89 m/cmm . This shows a tendency for decreasing the number of erythrocyte as the level of copper is increased.

6 . 1 . 2 . 2 Haemoglobin

The mean haemoglobin level of ducks is depicted in Table 4. The treated animals shows a decreasing tendency of haemoglobin values. It is evident from the data that 100, 200, and 300 ppm of copper supplementation in the feed did not affect the haemoglobin level significantly. Goldberg *et al.* (1956) got an adverse effect on haemopoietic system of chicks fed with 100 ppm copper in the feed for a long period (until the birds developed toxicity) Radzanowska (1989) also got similar observations when adult hens were fed with 100 ppm copper supplemented diet for 6 weeks. Rangachar and Jayaprakash (1979) observed a stimulatory effect on haemaglobin synthesis when 10 weeks old birds were fed with 100 ppm copper supplemented diet

for 75 days along with 100 ppm zinc, but at 105 days copper and zinc did not influence the haemogram of chicks.

It is also evident from the table that haemoglobin is increased as age advances. It is in agreement with the observation of Sreenivasan and Rao (1965). In 1-2 year old male ducks they got 12.69 g% of haemoglobin which is partially in agreement with the result of the present study. The haemoglobin percentage of control group varies from 11.21 to 13.07 g% which is also in agreement with the observation of Surendranathan (1966). He reported haemoglobin level of 13.27 g% in males and 12.3 g% in females. Sreeraman *et al.* (1979) also reported a haemoglobin value of 13.83g% in adult male ducks.

5 . 1 . 2 . 3 Erythrocyte sedimentation rate (ESR)

The mean erythrocyte sedimentation rate is shown in Table 5. Significant difference was not noticed between treated and control values. This indicates that the level of copper used in the present experiment does not have any influence on the ESR of ducks. Erythrocyte sedimentation rate can mainly be influenced by many factors like pathological conditions, alterations in specific gravity of plasma, change in viscosity , size of the corpuscles and fibrinogen content of plasma. The ESR of control animal in the present study varies from 0.11 to 0.20 mm / hr . This is in agreement with the observation of Sreeraman *et al.* (1979). In the present study younger birds show a higher ESR values and as age advances it decreases. Similar observation was also made by Surendranathan (1966). He recorded 0.29 mm / hr for one month old and 0.13 mm / hr for adult female ducks.

5 . 1 . 2 . 4 Packed cell volume (PCV)

The packed cell volume of ducks is shown in Table 6. There is no significant difference in these values between control and treated, indicating that PCV is not

influenced by copper supplementation at these levels. The values for control birds vary from 41.13 to 42.80% which is partially in agreement with the observation of Surendranathan (1966) who got 40.70% for male and 38.10% for female ducks.

5 . 1 . 2 . 5 Leucocyte count

The mean leucocyte count is presented in Table 7. There is no significant difference in values of treated groups from those of controls. The results indicate that copper (100-300 ppm) in the feed has no influence on the leucocyte count of ducks. In the present investigation, the leucocyte count of control birds ranges from 28.0 to 33.6 thousands/cmm. which is in agreement with the findings of Surendranathan (1966). He observed 31.49 and 28.96 thounds/cmm for male and female ducks respectively. Several factors like folic acid deficiency , viraemia, age, inflammation, bacterial disease. tissue necrosis. environment and time of the day can cause fluctuations in leucocyte count.

5 . 1 . 2 . 6 Differential count

5 . 1 . 2 . 6 . 1 Lymphocyte

The lymphocyte percentage of all groups of ducks is shown in Table 8 and Fig.2. In the third month group I and II showed a significant increase and in the sixth month group I and III showed a significant reduction in lymphocyte count. As age advances, an increase of lymphocyte percentage is seen in all the groups. Even though there is an increase in the percentage of lymphocytes of group I and II in third month and a decrease in the percentage of lymphocytes of all the group in sixth month. these values are in the normal range. Hence this can not be considered as pathological. The value of control birds vary from 61.2 to 67.6 %. This tallies with the observations of Surendranathan (1966). He recorded 68 % lymphocyte in males and 62.6 % in

females. He also noticed an increase in lymphocyte count as age advances. Devanand (1991) observed 69 % lymphocyte in adult desi ducks.

5 . 1 . 2 . 6 . 2 Heterophils

The mean heterophil percentage of four groups of ducks is depicted in Table 9 and Fig. 2. In group I and II there was a significant reduction in heterophil percent. but at sixth month a significant increase is also seen in all the treated groups. The proportionate increase is due to a reduction in lymphocyte count. The values for control birds vary from 23.4 to 29.8 percent. A similar observation was made by Surendranathan (1966) and Devanand (1991).

5 . 1 . 2 . 6 . 3 Eosinophils

The mean eosinophil count of four group of ducks is shown in Table 10 and Fig.2. No significant difference could be noted between control and treated birds. The values for control birds vary from 4.5 to 5.3 percent which tally with the observation of Surendranathan (1966) and Devanand (1991) who got 4.6 and 4.8 percent eosinophil respectively in ducks.

5 . 1 . 2 . 6 . 4 Monocyte

The mean monocyte count of ducks is shown in Table 11 and Fig 2. Though there was an increasing tendency in the monocyte percentage in all the treated group. none of the value is significantly different from that of controls. The monocyte percentage of control group ranges from 2.87 to 4.27. However Surendranathan (1966) observed a value of 7.5 percent in desi ducks.

5 . 1 . 2 . 6 . 5 Basophils

The mean basophil percentage of four groups of ducks is shown in Table 12 and Fig.2. None of the values are significantly different from those of controls. The basophil percentage of control group ranges from 0.67 to 0.93 a similar observation was made by Surendranathan (1966). The results of the present study indicates that copper supplementation at 100 to 300 ppm in the feed does not influence the differential leucocyte count of ducks.

5 . 1 . 3 Enzyme profile

5 . 1 . 3 . 1 Aspartate Amino Transferase (AST)

The AST level of four groups of ducks is shown in Table 13 and Fig.3. Only very few values show significant difference from controls. In group I significantly lower value was noticed in the third and fifth month, 8.20 and 8.27 units as compared to control values (10.0 and 13.3). Group II also showed a significantly lower value in fifth month 9.73 and 13.3 units for treated and controls respectively. Birds fed with the highest amount (300 ppm) of copper showed a significantly higher value (18.7) than that of controls (13.3)in fifth month. Usually damage to heart, liver, skeletal muscles, kidney and brain tissues, haemolysis and stress causes an increase in the serum AST level in ducks. In tissue injury there will be 2 to 4 fold increase of enzyme level in the serum (Coles, 1986). Hence it is evident that copper 100 to 300 ppm does not cause any apparent damage to these tissues. Stevenson and Jackson (1980) also could not observe any effect on these enzymes in hens , fed with 500, 1000 and 2000 ppm copper for eight weeks.

5 . 1 . 3 . 2 Alanine Amino Transferase (ALT)

The mean ALT level of treated and control birds is shown in Table 14 and Fig.4. In the first five months all the treated groups except group I and II in the fourth month

showed a tendency for higher values. In the sixth month all the treated groups showed lower values (5 units) than that of controls (13.6 units).

The ALT level will vary with species, in ducks, kidney has the maximum level, erythrocyte also has substantial amount. Heart, skeletal muscle, liver and lung have low ALT level. Membrane damage to these organs causes 3 to 8 fold increase in the level of this enzyme (Coles, 1986). Since the difference seen in the present experiment is below three fold during the entire treatment periods, it indicates that there is no damage to these organs.

5 . 1 . 3 . 3 Alkaline Phosphatase (ALKP)

The mean ALKP level of ducks is depicted in Table 15 and Fig. 5. All the treated birds show an increasing tendency of the enzyme level when compared with those of controls. Wide variation is seen between normal individual of the same species, and with repeated sampling from the same bird. If there is 4 fold increase in ALKP it is suggestive of some involvement of bone. Intestinal disturbances and inappetance reduces the level of ALKP. (Coles,1986) In the present experiment the greater variation seen is in the third month *i.e.* 25.31 against 9.03 in controls. It is only less than 3 fold increase, suggestive of no damage to skeletal system. The increased level of treated birds may be due to growth greater than control birds as evidenced by the body weight.

5 . 1 . 4 Phytohaemagglutinin-P test. (PHA-P)

The mean wing web thickness of ducks injected with PHA-P test is shown in Table 16. Significant difference is not seen between control and treatment and reveals that copper 100 to 300 ppm does not have any significant change in cell mediated immunity.

5 . 1 . 5 Antibody titre.

The mean antibody titre in the serum of ducks is detailed in Table 17 and Fig 6. No significant difference could be observed between treated and control. This is in agreement with the observation of Stahl *et al.* (1989), who could not get any response in antibody production with 150 ppm copper supplemented diet for 3 weeks. However the present observation is not in agreement with the findings of Rangachar and Hegde (1974), who reported a greater primary response to *Salmonella gallinarum* with 100 ppm copper supplemented diet. Rangachar *et al.* (1978) noticed a significant increase in the synthesis of Ig G by feeding 100 ppm copper or zinc or both for 3-5 months in poultry. From the figure it is evident that feed supplemented with 100 ppm copper produces an increase (not significant) in antibody response after booster dose of vaccine. On increasing the copper level in the feed a corresponding reduction in the stimulation of antibody level is also noticed.

5 . 1 . 6 Copper content in the serum.

The copper content in the serum of birds is shown in Table 18 and Fig. 7. The copper level is increased from one month to the sixth month of treatment in all the treatment groups. The increase was greater in the groups fed with more copper. A significant increase could be observed only in 4th, 5th and 6th month in group III (fed with 300 ppm copper). Relationship between dietary copper and concentration of copper in plasma was also recorded by Ekpergin and Vohra (1981).

Panic *et al.* (1974) observed a plasma level of 0.12 ppm in hens and 0.1 ppm in cocks, at 94th day, fed with a normal diet. However in the present study, on 90th day the control animals showed a level of 0.3ppm copper in the serum. The present observation is not in agreement with the observation of Ward *et al.* (1994), who could not get any change in plasma copper concentration when copper at 204 ppm was fed to chicks. In the serum of control group also a gradual increase in the copper content is seen.

Markarova (1978) also reported that the plasma copper level of chicks will be increased with age.

5 . 1 . 7 Copper content in the tissues.

The mean copper content of the liver, kidney and muscle tissues is shown in Table 19 and Fig.8. Liver tissue shows the greater concentration of copper than kidney and muscle. All the treated groups show a higher value in a dose dependent manner. but significantly higher value is noticed in liver tissue of group II and III. Jackson *et al.* (1979) noticed a sharp rise in liver copper concentration by feeding 600 - 800 ppm copper for 336 day. Similar observation was also made by Jensen and Maurice (1979). A relationship between dietary copper and the concentration of copper in the liver was also recorded by Ekpergin and Vohra (1981), Southern and Baker (1982) and Proske *et al.* (1991).

Owood and Worden (1973) noted 140 ppm copper in the liver of ducklings after feeding 200 ppm copper in the feed for 28 days. In the present study after feeding 200 ppm copper. mean liver copper level was elevated to 822 ppm after six months. The liver copper level recorded after feeding 30 ppm copper (control group) for six months was 523 ppm.

In kidney also significantly higher concentration of copper is seen in all treated birds. Even though there is no significant difference, a higher level is noticed in muscle tissues of all treated birds. In the muscle tissue of the control birds the copper content is 20.8 ppm. Martynyvk *et al.* (1980) recorded 0.61 to 4.2 ppm copper in chicken. They stated that duck and quail meat contains more copper. While hens and broiler chicken contain lesser copper.

5 . 1 . 8 Weight of organs.

The mean weight of liver and spleen of sacrificed ducks is shown in Table 20. There is no significant difference between the treated and controls revealing that copper supplementation (100-300 ppm) will not significantly affect the weight of liver and spleen.

5 . 1 . 9 Gross and histo-pathological changes.

Literature is scanty on the histo-pathology of copper toxicity in birds. In the present investigation copper supplementation (100-300 ppm) in the feed for six months was unable to produce any gross lesions in the liver, kidney, spleen and muscle. This is in agreement with the observations of Anjum *et al* (1992), who could not observe any change in clinical appearance of internal organs of one week old chicks fed with diet containing 500 – 1000 ppm copper.

Histo-pathologicaly no lesions could be detected in the liver of any birds. However, Kamel *et al* (1971) noticed acute toxic dystrophy of liver in chicks died in 2-4 days after administration of copper sulphate 0.5 to 1 g per Kg body weight. Anjum *et al* (1992) also observed mild degenerative changes in liver when one week old chicks were fed with diet containing 500 - 1000 ppm copper.

Focal vacuolation and necrosis of tubular epithelium were noted in the kidney of birds in group III (Fig.8a). Thickening and congestion of small blood vessels were also noticed. Kamel *et al* (1971) also observed acute tubular nephrosis and necrotic changes in the kidney of chicks sacrificed at 24 hours after the administration of copper sulphate, at the rate of 0.5 to 1 g per Kg body weight. Those chicks died in 2 - 7 days also showed advanced degenerative changes in the kidney.

In the spleen, mild depletion of lymphoid cell in the peri arteriolar aggregation were detected in group II. A reduction in the lymphocyte count of the blood collected just before slaughter, correlates with the depletion. This depletion of lymphoid element is not an indication of any significant suppression of immune system as there is no difference in the antibody titer at this stage.

In all the birds, including controls, thymus showed moderate fibrous tissue proliferation. In the bursa, depletion of lymphoid follicle was noticed in all the birds (Fig. 8b to 8d.)These changes are not suggestive of any pathological condition as these organs are in the process of regression.

5 . 2 . ZINC

5 . 2 . 1 Body weight

The mean body weight of four group of ducks fed with 100, 200 and 300 ppm zinc supplemented diet and control diet are shown in Table 21 and Fig. 9. In all the groups body weight is increased up to 90-105th day and later there is a decrease. There is no significant difference between the treated and the controls except for the last observation (180th day) in Group I and III, in which a significantly lower value is seen. The mean value of group III showed a decreasing tendency in body weight gain through out the experiment. Group I also showed a decreasing tendency in body weight gain after 75th day. However group II showed an increasing tendency in body weight gain after 15th day.

The present observation is in agreement with those of several authors who could observe a reduction in body weight gain by feeding zinc at a level higher than 300 ppm. Robertson and Schaible (1960) noticed a reduction in growth rate and feed efficiency in day old chicks only by feeding 500 ppm or more zinc in the ration for 4 weeks. Similar observation was also made by Vohra and Kratzer (1968). Hermeyer (1977)

Pava (1980) Southern and Baker (1983), Charles *et al.* (1987) and Andrews *et al.* (1988).

In Mallard ducks zinc at a level of 3000 ppm in the feed reduced the weight gain (Gasaway and Buss, 1972). From the studies of Kincaid *et al.* (1976), they concluded that homeostatic mechanism of young chicks is effective up to 1200 ppm of dietary zinc.

The present study reveals that zinc up to a level of 380 ppm of feed (300 supplemented and 80 in the control feed) had no significant influence on the body weight gain in ducks.

5 . 2 . 2 Haemogram

5 . 2 . 2 . 1 Erythrocyte count

The mean erythrocyte count is given in Table 22. There is no significant difference between treated and controls. However in group I all the mean values show an increasing tendency and group III show a decreasing tendency. It indicates that supplementation of feed with 100 ppm zinc is ideal for a higher erythrocyte count. The erythrocyte count of control birds ranges from 2.97- 3.19 m/cmm which is in agreement with the observation of Sreeraman *et al.* (1979) who got an average of 3.02 m/cmm. Surendranathan (1966) recorded 2.96 and 2.42 m/cmm in adult male and female ducks respectively. The overall mean of groups I, II, III and IV are 3.16, 3.10, 3.04 and 3.10 m/cmm respectively, also shows a better performance of group I than others. It can be concluded that 100 ppm supplementation of zinc in the ration has a comparatively favourable influence on the erythropoietic system

5 . 2 . 2 . 2 Haemoglobin

The mean haemoglobin level of ducks at monthly intervals are shown in Table 23. The group I, fed with 100 ppm zinc supplemented diet showed a significantly higher

value in first, second and fourth month and in group II significantly higher value only in the first month. The overall average of group I, II, III, and IV are 12.46, 11.63, 11.73 and 11.44 g% respectively. It is concluded that feeding ducks with 100 ppm zinc supplemented diet had a favorable influence on haemoglobin production than other groups. The mean haemoglobin level of control group is 11.44. This is not in agreement with the observation of Surendranathan (1966) who got haemoglobin value of 12.2 in desi ducks. The difference may be due to the variation in the species of ducks or a difference in the minerals in the feed.

5 . 2 . 2 . 3 Erythrocyte sedimentation rate (ESR)

The mean erythrocyte sedimentation rate of ducks are shown in Table 24. No significant difference is seen between the treated and the controls. The over all mean ESR of control bird is 0.115 cm/hr. This is in agreement with the observation of Sreeraman *et al.* (1979), who reported an ESR of 0.1 in adult male ducks. Devanand (1991) recorded an ESR of 0.12 in adult desi ducks. This indicates that zinc at this level (100-300 ppm) has no influence on the ESR of ducks.

5 . 2 . 2 . 4 Packed cell volume (PCV)

The mean packed cell volume of ducks at monthly intervals are shown in Table 25. All the treated groups show a significantly higher value by the last month of experiment. There is a tendency among treated animals to have a high PCV value. The PCV of control birds vary from 39.67 to 41.50 %. The over all mean is 40.37. This is in agreement with the observation of Surendranathan (1966), who got a mean value 39.40 % in desi ducks.

5 . 2 . 2 . 5 Leucocyte count

The mean leucocyte count of ducks treated with zinc is shown in Table 26. Even though all are not significant, treated groups are showing a tendency for higher value.

However, in group I and III the value at 3rd, 5th, and 6th month is significantly higher. This reveals that leucopoiesis is stimulated by all these levels of zinc supplementation in the feed. The leucocyte count of group IV ranged from 27.08 to 32.00 thousands/cmm, which is in agreement with the observation of Surendranathan (1966), who got 31.49 and 28. 96 in male and female adult ducks respectively.

5 . 2 . 2 . 6 Differential count

5 . 2 . 2 . 6 . 1 Lymphocyte

The mean lymphocyte count of four group of ducks at monthly intervals are detailed in Table 27 and Fig. 10. In the first month groups I and II, and in the second month groups II and III only shows a significantly higher values. It is evident from the data that zinc at 100 to 300 ppm in the feed is not having any influence on lymphocyte count on ducks. As age advances an increase in percentage of Lymphocyte is seen in all the groups. The overall mean value of control group vary from 56.58 to 63.50 %. The value is slightly less than that observed by Devanand (1991) who got 69 % and Surendranathan (1966) who got 68% and 62 % in male and female ducks respectively.

5 .2 . 2 . 6 . 2 Heterophils

The mean heterophil count of ducks is shown in Table 20 and Fig. 10. Groups I and II show a significantly lower heterophil percentage in the first and second month. However group III shows a significantly lower value only in the second month. No other value is significantly different from controls. As there is no significant difference in the total leucocyte count during this period the chances of immune suppression is not seen. The mean heterophil percent of control group vary from 26.42 to 33.17. This is in agreement with the observation of Devanand (1991) who got a heterophil count of 27.6% in desi ducks. Surendranathan (1966) got a value of 22% and 26% in male and nonlaying female ducks respectively.

5.2.2.6.3 Eosinophils

The mean eosinophil count of ducks is depicted in Table 29 and Fig. 10. There is no significant difference between treated and control groups. The mean value of control group ranged from 5.00 to 6.33%, which is in agreement with observation of Surendranathan (1966) he recorded an eosinophil percent of 5.2 in adult ducks. Sreeraman *et al.* (1979) observed an eosinophil count of 7% in male Mallard ducks. Lahir (1982) got 4.8 % eosinophils in ducks.

5.2.2.6.4 Monocytes

The mean monocyte count of four group of ducks is given in Table 30 and Fig. 10. Significant difference is not observed between the treated and control. The value for control birds ranged from 3.42 to 4.25, which is slightly less than that observed by Surendranathan (1966) and Sreeraman *et al.* (1979).

5.2.2.6.5 Basophils

The mean basophil count of ducks fed with zinc supplemented diet and control diet is detailed in Table 31 and Fig.10. None of the values were significantly different from those of the controls. The mean value of the group IV ranged from 0.63 to 0.88, which is in agreement with the observation of Surendranathan (1966). He got 0.5 to 0.61 % basophils in ducks.

5.2.3 Enzyme profile

5.2.3.1 Aspartate Amino Transferase (AST)

The mean AST level of four group of ducks is shown in Table 32 and Fig. 11 The mean AST level of treated groups in first and second month was significantly less than that of the control values, indicating that no tissue damage has occurred by these levels of zinc. By sixth month, as zinc level is increased in the serum and tissues an

increase in the level of AST was noted. Data of zinc status on AST level are scanty. It can be concluded that the zinc fed at 100 to 300 ppm for six months period will not affect the integrity of the tissues as the increase is not beyond 2 fold (Coles, 1986)

5 . 2 . 4 . 2 Alanine Amino Transferase (ALT)

The mean ALT levels of four groups of ducks at monthly intervals are shown in Table 33 and Fig. 12. Group I showed a significantly lower value in second, third and fourth month. Group II also showed a significantly lower value in first month. In sixth month all the treated group showed a significantly higher value. Through out the period of experiment group III showed a tendency for higher ALT level than control However significant difference was noticed only in the first, the fifth and the sixth month. This shows that higher levels of zinc adversely affects the ALT level. since by sixth month all the groups are showing significantly higher enzyme values. Cumulation of zinc in the tissues or serum also influence the ALT levels. As age advances a gradual reduction in ALT level is noticed in both control and treated groups. In membrane damage a 3 to 8 fold increase in enzyme level is seen (Coles 1986). In the present study none of the values were near the 3 fold increase in the serum level of ALT, indicating that no membrane damage is induced by feeding higher levels of zinc up to 300 ppm in the diet.

5 . 2 . 3 . 3 Alkaline phosphatase (ALKP)

The mean alkaline phosphatase level are depicted in Table 34 and Fig. 13. It shows that all the values of treated groups are higher than the control groups, even though all are not significant. There is a gradual reduction in ALKP level as the birds advances in age. Serum ALKP level relates to the rate of growth of bone tissues This may be the reason for the reduction in ALKP as the age advances. There is a drop in the level of enzyme in all the treated groups in fourth month. Intestinal disturbances or mappetance reduced the level of ALKP (Coles, 1986). For an involvement of any

system there must be at least 3 fold increase in the enzyme level. Since there is no such increase in the serum level of ALKP in the present study, indicates that zinc at this level (100 to 300 ppm) will have no pathological effect on such organs.

5 . 2 . 4 Phytohaemagglutinin –P test (PHA-P)

The mean value of PHA-P test is shown in Table 35. From the data it is evident that supplementation of zinc 100 to 300 ppm in the diet will not significantly affect the cell mediated immunity. This is in agreement with the observations of Pimental and Cook (1988), who had observed no influence on the delayed hypersensitivity to PHA-P test by supplementation of zinc (30- 80 ppm) in the diet of chicks. Pimental *et al.* (1991) also had similar observation , when they fed zinc for 7 weeks at the rate of 8-88 ppm of feed in chicks. However Kidd *et al.* (1993) noticed an increase in basal hyper sensitivity to PHA-P in the progeny of hens fed with supplemented zinc-methionine in corn-soyabean meal.

5 . 2 . 5 Antibody titer

The valuesof antibody titer are shown in Table 36 and Fig. 14. None of the values were significantly different from controls, except 9th observation (135th day) onwards of group III. This indicated that zinc at 300 ppm when fed beyond 4 months stimulated the antibody production in ducks.

Burns (1983) stressed the need of zinc for immune system, but Pimentel and Cook (1988) and Pimentel *et al.* (1991) could not observe any change in immune response when chicks were fed with zinc deficient diet. The present observation is in agreement with the observation of Rangachar *et al.*(1978).They could not observe any change in the immuno globulin level by feeding zinc at the rate of 100 ppm in the feed for 3-5 months in cockrels. Shoyinka and Dandu (1986) got maximum antibody response with 200 ppm zinc than with 50 or 400 ppm in the ration of chicks. Kidd *et al.*

(1992 and 1993) recorded an increase in antibody titer of progeny when dams were fed with 72 152 ppm zinc supplemented ration as zinc methionine for one month.

5 . 2 . 6 Zinc content in the serum

The serum zinc levels in ducks are detailed in Table 37 and Fig. 15. Significantly higher value was noticed in all the treated groups only in the 4th, 5th and 6th month. A gradual increase in the serum concentration of zinc is seen in control group as age advances. In the treated group a more rapid increase proportional to the level fed is seen. This tally with the observation of Makarova (1978) who noticed an increase in zinc level in serum as age increased, and the peak concentration was recorded when egg laying was in full swing *i.e.* 5-7 months. Makarova and Chekalova (1979) found that during egg laying the zinc content in the serum is increased to 4.4 µg/ml. This is in agreement with the present observation in controls. The serum zinc level observed at 4th to 5th month was 3.44 to 3.99 µg/ml. Bettger *et al.* (1981) observed a plasma zinc level of 1.3 µg/ml when 100 ppm zinc supplemented diet was fed to chicks.

5 . 2 . 7 Zinc content in the tissues

The mean zinc content of liver, kidney, and muscle of ducks is shown in Table 38 and Fig. 16. A significantly higher value is noticed only in the case of liver in group I, but the tissue zinc level is not proportional to the serum zinc level. Oh *et al.* (1979) stressed the involvement of metallothionine in zinc homeostasis. Richard *et al.* (1980) opioned that metallothionine may function as a conservation mechanism to prevent the loss of zinc and copper during tissue catabolism. They noticed an increase in the level of zinc in liver and kidney on fasting the turkey. Refeeding resulted in a decline in zinc level in these organs. It is revealed that during fasting a zinc copper binding protein metallothionine is increased and decline with feeding. Dressel *et al.* (1988) recorded a

liver zinc concentration of 154.1ppm in ducks. However, in the present study the higher liver zinc concentration observed was 148.1ppm in kidney of treated groups. The zinc content was not significantly different from controls. The highest level was seen with 100 ppm and lower level with 300 ppm zinc supplemented diet.

In the muscle tissue also no significant difference was observed in zinc level between the control and treatement groups. A higher concentration of zinc was noted with 300 ppm and lower concentration with 200 ppm zinc supplemented diet.revealing the involvement of some regulatory mechanism in these tissues also.

5 . 2 . 8 Weight of organs

The mean weight of liver and spleen are shown in Table 39 and Fig. 17. There was no significant difference in the weight of these organs between treated and controls.

5 . 2 . 9 Histo-pathological examination

In birds fed with zinc no gross lesions could be seen except for diffuse petechiae in the liver of birds in group III. Breeding *et al* (1992) could not observe any lesions in chicks fed with 2800 ppm zinc in the diet. However Gasaway and Buss (1972)reported yellowish red colouration of kidney when 3000 – 12000 ppm of zinc was fed to domestic Mallard ducks.

Birds belonging to group III showed mild lymphoid infiltration in the liver. In the spleen, mild depletion of lymphoid cell was noticed, whichwasnot suggestive of any immune suppression as there was no reduction in the antibody production during this stage. Congestion and diffuse calcification of endothelium of small blood vessels in the kidney were noticed in group II and III (Fig.16a). Depletion of lymphoid follicle in the bursa and fibrous tissue proliferation of thymus were noted in all the birds

(Fig.16b to 16d).This can not be considered as pathological as these are normal features of regression.

5.3 MANCOZEB

5.3.1 Body weight

Mean body weight of four groups of ducks reared on feed added with Mancozeb at the rate of 1000, 1500 and 2000 ppm, and control feed with out Mancozeb is shown in Table 40 and Fig. 17. In group II and III the body weight is lower than the corresponding controls, with significant difference from 90th to 135th day of the experiment. Group I fed with 1000 ppm Mancozeb showed no significant difference in the body weight with controls. This indicates that 1000 ppm Mancozeb has no adverse effect on the growth of ducks. However 1500-2000 ppm causes adverse effect on the growth.

The weight of birds increased up to 90th, 75th and 75th day of the experiment in group I, II and III respectively. In control group this value is 75th day, indicating that Mancozeb does not have any significant influence on the period of peak gain in body weight of ducks. Literature on the influence of Mancozeb on body weight of birds are scanty. Some studies on related compounds have been conducted in other species. Begliomini *et al.* (1967) noticed a reduction in weight gain in broiler chicks when Ziram was fed at the rate of 1000 ppm of the feed. Hore *et al.* (1997) also noticed a reduction in body weight in rats when Mancozeb was fed at a rate of 400 mg per kg body weight for 49 days. Carlonebbia *et al.* (1991) observed a reduction in body weight in calves fed with Zineb at a rate of 200 mg per Kg body weight for 80 days. These observations, even though related to other species, are in agreement with the present findings.

5 . 3 . 2 Haemogram

5 . 3 . 2 . 1 Erythrocyte count

The erythrocyte count (millions per cmm) of ducks treated with Mancozeb is shown in Table 41. The overall mean value of three treated and the control groups are 2.89, 2.90, 3.04 and 2.88 respectively. All the values fall in the normal range. This indicates that there is no adverse effect on the erythropoisis in treated groups. Much investigation has not been done on the effect of Mancozeb on erythrocyte of ducks. Sreeraman *et al.* (1979) recorded value of 3.02 and Surendranathan (1966) recorded 2.92 and 2.42 in adult male and female ducks respectively which is partially in agreement with the present observation.

5 . 3 . 2 . 2 Haemoglobin

The mean haemoglobin levels (g %) of ducks are shown in Table 42. Data in the present study indicate that Mancozeb 1000, 1500 and 2000 ppm does not have significant effect on the haemoglobin level in ducks. The overall mean haemoglobin level of Group I, II and III are 11.45, 11.47 and 11.49 respectively and of control group is 11.50. The mean haemoglobin value observed in the present study for control bird varies from 10.5 to 12.3 which partially agrees with the observation by Sreenivasan and Rao (1965), Sreeraman *et al.* (1979) and Surendranathan (1966). They observed haemoglobin level s of 12.8, 13.8 and 12.2 respectively.

5 . 3 . 2 . 3 Erythrocyte sedimentation rates (ESR)

The mean ESR (mm per hour) is shown in Table 43. The mean values were not significantly different from those of controls, revealing that treatment has no significant effect on the ESR of ducks.

5 . 3 . 2 . 4 Packed cell volume (PCV

The mean packed cell volume (percent) is detailed in Table 44.The overall mean value of treated groups I, II, III and control are 41.90, 42.37, 42.60 and 41.86 respectively. These values indicate that the treatment with Mancozeb has no influence on packed cell volume. However, Hore *et al.* (1997) noticed a reduction in PCV when rats were fed with Mancozeb This may be due to species difference. The packed cell volume of control birds is 41.86 which is not significantly different from the value observed by Surendranathan (1966), who observed 40.7 in male desi ducks.

5 . 3 . 2 . 5 Leucocyte count

The mean leucocyte count (thousands per cmm) of ducks is shown in Table 45. Most of the values in the treated group show an increasing tendency in the number of leucocyte count. However towards the end of the experiment ducks in group III show a reduction in the number of leucocyte indicating adverse effect of higher amount of Mancozeb on leucocyte production. Hore *et al.* (1997) also noticed a reduction in total leucocyte count in rats fed with Mancozeb. The overall mean of treated groups I, II, III and control group are 37.9, 42.5, 40.9 and 36.8 respectively. The mean value of control group is slightly higher than that observed by Surendranathan (1966) who got 31.49 and 28.96 in male and female ducks respectively. Krishnan Nair (1990) noticed mean value of 24.8 to 36.1. However the present observation endorses the observation of Sreenivasan and Rao (1965) who observed 37.4 in ducks.

5 . 3 . 2 . 6 Differential count

5 . 3 . 2 . 6 . 1 Lymphocyte

The lymphocyte percentage is shown in Table 46 and Fig. 18. It is evident from the table that the lymphocyte percentage is not significantly affected by the treatment. The overall mean of group I, II, III and control are 71.15, 70.82, 71.0 and 70.71

respectively, indicating no significant difference. The control group shows a range of 67.67 to 74.83 percent. This is in agreement with the value observed by Devanand (1991), who reported 69 percent lymphocyte in adult desi ducks. Surendranathan (1966) noticed 68 percent lymphocyte in males and 62.6 percent in females, also noticed an increase in lymphocyte count as age advances. The present study also agrees with the above observations.

5.3.2.6.2 Heterophils

The mean heterophil percent is detailed in Table 47 and Fig. 18. It indicates that the treatment does not have any influence on the heterophil percent in ducks. The overall mean of group I, II, III and control are 19.61, 18.98, 19.41 and 20.38 respectively. Only narrow difference is seen between groups. In control group the value ranges from 19.00 to 22.08, which is partially in agreement with the previous observations. Surendranathan (1966) observed 22 percent heterophil in males and 26 percent in non laying female ducks. Devanand (1991) got a heterophil percent of 27.6 in desi ducks.

5.2.6.3 Eosinophils

The Mean eosinophil percentage of four groups of ducks is shown in Table 48 and Fig. 18. No significant difference is seen between control and treated birds. This proves that eosinophil percentage is not influenced by the treatment. The overall mean shows narrow difference. In group I, II, III and IV the values are 5.08, 6.29, 5.65 and 5.43 respectively. The percentage of eosinophils for control birds varies from 4.92 to 6.33. This is partially in agreement with the observation of Surendranathan (1966), who reported an eosinophil percentage of 2.40 to 5.20 in different age groups. Lahir (1982) also noticed a value of 4.8 percent eosinophil in ducks. However, the present observation is slightly lower than the value noted by Sreenivasan and Rao (1965) and Sreeraman *et. al* (1979) they noticed an eosinophil percentage of 6.8 and 7 in ducks.

5.3.2.6.4 Monocytes

The mean monocyte percent of four groups of ducks is detailed in Table 49 and Fig. 18. None of the values are significantly different from controls. The overall mean is 3.39, 3.11, 3.18 and 3.29 for group I, II, III and IV respectively which also indicates the non significance between control and treatment. The values of control birds range from 2.92 to 3.83. The present observation is less than those observed by Surendranathan (1966) who reported a mean count of 7.5 percent in desi ducks.

5.3.2.6.5 Basophils

The mean basophil count of ducks is shown in Table 50 and Fig 18. No significant difference can be seen between control and treatment. The overall mean is 0.82, 0.78, 0.77 and 0.75 for group I, II, III and control respectively. In controls the value ranges from 0.63 to 0.92. this is in agreement with the observation of Surendranathan (1966) who observed a mean basophil percent of 0.5 to 0.6 in desi ducks.

5.3.3 Enzyme profile

5.3.3.1 Aspartate Amino Transferase (AST)

The AST levels of ducks fed with Mancozeb added diet are shown in Table 51 and Fig. 19. In the first two months all the treated groups show higher levels of enzymes and for the rest of the period a tendency for more reduced level than control is noticed. However, none of the values of treated groups are 2 to 4 fold than the normal values which indicates no apparent damage to the tissues. Studies of Mancozeb on AST level of bird are scanty. However, *in vitro* studies in human serum enzyme showed an inhibition of AST level by Mancozeb (Kara and Celik, 1997).

5 . 3 . 3 . 2 Alanine Amino Transferase (ALT)

The mean ALT levels of four groups of ducks are shown in Table 52 and Fig. 20 Even though majority of the values are significantly higher than the controls, only one value (second month of group III) shows more than 3 to 8 fold increase than the normal level, which indicates some damage to the membranes (Coles , 1986).

5 . 3 . 3 . 3 Alkaline Phosphatase

The mean alkaline phosphatase levels of four groups of ducks are depicted in Table 53 and Fig. 21. A tendency for higher enzyme levels in treated groups than controls is noticed for the first three months. Except for the last month of the experiment a gradual reduction in the enzyme level is seen from the first to the fifth month. It can be due to normal reduction in the bone tissue formation as age advances. During the fourth to the sixth month a tendency is seen to reduce the enzyme level than controls. It may be due to intestinal disturbances or inappetance (Coles, 1986). Studies on the effect of Mancozeb on Alkaline Phosphatase of birds are scanty However , Kara and Celik (1997) noticed a reduction in the level of human serum Alkaline Phosphatase in vitro by Mancozeb.

5 . 3 . 4 Phytohaemaglutinin P. skin sensitivity test (PHA-P)

The effect of Mancozeb on PHA-P skin sensitivity test is detailed in Table 54. Only few values show significant change with controls, indicating that treatment had only negligible influence on the cell mediated immunity.

5 . 3. 5 Antibody titre

The mean antibody titre of four groups of ducks is shown in Table 55 and Fig. 22 . From the data it can be concluded that Mancozeb does not have any immune-suppressant action.

5 . 3 . 6 Zinc content in the serum

The mean serum zinc level of the experimental and control ducks is shown in Table 56 and Fig. 23. There is a gradual increase in the serum concentration of zinc in all the groups including controls. As age advances, proportional to the level in the feed a higher increase is noted in the treated groups. This reveals that feeding of mancozeb will increase the serum content of zinc.

5 . 3 . 7 Manganese content in the serum

The mean manganese level in the serum of ducks is shown in Table 57 and Fig. 24. In all the treated groups, as age advances there is a tendency for increased serum level of manganese. The highest level is seen by the fourth month and then there is a reduction probably due to egg laying. From the data it can be concluded that it will accumulate in the body on continuous feeding.

5 . 3 . 8 Zinc content in the tissues

The mean zinc content in Liver, Kidney and Muscle of treated group is shown in Table 58 and Fig. 25. There is no significant difference between the treated and the controls. Accumulation is more in the muscles than in the liver or in the kidney . In group I more concentration is seen in the liver. It can be concluded that Mancozeb addition to the diet will increase the accumulation of zinc in the Muscles.

5 .3 . 9 Manganese content in the tissues

The mean manganese content is shown in Table 59 and Fig. 26. There is no significant difference between treated and controls. This indicates that Mancozeb addition to the diet has no effect on the manganese accumulation in the tissues. In all the groups including controls more concentration of manganese is seen in liver tissue.

than in muscles. revealing that manganese will accumulate more in the liver tissue than in muscles.

5 . 3 . 10 weight of liver and spleen

The mean weight of liver and spleen is shown in table 60. The weight of liver is significantly higher in group I. than controls. Even though not significant. group II also shows higher liver weight. However, group III (fed with 2000 ppm Mancozeb) shows a reduced liver weight. The results indicate that 1000 and 1500 ppm Mancozeb has no adverse effect on liver weight but 2000 ppm adversely affects it.

In the case of spleen group III shows significantly lower weight than controls. However. group I and II show better weight of spleen. This reveals that 2000 ppm Mancozeb will have adverse effect on the weight of spleen.

5 . 3 . 11 Gross and histo pathological examination

Birds fed with Mancozeb showed gross lesions in many of the internal organs. Focal area of sub capsular haemorrhage and diffuse grayish streaks of necrosis in the borders of liver were noted in birds belonging to group I and II. Liver tissue of birds belonging to group III showed linear streaks of petechiae. haemorrhage and haematoma in addition to the lesions in group II.

In spleen and kidney also focal area of petechiae were noted. During this period animal showed significantly higher level of AST and Alkaline phosphatase. Beghomini *et al* (1967) noticed enteritis, renal and hepatotrophy and bone fragility in broiler chicks after feeding 0.1- 1.0 percent Ziram, a related compound to Mancozeb. The present finding revealed that Mancozeb is toxic if fed for six months. at the rate of 1000 - 2000 ppm in the feed.

Histo pathological examination of liver, kidney, spleen, thymus and bursa of treated birds showed lesions (Fig. 26a to 26f). Necrotic changes were seen in the liver tissue of all the treated birds. These changes were more in group III (fed with more Mancozeb). Hore *et al* (1997) noted sinusoidal congestion of liver, congestion and haemorrhage on spleen, kidney and heart in rats after feeding Mancozeb (at the rate of 400 mg per Kg body weight)for 49 days. In the present study also, spleen showed dilatation and thickening of blood vessels, fibrous tissue proliferation and depletion of germinal centre.

Extensive vacuolation of tubular epithelium, haemorrhage in the interstetial tissue and calcification of endothelium of small arterioles were recorded in the kidney of treated birds. These changes are suggestive of Mancozeb toxicity. In thymus and bursa depletion of lymphoid element and fibrous tissue hyperplasia was seen.

SUMMARY

Chapter 6

SUMMARY

An investigation into the toxicity of dietary levels of copper sulphate, zinc sulphate and Mancozeb in ducks was carried out at the Department of Pharmacology and Toxicology, college of Veterinary and Animal Sciences, Mannuthy. One hundred and fiftysix, one month old clinicaly healthy White Peckin ducks from the same hatch were used for the study. The investigation was carried out in three phases with copper sulphate, zinc sulphate and Mancozeb supplemented feed.

In phase one, sixty ducklings were used. They were divided in to four groups of fifteen each. Group I, II and III were reared on feed supplemented with 100, 200 and 300 ppm elemental copper (as copper sulphate) respectively. Group IV was served as control and reared on the same feed of experimental group with out copper supplementation.

In phase two, fortyeight ducklings were used and they were divided into four groups of twelve each. The treatment groups I, II and III were reared on feed supplemented with zinc (as zinc sulphate) 100, 200 and 300 ppm respectively. Group IV was served as control and fed with the same feed of experimental groups with out zinc supplementation.

In phase three, forty eight ducklings were used. The treatment groups I, II and III were reared on feed added with Mancozeb 1000, 1500 and 2000 ppm respectively. The control group was reared on the same feed of experimental groups with out the addition of Mancozeb.

The parameters observed include 1) Body weight at fortnight intervals till the end (six months) of the experiments. 2) Haemogram which include total erythrocyte

count, total and differential leucocyte count, haemoglobin, erythrocyte sedimentation rate and packed cell volume. These were recorded at one month intervals. 3) Enzyme profile-include Aspartate amino transferase, Alanine amino transferase and Alkaline phosphatase in serum at monthly intervals. 4) The cell mediated immunity of birds were assessed at intervals of 45 days by PHA-P skin sensitivity test. 5) Humoral immunity was assessed by estimating the antibody production against ED-76 viral vaccine at 15 days intervals by HA and HI test. 6) The element content in the serum was estimated at monthly intervals 7) The element content in the tissue was estimated at the end of the experiment after sacrificing the birds. 8) Gross lesions of the internal organs and histo pathological lesions of lymphoid organs were noted .

The results revealed that the body weight of the birds were not significantly affected by feeding copper (100, 200 and 300 ppm) supplemented diet. However, all the treated birds showed a tendency for higher body weight gain than the controls. In all groups including the controls peak gain in body weight was seen on the 90th day of observation (ie the 4th month of age) which was also not significantly affected by copper.

Stimulation of erythropoiesis in ducks reared on feed supplemented with 100 ppm copper was observed. However, in group II and III (supplemented with 200 and 300 ppm copper respectively) this effect was reversed, revealing that supplementation of copper at 100 ppm level in the feed is ideal for erythropoiesis if fed for a longer period. The haemoglobin level was not significantly affected by copper at these levels. However, a decreasing tendency in haemoglobin value was observed in treated groups. The packed cell volume and leucocyte count were not significantly affected by these levels of copper. The variation in the differential count noticed between groups are within the normal ranges.

The enzyme Aspartate amino transferase, Alanine amino transferase and Alkaline phosphatase were showed differences between treated and controls at certain stages of experiment. This observation has no clinical significance, as these values have not deviated from the normal range (less than 3-4 times that of the controls).

The cell mediated immunity as evidenced by the PHA-P test did not show any significant difference between treated and controls. The humoral immunity assessed by HA and HI test was also not significantly different from controls. These data suggest that copper 100-300 ppm supplementation in the feed has no significant influence on the antibody production in ducks. However,an increasing tendency was seen in group I ,after booster dose of vaccination.

The serum copper level showed proportionate increase with respect to the duration of treatment and level of copper in the feed . Among tissues analysed for copper content the liver showed greater concentration than the kidney and the muscle. All the treated groups showed a dose dependent higher values. The weight of the liver and spleen was not significantly altered by the treatment.

In liver, kidney and spleen no gross lesion could be detected. Histo-pathological examination showed focal area of vacuolation and necrosis of tubular epithelium and thickening and congestion of small blood vessels in kidney of birds belonging to group III. In other birds no lesion could be detected.

Feeding of zinc at the rate of 100, 200 and 300 ppm as zinc sulphate in the diet showed no significant influence on the body weight. However, group II fed with 200 ppm zinc showed an increasing tendency in body weight gain. Group fed with 100 and 300 ppm zinc supplemented diet showed a decreasing tendency in body weight gain, indicating that 200 ppm supplementation is ideal for growth.

On erythrocyte count no significant difference was noticed. However group I showed an increasing and group III showed a decreasing tendency. This reveals that 100 ppm zinc supplementation is better for erythropoiesis. Haemoglobin level also showed a favourable influence in group I (fed on 100 ppm) more than the other groups. The erythrocyte sedimentation rate was not affected by zinc supplementation. Packed cell volume of all the treated group showed an increasing tendency . All levels of zinc supplementation showed a stimulating tendency on leucopoisis. Differential count was not affected by the treatment.

Zinc supplementation at the rate of 100 – 200 ppm for six months or 300 ppm for one month showed an increasing tendency on Alanine amino transferase level. The alkaline phosphatase level also showed an increasing tendency in all the three groups. The Aspartate amino transferase level was not influenced by these levels of zinc in the feed. This increase is not indicative of any tissue damage as differences are not enough for clinical significance. The cell mediated immunity showed no difference between treated and control group. However, in group III humoral immunity showed a favourable response after the fourth month.

The serum zinc levels of control group showed a gradual increase as age advanced. In the treated group a more rapid increase of the zinc level proportional to the level fed was noticed . Tissue concentration of zinc was significantly higher in liver of group I but it was not proportional to the serum zinc level. There was no significant difference in the kidney and muscle zinc content between treated and controls. The weight of the liver and spleen of treated groups was also not affected by treatment.

One animal in group III showed diffuse petechiae and moderate nodularity of the surface of the liver. Mild lymphoid infiltration in the liver , mild depletion of lymphoid cells in the spleen were observed in group III . Congestion and diffuse calcification of

endothelium of small blood vessels in the kidney of birds belonging to group II and III were also noted.

The body weight of ducks fed with 1000 ppm Mancozeb (group I) was not affected. However, in groups II and III fed with 1500 and 2000 ppm, the body weight was adversely affected. The erythrocyte count, haemoglobin, erythrocyte sedimentation rate and packed cell volume were not affected by the treatment. The leucocyte count of group I and II showed a tendency for stimulation for a short period. However, group III showed an adverse effect . The differential count was not affected by these levels of Mancozeb. The Aspartate amino transferase level of treated groups showed a tendency to increase for the first two months, and then there was a decreasing tendency. However, this has no clinical importance as the increase is less than 2-4 fold. Clinically significant higher level of Alanine amino transferase is noticed in group III which indicates that 2000 ppm Mancozeb causes some damage to membranes. The Alkaline phosphatase level of treated groups showed a tendency to increase for the first three months. The humoral and cell mediated immunity was not significantly affected by the Mancozeb 1000 - 2000 ppm .

The serum zinc concentration of the treated groups showed an increase proportional to the level of Mancozeb in the feed and the duration of treatment. This reveals that feeding of Mancozeb will increase the serum level of zinc. The manganese level in the serum was also increased up to the fourth month and then there was a reduction. Accumulation of zinc in the tissue was not significantly affected by the treatment. The accumulation of zinc was more in the muscle than in the kidney or in the liver. The manganese content in the tissue was also not significantly affected , though more concentration of manganese was seen in the liver tissue.

The weight of liver and spleen of ducks fed with 1000 and 1500 ppm Mancozeb showed a tendency to increase, but group III showed a decrease in the weight of these organs revealing that higher level of Mancozeb adversely affected the weight of the organs.

Birds belonging to all treatment groups showed gross lesions in internal organs. These include varying degrees of focal area of subcapsular haemorrhage, diffuse greyish streaks of necrosis in the borders of liver, linear streaks of petechiae, and haematoma in the liver, focal area of petechiae in the spleen and kidney. Histopathologically necrotic changes were seen in the liver tissue. Kidney showed extensive vacuolation of tubular epithelium, haemorrhage in the interstitial tissue and calcification of endothelium of small arterioles.

Conclusion

1. The body weight gain of ducks was not significantly affected by rearing on feed supplemented with 100, 200 and 300 ppm copper for six months. However, all the treated birds showed a tendency for higher body weight gain than controls.
2. For erythropoiesis, supplementation of copper at 100 ppm in the feed was found to be better.
3. The haemoglobin level, packed cell volume and leucocyte counts were not significantly affected by any of these levels of copper tested.
4. The enzyme, Aspartate amino transferase, Alanine amino transferase and Alkaline phosphatase levels were having differences between groups ,but they were not clinically significant.
5. The cell mediated and humoral immunity was not significantly affected by copper up to 300 ppm in the feed. However, an increasing tendency for antibody production was noticed in group fed with 100 ppm copper, after the booster dose of vaccination.

6. Rearing of ducks on feed supplemented with zinc at the rate of 100, 200 and 300 ppm showed no significant influence on body weight gain. However, group fed with 200 ppm zinc showed a tendency for higher body weight gain.
7. The erythrocyte count and haemoglobin level was better in group fed with 100 ppm zinc but not statistically significant.
8. The erythrocyte sedimentation rate and differential count was not affected by zinc at these dose levels.
9. The leucopoiesis showed a stimulating tendency in all the treated groups.
10. The humoral immunity showed a favorable response after fourth month in group fed with 300 ppm zinc.
11. It can be inferred that copper and zinc supplemented up to 300 ppm in the feed will not adversely affect the health status of the ducks.
12. The body weight of ducks fed with 1500 and 2000 ppm Mancozeb was adversely affected.
13. The erythrocyte count, haemoglobin, erythrocyte sedimentation rate, packed cell volume and differential counts were not affected by Mancozeb.
14. The serum level of Aspartate amino transferase and Alkaline phosphatase was not affected by the dose levels of Mancozeb used. However, Alanine amino transferase level showed a clinically significant increase at 200 ppm in feed indicating some damage to cell membranes.
15. High levels of Mancozeb above 1500 ppm adversely affected the weight of the internal organs. Gross and histopathological lesions were noticed in all the organs examined in the treated groups. It can be inferred that Mancozeb above 1000 ppm are deleterious to the health of ducks.

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COPPER SULPHATE, ZINC SULPHATE AND MANCOZEB TOXICITY IN DUCKS

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ABSTRACT OF THE THESIS

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ABSTRACT

An investigation was undertaken to assess the chronic toxicity of copper sulphate, zinc sulphate and Mancozeb in ducklings. The experiment was carried out in three phases. In the first phase four groups of ducklings were used. Birds in group I, II and III were reared on feed supplemented with 100, 200 and 300 ppm copper respectively. Group IV was reared on control feed. In the second and third phases the experiment was repeated with zinc and Mancozeb respectively. The latter was added at a rate of 1000, 1500 and 2000 ppm to diet.

The body weight of the ducklings was assessed at fortnight intervals. The erythrocyte count, haemoglobin, ESR, PCV, total and differential leucocyte count, serum AST, ALT and Alkaline phosphatase were noted at monthly intervals. The cell mediated immunity was recorded at intervals of forty five days. Humoral immunity was assessed by estimating the antibody titer at 15 days intervals. The element content in the serum and tissue was also recorded. The gross and histo-pathological lesions were noted at the end of the experiment after six months.

The results revealed that body weight of the ducklings was not significantly influenced by feeding copper at the above levels. However, all the treated birds showed a tendency for higher body weight gain than controls. Erythrocyte count was more in the group fed with 100 ppm copper and less in the group fed with 200 and 300 ppm. All the treated birds showed a tendency for low haemoglobin value. The PCV,ESR,total and differential leucocyte count were not significantly affected.

In all the groups the difference in the level of AST, ALT and Alkaline phosphatase was not clinically significant. Cell mediated and humoral immunity were not significantly altered by copper at the above levels. The serum copper level showed

an increase proportional to the duration of treatment and level of copper in the feed. Liver showed more concentration of copper than kidney and muscle. The birds in the group III showed histo-pathological lesion in the kidney.

Zinc supplementation in the feed also has no significant influence on the body weight gain. However, the group fed with 200 PPM zinc showed a tendency towards an increase of the body weight gain and the group fed with 100 and 300 PPM zinc showed a tendency towards a decrease in the body weight gain. The erythrocyte count and haemoglobin level showed an upward trend in group I. In all the treated groups the PCV, leucocyte count, serum ALT and Alkaline phosphatase showed an upward trend. In group III humoral immunity showed a favourable response.

A rapid increase in the level of zinc in the serum proportional to the level in the feed and period of treatment was observed. The tissue zinc concentration was significantly higher in the liver in group I, but it was not proportional to the serum zinc. Histopathologically, mild depletion of lymphoid cells in the spleen of group III, congestion and diffuse calcification of small blood vessels of kidney in group II and III were noticed.

The body weight of birds reared on Mancozeb added diet was affected adversely in groups II and III. The leucocyte count was reduced in group III. The erythrocyte count, Haemoglobin, ESR and PCV were not affected by the treatment. The serum ALT was increased significantly in group III. Alkaline phosphatase was increased for the first few months. Humoral and cell mediated immunity was not affected. Serum zinc and manganese level showed an upward trend. The accumulation of zinc was more in the muscles than in the kidney or liver. Most of the treated birds showed focal area of sub- capsular haemorrhage and streaks of necrosis in the borders of liver. Histo-pathologically necrotic changes were seen in the liver, kidney and spleen.

To

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As per the reference cited above the evaluation of thesis and the final viva-voce examination of Dr. Chandrasekharan Nair, A.M.was conducted on 24-9-99 and he has successfully passed the examination. The thesis with necessary corrections made along with the evaluation reports received from the external examiners (Dr Honne Gowda and Dr .Jayasekharan Samual) are forwarded herewith for necessary action.

Yours faithfully

Mannuthy.
24-9-99

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