PATHOLOGY OF ASCITES SYNDROME IN BROILER CHICKEN

By
JACOB ALEXANDER

THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
KERALA AGRICULTURAL UNIVERSITY

Department of Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY - THRISSUR
KERALA

1996

ASCITES SYNDROME

AN EMERGING THREAT TO BROILER INDUSTRY.....

DECLARATION

I hereby declare that the thesis entitled **"PATHOLOGY OF ASCITES SYNDROME IN BROILER CHICKEN"** 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.

Mannuthy
16.12.1996

JACOB ALEXANDER

CERTIFICATE

Certified that the thesis, entitled **"PATHOLOGY OF ASCITES SYNDROME IN BROILER CHICKEN"** is a record of research work done independently by **Sri. Jacob Alexander**, 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.

Dr. K.M. Ramachandran
(Chairman, Advisory Committee)
Professor and Head
Department of Pathology

Mannuthy

16.12.1996

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sri. Jacob Alexander**, a candidate for the degree of Master of Veterinary Science in Pathology, agree that the thesis entitled **"PATHOLOGY OF ASCITES SYNDROME IN BROILER CHICKEN"** may be submitted by Sri. Jacob Alexander, in partial fulfilment of the requirement for the degree.

Dr. K.M. Ramachandran
(Chairman, Advisory Committee)
Professor and Head
Department of Pathology

Dr. K.V. Valsala
Professor
Department of Pathology
(Member)

Dr. G. Krishnan Nair
Associate Professor
Department of Microbiology
(Member)

Dr. C.B. Manomohan
Associate Professor
Department of Pathology
(Member)

External Examiner

ACKNOWLEDGEMENT

I express my sincere gratitude to my guide and chairman of the Advisory Committee Dr. K.M. Ramachandran, Professor and Head, Department of Pathology, for his affectionate guidance, valuable suggestions and the strong support and inspiration during the entire period of my study.

I extend my heartfelt thanks to Dr. K.V. Valsala, Professor, Department of Pathology and Member of the Advisory Committee for her interest and encouragement evinced during my period of study and experimentation.

Special thanks are due to Dr. C.B. Manomohan, Associate Professor, Department of Pathology and Dr. G. Krishnan Nair, Associate Professor, Department of Microbiology, Members of the Advisory Committee, for their scholarly suggestions and guidance.

I am immensely grateful to Dr. A. Rajan, Dean, College of Veterinary and Animal Sciences, for the scientific enthusiasm and inspiration which evoked an interest in this research.

I am indebted to Dr. T. Sreekumaran, Professor and Dr. Koshy Verghese, Assistant Professor, Department of Pathology for their painstaking co-operation generously offered on many occasions.

My sincere thanks are due to Dr. N. Divakaran Nair, Dr. T.V. Anilkumar, Dr. B. Gangadharan, Dr. N. Vijayan, Dr. Mammen J. Abraham and Dr. T. Leena Devi, Assistant Professors and

Dr. C.R. Lalithakunjamma, Associate Professor, Department of Pathology for sharing their vast knowledge and rich experience.

I offer my thanks to Dr. Chaudhary, Dr. K.C. George and Dr. S.M. Vyas, Ph.D. Scholars, and to all staff members of the Department of Pathology for their valuable assistance, encouragement and friendship which enabled a fairly strenuous task to remain a pleasure throughout.

I am grateful to Dr. S. Sulochana, Professor and head, Department of Microbiology for the help rendered.

I offer my heartfelt thanks to Dr. S. Vigil Anbiah, Dr. Anilkumar, Dr. Ajith Jacob George, Dr. P. Biju, Dr. S. Silamban, Dr. S. Nándakumar and Dr. S. Mohan, M.V.Sc. Scholars, Department of Pathology and Mr. N.H. Mohan B.V.Sc. & A.H. scholar for their unhesitating response and timely help.

I wish to place on record my gratitude to Mr. Vimalan, Lecturer, LBS Computer Centre, for his expertise and help rendered in data processing and programming.

I profoundly appreciate with thanks, all my teachers, colleagues, fellow veterinarians and friends for their inspiration, goodwill and co-operation.

I am immensely grateful to my loving parents and to all members of my family for their constant encouragement and support in fulfilling this endeavour.

JACOB ALEXANDER

Dedicated to my beloved parents

CONTENTS

LIST OF ILLUSTRATIONS

GRAPH/PHOTOGRAPH

LIST OF TABLES

LIST OF ABBREVIATIONS

A	Auricle
AS	Ascites Syndrome
BW	Body Weight
CT	Connective Tissue
D	Day
DF	Degrees of Freedom
dl	decilitre
EM	Electron Microscope
ESR	Erythrocyte Sedimentation Rate
FI	Feed Intake
FC	Feed Conversion
GZD	Gizzard
g	grams
Hb	Haemoglobin
L	Litre
LNG	Lung
LV	Left Ventricle
ME	Metabolisable Energy
mEq	milli Equivalents
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MS	Mean Sum of squares
PALS	Peri Arterial Lymphoid Sheath
PAS	Periodic Acid Schiff
PCV	Packed Cell Volume
PELT	Peri Ellipsoidal Lymphoid Tissue
PH	Pulmonary Hypertension
pH	hydrogen ion concentration
PI	Post Inoculation
ppb	parts per billion
ppm	parts per million
PVS	Proventriculus
RBC	Red Blood Corpuscle
RE	Reticulo Endothelial
RV	Right Ventricle
RVF	Right Ventricular Failure
RVH	Right Ventricular Hypertrophy
RV:LV	Right Ventricle:Left Ventricle
RV:TV	Right Ventricle:Total Ventricle
SE	Standard Error
SPF	Specific Pathogen Free
SDS	Sudden Death Syndrome
USDA	United States Departments of Agriculture
WBC	White Blood Corpuscle

Introduction

1. INTRODUCTION

Ascites syndrome (AS) in broiler chicken is characterized by the development of tense fluid filled abdomen, respiratory distress, dilatation and hypertrophy of the right ventricle, increased haematocrit and haemoglobin values. This disease has emerged as one of the major causes for mortality in the broiler flocks of many countries. (Coello *et al.*, 1985; Hernandez, 1987). Chick oedema disease, toxic fat syndrome (dioxin toxicity), high altitude disease, oedema disease, water belly, ascites, ascites syndrome and pulmonary hypertension syndrome are synonyms. However, the name "ascites syndrome" appears to have more popularity. It was initially described from areas of high altitude and later on this was also reported from low altitude areas from different parts of the world including India. Recently the disease was recorded in several broiler farms from different parts of Kerala.

AS is the major cause of economic loss in broiler flocks in several countries. In Mexico the loss due to mortality alone was 40,000,00 dollars in 1984 (Coello *et al.*, 1985). In India, at present there is rapid growth in the organised broiler sector. According to available data, poultry production in India in 1993 was estimated to be 235 million birds and poultry meat production was 454,000 tonnes. The broiler production is expected to double itself in 1996 in a span of three years.

Poultry meat accounts for 45 per cent of the total meat output. As the changing life style of the population with increase in intake of non vegetarian food is already resulting in an unprecedented consumer boom, a considered growth of the annual production is expected. The mortality due to AS can fluctuate from below one per cent to upto 30 per cent in a flock. This can cause serious economic loss and instability in broiler production, hampering the achievement of set targets. In Kerala also the prevalence of ascites syndrome is increasing. Hence an investigation on the disease was taken up.

The broiler is more predisposed to this disease genetically and the constraints of environment in housing increases the prevalence. Certain feed additives, feeds and type of feeding have always been suspected/proved to precipitate this disease. A preliminary investigation conducted at the Department of Pathology also suggested this fact. Hence a study on the incidence, managemental aspects, symptoms, pathology and microbiology were carried out in spontaneous field cases and the same was compared by feeding excess of different feed additives to ascertain their role in precipitating ascites syndrome.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Epidemiology

Ascites as a sequelae to right ventricular failure occurs world wide in growing broiler chicken and is a significant cause of mortality in many flocks. The disease was first reported in high altitude areas and more recently in low altitude areas (Riddel, 1991).

2.1.1 High altitude

Hall and Machicao (1968) reported this disease in broiler chicks reared at high altitudes in Bolivia, subsequently Cueva *et al.* (1974) reported from Peru; Buys and Barnes (1981), Dupreez (1985) and Huchzermeyer (1985) from South Africa; Coello *et al.* (1982) from Mexico; Ashfeque *et al.* (1983) from Pakistan, Julian (1983) from Canada; Power *et al.* (1984) from Ireland; Maxwell *et al.* (1989) from Kenya and Banday and Maqbool (1992) and Kannan and Singh (1994) from India.

2.1.2 Low altitude

Swire (1980) reported ascites in flocks at low altitude in the UK, subsequently Riddel (1985) and Julian *et al.* (1986) reported from Canada; Huchzermeyer *et al.* (1988) from South Africa; Sarakbi (1988) from Yemen; Cowen (1989) from Italy; Gowda *et al.* (1989, 1990); Prajapathi and Solanki (1994) and

Biswas *et al.* (1995) from India; Shlosberg *et al.* (1991) from Israel; Lu *et al.* (1992) from Taiwan; Griffith (1993) from Barbados; Madej *et al.* (1992) from Poland and Martinez *et al.* (1993) from Urugay.

2.2 Incidence

Hall and Machicao (1968) reported an incidence of 3.5, 6.1, 11.8 and 12.0 per cent in four lots of birds on a single farm situated at an altitude of 10,800 ft. Julian (1983) reported the incidence as one per cent in broiler flocks and in roaster flocks occasionally more than 10 per cent in Ontario. Coello *et al.* (1985, 1987) reported 15 to 30 per cent flock mortality in Mexico. In an experimental rearing to find out the incidence, mortality was 10 per cent with a peak occurring at seven weeks of age (Coello *et al.*, 1987).

Fitz-Coy and Harter-Dennis (1988) reported that ascites accounted for approximately 4.62 per cent of the causes of mortality. The highest incidence was seen in the broilers during the cooler months and following medication with furazolidone. The incidence was four times greater than hatch mates brooded under warmer temperatures. Twice as many male broiler chicks had ascites as female hatch mates.

Sarakbi (1988) reported an incidence of 10 to 30 per cent with mortality upto 30 per cent.

Gowda *et al*. (1989) reported that the occurrence was more frequent in the age group of four to five weeks.

Julian (1990b) reported that the incidence of ascites was above one per cent in some broiler flocks and occasionally 15 to 20 per cent in roaster flocks.

Witzel *et al*. (1990) recorded an incidence of 13, 27 and 80 per cent at simulated altitudes of 1980, 2438 and 2896 m respectively.

The incidence appeared to be on the increase (Riddell, 1991).

Shlosberg *et al*. (1991) reported that ascites syndrome in broilers took a very high toll in the industry during winter in Israel. The condition was prevalent in fast growing strains of broilers, particularly males from 4 weeks of age.

Banday and Maqbool (1992) reported a mortality of 3, 1, 5, 1 and 3 per cent for the 2nd, 3rd, 4th, 5th and 6th weeks respectively in a farm in Srinagar.

Prajapathi and Solanki (1994) recorded 5.5 to 10.6 per cent with an average of 7.17 per cent mortality in nine broiler flock ranges. Analysis of poultry autopsies in 1992-94 showed 9.5 per cent cases. Cases were recorded from 1st week to 7th week with peak incidence at 5th week of age. Males were more

susceptible (86.6%) compared to females. Most cases (75.7%) were recorded during winter months.

The syndrome occurred most frequently in rapidly growing birds with approximately 70 per cent of the affected being males. Peak mortality occurred between five to seven weeks of age (Walker, 1994).

Biswas *et al.* (1995) recorded four per cent cases in selected areas in West Bengal, which was most common in the age group of four to eight weeks. The mortality was 100 per cent. The occurrence was higher in the saline belt of West Bengal during winter months.

2.3 Symptomatology

The death of birds in ascites syndrome was sudden (Swire, 1980).

The affected birds were listless and smaller than normal with ruffled feathers and cutaneous congestion. Severely affected birds had abdominal distention, dyspnoea and were reluctant to move (Maxwell *et al.*, 1986a; Fitz-Coy and Harter-Dennis, 1988; Wilson *et al.*, 1988; Albers *et al.*, 1990; Gowda *et al.*, 1990; Banday and Maqbool, 1992; Squires and Summers, 1993; Biswas *et al.*, 1995).

Julian *et al.* (1989) experimentally induced ascites syndrome with low ambient temperature and noted that almost half of the chicken that died from right ventricular failure (RVF) became cyanotic before fluid appeared in the abdomen or hepatoperitoneal cavities. A few of these chicken and 30 per cent of the total number that died from RVF, died without showing any other premonitory signs. About half of this group had no ascites. In chicken that developed ascites, fluid accumulated very rapidly once it was noted.

Julian (1990b) suggested that not all broilers that died from pulmonary hypertension (PH) had ascites. Death occurred suddenly before clinical signs were observed or after a short period of dyspnoea and the affected broilers frequently died on their back.

Banday and Maqbool (1992) also reported that some of the affected birds died suddenly during handling.

Squires and Summers (1993) also noted that the affected birds had pale shrunken combs and red skin due to congestion of the peripheral vessels. They had decreased tolerance to exercise.

Odom (1993) stated that many broilers died without having a moderate to severe accumulation of fluid in the abdominal cavity. Broilers which died rather acutely did not exhibit the

same degree of fluid accumulation and severity of other lesions as the broilers exhibiting chronic ascites syndrome.

Prajapathi and Solanki (1994) reported that the clinical signs were suggestive of cardiac and pulmonary insufficiency.

Biswas *et al.* (1995) also reported that the affected birds showed shrunken comb, loss of feathers in the abdominal region and oedema of head.

2.4 Susceptibility of broilers

Hall and Machicao (1968) suggested that broiler breeds were more susceptible to heart failure than layer breeds. Some broiler strains were more susceptible than other strains (Coello *et al.*, 1985; Huchzermeyer *et al.*, 1988; Julian *et al.*, 1989; Martinez *et al.*, 1991; Odom *et al.*, 1992).

The low hypoxia resistance of chicken was related to their relatively low haematocrit values, low oxygen carrying capacity (Rostofer and Rigdon, 1947) and to the inefficient oxygenation of the fowl lung (Sykes, 1960).

Atland (1961) found that domestic chicken had a much lower altitude tolerance than other small warm-blooded animals previously studied.

Morphometric parameters of the lung of the domestic fowl were remarkably out of line with those of bird in general (Maina, 1982).

Julian *et al.* (1987) indicated that fast-growing chicken exhibited elevated metabolic rates and a faster blood flow together with higher oxygen requirements.

The basal metabolic rate per gram of tissue of a young leghorn chicken was actually higher than a broiler of the same age, but the total tissue mass of the broiler was greater. Assuming that the respiratory capacity of both chicken was about equal, the amount of energy required to meet the growth potential of broiler chicken might be creating oxygen demands near physiologic limit of the respiratory system. Birds differ from mammals in that portal blood flow also passes through the kidneys. Thus renal disease or any alteration in intra renal portal blood pressure might also contribute to ascites in birds (Hoerr, 1988).

The right ventricle (RV) in birds was very thin walled and had developed as a volume pump, not a pressure pump. It responded to increased work load by dilatation and hypertrophy. The right atrioventricular valve was a muscle flap made up mainly from fibres from the RV. It also thickened along with the RV wall in response to increased work load and this

probably predisposed the heart of birds to RVF from valvular insufficiency (Julian, 1990a).

Vidyadaran *et al.* (1990) found out that the volume of the lung per unit body weight of the domestic bird was 20 to 33 per cent smaller than that of the wild bird. The domestic fowl had partly compensated for this by increasing the surface area for gas exchange per unit volume of exchange tissue. However the blood gas tissue barrier was about 28 per cent thicker in the domestic fowl than in the red Jungle Fowl and this had lead to a 25 per cent lower anatomical diffusing capacity for oxygen of the blood gas tissue barrier per unit body weight in the domestic fowl. These structural characteristics may make the modern domestic fowl vulnerable to stress factors such as altitude, cold, heat or air pollution by predisposing to hypoxemia and perhaps thence to ascites.

Mirsalimi and Julian (1991) stated that the lung was a fixed organ in the rib cage in birds. It expanded and contracted very little compared with the mammalian lung. The blood capillaries were small and dilated very little to allow more blood flow. They also stated that broilers had larger erythrocytes and reduced erythrocyte deformability, when compared to leghorns and this, they suggested was one of the predisposing factors that increased resistance to blood flow, and altered the rheology of blood in the microcirculation in

the lung which resulted in pulmonary hypertension, heart failure and ascites in broiler chicken. They also attributed fast growth, and hypoxaemia to the increased susceptibility of broiler chicken.

Scheele *et al.* (1991) noted that selection created birds with less tolerance to high fat diets which required more oxygen for combustion than carbohydrate.

The lungs of chicken grew much less rapidly than the rest of the body and lung capacity was not proportional to the very rapid growth of muscle in fast growing broiler chicken (Julian, 1990c).

Julian and Mirsalimi (1992) recorded that the per cent oxygen saturation was significantly higher in light chicken than in heavy chicken and that the per cent oxygen saturation was significantly higher in groups with normal hearts than in the group with RVF from valvular insufficiency.

Air sacs located in the abdominal cavity were of great importance in regulating the air flow rate entering and leaving the avian lung (Akester, 1978). Fast growing meat-type chicken were hypoxaemic, because the abdominal mass interfered with the bellous effect of the air sacs, causing hypoxic hypoxaemia by reducing tidal volume (Julian and Mirsalimi, 1992).

Martinez *et al.* (1992) compared the diameter of the right atrioventricular valve, left atrioventricular valve, aortic semilunar valve and pulmonary semilunar valve in Arbor Acres and Indian River which were considered susceptible to ascites syndrome, with single comb White Leghorns. The latter had significantly less diameter than the former two. However at three weeks of age the diameter of the pulmonary semilunar valve was significantly higher in the leghorns.

Yersin *et al.* (1992) suggested that two populations of chicks existed within any given hatch; good quality chicks which exhibited no cardiac or respiratory dysfunction and poor quality chicks, which exhibited right ventricular dilatation and abnormal electrocardiogram.

2.5 Pathological features

2.5.1 Haematology

There was an increase in total RBC count, packed cell volume (PCV), haemoglobin and mean cell volume (MCV) in ascitic birds (Hall and Machicao, 1968; Sandino and Hernandez, 1985; Maxwell *et al.*, 1986a; Hernandez, 1987; Odom *et al.*, 1989; Maxwell *et al.*, 1990; Owen *et al.*, 1990; Witzel *et al.*, 1990; Banday and Maqbool, 1992; Shlosberg *et al.*, 1992; Yersin *et al.*, 1992; Mirsalimi and Julian, 1993; Julian and Squires, 1994; Kannan and Singh, 1994 and Biswas *et al.*, 1995.

Witzel *et al.* (1990) also reported an increase in MCH and MCHC with ascites in increasing altitude.

There was a marked increase in blood volume in RVF (Burton and Smith, 1972; Cai *et al.*, 1984).

Maxwell *et al.* (1986a) found that the WBC counts were significantly raised in five week old birds suffering from ascites. Heterophils and monocytes were also increased in these birds at the expense of lymphocytes.

Banday and Maqbool (1992) also reported that the number of heterophils showed a significant increase in ascitic birds. The increase in MCV they got was not statistically significant.

USDA researchers found a lower blood pH and per cent oxygen saturation (Walker, 1994).

Biswas *et al.* (1995) reported that the leucocyte count was high due to absolute increase of lymphocyte.

2.5.2 Biochemistry

2.5.2.1 Blood/plasma/serum and tissues

2.5.2.1.1 Protein and albumin

Broilers with ascites from RVF showed low blood protein, mainly due to reduced albumin (Cardenas *et al.*, 1985).

Odom *et al.* (1989) noted an increase in plasma protein in their electrocardiographic studies in the development of ascites in broilers.

2.5.2.1.2 Enzymes and minerals

Maxwell *et al.* (1990) found that lactate dehydrogenase was increased in the heart muscle of ascitic broilers. Witzel *et al.* (1990) reported that the aspartate aminotransferase, gamma glutamyl transferase and corticosteroid activities were significantly reduced at high altitude of 2,896 m, perhaps reflecting a terminal condition in the chicken. Earlier peak values were not determined.

Yersin *et al.* (1992) experimentally induced ascites in simulated high altitude and found that the enzyme activities of gamma glutamyl transferase, aspartate transferase, alkaline phosphatase and the concentrations of cholesterol and albumin were depressed. There was an increase in plasma corticosterone. Serum inorganic phosphorous levels were elevated but total serum calcium was depressed.

Enkvetchakul *et al.* (1993) found that the lung and liver concentration of ascorbic acid, tocopherol, glutathione and uric acid were lower in birds which showed overt symptoms of ascites due to low ventilation. However uric acid

concentration was higher in the lung and serum at fifth week and in the liver at seventh week.

Maxwell *et al.* (1995) found that the serum troponin T values increased but serum creatine showed no change in 30 day old ascitic birds when compared with control.

2.5.2.2 Ascitic fluid

Hall and Machicao (1968) reported that the specific gravity of ascitic fluid was 1.016 or more.

Prajapathi and Solanki (1994) reported that the ascitic fluid was having a neutral pH, with an average protein content of 3.01 g which increased with age.

2.5.3 Gross pathology

There was variable amount of straw coloured fluid and clots of fibrin in the abdomen. The liver was swollen and congested, or firm and irregular with oedema and had clotted protein adherent to the surface; it might be nodular or shrunken; it might be white with oedema and show fibrosis under the capsule, or had large or small blobs of oedema in the hepatoperitoneal sacs. There was mild to marked hydropericardium and, occasionally, there was pericarditis with adhesions. There was right ventricular dilatation and mild to marked hypertrophy of the right ventricular wall. The right

atrium and vena cava were very dilated. Occasionally, there was thinning of the left ventricle. The lungs were extremely congested and oedematous (Hall and Machicao, 1968; Coello *et al.*, 1985; Fitz-Coy and Harter-Dennis, 1988; Wilson *et al.*, 1988; Gowda *et al.*, 1989, 1990; Julian *et al.*, 1989; Albers and Frankenhuis, 1990; Fraser, 1991; Banday and Maqbool, 1992; Squires and Summers, 1993).

Hall and Machicao (1968) also reported acute myocarditis and nodular thickening of the endocardium, particularly in the region of the right atrio-ventricular value. Coello *et al.* (1985) also reported lung damage accompanied by pneumonia and atrophy of the bursa. Gowda *et al.* (1989) also reported atrophy and narrowing of the lumen of intestine with thick slimy brownish mucus. The mesenteric blood vessels were engorged and contained watery unclotted blood. Gowda *et al.* (1990) also reported that there was mosaic appearance of spleen.

Banday and Maqbool (1992) reported that there was moderate to severe skeletal muscle congestion and that the spleen was small.

Griffith (1993) reported that the pancreas usually showed a whitened marbled appearance.

2.5.4 Histopathology

Changes in the myocardium were mild. There was oedema of the myocardium and slight proliferation of loose connective tissue in some areas. Myocardial fibres showed pallor of cytoplasm and atrophy or hypertrophy, with variation in size of myofibres and their nuclei. There was an increase in the number of heterophils between myocardial fibres and fibrosis of the atrial endocardium (Hall and Machicao, 1968; Maxwell *et al.*, 1986a).

There was diffuse oedema and congestion in the lung and an increase in interstitial connective tissue. Hypertrophy of smooth muscle within the parabronchial wall was prominent and was accompanied by collapse of the atria. Increased number of cartilaginous and bony nodules were present in the pulmonary parenchyma (Hall and Machicao, 1968; Maxwell *et al.*, 1986a).

In the liver there was dilatation of periacinar sinusoids and atrophy of intervening hepatocytes. There was a decrease in the amount of vacuolation of hepatocytes, and cholangioles were frequently filled with bile. Focal necrosis of hepatocytes was present. Proliferation of fibrous tissue within the capsule was often accompanied by dilatation of capsular lymphatics. Similar changes occurred in the capsule of kidney, spleen and pancreas (Hall and Machicao, 1968; Maxwell *et al.*, 1986a, 1988; Hernandez, 1987; Wilson *et al.*, 1988).

Hall and Machicao (1968) reported focal haemorrhages in the myocardium and acute haemorrhagic myocarditis which was occasionally associated with oedema of the endocardial leaflets. There was moderate infiltration of leucocyte in the periglomerular and interstitial region of the kidney.

Maxwell *et al.* (1986a) also reported that the kidneys might have congested glomeruli with thickened basement membranes and scattered foci of lymphocytes.

Maxwell *et al.* (1988) reported that histologically the lung nodules contained more chondroitin sulphate, proteoglycans than keratin sulphate. Haemosiderin was also demonstrated. In lungs of ascitic birds, when there was a high incidence of nodules, large areas of the parenchyma contained bundles of non mineralised collagen fibres. Osteoclast were not associated with any type of nodules.

Gowda *et al.* (1990) reported bile duct and endothelial proliferation, fatty changes in the liver, congestion and degenerative changes in kidneys and haemorrhages with edema in lungs.

2.5.5 Ultrastructural studies

Maxwell *et al.* (1986b) reported a degeneration of the cardiac myofibrils with heterophil infiltration and a marked reduction in myocardial glycogen. There was dilatation of the

lung parabronchi and associated air spaces with thickening of the alveolar and capillary walls. The kidneys contained elongated or ring-shaped mitochondria and there was excess lipid in the tubules and thickening of the glomerular basement membranes. The livers were depleted in glycogen and showed light and dark degenerative cells and infiltration of heterophils.

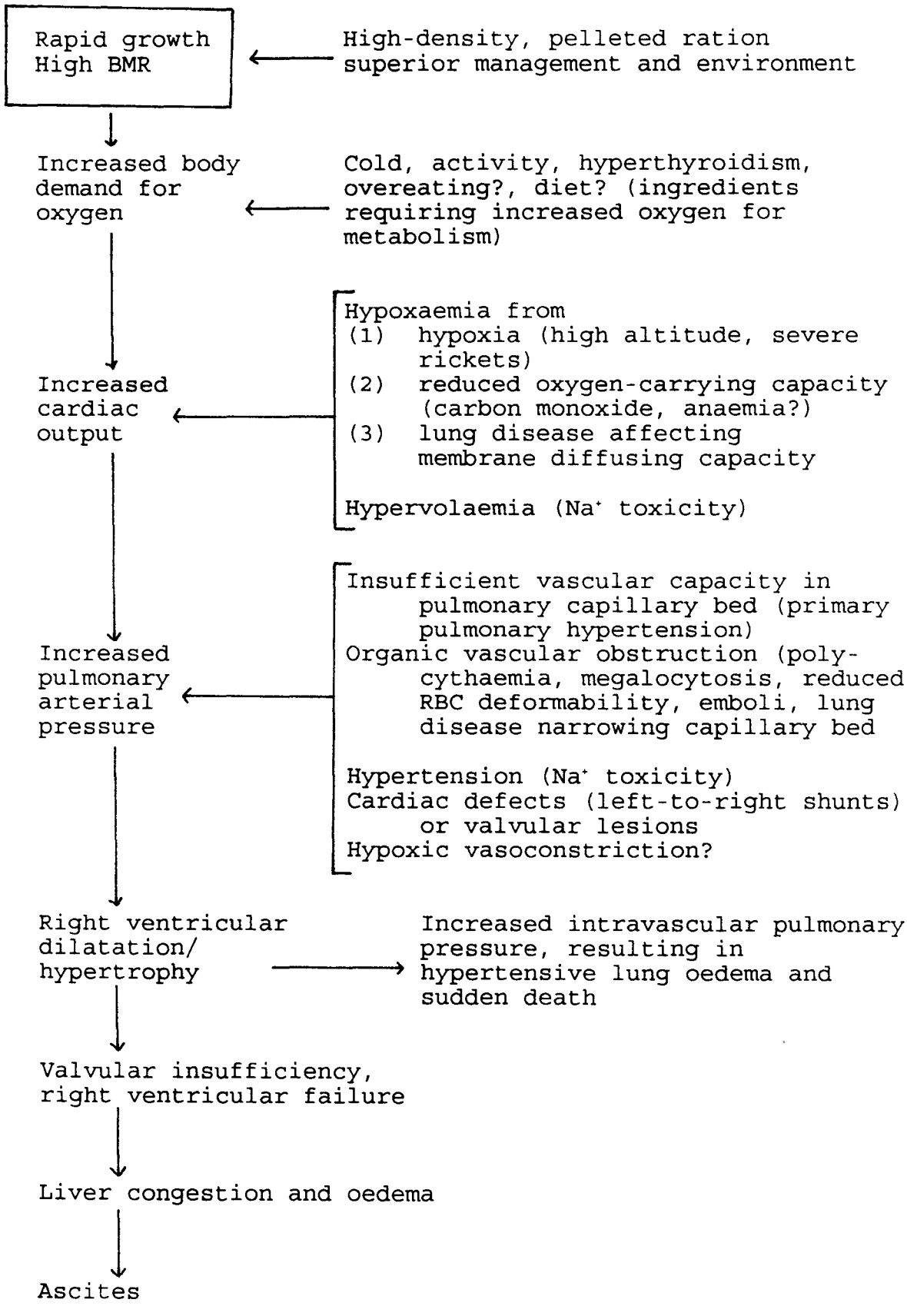
Virus particles were also seen by Maxwell *et al.* (1986b) in broilers with ascites. These particles were identified as retrovirus by Payne *et al.* (1991).

Maxwell *et al.* (1988) studied the ultrastructure of the lung nodules in ascitic birds and described the structure of four types namely hyaline, fibrous, mineralised cartilaginous and the osseous type.

Maxwell *et al.* (1993) reported deposits of Ca^{2+} located extensively in the mitochondria, sarcoplasmic reticulum, golgi apparatus and sometimes in myocyte and endothelial cell nuclei.

2.5.6 Pathogenesis

Since the aetiology of ascites was of multifactorial origin, pathogenesis also varied. However development of pulmonary hypertension and heart failure could be considered to be a major factor in the development of ascites syndrome in broilers which was depicted diagrammatically (Julian, 1990a).


Rapid growth
High BMR
High-density, pelleted ration
superior management and environment
Increased body demand for oxygen
Cold, activity, hyperthyroidism, overeating?, diet? (ingredients requiring increased oxygen for metabolism)
Increased cardiac output
Hypoxaemia from
(1) hypoxia (high altitude, severe rickets)
(2) reduced oxygen-carrying capacity (carbon monoxide, anaemia?)
(3) lung disease affecting membrane diffusing capacity
Hypervolaemia (Na+ toxicity)
Increased pulmonary arterial pressure
Insufficient vascular capacity in pulmonary capillary bed (primary pulmonary hypertension)
Organic vascular obstruction (polycythaemia, megalocytosis, reduced RBC deformability, emboli, lung disease narrowing capillary bed
Hypertension (Na+ toxicity)
Cardiac defects (left-to-right shunts) or valvular lesions
Hypoxic vasoconstriction?
Right ventricular dilatation/ hypertrophy
Increased intravascular pulmonary pressure, resulting in hypertensive lung oedema and sudden death
Valvular insufficiency, right ventricular failure
Liver congestion and oedema
Ascites

2.6 Experimental studies

2.6.1 Sodium chloride/Common salt

Seyle (1943) reported that sodium chloride produced nephrosclerosis in chicks due to the extreme sensitivity of chicks to the presence of comparatively small doses of sodium chloride in the drinking water.

Doll *et al.* (1946) observed the toxic effects of sodium chloride solution in baby chicks.

Scrivner (1946) produced experimental oedema and ascites in poults by administering sodium chloride and sodium bicarbonate in feed or water in certain proportions.

Bressler *et al.* (1951) experimented in turkey poults with different doses of salt levels in the mash and found that added salt at the 0.9 per cent levels and above resulted in a considerable mortality from ascites and visceral edema. The addition of salt to the ration upto the 1.8 per cent level had a favourable effect on body weight. Levels of 3.6 per cent salt were definitely detrimental to body weight. Increased water consumption was associated with increased salt level. Increases in the levels of salt tended to reduce the feed intake during the first and second weeks of age. During the third week, groups upto the 1.8 per cent salt level seemingly made an adjustment to the excess salt in the mash. Feed

consumption in the 3.6 per cent salt groups was definitely below normal at all times, particularly during the second and third weeks.

Mohanty and West (1969) gave 1 per cent sodium chloride in distilled drinking water for 10 days and then 1.5 per cent for the remainder of the six week experimental period in Babcock strain of chicks and made the following observations. Water consumption increased gradually and was accompanied by marked deterioration in condition and anorexia. Most of the chicks exhibited nervous disorders. By the third week, the mean average weight of the surviving experimental chicks was only about half that of the control group. All chicks had poor musculature, oedematous subcutaneous tissue, and variable amounts of fluid in the pericardial sac, thoracic cavity, and peritoneal cavity. Microscopic changes in kidney, liver, spleen, pancreas, adrenals, esophagus, crop, proventriculus, small intestine, lungs, heart, skeletal muscles, and central nervous system included degeneration with very little inflammatory reaction. Encephalomyopathy consisted of neuronal vacuolation, degeneration and necrosis, perineuronal and periglial oedema, and proliferation of oligodendroglial cells. Eosinophilic meningitis, primarily involving the lumbar segment of the spinal cord, was observed in only four cases. The cellular reaction was principally eosinophilic, with some lymphocytes, large mononuclear cells, and plasma cells.

Vacuolar and necrotic changes were also seen in the ganglionic cells of the adrenal medulla.

Onderka and Bhatnagar (1982) induced round heart disease and ascites in eight-day old turkey poults by administering 0.75 per cent sodium chloride in distilled water. Clinical signs included increased water consumption, loose feaces, depression and increased respiratory rate. Mortality was noticed after three days in the saline solution. Light microscopic and electron microscopic studies were made on the right and left free cardiac ventricular walls. Massive glycogen accumulations were demonstrated by periodic acid - Schiff staining and malt diastase digestion. electron microscopic studies further showed the presence of extensive intra fibrillar glycogen, myofibrillar disarray, lysis of myofilaments, Z-band streaming, and disruption of intercalated discs.

Swayne *et al.* (1986) reported that a four per cent mortality in five to eleven day old turkey poults was attributed to 1.85 per cent sodium chloride in the feed. The syndrome included peracute respiratory distress, ascites and sudden death that resembled peracute heart failure. Clinical signs were observed only in the final phase of the toxicity, but progressive histologic lesions were found. Live, apparently unaffected poults showed increases in intracellular glycogen and cytoplasmic granularity, loss of striation and

early mild intercellular myocardial oedema. Similar but more severe histologic lesions were seen in live, ascites affected poults. The ascites-affected poults had hydropericardium and hydrothorax which seemed to develop just minutes before death. Ultrastructurally, focal areas of myocardial cells exhibited myofibrillar disarray, lysis of myofilaments, widened Z bands, and dilatation of sarcoplasmic reticulum.

Julian (1987) grew one hundred commercial male broiler chickens upto 27 days in four floor pens on a commercial diet containing 0.14 per cent sodium (Na^{++}). From day six each pen received different levels of sodium chloride in the drinking water, 0.0 per cent, 0.15 per cent (0.06% Na^{++}), 0.3 per cent (0.12% Na^{++}) and 0.6 per cent (0.24% Na^{++}). Eight chicks from each group were killed at 13, 20 and 27 days and examined for right ventricular hypertrophy (RVH) and RVF. By day 27 as little as 0.06 per cent added Na^{++} caused ten cases of RVH and one case of RVF with ascites, typical of the ascites caused by RVF in commercial broilers.

Mishra *et al.* (1987) conducted an experiment with White Leghorn male chicks to study the effects of high level of salt in the diet. The dietary salt level ranged from 0.41 to 6.51 per cent. The weight gain in chicks was affected significantly by the dietary treatments. The water intake was proportionately increased with more intake of sodium in the diet. Higher intake of dietary salt caused diarrhoea, ascites

and generalised oedema. Chicks were more susceptible within first two weeks of age. Diet with 6.51 per cent salt had highest mortality. With increase in the intake of sodium there was proportionate increase in plasma sodium concentration. The plasma potassium level exhibited a reverse relationship with plasma sodium. Extensive damage was done to liver, kidney, skeletal muscle, heart and brain of chicks fed more than 5 per cent salt in the ration.

Pimental and Cook (1988) suggested that salt improved immune levels and growth at 0.25 per cent.

Fields *et al.* (1991) reported that sodium causes hypertension induced left ventricular hypertrophy in human beings.

Howell and Gumbrell (1992) reported salt poisoning in broiler chicken. Thirst, diarrhoea, weakness, laboured, breathing, hydropericardium and non fibrinous ascites, lung congestion and oedema, and cystic testes were noticed.

2.6.2 Furazolidone

Blount (1955) described patchy enteritis and cardiac degeneration in chicks with nitrofurazone toxicity. Newberne and McEven (1957) observed nervous symptoms in chicks with nitrofurazone toxicity.

Harwood *et al.* (1958) found 563 ppm (512 g/ton) furazolidone toxic to male chicks. They reported that 30 per cent died when fed this concentration continuously for the first four weeks of life. Ascites was observed.

Klimes and Kruza (1962) reported field cases of furazolidone induced cardiomyopathy and ascites in ducks.

Feron and Van Stratum (1966) reported a syndrome of cardiomyopathy associated with cardiac dilatation and ascites in chicks fed furazolidone at 400 ppm.

Webb and Vanvleet (1971) and Reed *et al.* (1987) stated that in furazolidone induced pathogenic mechanism of ascites, right ventricular failure was due to degenerative change in the muscle of the right ventricular wall and there was no evidence that pulmonary hypertension was involved.

Jensen *et al.* (1975) reported that turkeys and chicks differed markedly in cardiac response to toxic levels of furazolidone. Furazolidone did not induce anaemia in either species. Added furazolidone of 300 or more ppm significantly depressed body weight below than that with 100 ppm furazolidone in chicks.

Vanvleet and Ferrans (1983) induced cardiomyopathy and ascites in ducklings fed graded amounts of furazolidone. Reed and Vanvleet (1988) reported furazolidone induced cardiomyopathy and ascites in broilers.

Bahgat *et al.* (1990) on an investigation in ascites outbreak showed a high level of furazolidone (400-500 ppm) in feed analysis. Ascites, hydropericardium, congested liver,

pale kidney, flabby and enlarged heart were the most common gross lesions. Microscopical examination revealed perivascular fibrosis, degenerative changes of the hepatic cells and focal lymphocytic aggregation, together with hyperplasia of the bile ducts in the liver. The kidneys revealed severe degenerative changes of the tubular epithelium, focal lymphocytic aggregations in the interstitial tissue and hypercellularity of the glomerular tuft. In the heart there was oedema, eosinophilic cellular infiltration and occasionally hyalinization and necrosis of the myocardial muscles. The intestinal mucosa showed catarrhal inflammation.

Webb *et al.* (1991) found significant difference between control and treated group in creatine kinase MB (CK-MB, cardiac muscle origin) isoenzymic activity and bilirubin, potassium, calcium and total carbon dioxide concentrations in the sera of ducklings fed 700 ppm furazolidone in feed for 28 days.

O'Brien *et al.* (1993) observed mild increases in serum urate concentration, liver and muscle enzyme activities, but moderately increased sodium concentration with decreased chloride concentration in turkey poults with furazolidone cardiomyopathy. They also attributed the moderate to marked hypoproteinemia to decreases in albumin and globulin concentrations.

2.6.3 Monensin

Stuart (1978) reported that monensin fed in excess of 200 ppm caused mortality in turkeys aged 23 weeks and above. The birds showed signs of drowsiness, excessive thirst and panting, later they developed a flaccid paralysis and became prostate. Most birds had small pale spleen, pale areas in the myocardium and haemorrhages on the heart fat. Other birds had pale kidney and a few had congested lungs.

Hanrahan *et al.* (1981) noted that chicken treated at seven weeks of age with 150, 200 or 230 mg of crystalline monensin sodium per kg body weight had signs of toxicosis including extreme weakness, anorexia, paralysis and death. Gross lesions included emaciation, generalized congestion, myocardial enlargement and pallor and hydropericardium. Intermyofibrillar vacuolation, histochemically positive for neutral fat, was severe in the myocardium and red muscle fibres. Mitochondrial degeneration was apparent in myocardial sections from several chickens. Interstitial infiltration by macrophages and heterophils was common in the myocardium and aerobic skeletal muscle.

Gregory *et al.* (1992) reported a case of monensin poisoning in ostriches. Four mature ostriches collapsed and were unable to stand or walk. Two died within 48 hours.

Cardona *et al.* (1993) produced necrotizing skeletal myopathy in turkeys with monensin.

Cash (1993) reported an unsuccessful attempt to reproduce knock down syndrome in turkeys by feeding monensin upto 140 mg/kg of feed.

Khan *et al.* (1993a) reported monensin slightly increased Se and significantly increased Pb and Fe concentrations in the liver of broiler chicks fed 240 and 250 ppm in feed.

Khan *et al.* (1993b) reported that concurrent administration of selenium and monensin at toxic levels resulted in greater toxicity in broiler chicken.

Szarek and Khan (1993) suggested that there was an interaction between Pb, Se or monensin in elevation of porphyrins in the liver tissue of broiler fowls.

Watkins *et al.* (1993) reported that monensin fed to toms at either 100 or 140 ppm reduced the feed intake in birds fed ad libitum. Feed efficiency was linearly improved by increasing levels of dietary monensin regardless of feeding regimen. Monensin had no influence on the incidence of mortality.

Watkins and Novilla (1994) reported that when toms were fed excess monensin, there was no effect on feed intake

regardless of feeding and watering regimen. It had no effect on the weight gain and feed efficiency of ad libitum fed birds.

Bartov (1994) fed seven to twenty eight days old male broiler chicken diet containing monensin 150 mg/kg. Monensin reduced feed intake, weight gain and feed conversion efficiency. Monensin did not affect utilization of dietary dry matter, fat or energy, but significantly decreased nitrogen utilization. The relative length of the small intestine was increased but decreased its specific weight. It did not affect yield of the defeathered eviscerated carcass or relative liver size.

Khan *et al.* (1994) reported that 40 mg of monensin per kg body weight on alternate days for four weeks in broiler chicks caused a reduction in haematocrit and an increase in blood serum levels of alanine amino transferase, aspartate amino transferase and cholesterol.

Weisman *et al.* (1994) reported monensin toxicity in laying hens in which they were fed 800 ppm for four days. The birds lost appetite and were not able to stand, eat or drink and their combs became blue. The egg production declined, with severe mortality.

2.6.4 Cobalt

Cobalt induced polycythemia in both man and animals (Miller *et al.*, 1974; Underwood, 1975) and it was demonstrated that cobalt exerted its effect by stimulating the production of erythropoietin.

Cobalt induced cardiomyopathy in man and in animal species including swine, dog, and rats (Underwood, 1975; Van Vleet *et al.*, 1977; Unverferth *et al.*, 1983).

Van Vleet *et al.* (1977) reported cardiomyopathy and moderate mortality in pigs given three daily doses of cobalt as sulphate. Myocardial damage was more prominent in the atria and ultrastructural alterations included mitochondrial swelling, dilatation of the segments of sarcoplasmic reticulum, interstitial oedema, lipid deposition, myofibrillar degeneration and fibrosis. Gross examination showed mild to moderate hydropericardium and the hearts were either mottled or pale.

Ducklings fed cobalt (200 or 500 mg/kg as chloride) developed necrosis of skeletal muscle and smooth muscle of the gizzard and intestine, similar to that induced by Vitamin E and selenium deficiency. No lesions were seen in cardiac muscle (Van Vleet *et al.* , 1981).

Microscopic analysis of myocardium of cobalt treated dogs revealed increased subendocardial and subepicardial fibrosis (Unverferth *et al.*, 1983).

Levels of 50-500 ppm of cobalt in the diet of chicken are considered toxic, specially if the diet is low in iron (Puls, 1988). Depressed growth rate had been reported as the most important sign of excessive cobalt intake in chicken (Southern and Baker, 1981; Hill, 1979).

Diaz *et al.* (1994) induced right ventricular hypertrophy and right ventricular failure and ascites (18.3%) by feeding cobaltous chloride at 500 ppm in feed to meat-type chicken from one day old for 42 days. There was significant increase in haemoglobin content and, to a lesser extent RBC count and PCV. No effect was observed on MCV. Increased haemoglobin content was linearly correlated with PH as measured by the right ventricle weight to total weight ratio (RV:TV). Levels of malondialdehyde in cardiac tissue were also correlated with the RV:TV ratio, suggesting that peroxidative damage might be related to ventricular hypertrophy.

2.6.5 Transmission studies

2.6.5.1 Ascitic fluid

A virus was isolated from the caecal tonsils of three to six week old white leghorn pullets with ascites. The virus was

recovered in seven day old specific pathogen free (SPF) embryonating chicken eggs and typically caused embryo deaths between three to nine day post inoculation (PI). Pathological alterations in the embryo consisted of ascites, hydropericardium, enlargement of the heart and diffuse non suppurative myocarditis. Inoculation of one day old SPF chicks with embryo ascitic fluid resulted in clinical and pathological alterations identical to those observed in the white leghorn pullets. Ten days PI the birds exhibited swollen abdomens, had dyspnoea and became prostrate after exertion. The heart was pale and flabby, there was marked pulmonary edema, hydropericardium and ascites. Liver was small and turgid. Myocarditis was considered the principal lesion and other lesions were consistent with congestive heart failure (Reed and Winterfield, 1985).

2.6.5.2 Liver suspension

Ahmad *et al.* (1992) investigated the routes of transmission of Angara disease (also known as hydropericardium syndrome). Two week old broiler chickens were inoculated with a 20 per cent suspension of infected liver filtrate subcutaneous and intra muscularly causing 80 per cent and 50 per cent mortality respectively.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Survey studies

Chicken brought for autopsy to the Department of Pathology from various farms during the period 1993-95 were examined in detail for AS. The post mortem reports maintained at this centre during the period 1985-95 were also made use of for the study.

3.2 Field case study

Field cases were studied at their farm premises. The management practices, nature of disease and symptoms were closely watched.

Blood and ascitic fluid samples were taken from ailing birds. Detailed post mortem examination was done in dead and sacrificed birds and their organs were collected for histopathology. Pieces of liver were collected aseptically for transmission studies.

Samples were taken for bacteriological study from the ascitic fluid, pericardial fluid, liver and heart blood and streaked on to Mueller Hinton agar (Himedia, Bombay). The isolates obtained were subcultured and characterised by the methods described by Cowan (1974).

Feed samples were collected and the aflatoxin content was estimated as per the method of Pons and Goldblatt (1969).

Water samples collected from wells were estimated for their sodium content by the procedure given in the manual of Perkin Elmer Company, USA in the Perkin Elmer 2380 atomic absorption spectrophotometer.

3.3 Experimental studies

3.3.1 Experimental design

Two separate experiments were conducted at different periods (October-December and April-June).

3.3.2 Experiment - 1

Fifty one-day-old Vencob strain of broiler chicks were maintained on a standard compounded broiler feed free of aflatoxin. Samples of feed were taken from different bags before procurement and analysed for aflatoxin as per the method of Pons and Goldblatt (1969).

The chicks were divided at random at two weeks of age into four groups. Three groups consisted of twelve birds each and the fourth one had fourteen chicks. The latter was kept as control. The treatment groups were given furazolidone 800 ppm (Merind, India Ltd.), sodium chloride 25,000 ppm (Analytical grade (99.9% pure, E. Merck India Ltd.) or monensin sodium 360 ppm (Coban 100 premix Venky's) in feed.

3.3.3 Experiment - 2

Eighty one-day-old Vencob strain of broiler chicks were maintained on a standard compounded broiler feed free of aflatoxin as in experiment 1.

The chicks were divided at random at two weeks of age into eight groups of ten each. The treatment groups I-V were given furazolidone 1000 ppm (Merind India Ltd.), common salt 25,000 ppm (Vinayaka Salt Company, Tuticorin), cobalt chloride 600 ppm (Glaxo Laboratories, India Ltd.) (cobaltous chloride - 97% pure), cobalt nitrite 600 ppm (E. Merck India Ltd. 97% pure) or cephalexin 800 ppm (Glaxo India Ltd.) in feed. Ascitic fluid (see 3.3.8) and liver suspension (see 3.3.9) were given to groups VI and VII at a graded dose (two birds each were given this preparation at a dose of 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml at the second week as a single intraperitoneal injection). Group VIII was kept as control.

Blood was collected by jugular venipuncture at fortnightly intervals on starting the treatment.

All groups were maintained under identical condition except for the feed additives. Feed and water were given *ad libitum*. The birds were regularly watched for clinical symptoms. The body weight was taken at weekly intervals.

3.3.4 Haematological studies

Five ml of blood was collected from each bird and one ml in 10 per cent EDTA solution was used for haematological studies. Procedures described by Schalm (1975) were followed for the determination of erythrocyte sedimentation rate (ESR) and PCV. Haemoglobin was estimated by the cyanmethaemoglobin method described by Miale (1972).

3.3.5 Serum studies

Blood (3-4 ml) was used for serum separation. The separated serum was centrifuged and stored at -70°C. Biuret assay method of Inchiosa (1964) was used for estimating total protein and BCG dye binding method for albumin. The albumin content was subtracted from that of total protein to get the globulin content. Sodium and potassium were estimated by the procedure given in the manual of Perkin Elmer Company, U.S.A. in the Perkin Elmer 2380 atomic absorption spectrophotometer.

3.3.6 Studies on ascitic fluid

Total protein, albumin, sodium and potassium content in the ascitic fluid was estimated by the same procedures followed in serum studies. The specific gravity was determined and the centrifuged sediments were examined microscopically.

3.3.7 Preparation of glasswares and utensils

All glasswares and utensils used for blood and ascitic fluid collection, serum separation and dilution for the

estimation of sodium and potassium were cleaned and subsequently washed thoroughly in Extran neutral (E. Merck, India Ltd.), rinsed in deionised water and then in double distilled water and dried in hot air oven.

3.3.8 Preparation of ascitic fluid

Ascitic fluid from field cases was collected under sterile conditions and was stored at -70°C. Pooled samples of this were taken and an antibiotic-antifungal solution containing streptomycin, penicillin, gentamicin and nystatin were added at the rate of 250 μg, 250 IU, 100 μg and 250 IU respectively per millilitre of ascitic fluid and incubated for half an hour at 30°C.

3.3.9 Preparation of liver suspension

Pieces of the liver from field cases were collected under sterile conditions and stored at -70°C. A 10% suspension of the pooled sample was made in sterile normal saline solution, centrifuged and the supernatent was processed as for ascitic fluid.

3.3.10 Gross and histopathological studies

The live birds at the eighth week were killed by exsanguination. Detailed postmortem examination was conducted on all the dead as well as killed birds. Tissues were

collected and preserved in 10 per cent formalin and processed by routine paraffin embedding technique (Luna, 1968). All the tissue sections were stained with hematoxylin and eosin (Bancroft and Cook, 1984) and wherever necessary, duplicate sections were also stained with special stains namely PAS, Van Gieson's, Trichrome and Phosphotungstic acid hematoxylin (PTAH) (Disbrey and Rack, 1970).

3.4 Organ weight

The weight of different organs was taken to correlate the effect of the treatments. The weights of liver, intestine, and that of proventriculus and gizzard together were taken immediately on slaughter with their contents.

Other organs comprising of heart, right and left lung, spleen, brain, bursa and kidney were collected and preserved in 10 per cent formalin. They were rinsed in three changes of tap water, their adnexa trimmed and blotted gently in a filter paper until no water came out and then weighed.

The weight of the liver comprised of gall bladder with its contents; spleen - with its capsule; kidney - both right and left together and in lungs - the bronchi was cut close to the lung and the air sacs removed. Weight of the right ventricle; heart without blood and heart without auricles were weighed separately.

The weight of the right ventricle was subtracted from heart without auricles to get the weight of left ventricle with septa.

The weight of heart without auricles was subtracted from the weight of heart without blood to get the weight of auricles.

3.5 Relative organ weight

The weight of bursa, spleen, brain, left lung, right lung, kidneys, liver, proventriculus + gizzard and intestine were divided by the weight at day 56 (D56) and multiplied by 100 to get their relative organ weights. The percentage of weight of heart, right ventricle, left ventricle, total ventricle and auricle to body weight and the heart were taken respectively. The weight of right ventricle was divided with that of left ventricle to get RV/LV and right ventricle was divided with the value of total ventricle to get RV/TV.

3.6 Virological study

Pooled samples of ascitic fluid were centrifuged at 3,000 rpm for 30 mts at 4°C and the sediment (debris) was discarded. The supernatent fluid was centrifuged at 35,000 rpm for 90 mts in the Sorvall Combi Plus fixed angle refrigerated ultracentrifuge. The pellet formed was broken and resuspended in 0.5 ml of phosphate buffered saline and charged on to formvar coated EM grids. These grids were stained with potassium phosphotungstic acid (pH 7) and screened in the Hitachi H600A transmission electron microscope upto 1 lakh magnification to detect virus.

3.7 Analysis

The data were analysed using the methods described by Snedecor and Cochran (1967).

Results

4. RESULTS

4.1 Survey studies

The prevalence of ascites syndrome in broiler chicken based on the records maintained at the Department of Pathology during the period 1985-1995 is shown in Fig.1.

4.2 Field case study

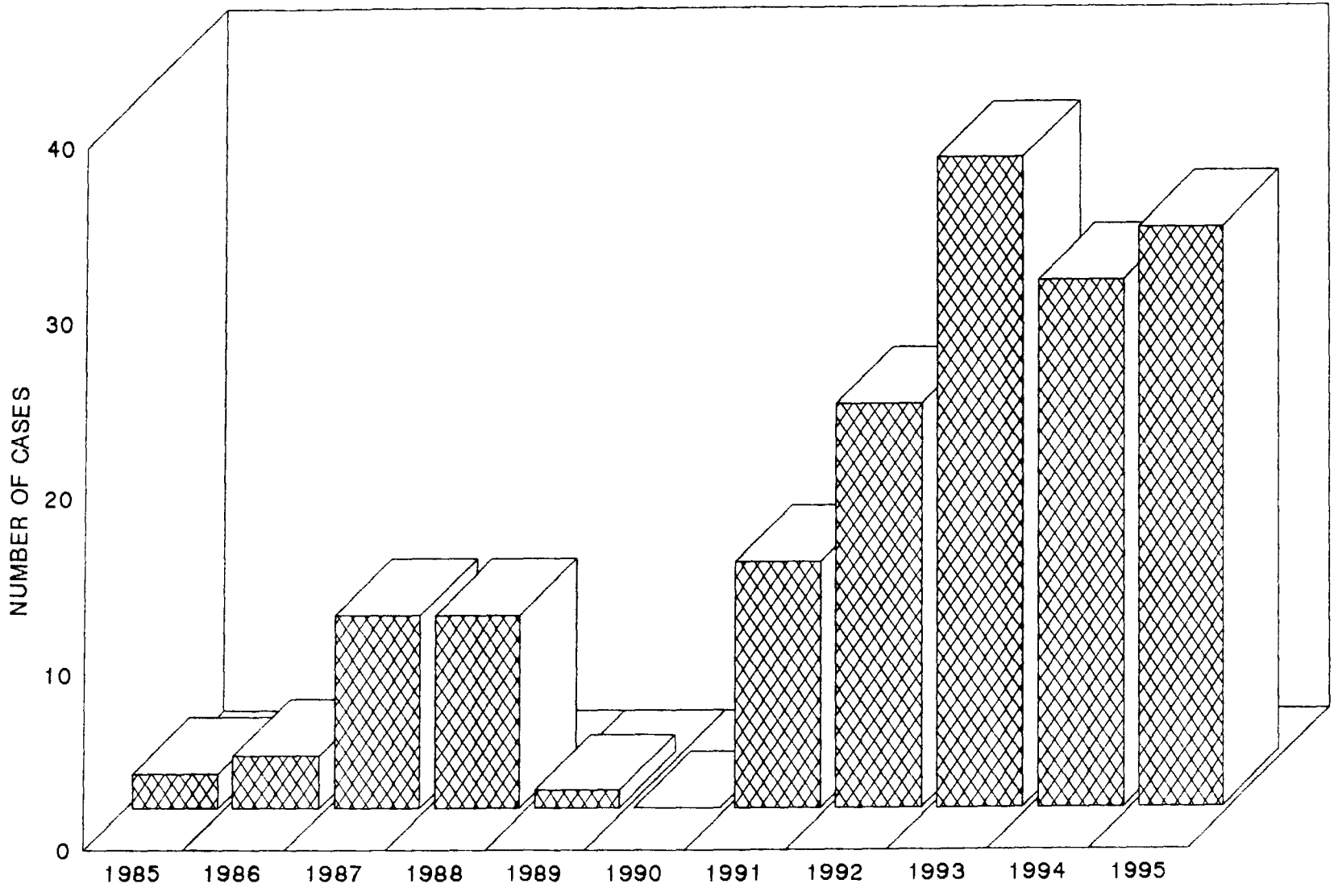
During the period of investigation (1993-95) the ascitic birds and feed samples which were brought for examination from various farms to the Department of Pathology formed the basis of this study. Fifty four cases were presented. Out of these ten farms were visited personally for further investigation.

4.2.1 Management practises

In all the farms visited, there was not much deficiency in the management practises, on the contrary they were better. Most of the farms had thatched roof which controlled excessive heat. There was no over crowding in the chicken houses.

In one case the novice farmer tried an experimentation with his one week old chicks. He divided the flock of 100 birds into two groups of 50 each. One group was given a commercial starter feed while the other was given the feed he

Fig.1 PREVALENCE OF ASCITES SYNDROME IN BROILER CHICKEN FROM 1985 TO 1995



compounded. There was 36 per cent mortality due to ascites syndrome in the group fed home made feed. On questioning he revealed that he had added salted fish available in the local market instead of unsalted fish and five per cent of common salt instead of 0.5% in the feed. The feed also contained 200 ppb of aflatoxin.

In all other farms, aflatoxin level was either nil or well within the permissible limits. The well water in all the farms was tested for sodium content and was found to be within limits.

4.2.2 Clinical symptoms

The affected birds showed distended abdomen and difficulty in breathing. The few recovered ones had stunted growth. The mortality rate was high in summer and winter seasons. In most cases birds were seen dead while in some cases death occurred within one or two days after ascites was noticed. The mortality rate varied from one to thirty per cent in different farms.

4.2.3 Clinical pathology

The haemoglobin and haematocrit, protein, albumin, globulin, albumin-globulin ratio, potassium and sodium content in both serum and ascitic fluid, specific gravity of the

ascitic fluid, aflatoxin level in the feed and sodium content in well water are shown in Table 1.

4.2.4 Gross pathology

The body condition of those birds which died suddenly was good while those which died later after development of ascites were poor. There was passive venous congestion all over the body including the breast and thigh muscles. The head was oedematous and sometimes haemorrhage was seen subcutaneously above the occipital bones. In some cases haemorrhage was also seen in the occipital bones. The venous plexus in between the abdomen and the cloaca was congested in all cases and bled while the skin was peeled (Fig.24).

The lungs were severely congested and oedematous. In one case a hard thrombus was noticed in an artery when the lung was cut.

There was hydropericardium in most of the cases. The fluid was clear straw coloured and the amount ranged from two to four ml. There was right ventricular dilatation and mild to marked hypertrophy of the right ventricular wall. The auricles were distended with blood (Fig.25). The sinus venosus was also distended with blood in all the cases. Pale areas were noticed in the myocardium of two birds.

Liver was swollen and congested and was firm to cut. In one case it was nodular. Focal pale areas and thickened capsule were noticed in most cases (Fig.25). In few cases liver surface had fibrinous clots over its surface which easily got detached.

Kidneys were severely congested with focal areas of haemorrhage in some birds. There were also pale nephrotic patches in most of the birds. In one bird there was collection of clear fluid under the capsule.

Spleen appeared normal in most birds but was severely congested in very young chicks.

Bursa showed congestion in most birds. The plicae were hyperaemic and oedematous in one bird.

The blood vessels of the intestinal serosa and mesentery were congested.

The meningeal blood vessels were congested.

4.2.5 Histopathology

Lungs

Lungs showed severe diffuse congestion, haemorrhage and emphysema. Groups of chondroblasts of varying sizes (8-70 μ) were seen in between the alveoli as well as the secondary and

tertiary bronchial structures (Fig.34). These structures took red colour in Van Gieson's staining while they were not positive for PAS staining. There was mild proliferation of loose alveolar connective tissue along the interlobular space, peripheral to the bronchi and around the blood vessels (Fig.35).

Pink stained albuminous material was noticed in the inter-parabronchial septa. Complete loss of bronchial epithelium was observed in some birds. A thrombus was present in a major pulmonary vessel in one case (Fig.36). Hypertrophy of smooth muscle within the parabronchial walls was noticed in most cases.

Heart

Moderate congestion and loss of striation at few places were noticed in the left ventricle.

The fibres of the right ventricle showed separation and loss of striation at certain places. Nucleus appeared to be highly vesicular in some areas. Mononuclear infiltration, congestion and mild haemorrhages were seen in isolated areas.

Liver

The sinusoids and portal as well as central veins were congested and dilated. Vacuolar degenerative changes of the

hepatocytes were seen at focal areas. Nuclear changes like pyknosis were prominent (Fig.37). Thickening of the Glisson's capsule due to vascular connective tissue proliferation was seen in most cases and these were seen as red areas in Van-Gieson's staining (Fig.38). PAS positive granules were seen diffusely in the cytoplasm of the hepatocytes in the sub capsular areas and occasionally in patchy areas within the parenchyma (Fig.39).

Kidney

Congestion of all blood vessels including interstitial as well as glomerular capillaries was seen. Varying degrees of vacuolar degeneration and desquamation of the tubular epithelial cells were also noted. Dilatation of Bowman's space and thickening of the basement membrane of the glomerular tuft were noticed in some birds.

Spleen

Congestion of all blood vessels including the sinusoids was noticed in most of the birds. Lymphoid depletion and decrease in the size of white pulp was also seen. Thickening of the blood vessel wall was also noticed.

Bursa

Congestion was noticed in all blood vessels including those in the cortico-medullary junction. Uniform mild to moderate lymphoid depletion was seen in the follicles.

Brain

There was severe congestion and oedema of the cerebrum and cerebellum (Fig.40). Congestion was also noticed in the meninges and choroid plexus.

4.3 Bacteriological study

Isolates were got from only two birds which were identified to be *Bacillus subtilis* and *Bacillus cereus*. The rest of the samples were sterile.

4.4 Virological study

On scanning the charged EM grids, no virus was detected.

4.5 Transmission study

Ascites syndrome was not reproduced in both the groups given ascites fluid and liver suspension.

EXPERIMENT - I

4.6 Clinical symptoms

4.6.1 Sodium chloride

The birds developed shooting diarrhoea within six hours of ingestion of feed. Their appetite, water and feed intake were more than the other groups. However, this declined after the seventh week. Their skin was hyperaemic and their abdomen appeared to be fluid filled, partly due to the fluidity of the contents in the intestine. As the birds become aged the hyperaemia disappeared. On post mortem examination at the eight week, none showed ascites.

4.6.2 Furazolidone

These birds were notably susceptible to stress. Some of the birds developed inco-ordinated gait and twisting of head subsequent to the stress of weighing them. Two birds developed a peculiar wheezing sound and on palpation they had fluid filled abdomen. One of them died showing typical lesions of ascites syndrome at the 45th day of treatment (Fig.28) while the other bird recovered. Two birds developed weakness of leg.

4.6.3 Monensin

This group exhibited reduced feed intake and body weight gain. Their droppings had lesser water content and hence the litter was always dry. None of them developed symptoms of ascites syndrome.

4.6.4 Control

Eventhough they had the highest growth rate none of them developed ascites.

4.7 Body weight

At D21, D28 and D56 the control group had more body weight than all other groups ($P<0.01$). At D35, D42 and D49 the furazolidone and monensin groups had lesser weight than the control and sodium chloride groups ($P<0.01$) (Fig.2); (Table 2 and 16).

4.8 Clinical pathology

4.8.1 ESR

No significant variation was noticed at D15 and D28, eventhough the ESR varied at D42 which was proportional to their body weight ($P<0.01$). However this variation differed at D56 (Fig.3); (Table 3 and 17).

4.8.2 PCV

Sodium chloride group had higher PCV than all other groups at D28 and D42 ($P<0.01$). Furazolidone group had the lowest PCV but it shot up on D56 ($P<0.05$) (Fig.4); (Table 3 and 17).

4.8.3 Haemoglobin

Sodium chloride group had higher haemoglobin at D28 and D42 and it declined at D56. The haemoglobin of control had a steady increase. Furazolidone group had the lowest haemoglobin ($P<0.01$) (Fig.5); (Table 4 and 17).

4.8.4 Serum protein

The protein of sodium chloride group declined gradually upto D42 and then got slowly raised. Control group had the highest protein value all throughout. Protein value of furazolidone decreased steadily after D28 while that of monensin fluctuated (Fig.6); (Table 4 and 17).

4.8.5 Serum albumin

Albumin of sodium chloride group decreased while that of monensin increased. The value for the control group was almost steady. In furazolidone group, it fluctuated (Fig.7); (Table 5 and 17).

Fig.2 BODY WEIGHT OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

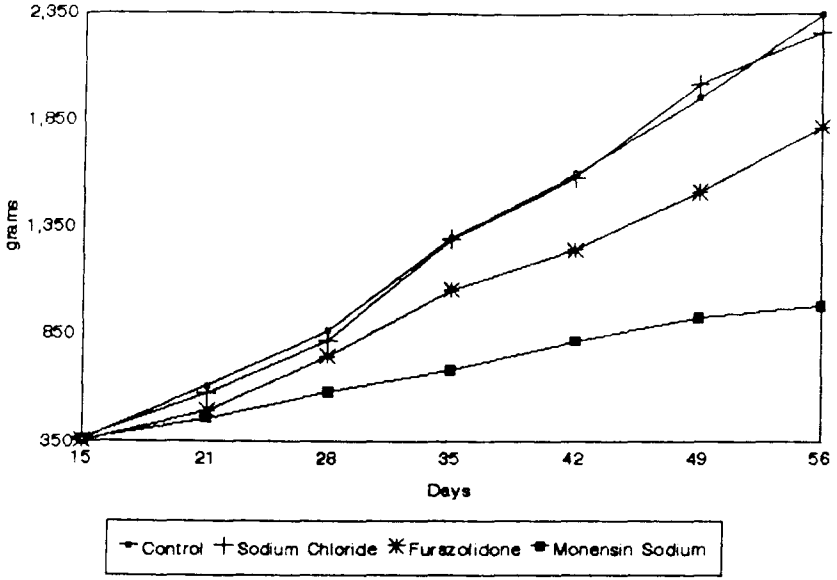


Fig.3 ESR OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

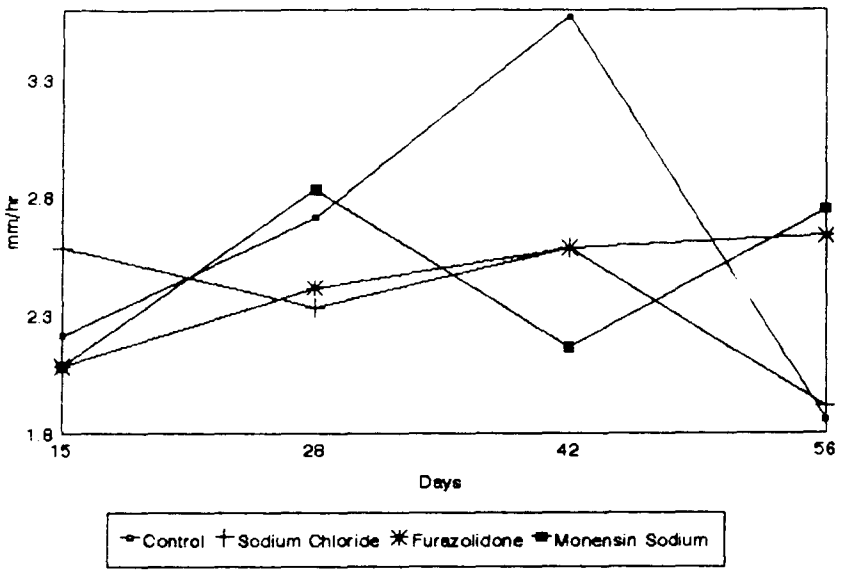


Fig.4 PCV OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

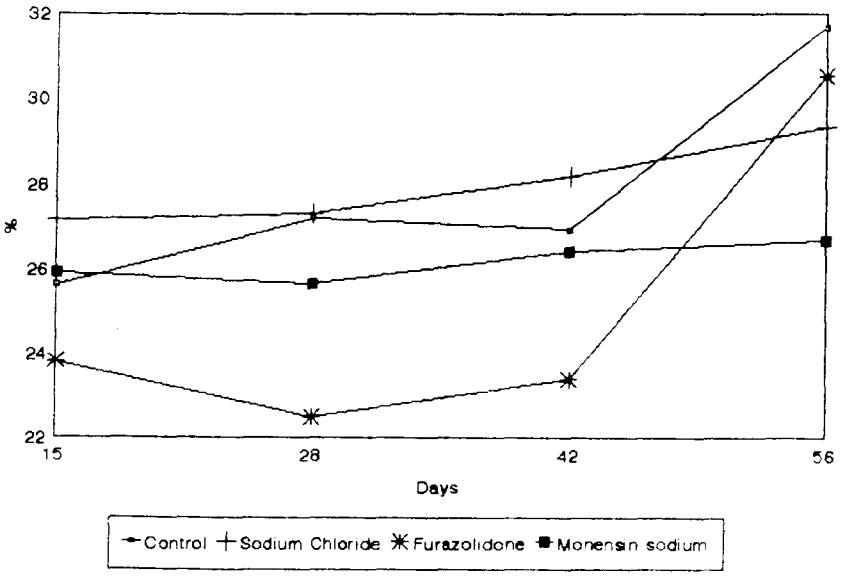
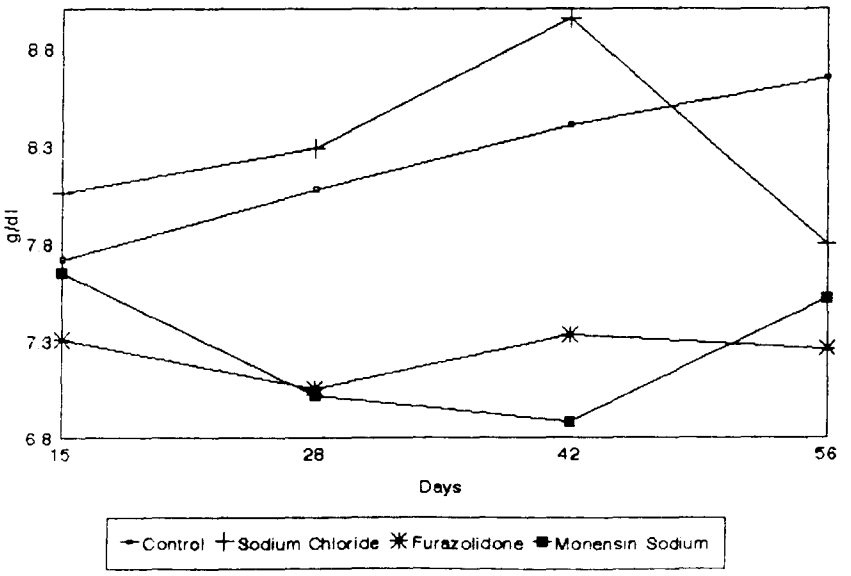


Fig.5 HAEMOGLOBIN OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM



4.8.6 Serum globulin

Globulin value of sodium chloride group decreased steadily while that of furazolidone group had a steady increase till D28 and then gradually decreased. Control group had the highest values while that of monensin group fluctuated (Table 5 and 18).

4.8.7 Albumin-globulin ratio

The albumin-globulin ratio did not vary significantly. The ratio in monensin group increased but was not significant (Table 6 and 18).

4.8.8 Serum sodium

Generally sodium chloride and monensin group had higher values than the control group which kept on increasing with age except for sodium chloride at D56 (Fig.8); (Table 6 and 18).

4.8.9 Serum potassium

Serum potassium levels of monensin increased on D28 and D42 and then decreased. All other groups had a steady increase with age. Generally monensin and sodium chloride groups had higher values than the control group except at D56 (Fig.9); (Table 7 and 18).

Fig.6 SERUM PROTEIN OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

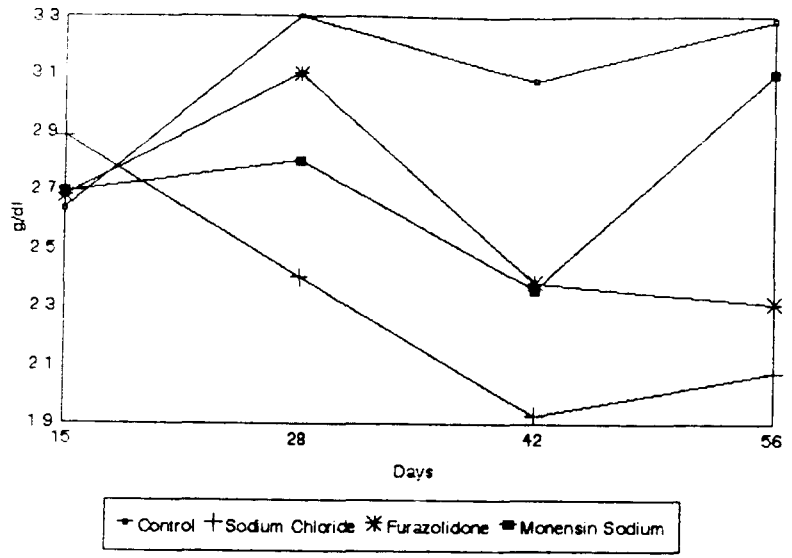


Fig.7 SERUM ALBUMIN OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

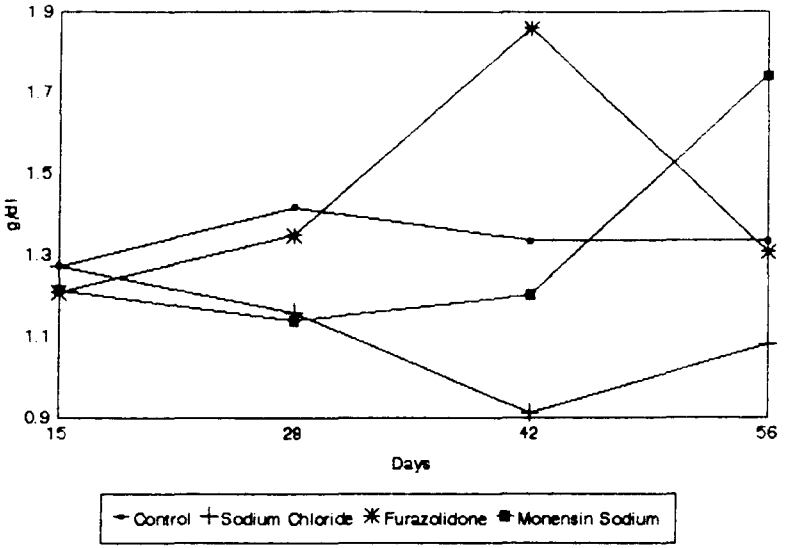


Fig.8 SERUM SODIUM OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

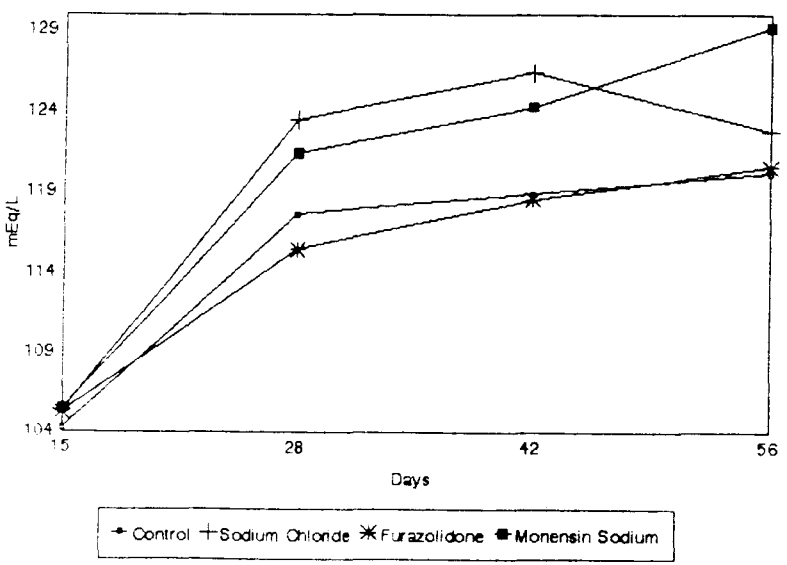
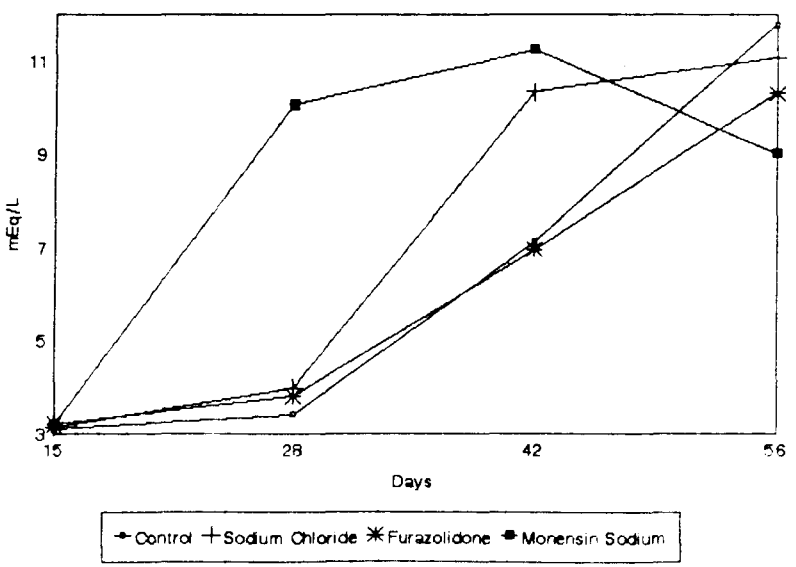


Fig.9 SERUM POTASSIUM OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM



4.9 Gross pathology

4.9.1 Sodium chloride

The body condition of the birds were fair but not as juicy and glossy as the control birds. There was moderate congestion and oedema of the lungs. The epicardial vessels were seen more prominently and in a few cases moderately increased amount of pericardial fluid was noticed. The liver was enlarged and showed diffuse areas of degeneration, oedema and congestion. Kidney also showed areas of nephrosis, mild congestion and oedema. The tubules were prominently seen. Spleen appeared to be bigger in size. Bursa was small in most of the birds. The intestinal contents were watery. The brain showed moderate congestion and oedema.

4.9.2 Furazolidone

The body condition of the birds were fair but not as good as the control birds. Lungs of few birds showed severe congestion. Left ventricle of certain birds showed thickening. The liver was friable and showed diffuse areas of degeneration and congestion. Kidney showed patchy areas of nephrosis and congestion. One bird showed a nodular mass in the upper lobe of the left kidney. The bird that died showed all typical

lesions of ascites syndrome (Fig.28 and 29). The ascitic fluid in this bird was straw coloured.

4.9.3 Monensin

The body condition of these birds were poor. Lungs of two birds showed consolidated areas. All the organs were small. The gizzard was not muscular and hence pliable (Fig.30). There were focal areas of nephrosis in a few birds.

4.10 Histopathology

4.10.1 Sodium chloride

Lungs

Severe congestion was noticed in the alveolar capillaries with few erythrocytes and oedematous fluid in the lumen of the alveoli and parabronchi. Oedematous fluid was also seen around the perivascular tissue (Fig.46) and in the interlobular spaces (Fig.47). Connective tissue proliferation was evident especially around the blood vessels. Infiltration of mononuclear cells in the submucosa of the primary bronchiole was noticed in two birds.

Liver

There was moderate congestion of the portal vessels. Mild connective tissue and biliary tissue proliferation were seen in

the portal areas of some birds. Severe diffuse granular degeneration of the hepatocytes with multifocal areas of necrosis and oedema were evident. Hepatocytes showed loss of chord-like arrangement assuming an acinar pattern in many places (Fig.48,49). Nuclear changes like pyknosis were seen occasionally. Thickening of the sinusoidal endothelium with separation from the hepatocytes leading to dilatation of the space of Disse was also seen. The thickened sinusoidal endothelium appeared to be mildly PAS positive.

Heart

There was moderate degree of congestion. Vacuolation and separation of the muscle fibres were noticed in four birds.

Kidney

There was moderate congestion with severe diffuse vacuolar degeneration and desquamation of epithelial cells of the tubules, the intensity of which was more prominent towards the collecting tubules. Nuclear changes like pyknosis, karyolysis etc. were also seen. Increase in the Bowman's space and glomerular hypertrophy were seen in some areas.

Spleen

Disorganisation of the lymphoreticular cells with depletion of lymphoid cells was noticed.

Bursa

Lymphoid depletion was evident. The bursal epithelial cells were showing degenerative changes. Separation of the follicles with oedematous fluid was seen in some birds.

Brain

Multivacuolation of the molecular layer and oedema surrounding the purkinje cells of cerebellum were seen in most of the birds. Glial cell proliferation, oedema in the white matter, perineuronal oedema, perivascular oedema and congestion were seen in the cerebellum of the birds.

4.10.2 Furazolidone

Lung

Lungs of few birds showed severe congestion and haemorrhage. Erythrocytes were seen in large numbers in the lumen of the secondary and parabronchioles. Perivascular and interlobular tissue showed the presence of oedema fluid (Fig.41). Ectopic cartilaginous nodules of varying sizes from 10-60 μ were seen in between the alveoli as well as the secondary and tertiary bronchial structures (Fig.42). Interatrial smooth muscle showed hypertrophy and some of these muscles were slightly PAS positive. The smooth muscle bundles of the blood vessels were hypertrophied. Emphysematous

areas were seen in some birds. There was a tendency for proliferation of the lymphoreticular tissue beneath the mucosa of the secondary bronchioles and the interatrial tissue in parabronchioles.

Heart

Moderate degree of congestion and loss of striation of myofibrils were noticed.

Liver

Severe diffuse granular degeneration of hepatocytes and moderate congestion of portal and central veins and sinusoids were seen. Focal infiltration of mononuclear cells and early nuclear changes like pyknosis were seen.

Kidney

Mild to moderate congestion, diffuse degenerative changes like cloudy swelling and vacuolar degenerative changes were seen in the tubules.

The lower parts of the nephrons showed severe vacuolar degeneration and nuclear changes like pyknosis. Collecting tubules at places showed complete degeneration and desquamation leaving only the basement membrane (Lower nephron nephrosis) (Fig.43).

The nodular mass in the left upper lobe of the kidney seen in one of the birds in this group was identified to be nephroblastoma (Fig.44).

Spleen

Moderate reticulo-endothelial cell hyperplasia was prominent especially around the ellipsoids. Uniform sheet-like cells with eosinophilic cytoplasm was seen to be very prominent within the periellipsoidal lymphoid tissue (PELT).

Bursa

Uniform sheet-like cells with eosinophilic cytoplasm were seen to be very prominent within the follicles. There was moderate lymphoreticular hyperplasia and mature lymphocytes were fewer in number. Bursal follicles towards the tip were depleted of lymphocytes when compared to basal follicles.

Brain

There was mild degree of congestion and oedema in the white matter.

4.10.3 Monensin

Lung

Mild to moderate pulmonary congestion was noticed. Mild proliferation of loose aereolar connective tissue in the interlobular septum with infiltration of few mononuclear cells was seen. Mild oedema of the perivascular and interlobular tissues and mild thickening of the adventitia of pulmonary vessels with loose areolar tissue was noticed in few birds.

Heart

There was mild vacuolation in the sarcoplasm of the myofibrils in four birds.

Liver

Severe vacuolation of the hepatocytes and early nuclear changes like pyknosis were seen in most places. Mild congestion of the portal and central veins and focal necrosis of hepatocytes around the bile duct were evident at few places (Fig.50). Mild proliferation of the biliary epithelial cells and connective tissue fibres together with sparse number of mononuclear infiltrating cells were seen in portal areas. Few foci of necrosis with infiltration of mononuclear cells were seen at places.

Kidney

Congestion and dilatation of the veins as well as interstitial capillaries and vacuolar degeneration of the tubular epithelium with desquamation was seen at certain places. The degenerative changes were more prominent in the distal convoluted tubules and collecting tubules. Periveinular tubules showed advanced vacuolar degeneration with some of the tubules left with only the basement membrane. Tubular epithelial cells at few places showed nuclear changes like pyknosis, karyorrhexis and karyolysis.

Spleen

Periellipsoidal tissue in the cortical region showed mild to moderate RE cell hyperplasia. A mild lymphoid depletion was noted from the PALS in the medullary region.

Bursa

Mild lymphoid depletion was seen in certain follicles of the bursa.

Brain

Mild congestion and oedema were noticed. Virchow-Robin space was dilated. Satellitosis and neuronophagia were also noticed.

4.11 Organ weight

Heart (H); Right ventricle (RV)

Furazolidone and Monensin groups had lesser weight than the control group (P<0.01) (Fig.10); (Table 8 and 19).

Left ventricle (LV); Total ventricle (TV)

Monensin group had lesser weight than all other groups (P<0.01) (Fig.10); (Table 8 and 19).

Auricle (A)

Monensin group had lesser weight than the control group (P<0.01) (Fig.10); (Table 8 and 19).

Bursa

Monensin and sodium chloride groups had lesser weight than furazolidone and control groups (P<0.01) (Fig.12); (Table 9 and 22).

Spleen, Right lung, Liver, Intestine

Sodium chloride group had higher weight than all other groups while furazolidone and monensin had lesser weight than the control group (P<0.01) (Fig.12,13); (Table 9, 22 and 23).

Brain, Left lung

Sodium chloride group had more weight than all other groups (P<0.01) (Fig.12); (Table 9 and 22).

Kidney

Sodium chloride group had more weight compared to all other groups. Monensin group had lesser weight than the control (P<0.01) (Fig.13); (Table 9 and 23).

Proventriculus + gizzard (PVS+GZD)

Furazolidone and monensin groups had lesser weight than the control and sodium chloride groups (P<0.01) (Fig.13); (Table 9 and 23).

4.12 Relative organ weight

H/Body weight (BW)%, TV/BW%

Control group had lesser value than all other groups (P<0.05) (Table 8 and 20).

LV/BW%

Control group had lesser value than all other groups (P<0.01) (Table 8 and 20).

RV/BW%, RV/H%, A/H%

There was no significant variation between the groups (Table 8,20 and 21).

LV/H%

Furazolidone group had higher value than the control while monensin and sodium chloride group had lesser value than the control group ($P<0.01$) (Table 8 and 21).

RV/LV

Sodium chloride group had higher value than the control group while monensin and furazolidone groups had lesser value than the control group ($P<0.05$) (Fig.11); (Table 8 and 21).

RV/TV

Sodium chloride group had higher value than all other groups while furazolidone group had lesser value than the control and monensin groups ($P<0.05$) (Fig.11); (Table 8 and 21).

Bursa/BW%

Furazolidone group had higher value when compared to control and monensin groups while sodium chloride group had the least value ($P<0.01$); (Table 9 and 24).

Fig.10 ORGAN WEIGHT OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

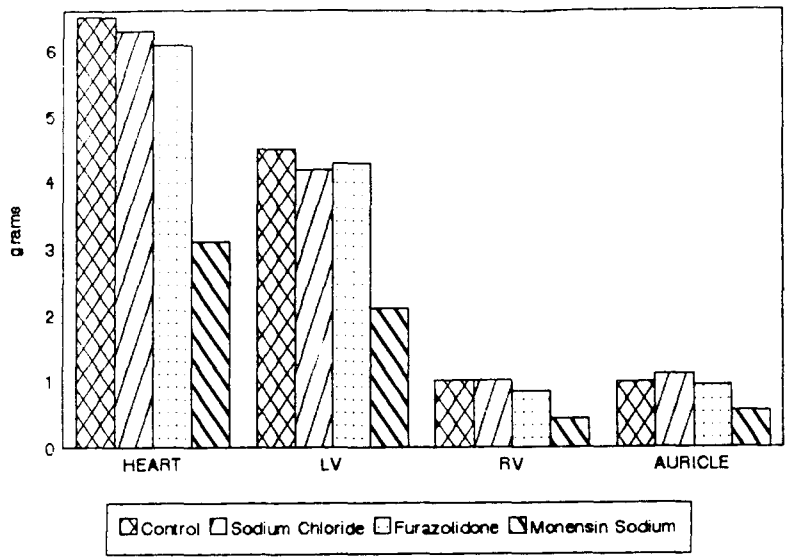


Fig.11 RV/LV & RV/TV OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

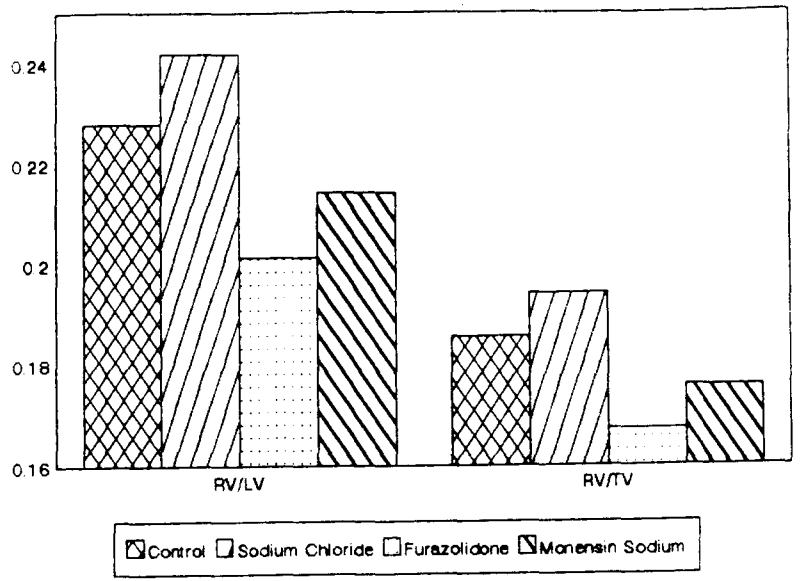


Fig.12 ORGAN WEIGHT OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM

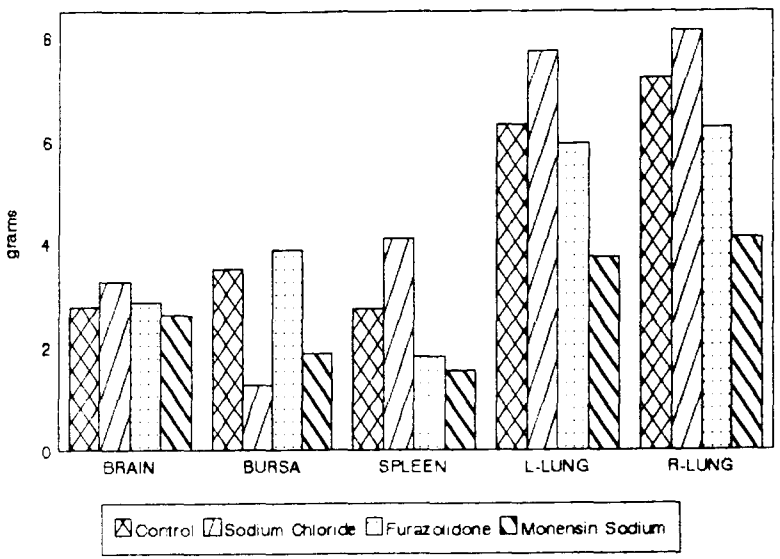
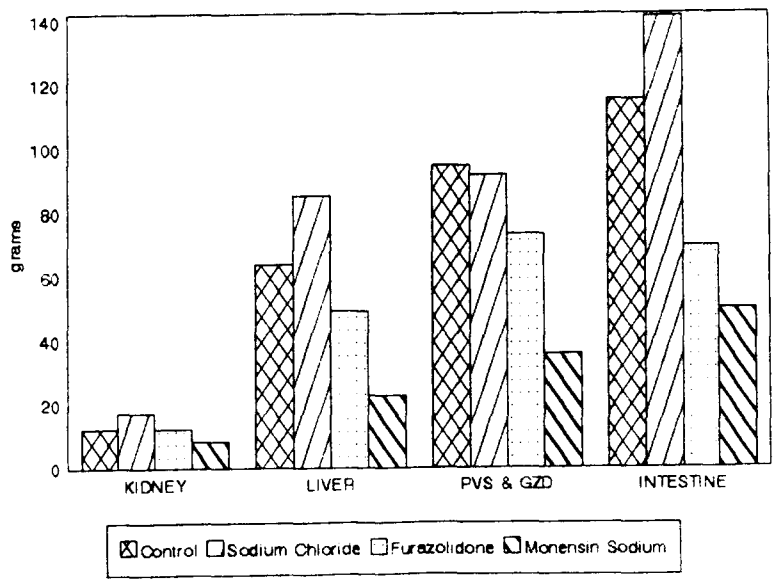


Fig.13 ORGAN WEIGHT OF BROILERS TREATED WITH SODIUM CHLORIDE, FURAZOLIDONE & MONENSIN SODIUM



Spleen/BW%

Sodium chloride and monensin groups had higher value than the control group while furazolidone group had lesser value ($P<0.01$) (Table 9 and 24).

Brain/BW%, Left lung/BW%, Right lung/BW%, Kidney/BW%

The control group had lesser value than all other groups ($P<0.01$); (Table 9).

Liver/BW%

Sodium chloride group had higher value than all other groups while monensin group had lesser value than the control group ($P<0.01$) (Table 9 and 25).

Proventriculus + gizzard/BW%

There was no significant variation between the groups (Table 9 and 25).

Intestine/BW%

Sodium chloride group had higher value than all other groups while furazolidone group had lesser value than the control group ($P<0.01$) (Table 9 and 25).

EXPERIMENT - II

4.13 Clinical symptoms

4.13.1 Common salt

The birds developed excessive thirst and diarrhoea started within six hours. Their feed and water intake were high when compared to other groups. Seven birds developed distended abdomen with fluid feeling within two weeks. Three birds died on the 5th, 11th and 24th days of treatment.

The first bird that died was the best in the group and was especially noted at night as it was standing alone at the feeder and was pecking at the feed at an unusual pace. The bird was found dead the very next morning. The second bird was noted for its respiratory distress and a peculiar wheezing sound in an afternoon. The bird was rocking on inhalation and exhalation. On examination, the abdomen was very tense with fluid. The bird exhibited shooting diarrhoea but the vent region was not soiled. There was a haematoma under the wing band of the left wing. The heart rate was increased and venous pulse was also present. An attempt to collect blood caused excessive (subcutaneous) extravasation of dark blood from the single punctured point in the jugular vein and it died. The third bird which showed maximum fluid feeling on palpation of

the abdomen among the other birds was found dead on the 24th day (Fig.31).

4.13.2 Furazolidone

These birds consumed only less feed and water and became stunted. Their droppings were dry. Leg weakness was noticed in one bird. Eventhough their abdomen were taut, slight fluid feeling was felt in three birds. During blood collection, one of the birds died and it was confirmed to have ascites on post mortem examination. The birds were also noted for their excitation when disturbed.

4.13.3 Cephalexin

These birds were normal except for a feathering problem noticed in one bird. None of them died of ascites.

4.13.4 Cobalt chloride

These birds showed a hyperaemic skin on the third, fourth and fifth week which gradually declined. None of them died of ascites.

4.13.5 Cobalt nitrite

These birds also showed a hyperaemic skin on the third and fourth week which gradually reduced and none of them died of ascites.

4.13.6 Liver suspension group

None of the birds had ascites.

4.13.7 Ascitic fluid group

One of the birds developed a flabby, gas filled abdomen. None of the birds had ascites.

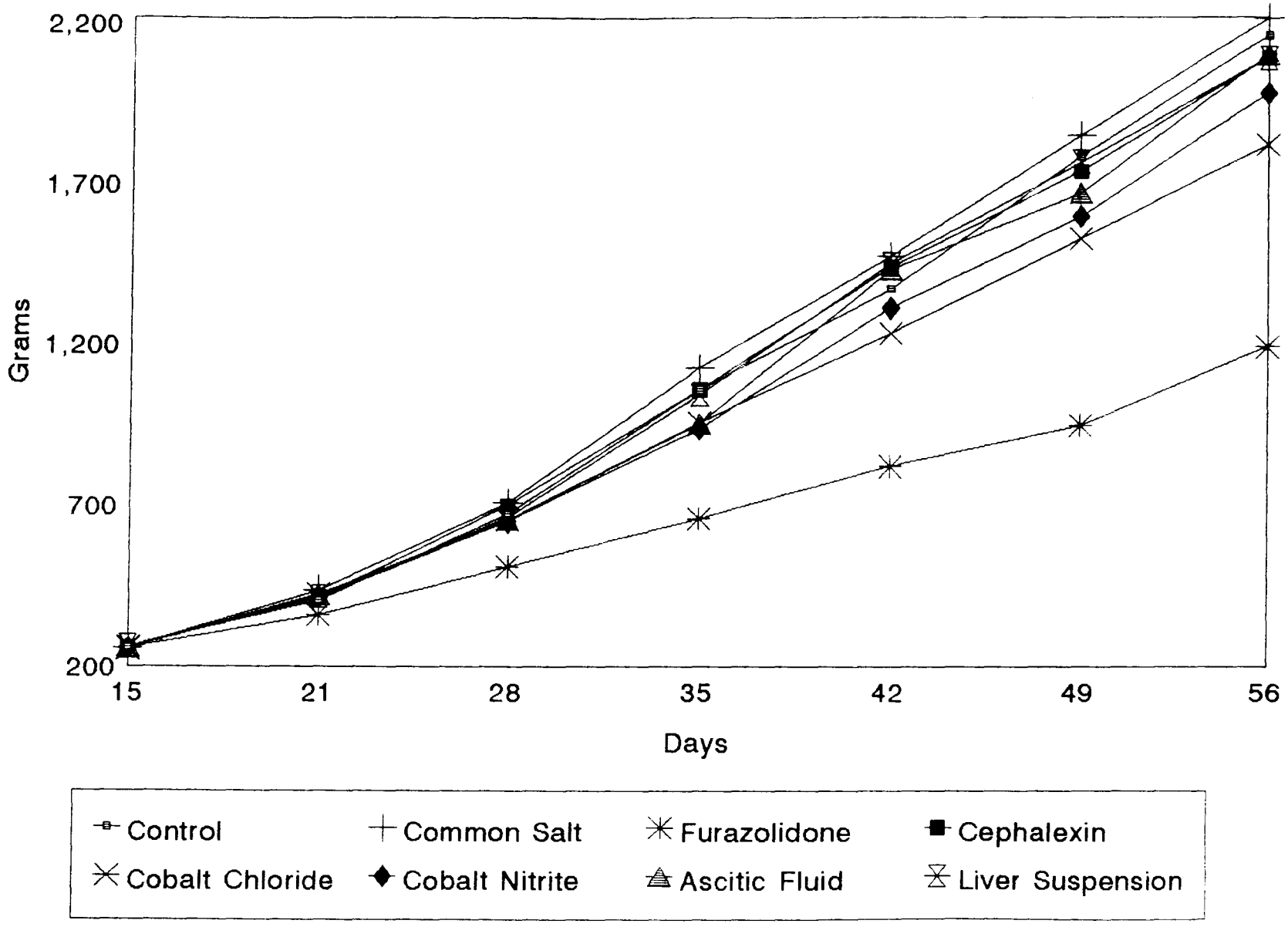
4.13.8 Control

None had ascites.

4.14 Body weight

The body weights did not differ significantly during the first week of treatments. Common salt group had more body weight than all other groups and this increase was significant ($P<0.01$) on D35 and D42. Furazolidone group had lesser weight than all other groups from D28 onwards ($P<0.01$). Cephalexin group did not vary significantly from the control. Cobalt chloride and nitrite groups were lesser than the control from D35 onwards ($P<0.01$) except at D42 where the nitrite group did not vary significantly. On D35 and D49 ascitic fluid group had lesser body weight than the control but regained weight

Fig.13aBODY WEIGHT OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION



on D56 (P<0.01). The liver suspension group did not vary significantly from the control (Fig.13a); (Table 10,16).

4.15 Clinical pathology

4.15.1 ESR

Furazolidone group had higher ESR than the control at D42 (P<0.01). At D56 cobalt chloride and cobalt nitrite group had lesser ESR than control (P<0.05); (Table 11,26).

4.15.2 PCV

Cobalt chloride and common salt group had significantly higher PCV than the control throughout the experiment except at D56 when the increase in the common salt group was not significant (P<0.01). Furazolidone had significantly lesser PCV than the control throughout the experiment (Fig.14); (Table 11,26).

4.15.3 Haemoglobin

Cobalt chloride and common salt group had higher haemoglobin than the control throughout the experiment (P<0.01) except at D28 (P<0.05) when the hike seen in the common salt group was not significant. Though, cobalt nitrite group had higher values than the control it was significant only at

Fig.14 PCV OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION

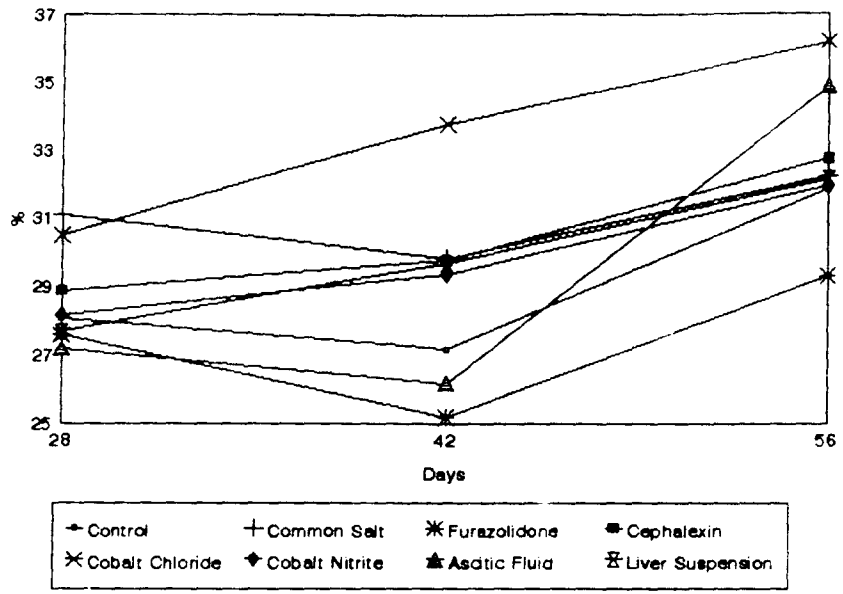
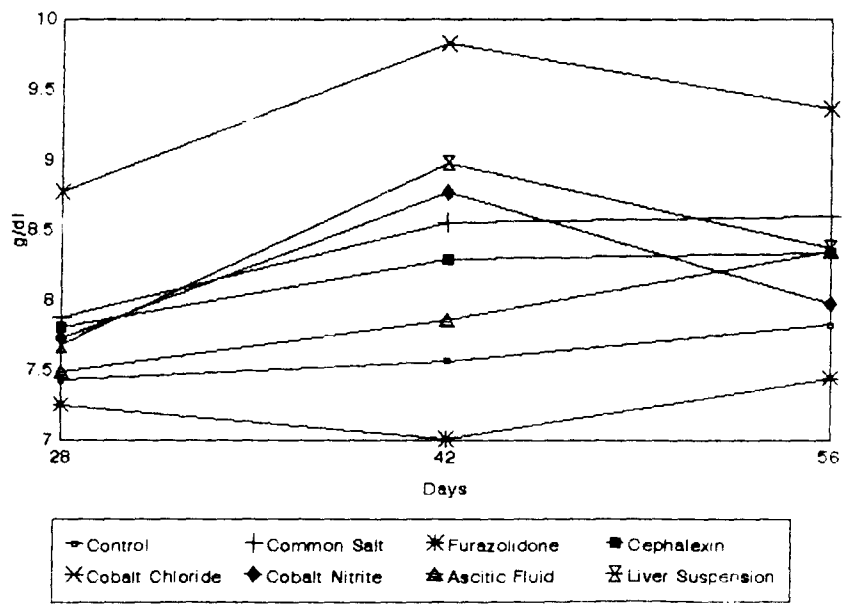


Fig.15 HAEMOGLOBIN OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION



D42 ($P<0.01$) Though furazolidone group had the lowest haemoglobin throughout the experiment, it was significant only at D42 when compared to the control ($P<0.01$). At D42 liver suspension group had higher haemoglobin than the control and ascitic fluid group ($P<0.01$) (Fig.15); (Table 11,26).

4.15.4 Serum protein

Furazolidone group had lesser serum protein than the control at D42 ($P<0.05$) and D56 ($P<0.01$) while cobalt nitrite and ascitic fluid group had greater serum protein than the control at D56 ($P<0.01$) when the value for the control group slumbered (Fig.16); (Table 12,26).

4.15.5 Serum albumin

Serum albumin was less for furazolidone group at D56 ($P<0.05$). Though cobalt nitrite group had a lesser albumin content than the control at D28 ($P<0.05$), the albumin content for both cobalt nitrite and chloride was higher at D56 ($P<0.05$) (Fig.17); (Table 12,26).

4.15.6 Serum globulin

Though common salt group had an initial decrease in the serum globulin at D28 ($P<0.01$) it picked up and at D56 it was higher than the control ($P<0.05$). Furazolidone group had lesser globulin content throughout the experiment but was not

significant than the control at D28. Cephalexin group had a decrease in globulin content than the control group at D28 ($P<0.01$) (Table 12 and 27).

4.15.7 Albumin-globulin ratio

On D28 the albumin-globulin ratio of cobalt nitrite and liver suspension groups were greater than the control group ($P<0.01$). Then onwards the difference was not significant (Table 13 and 27).

4.15.8 Serum sodium

Common salt group had a higher Na^{++} content in the serum throughout experiment ($P<0.01$). Though furazolidone group had lesser Na^{++} content at D28 ($P<0.01$), it was higher than the control group at D42 and D56 ($P<0.01$). Though cephalexin had a lower Na^{++} profile than the control group throughout the experiment ($P<0.01$), it was not significant at D56. Cobalt nitrite and cobalt chloride groups had higher Na^{++} content at D28 and D42 but cobalt nitrite group showed lesser Na^{++} content than the control group at D56 ($P<0.01$). Liver suspension group had a higher Na^{++} content than the control and ascitic fluid groups at D28 and D42 ($P<0.01$), but was lesser than the control group at D56. Ascitic fluid group had a steady low Na^{++} level and was lesser than the control group at D42 and D56 ($P<0.01$) (Fig.18); (Table 13 and 27).

4.15.9 Serum potassium

Common salt group had the highest K^+ content all throughout the experiment ($P<0.01$). Furazolidone group had lower values than the control throughout ($P<0.01$) but was not significant at D42. Though cephalexin group had lesser values, it was significant only at D56 ($P<0.01$). Cobalt chloride and nitrite groups had lesser values at D42 and D56 than the control ($P<0.01$). Liver suspension group had an initial increase in K^+ content at D28 but at D42 it decreased ($P<0.01$) than the control and ascitic fluid groups ($P<0.01$) but at D56 it recouped (Fig.19); (Table 13 and 27).

4.16 Gross pathology

4.16.1 Common salt

The male bird that died on the fifth day of treatment had very good body condition and was the heaviest among the lot. There was generalized congestion of the skin and the mouth was full of feed. There was a pale patchy area on the breast muscle of the left side. The venous plexus in between the abdomen and the cloaca was severely congested and haemorrhagic. The occipital bones covering the brain were congested and haemorrhagic. There was moderate congestion of the brain. The lungs were severely congested and oedematous. The trachea also showed moderate congestion. There was congestion of the heart

Fig 16 SERUM PROTEIN OF BROILERS TREATED WITH COMMON SALT, FURAZOLIDONE, CEPHALEXIN, COBALT CHLORIDE, COBALT NITRITE, ASCITIC FLUID AND LIVER SUSPENSION

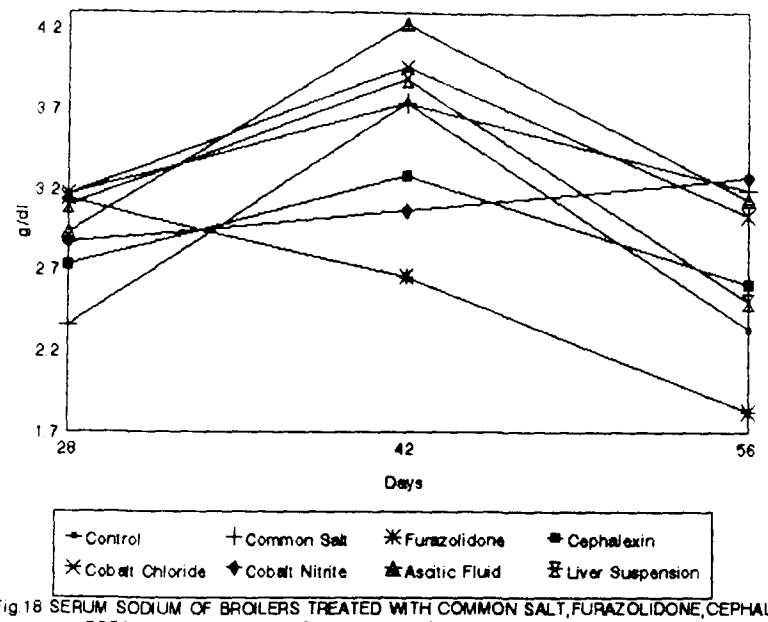


Fig 17 SERUM ALBUMIN OF BROILERS TREATED WITH COMMON SALT, FURAZOLIDONE, CEPHALEXIN, COBALT CHLORIDE, COBALT NITRITE, ASCITIC FLUID AND LIVER SUSPENSION

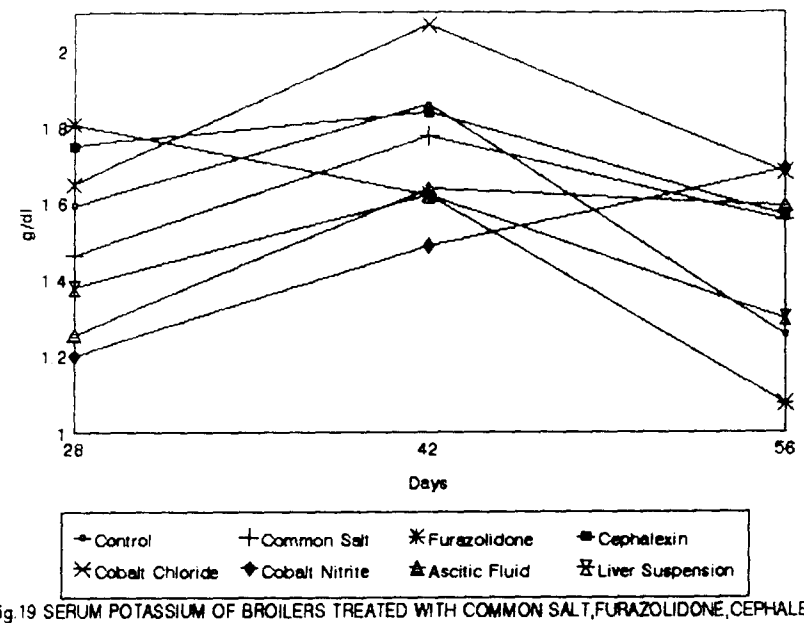


Fig 18 SERUM SODIUM OF BROILERS TREATED WITH COMMON SALT, FURAZOLIDONE, CEPHALEXIN, COBALT CHLORIDE, COBALT NITRITE, ASCITIC FLUID AND LIVER SUSPENSION

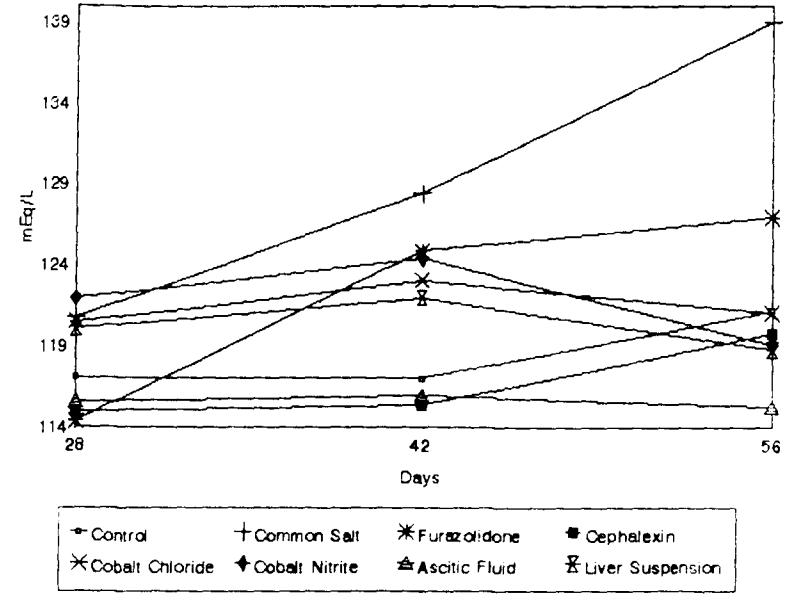
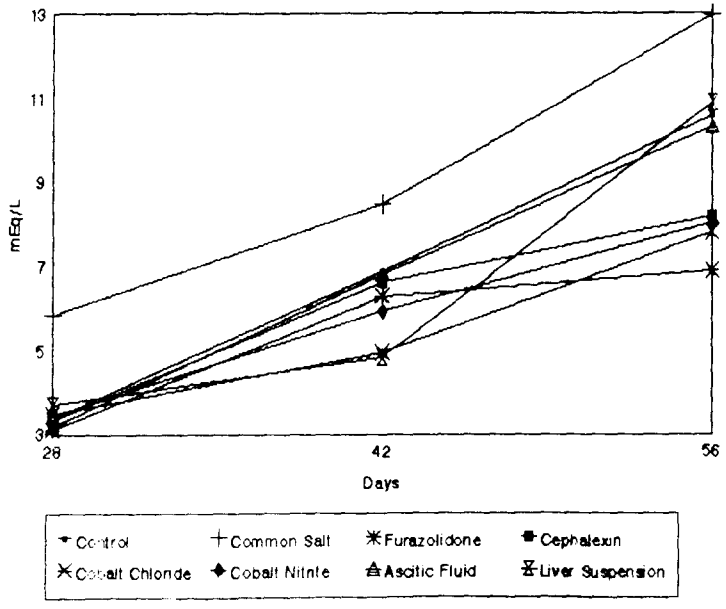


Fig 19 SERUM POTASSIUM OF BROILERS TREATED WITH COMMON SALT, FURAZOLIDONE, CEPHALEXIN, COBALT CHLORIDE, COBALT NITRITE, ASCITIC FLUID AND LIVER SUSPENSION



and both the auricles were dilated and filled with blood. Hydropericardium with straw coloured fluid amounting to 2.5 ml was noticed. Feed mixed with plenty of water was noted in the crop, proventriculus and intestine. The proventriculus and intestines (Fig.33) were congested. The duodenum, pancreas and thyroids also showed severe congestion. The friable liver showed severe congestion with focal areas of hepatosis and each lobules were clearly demarcated like the veins of a decayed leaf (Morocco leather appearance) but no ascites was noticed. Congestion was noticed in the kidneys.

The second female bird which died on D11 showed all the lesions similar to the first bird. All the hepatoperitoneal sacs were filled with nearly 12 ml of clear straw coloured fluid. The digestive tract except the intestines was comparatively empty. The heart was dilated and had hydropericardium with approximately three ml of clear fluid. Pale areas were also noticed on the heart. The lobular markings of the liver were very clear and they protruded out of the surface making it rough. The liver was tough to cut. Thyroid was not so congested as in the first case. The kidney showed moderate congestion and focal pale areas.

The third bird (female) which died on the 24th day showed more chronic lesions. The body condition was fair. The skin was hyperaemic and all the cutaneous vessels were congested and

engorged. The head was oedematous and swollen. There was clotted blood, approximately two ml, above the occipital bones. All the organs were congested. There was about 200 ml of ascitic fluid in different hepatoperitoneal sacs. The lungs were more oedematous than the former birds. The ventricles and auricles of the heart were extremely dilated. There was hydropericardium amounting to 3.5 ml of clear watery fluid (Fig.31).

There were nodules on the liver which made the surface irregular (Fig.32) and it was tough to cut. The spleen was severely congested and was bluish black like a black grape. There was collection of clear fluid beneath the capsule of the kidney which also showed severe congestion and focal pale areas. There was blood tinge in the articular surface of the joint cavities. The ovaries were also congested.

The rest of the birds which were slaughtered on the 56th day did not have ascites. They had severe diffuse hepatosis and the liver had a cooked up appearance. The lungs were congested. There was enlargement of the heart especially the left ventricle and the auricles. One of them had bursitis of the hock and ureters filled with uric acid. The blood vessels in the brain were congested. These birds bled more when compared to birds of other groups.

4.16.2 Furazolidone

The birds were having poor body condition. Most of the organs were smaller in size. The lungs were moderately congested. Epicardial blood vessels were congested in some birds. The heart and auricles were enlarged and filled with blood. Hydropericardium was seen in two birds. Sinus venosus were also seen enlarged. The surface of the liver had markings between each lobules like that of the veins of a decayed leaf but they did not protrude. There were diffuse areas of hepatosis and nephrosis. The bird that died had 14 ml of straw coloured fluid.

4.16.3 Cephalexin

The birds were having fairly good body condition. There was mild congestion in the lungs. Two birds showed hydropericardium, rounded hearts with dilated auricles filled with blood. Liver showed moderate diffuse hepatosis. There was no ascites.

4.16.4 Cobalt chloride

These birds were having fair body condition. There was moderate congestion of the lung and liver. Liver showed areas of diffuse hepatosis. Hydropericardium was noticed in one

bird. Auricles were engorged with blood. Ventricles were also dilated in three cases. Greenish encapsulated streaks were noticed in the liver of one bird. Whitish spots were noticed on the spleen of another bird. Some birds showed diffuse areas of nephrosis with tubules projecting out clearly.

4.16.5 Cobalt nitrite

The body condition of these birds were fair. Two birds showed mild hydropericardium with two ml each of clear fluid. Diffuse areas of hepatosis and nephrosis were noticed in most birds. Blood vessels of the epicardium were seen congested in a few birds. The left auricle and sinus venosus were severely enlarged in two birds.

4.16.6 Ascitic fluid group

Mild hepatosis was noticed in some birds. In a few birds the auricles were dilated and filled with blood. Air-sacculitis was seen in one bird.

4.16.7 Liver suspension group

Mild diffuse hepatosis was noticed in some birds.

4.16.8 Control

Mild diffuse hepatosis was noticed in some birds. The blood vessels of the heart were engorged in a few birds.

4.17 Histopathology

4.17.1 Common salt

The birds that died on the 5th, 11th and 24th day of treatment showed definite progressive lesions.

Lung

The first bird showed severe pulmonary congestion and oedema of the perivascular tissue and the interparabronchial septa. The bronchiolar epithelium showed degeneration and desquamation and severe congestion and haemorrhage in the lamina propria. The bronchial lumen contained exudate mixed with blood and mucus.

The other two birds showed a chronic nature of the above lesions. There were many ectopic cartilaginous nodules of different sizes and shape - some were round to oval, others had irregular contour (Fig.52). Out of these nodules two were strongly PAS positive while others were not. Smooth muscle fibres in the atrial wall showed hypertrophy at many places. Degeneration of the bronchial epithelium were noticed at many

places. Mucosa showed severe congestion and haemorrhage with infiltration of mononuclear cells.

In those birds slaughtered at the eighth week, pulmonary congestion, haemorrhage, perivascular oedema, hypertrophy of the smooth muscles of para bronchi and proliferation of the interatrial and subatrial epithelial tissue were mainly noticed.

Heart

Congestion and vacuolar degeneration of the myocardium and separation of the muscle bundles were noticed in the first bird while the second and third birds in addition showed loss of striation and separation of the myocardial fibres. There was a tendency for hyalinisation in the right ventricle and in the right auriculo-ventricular valve. Other birds exhibited loss of striation and separation of muscle bundles.

Liver

The first bird showed severe congestion of all the blood vessels including the sinusoids. Parenchymatous degeneration, nuclear condensation and subcapsular coagulative necrosis of the hepatocytes were seen at certain places.

The second bird showed congestion and dilatation of all the portal veins and central veins. The central veins were

severely dilated in irregular shapes at certain areas and the hepatocytes surrounding them atrophied (Fig.53). There appeared to be an increase in the space of Disse with sinusoidal dilatation and rupture of the endothelial lining. There was loss of cord - like arrangement of hepatocytes with a tendency to form acinar pattern (Fig.54). Biliary hyperplasia in the portal areas along with connective tissue proliferation and mild thickening of the capsule were also noticed.

In the third bird these lesions were more chronic. The Glisson's capsule was thickened with three to four layers of cells. Three to four layers of hepatocytes just beneath the capsule showed atrophic changes. The connective tissue proliferation was more intense. Those birds which survived showed only a mild version of the above lesions. PAS positive material was found within the hepatocytes diffusely (Fig.55).

Kidney

The first bird showed moderate congestion, moderate mesangial cell proliferation and increase in the size of the glomerular tuft. Vacuolar degeneration, desquamation of tubular epithelium and nuclear changes like pyknosis were seen.

In the second bird in addition to severe venous congestion, the tubular lumen showed severe dilatation at many

places, with some of them containing proteinaceous fluid and some others erythrocytes. Mild vacuolar degenerative changes were noticed in the tubular epithelium. Most of the tubules showed loss of brush border. The was mesangial cell proliferation in the glomeruli, the basement membrane of which showed increased reactivity to PAS. There was mild to moderate thickening of the capsule. The tubular epithelium just beneath the capsule showed hyperplasia.

The third bird showed more chronic lesions. There was severe venous congestion and haemorrhage at certain places. The interstitial venules showed severe diffuse congestion and dilatation. Tubular epithelium showed vacuolar degeneration with many of the nuclei showing pyknotic changes. Glomeruli in general showed mild increase in the size and the basement membrane of the glomerular tuft was thickened (Fig.57). The capsule was thickened to about 80-100 μ. The tubules closer to the thickened capsule showed hyperplasia and hypertrophy with the tubular epithelium completely packing the lumen and showing papillary projections. Subcapsular vessels were severely engorged and thickened. Connective tissue around the major vessels within the kidney parenchyma showed thickening.

The survived birds showed congestion, haemorrhage, mesangial cell proliferation resulting in the increase in the size of the glomeruli, thickening of the basement membrane of

the glomerular tuft. There was moderate diffuse vacuolar degeneration of the tubular epithelium especially in the medullary cones.

Spleen

The first bird showed mild thickening of the tunica media and adventitia of the splenic artery in addition to congestion. Mild lymphoid depletion was also noticed.

In the second bird the congestion was severe and the PELT showed mild hyperplasia, while the third bird showed very severe congestion of all the blood vessels including the sinusoids (Fig.56). Severe lymphoid depletion and atrophy of the white pulp were noticed, however, germinal centres were prominently seen.

The congestion was only mild and the PELT showed proliferation in the survived birds.

Bursa

Congestion and mild lymphoid depletion in a few follicles were noticed in the first bird while in the second and third bird there was severe venous congestion. The capillaries in the cortico-medullary junction were also severely congested. Mild lymphoid depletion was also noticed. In survived birds a

mild lymphoid depletion especially in the follicles at the base of each plica was characteristic.

Brain

Congestion with mild oedema was prominent in the first bird. The second and third bird showed severe congestion and oedema of the white matter, increase in the Virchow-Robin space, perineuronal oedema and meningeal congestion. In survived birds, mild meningeal congestion and focal oedematous portions in the white matter of the pons were seen.

4.17.2 Furazolidone

Lung

Few ectopic cartilaginous nodules were seen in the parenchyma of the lung. Pulmonary congestion with emphysematous foci were seen in isolated areas. There was a general tendency for proliferation of alveolar connective tissue in the adventitia of pulmonary vessels as well as the inter tubular septa. RE cell proliferation along the submucosa of the bronchial tree with focal nodular infiltration of mononuclear cells were noticed in few cases.

Heart

Moderate degree of congestion and loss of striation of the myocardial fibres were noticed in certain birds. Separation of muscle bundles were also noticed in the bird that died of ascites.

Liver

Diffuse vacuolar degeneration with nuclear changes like pyknosis, karyorrhexis, karyolysis etc. and disorganization of hepatic cords with individualization of the hepatocytes were commonly noticed. Severe RE cell proliferation along the portal areas and the sinusoidal lining were also seen. Focal infiltration of mononuclear cells and mild biliary proliferation were noticed especially in portal areas.

Kidney

Tubules generally showed parenchymatous and vacuolar degenerative changes. Majority of the tubules showed advanced vacuolar degeneration with nuclear changes like pyknosis, karyorrhexis, karyolysis etc. The tubular epithelium showed desquamation from the basement membrane at many places. The regenerated tubules in between the degenerated tubules gave a mosaic appearance (Fig.45).

Increased cellularity of the glomerular tuft was noticed due to mesangial cell proliferation. The parietal layer of Bowman's capsule showed mild thickening and the epithelium of the collecting tubule showed a tendency for stratification.

Spleen

Spleen showed mild congestion, slight RE cell proliferation in the PELT and slight lymphoid depletion around the PALS.

Bursa

Mild to moderate lymphoid depletion was noticed in the follicles.

Brain

Mild gliosis, satellitosis and neuronophagia were noticed in the white matter.

4.17.3 Cephalexin

Lung

Pulmonary congestion with focal areas of haemorrhage, proliferation of interatrial epithelial tissue in the para-bronchi were seen.

Heart

The left ventricle showed mild heterophilic infiltration with fibrin deposits between the muscle fibres in isolated areas and vacuolar degeneration of the myocardium at certain areas.

The right ventricle showed mild congestion and fatty infiltration in the muscle bundles. The muscle bundles appeared to be mildly separated by loose areolar tissue.

Liver

Severe diffuse vacuolar degeneration of the hepatocytes with prominent nuclear changes like pyknosis was seen. Thickening of the sinusoidal endothelial lining which was weakly PAS positive was also observed.

Infiltration of mononuclear cells and mild proliferation of connective tissue were seen in the portal areas.

Kidney

Mild hypercellularity was noticed in the glomerular tuft. Maculadensa appeared to be proliferated in some areas.

Spleen

Mild lymphoid depletion from the PALS and mild proliferation in the PELT were seen.

Bursa

Mild lymphoid depletion was noticed from isolated follicles.

Brain

Mild meningeal congestion, spongy appearance of the white matter in few areas and mild gliosis were seen.

4.17.4 Cobalt chloride

Lung

Pulmonary congestion, presence of free erythrocytes in the para bronchial septa and proliferation of the loose areolar connective tissue in the inter para bronchial septa were seen frequently. Smooth muscle proliferation was also noted in the atria. Ectopic cartilaginous nodules were seen in almost all the para bronchi.

Heart

Mononuclear infiltration was seen focally in the myocardium of few birds.

Liver

Hepatocytes showed mild parenchymatous degeneration and some of the nuclei of the hepatocytes showed pyknosis.

Focal areas of liver tissue just beneath the capsule in one bird showed coagulative necrosis with encapsulation around the necrotic mass.

Kidney

Severe congestion of the interstitial capillaries was prominent. Tubular epithelium showed parenchymatous as well as vacuolar degeneration. Tubules around the inter lobular vein and in the medullary cone showed advanced degenerative changes and oedema (Fig.58).

Spleen, Bursa

There were no histological changes in the spleen and bursa.

Brain

Mild congestion, increase in the Virchow-Robin space and mild oedema were noticed in few birds.

4.17.5 Cobalt nitrite

Lungs

Histological changes were similar to those seen in the cobalt chloride group.

Heart

Focal lymphocytic infiltration was noticed in the myocardium of some birds. Myocardium of the right ventricle showed mild hyaline degeneration and uniform loss of striation. Separation of muscle fibres was also noted at some places.

Liver

Hepatocytes showed very mild parenchymatous degeneration and some of the nuclei of the hepatocytes showed pyknosis.

Kidney

There was severe diffuse congestion and few areas showed haemorrhages (Fig.59). Tubular epithelium of many of the tubules showed vacuolar degenerative changes and nuclear changes like pyknosis.

Spleen, Bursa

There were no histological changes in the spleen or bursa.

Brain

Histological changes were similar to those seen in the cobalt chloride group.

4.17.6 Ascitic fluid group

Histologic picture was almost similar to that of the control birds.

4.17.7 Liver suspension group

Histologic picture was almost similar to that of the control birds.

4.17.8 Control

The tissues were showing normal histologic picture. Congestion of the lungs and few ectopic cartilaginous nodules were noticed in few birds.

4.18 Organ weight

Heart (H)

Common salt group had more weight than the control group while cobalt nitrite, furazolidone, ascitic fluid and liver

suspension groups had lesser weight (P<0.01) (Fig.20); (Table 14 and 28).

Left ventricle (LV)

Common salt group had more weight than the control while the ascitic fluid, liver suspension, cobalt nitrite and furazolidone groups showed lesser weight (P<0.01) (Fig.20); (Table 14 and 28).

Right ventricle (RV)

Common salt and cobalt chloride groups had more weight than the control group while furazolidone group had lesser weight (P<0.01) (Fig.20); (Table 14 and 28).

Total ventricle (TV)

Common salt group showed more weight than the control group while furazolidone, liver suspension and ascitic fluid groups had lesser weight (P<0.01); (Table 14 and 28).

Auricle (A)

Common salt had greater weight than the control group while cobalt nitrite, cephalexin, liver suspension, ascitic fluid and furazolidone groups showed lesser weight than control group (P<0.01) (Fig.20); (Table 14 and 28).

Bursa

Cephalexin, cobalt chloride, furazolidone, liver suspension and ascitic fluid groups had lesser weight than the control group ($P<0.05$) (Fig.22); (Table 15 and 31).

Spleen

Common salt group had more weight than the control group while cobalt chloride, furazolidone, cobalt nitrite, liver suspension and ascitic fluid groups showed lesser weight than the control group ($P<0.01$) (Fig.22); (Table 15 and 31).

Brain

Cobalt nitrite, cobalt chloride, cephalexin, furazolidone liver suspension and ascitic fluid groups showed lesser weight than the control group ($P<0.01$) (Fig.22); (Table 15 and 31).

Left lung, Right lung

Common salt group had more weight than the control group while cobalt nitrite, cobalt chloride, cephalexin, furazolidone and ascitic fluid groups had lesser weight ($P<0.01$) (Fig.22); (Table 15 and 31).

Kidney

Cobalt nitrite, cobalt chloride, cephalexin, furazolidone and ascitic fluid groups showed lesser weight when compared to the control group ($P<0.01$) (Fig.23); (Table 15 and 32).

Liver, Intestine

Common salt group had more weight than the control group while the rest of the groups had lesser weight ($P<0.01$) (Fig.23); (Table 15 and 32).

Proventriculus + gizzard (PVS+GZD)

Cobalt nitrite, cobalt chloride, furazolidone,liver suspension and ascitic fluid groups had lesser weight when compared to the control group ($P<0.01$) (Fig.23); (Table 15 and 32).

4.19 Relative organ weight

H/BW%, LV/BW%

Furazolidone, common salt and cobalt chloride groups had higher value than the control group while liver suspension and ascitic fluid groups showed lesser value ($P<0.01$); (Table 14 and 29).

RV/BW%, TV/BW%

Furazolidone, common salt and cobalt chloride groups had higher value than the control group ($P<0.01$); (Table 14 and 29).

LV/H%

Cephalexin, common salt, furazolidone, liver suspension and ascitic fluid groups had higher value than the control group while cobalt chloride group had lesser value ($P<0.01$); (Table 14 and 30).

RV/H%

There was no significant variation between the groups (Table 14 and 30).

A/H%

Cobalt nitrite, cephalexin, common salt and furazolidone groups had higher value than the control group while liver suspension and ascitic fluid groups had lesser value ($P<0.01$); (Table 14 and 30).

RV/LV

Cobalt chloride group had higher value than the control group ($P<0.01$) (Fig.21); (Table 14 and 30).

Fig 20 ORGAN WEIGHT OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION

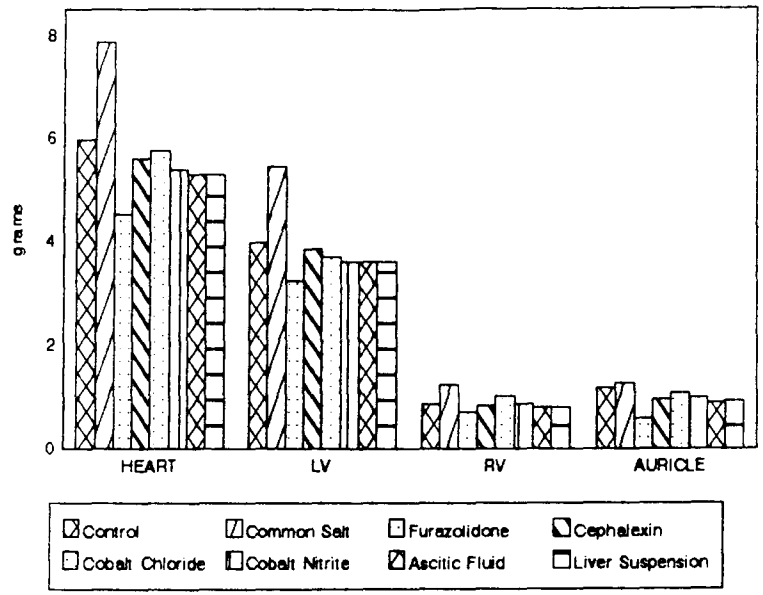


Fig 21 RV/LV & RV/LV OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION

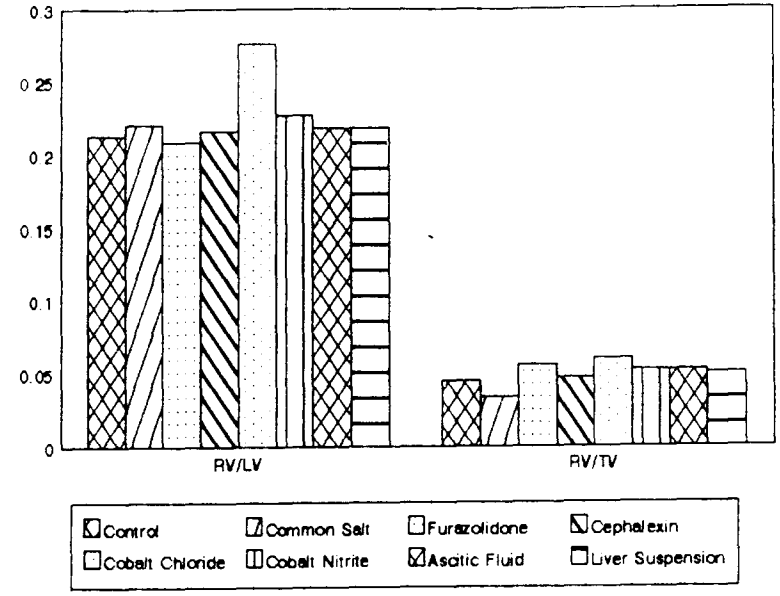


Fig 22 ORGAN WEIGHT OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION

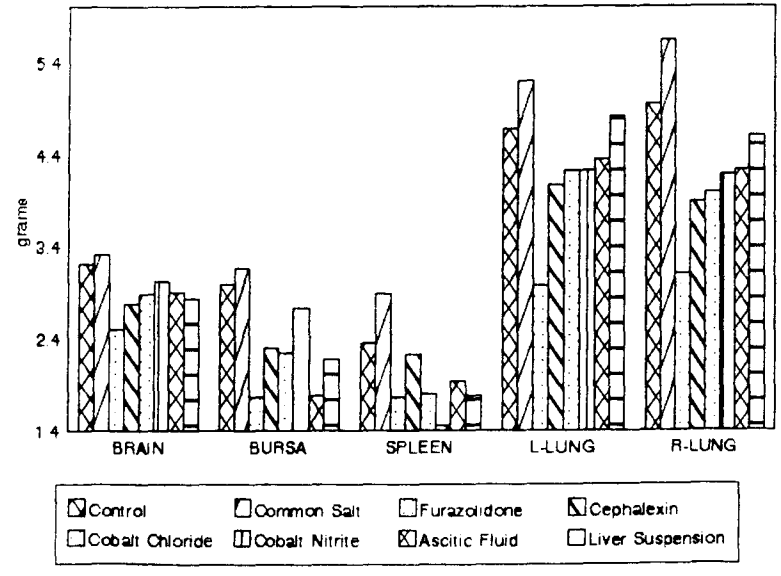
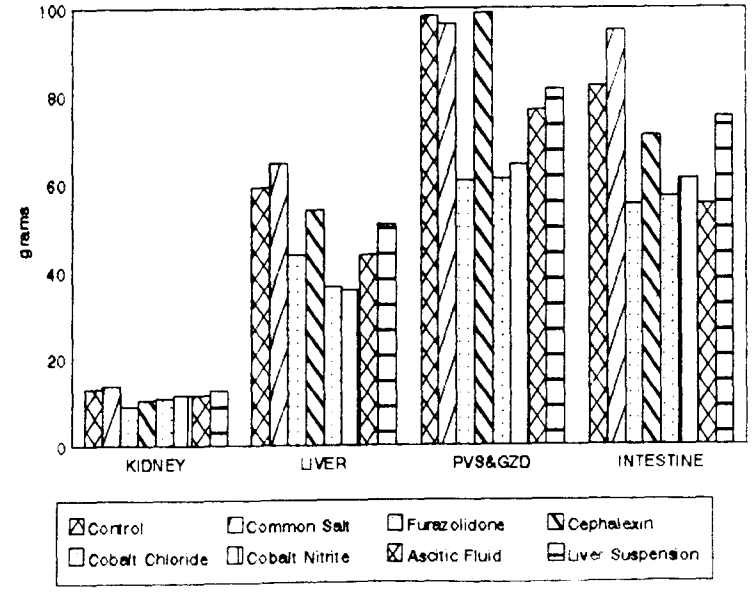


Fig 23 ORGAN WEIGHT OF BROILERS TREATED WITH COMMON SALT,FURAZOLIDONE,CEPHALEXIN, COBALT CHLORIDE,COBALT NITRITE,ASCITIC FLUID AND LIVER SUSPENSION



RV/TV

Cobalt chloride, furazolidone, cobalt nitrite, ascitic fluid and liver suspension groups had higher value than the control group while common salt group had lesser value ($P<0.01$) (Fig.21); (Table 14 and 30).

Bursa/BW%

There was no significant variation between the groups (Table 15 and 33).

Spleen/BW%

Furazolidone and common salt groups had higher value than the control group while cobalt nitrite, ascitic fluid and liver suspension groups had lesser value ($P<0.01$); (Table 15 and 33).

Brain/BW%

Furazolidone and cobalt chloride groups had higher value than the control group while cephalexin, liver suspension and ascitic fluid groups had lesser value ($P<0.01$); (Table 15 and 33).

Left lung/BW%

Furazolidone, common salt and cobalt chloride groups had higher value than the control group but the cephalexin group showed lesser value ($P<0.01$); (Table 15 and 33).

Right lung/BW%

Furazolidone, common salt and cobalt chloride groups had higher value than the control group while cobalt nitrite, cephalexin and ascitic fluid groups had lesser value ($P<0.01$); (Table 15 and 33).

Kidney/BW%

Furazolidone group had higher value than the control group while cephalexin and ascitic fluid groups had lesser value ($P<0.01$); (Table 15 and 34).

Liver/BW%, Proventriculus + gizzard/BW%

Furazolidone group had higher value than the control group while cobalt chloride, cobalt nitrite, liver suspension and ascitic fluid groups showed lesser value ($P<0.01$); (Table 15 and 34).

Intestine/BW%

Furazolidone and common salt groups had higher value than the control group while the rest of the groups had lesser value ($P<0.01$); (Table 15 and 34).

Table 1. The haemoglobin (g/dl), PCV (%) and protein (g/dl), albumin (g/dl), globulin (g/dl), potassium (mEq/L) and sodium (mEq/L) in serum and ascitic fluid; specific gravity of ascitic fluid; aflatoxin content (ppb) in feed and sodium content (mEq/L) in well water in field cases of AS

Sl. No.	Breed	Weeks						Blood		Serum						Ascitic fluid							Feed	Water
		2	3	4	5	6	7	Hb	PCV	Prot	Alb	Glob	Alb:Glob	Pot	Sod	Sp.Gra.	Prot	Alb	Glob	Alb:Glob	Pot	Sod	Afla	Sod
1.	H	*						16.38	37	1.384	0.704	0.680	1.035294	9.72	125.87	1.00321	0.791	0.328	0.463	0.708423	11.31	119.49	200	20.22
2.	H	*						15.29	33	2.123	1.215	0.908	1.138106	4.17	107.22	1.00097	1.254	0.112	1.142	0.098074	5.29	82.97	0	53.70
3.	H		*					16.14	36	2.629	0.927	1.702	0.544653	3.28	81.97	1.01215	2.861	0.971	1.890	0.513757	5.01	73.57	0	16.73
4.	V			*				17.13	34	3.177	1.801	1.376	1.308866	10.23	132.44	1.00732	2.647	1.637	1.010	1.620782	13.33	128.73	0	14.53
5.	V			*				18.39	37	2.398	1.012	1.386	0.730159	6.21	101.32	1.00978	1.919	0.711	1.208	0.588576	13.97	73.51	40	19.30
6.	V			*				16.68	32	2.587	1.130	1.457	0.775566	7.14	112.53	1.01637	1.874	1.023	0.851	1.202115	10.23	108.97	0	17.00
7.	V			*				12.97	39	2.689	1.001	1.688	0.593009	10.38	130.67	1.00983	2.171	1.132	1.039	1.089509	12.16	121.00	0	21.00
8.	V				*			18.79	40	2.792	1.134	1.658	0.683957	8.29	128.90	1.01238	1.175	0.687	0.488	1.407787	17.92	151.54	0	14.70
9.	V				*			17.04	38	2.688	1.072	1.616	0.663366	7.46	125.83	1.01427	1.146	0.759	0.387	1.961240	14.83	121.00	0	14.70
10.	H				*			18.82	42	3.759	2.168	1.591	1.362665	9.43	117.61	1.01500	2.013	0.916	1.097	0.835005	16.23	106.92	60	17.77
11.	H				*			19.88	48	2.024	0.836	1.188	0.703704	11.27	102.31	1.02001	1.983	1.013	0.969	1.045806	31.43	101.78	20	24.78
12.	V				*			19.91	48	3.463	1.763	1.700	1.037059	7.88	111.32	1.00093	2.262	1.144	1.118	1.023256	16.57	106.87	0	17.00
13.	H				*			16.69	34	2.391	0.981	1.409	0.695865	10.18	120.37	1.02123	2.331	1.081	1.250	0.864800	33.06	123.26	0	32.83
14.	V						*	17.58	27	2.985	1.147	1.838	0.624048	11.16	120.62	1.02723	2.864	1.275	1.589	0.802391	31.25	90.47	0	14.70
15.	H						*	19.77	40	3.234	1.982	1.252	1.583067	13.41	101.78	1.01824	1.871	0.354	1.517	0.233355	31.48	103.69	20	17.07
16.	H						*	20.15	46	3.422	1.798	1.624	1.107143	13.17	102.63	1.20183	2.989	0.848	2.141	0.396077	32.59	101.28	20	17.00
Mean ± SE								17.60 ± 0.47	37.8 ± 1.47	2.734 ± 0.147	1.291 ± 0.109	1.442 ± 1.075	0.924158 ± 0.079899	8.96 ± 0.69	113.96 ± 3.35	1.02442 ± 0.01158	2.009 ± 0.160	0.874 ± 0.092	1.134 ± 0.117	0.899435 ± 0.120328	18.54 ± 2.41	107.19 ± 5.01	22.5 ± 12.2	20.81 ± 2.48

H = Hubbard V = Vencob * = age of the bird

Table 2. Average (Mean ± SE) body weight in grams (Experiment 1)

Groups	D15	D21	D28	D35	D42	D49	D56
Control	353.214± 14.718	606.071± 23.727	861.786± 25.449	1295.714± 32.357	1597.857± 42.837	1954.286± 51.528	2342.857± 711.025
Sodium chloride	360.000± 14.142	573.75± 26.128	814.167± 35.249	1293.333± 45.515	1579.167± 55.449	1894.167± 62.103	2019.583± 71.955
Furazolidone	351.667± 13.045	492.500± 20.802	744.167± 28.510	1055.833± 42.756	1245.833± 47.296	1515.000± 61.649	1816.364± 78.245
Monensin	357.500± 9.604	455.417± 13.796	477.500± 27.335	681.667± 40.579	819.167± 55.261	928.333± 63.970	980.833± 70.864

Table 3. Average (Mean ± SE) ESR & PCV (Experiment 1)

Period	ESR (mm/hr)				PCV (%)			
	Control	Sodium chloride	Furazoli-done	Monensin	Control	Sodium chloride	Furazoli-done	Monensin
D15	2.214± 0.114	2.583± 0.745	2.083± 0.601	2.083± 0.601	25.643± 1.002	27.167± 0.935	23.833± 0.805	25.917± 0.609
D28	2.714± 0.114	2.833± 0.312	2.417± 0.008	2.833± 0.008	27.214± 0.826	27.333± 3.464	22.500± 0.338	25.667± 1.244
D42	3.357± 0.248	2.583± 0.147	2.583± 0.147	2.167± 0.112	26.929± 0.847	28.167± 0.621	23.417± 0.713	26.417± 0.620
D56	1.857± 0.206	1.917± 0.470	2.636± 0.151	2.750± 0.130	31.714± 0.917	29.333± 0.811	30.545± 2.002	26.667± 0.932

Table 4. Average (Mean ± SE) Haemoglobin & Serum protein (Experiment 1)

Period	Haemoglobin (g/dl)				Serum protein (g/dl)			
	Control	Sodium chloride	Furazoli-done	Monensin	Control	Sodium chloride	Furazoli-done	Monensin
D15	7.721± 0.179	7.817± 0.164	7.600± 0.141	7.650± 0.115	2.636± 0.182	2.882± 0.089	2.681± 0.182	2.693± 0.092
D28	8.079± 0.219	8.292± 0.320	7.058± 0.291	7.025± 0.317	3.279± 0.115	2.350± 0.122	3.100± 0.136	2.850± 0.141
D42	8.407± 0.211	8.950± 0.381	7.333± 0.326	6.883± 0.557	3.076± 0.184	1.928± 0.112	2.382± 0.138	2.360± 0.118
D56	8.650± 0.310	7.800± 0.222	7.264± 0.226	7.525± 0.277	3.285± 0.227	2.074± 0.159	2.312± 0.164	3.074± 0.291

Table 5. Average (Mean ± SE) Serum albumin & Serum globulin (Experiment 1)

Period	Serum albumin (g/dl)				Serum globulin (g/dl)			
	Control	Sodium chloride	Furazoli-done	Monensin	Control	Sodium chloride	Furazoli-done	Monensin
D15	1.239± 0.056	1.271± 0.031	1.207± 0.098	1.213± 0.023	1.396± 0.157	1.612± 0.081	1.474± 0.147	1.480± 0.089
D28	1.418± 0.067	1.158± 0.061	1.348± 0.063	1.137± 0.087	1.915± 0.096	1.242± 0.084	1.808± 0.127	1.760± 0.124
D42	1.333± 0.099	0.911± 0.075	1.186± 0.063	1.201± 0.095	1.744± 0.115	1.018± 0.066	1.196± 0.121	1.159± 0.101
D56	0.440± 0.171	1.077± 0.083	1.305± 0.078	1.740± 0.196	1.845± 0.211	0.997± 0.098	1.006± 0.114	1.359± 0.225

Table 6. Average (Mean ± SE) Albumin:globulin & serum sodium (Experiment 1)

Period	Albumin:Globulin				Serum Sodium (mEq/L)			
	Control	Sodium chloride	Furazoli-done	Monensin	Control	Sodium chloride	Furazoli-done	Monensin
D15	0.993± 0.091	0.814± 0.052	0.900± 0.095	0.856± 0.058	104.245± 3.742	105.379± 0.822	105.243± 0.851	105.422± 0.814
D28	0.773± 0.064	0.970± 0.081	0.799± 0.078	0.700± 0.081	117.529± 0.911	123.420± 1.362	115.368± 1.007	121.298± 1.885
D42	0.782± 0.056	0.930± 0.087	1.133± 0.147	1.150± 0.162	118.818± 1.013	126.363± 2.500	118.499± 0.768	124.248± 1.100
D56	0.922± 0.131	1.229± 0.188	1.536± 0.244	2.518± 1.111	120.094± 1.024	122.737± 1.718	120.505± 0.630	129.147± 0.906

Table 7. Average (Mean ± SE) Serum potassium (Experiment 1)

Period	Serum potassium (mEq/L)			
	Control	Sodium chloride	Furazoli-done	Monensin
D15	3.092± 0.088	3.157± 0.069	3.203± 0.072	3.202± 0.055
D28	3.419± 0.088	3.992± 0.583	3.818± 0.052	10.079± 0.583
D42	7.144± 0.190	10.331± 0.595	6.975± 0.199	11.249± 0.520
D56	11.772± 0.272	11.072± 0.652	10.296± 0.332	9.030± 0.208

Table 8. Average (Mean ± SE) Organ weight in grams and relative organ weight (Experiment 1)

	Control	Sodium chloride	Furazoli-done	Monensin
Heart	6.504± 0.281	6.291± 0.355	6.074± 0.338	3.099± 0.306
LV	4.484± 0.200	4.170± 0.240	4.281± 0.274	2.095± 0.216
RV	1.023± 0.053	1.010± 0.078	0.853± 0.054	0.444± 0.046
TV	5.507± 0.246	5.180± 0.309	5.134± 0.316	2.539± 0.257
Auricle	0.997± 0.059	1.111± 0.081	0.940± 0.030	0.560± 0.058
H/BW%	0.278± 0.008	0.310± 0.008	0.336± 0.015	0.313± 0.140
LV/BW%	0.191± 0.005	0.206± 0.009	0.236± 0.012	0.210± 0.009
RV/BW%	0.044± 0.003	0.050± 0.003	0.047± 0.003	0.045± 0.003
TV/BW%	0.235± 0.008	0.255± 0.008	0.283± 0.012	0.256± 0.011
LV/H%	68.939± 0.532	66.271± 0.961	70.201± 0.690	67.396± 0.958
RV/H%	15.709± 0.409	15.956± 0.597	14.091± 0.588	14.455± 0.768
Auricle/H%	15.352± 0.697	17.772± 0.929	15.709± 0.540	18.149± 1.432
RV/LV	0.228± 0.005	0.242± 0.011	0.201± 0.009	0.214± 0.011
RV/TV	0.185± 0.005	0.194± 0.006	0.167± 0.006	0.176± 0.006

Table 9. Average (Mean ± SE) Organ weight in grams and relative organ weight (Experiment 1)

Heart	Control	Sodium chloride	Furazoli-done	Monensin
Bursa	3.520± 0.473	1.259± 0.075	3.898± 0.434	1.894± 0.427
Spleen	2.741± 0.256	4.108± 0.433	1.826± 0.223	1.539± 0.199
Brain	2.799± 0.083	3.271± 0.133	2.873± 0.072	2.638± 0.095
L-LNG	6.325± 0.382	7.737± 0.369	5.937± 0.380	3.730± 0.424
R-LNG	7.226± 0.580	8.134± 0.467	6.241± 0.585	4.123± 0.473
Kidney	12.042± 0.625	17.066± 1.787	12.255± 0.736	8.454± 0.765
Liver	63.908± 4.148	85.192± 4.076	49.339± 2.903	22.770± 1.983
PVS±GZD	94.729± 4.760	91.787± 3.868	72.973± 7.682	35.582± 3.715
Intestine	115.142± 4.450	141.052± 11.936	69.355± 4.490	49.875± 2.615
Bursa/BW%	0.151± 0.021	0.063± 0.006	0.217± 0.024	0.177± 0.031
Spleen/BW%	0.119± 0.013	0.207± 0.023	0.101± 0.012	0.157± 0.017
Brain/BW%	0.120± 0.005	0.164± 0.866	0.160± 0.006	0.285± 0.023
L-LNG/BW%	0.272± 0.019	0.383± 0.009	0.331± 0.024	0.377± 0.029
R-LNG/BW%	0.308± 0.021	0.400± 0.011	0.344± 0.027	0.416± 0.032
Kidney/BW%	0.522± 0.035	0.832± 0.061	0.678± 0.036	0.853± 0.037
Liver/BW%	2.755± 0.198	4.241± 0.193	2.710± 0.102	2.327± 0.130
PVS+GZD/BW%	4.109± 0.259	4.552± 0.130	3.969± 0.298	3.651± 0.291
Intestine/BW%	4.933± 0.182	6.945± 0.465	3.848± 0.238	5.205± 0.470

Table 10. Average (Mean ± SE) body weight in grams (Experiment 2)

Groups	D15	D21	D28	D35	D42	D49	D56
Control	262.000± 10.198	408.000± 16.178	674.000± 23.151	1062.000± 34.086	1376.000± 42.979	1787.000± 56.194	2162.000± 71.562
Common salt	258.000± 13.335	437.000± 23.733	707.000± 36.733	1128.000± 38.611	1476.000± 70.353	1851.000± 64.930	2217.000± 68.990
Furazolidone	259.000± 12.212	360.000± 17.1702	511.000± 26.351	660.000± 36.667	822.000± 39.152	950.500± 37.233	1193.333± 65.356
Cephalexin	259.000± 11.542	415.000± 19.622	700.000± 32.559	1060.000± 40.986	1443.000± 48.212	1740.000± 43.994	2092.000± 50.018
Cobalt chloride	11.931± 15.217	426.000± 20.286	655.000± 32.084	956.000± 38.937	1237.000± 43.386	1531.000± 67.682	1823.000± 80.622
Cobalt nitrite	258.000± 15.476	414.000± 27.088	650.000± 35.870	939.000± 35.667	1316.000± 39.670	1600.000± 37.969	1982.000± 51.972
Ascitic fluid	261.000± 11.874	421.000± 18.404	656.000± 24.321	955.000± 31.980	1434.000± 51.773	1672.500± 55.131	2101.000± 65.326
Liver suspension	263.000± 11.931	418.000± 20.210	664.000± 28.330	1046.000± 43.620	1453.000± 51.532	1770.000± 67.954	2092.000± 61.911

Table 11. Average (Mean ± SE) ESR, PCV & Haemoglobin (Experiment 2)

Groups	ESR (mm/hr)			PCV (%)			Haemoglobin (g/dl)		
	D28	D42	D56	D28	D42	D56	D28	D42	D56
Control	2.200± 0.133	2.300± 0.212	2.400± 0.164	28.100± 0.639	27.200± 0.930	31.900± 0.851	7.425± 0.180	7.568± 0.290	7.821± 0.247
Common salt	2.111± 0.200	2.429± 0.200	2.000± 0.000	31.111± 0.873	29.857± 0.884	32.286± 0.748	7.871± 0.367	8.549± 0.333	8.596± 0.200
Furazoli-done	2.400± 0.164	3.400± 0.427	2.333± 0.167	27.600± 1.043	25.200± 1.040	29.333± 1.363	7.249± 0.303	7.007± 0.300	7.443± 0.310
Cephalexin	2.200± 0.133	2.700± 0.300	2.000± 0.000	28.900± 0.379	29.800± 6.999	32.300± 0.617	1.799± 0.117	8.294± 0.240	8.338± 0.262
Cobalt chloride	1.800± 0.133	1.900± 0.101	1.800± 0.133	30.500± 0.910	33.800± 1.132	36.200± 1.388	8.767± 0.490	9.823± 0.623	9.361± 0.436
Cobalt nitrite	2.200± 0.696	2.400± 0.164	1.800± 0.333	28.800± 1.141	29.400± 0.860	32.000± 0.870	7.722± 0.252	8.767± 0.272	7.975± 0.202
Ascitic fluid	2.200± 0.133	2.300± 0.152	2.300± 0.152	27.200± 0.771	26.200± 0.743	34.900± 0.876	7.480± 0.107	7.854± 0.200	8.349± 0.256
Liver suspension	2.000± 0.000	2.100± 0.101	2.100± 0.180	27.700± 0.667	29.700± 1.534	32.200± 0.917	7.676± 0.272	8.976± 0.319	8.371± 0.250

Table 12. Average (Mean ± SE) Serum protein, Serum albumin and Serum globulin (Experiment 2)

Groups	Serum protein (g/dl)			Serum albumin (g/dl)			Serum globulin (g/dl)		
	D28	D42	D56	D28	D42	D56	D28	D42	D56
Control	3.167± 0.050	3.739± 0.155	2.339± 0.199	1.593± 0.104	1.857± 0.098	1.256± 0.073	1.574± 0.107	1.882± 0.177	1.083± 0.095
Common salt	2.364± 0.110	3.736± 0.329	3.197± 0.374	1.462± 0.123	1.776± 0.163	1.556± 0.162	0.902± 0.127	1.960± 0.382	1.641± 0.306
Furazolidone	3.141± 0.281	2.659± 0.285	1.629± 0.203	1.806± 0.237	1.622± 0.237	1.076± 0.095	1.335± 0.092	1.037± 0.107	0.753± 0.140
Cephalexin	2.732± 0.120	3.284± 0.155	2.618± 0.247	1.748± 0.1047	1.838± 0.079	1.565± 0.136	0.984± 0.111	1.446± 0.098	0.993± 0.133
Cobalt chloride	3.171± 0.190	3.963± 0.256	3.040± 0.345	1.648± 0.110	2.067± 0.072	1.679± 0.205	1.523± 0.130	1.896± 0.218	1.361± 0.202
Cobalt nitrite	2.875± 0.256	3.064± 0.278	3.272± 0.275	1.199± 0.139	1.487± 0.161	1.687± 0.092	1.676± 0.168	1.577± 0.285	1.585± 0.269
Ascitic fluid	2.931± 0.253	4.233± 0.585	3.136± 0.278	1.255± 0.107	1.639± 0.294	1.593± 0.164	1.676± 0.231	2.594± 0.537	1.543± 0.161
Liver suspension	3.094± 0.221	3.886± 0.408	2.506± 0.332	1.378± 0.174	1.619± 0.149	1.297± 0.183	1.716± 0.190	2.267± 0.335	1.209± 0.168

Table 13. Average (Mean ± SE) Albumin:Globulin, Serum sodium and Serum potassium , (Experiment 2)

Groups	Albumin:Globulin			Serum sodium (mEq/L)			Serum potassium (mEq/L)		
	D28	D42	D56	D28	D42	D56	D28	D42	D56
Control	1.092± 0.139	1.068± 0.111	1.239± 0.114	114.406± 2.413	124.889± 1.938	127.016± 1.527	3.113± 0.183	6.303± 0.376	6.897± 0.353
Common salt	1.984± 0.373	1.071± 0.151	1.149± 0.223	121.947± 0.544	124.428± 1.217	119.136± 0.923	3.443± 0.149	5.928± 0.367	8.052± 0.534
Furazoli-done	1.368± 0.180	1.653± 0.243	1.927± 0.460	115.041± 1.464	115.449± 1.448	119.854± 1.107	3.321± 0.123	6.641± 0.278	8.198± 0.601
Cephalexin	1.940± 0.180	1.310± 0.082	1.772± 0.231	120.079± 0.825	122.012± 0.734	118.850± 1.050	3.693± 0.187	4.839± 0.259	10.881± 0.344
Cobalt chloride	1.170± 0.149	1.268± 0.199	1.400± 0.186	117.088± 0.996	117.046± 1.252	121.228± 0.670	3.363± 0.133	6.877± 0.291	10.581± 0.398
Cobalt nitrite	0.759± 0.088	1.551± 0.617	1.362± 0.243	120.562± 1.050	123.105± 0.730	121.114± 0.835	3.479± 0.145	4.964± 0.266	7.801± 0.503
Ascitic fluid	0.898± 0.149	0.897± 0.228	1.084± 0.111	120.789± 1.393	128.436± 1.070	139.011± 2.067	5.840± 0.357	8.500± 0.336	12.961± 0.779
Liver suspension	0.945± 0.202	0.808± 0.120	1.707± 0.104	115.657± 0.945	115.311± 1.126	115.311± 1.151	3.184± 0.158	6.822± 0.408	10.300± 0.670

Table 14. Average (Mean ± SE) organ weight in grams and relative organ weight (Experiment 2)

	Control	Common salt	Furazoli-done	Cephalexin	Cobalt chloride	Cobalt nitrite	Ascitic fluid	Liver suspension
Heart	5.947± 0.262	7.870± 0.854	4.494± 0.347	5.593± 0.199	5.726± 0.237	5.372± 0.256	5.268± 0.307	5.279± 0.212
LV	3.942± 0.171	5.418± 0.521	3.23 ± 0.233	3.834± 0.13	3.671± 0.18	3.582± 0.171	3.596± 0.221	3.595± 0.18
RV	0.845± 0.073	1.213± 0.166	0.679± 0.067	0.83 ± 0.035	0.997± 0.079	0.819± 0.054	0.781± 0.05	0.785± 0.054
TV	4.788± 0.228	6.631± 0.684	3.909± 0.297	4.664± 0.15	4.668± 0.202	4.402± 0.221	4.376± 0.262	4.38 ± 0.218
Auricle	1.16 ± 0.063	1.24 ± 0.185	0.585± 0.073	0.929± 0.076	1.059± 0.085	0.971± 0.057	0.891± 0.073	0.895± 0.047
H/BW%	0.276± 0.013	0.351± 0.026	0.375± 0.013	0.268± 0.006	0.320± 0.022	0.271± 0.013	0.250± 0.013	0.253± 0.006
LV/BW%	0.183± 0.006	0.242± 0.015	0.269± 0.01	0.183± 0.003	0.204± 0.013	0.181± 0.006	0.171± 0.009	0.172± 0.006
RV/BW%	0.039± 0.003	0.054± 0.007	0.056± 0.003	0.04 ± 0.003	0.057± 0.006	0.041± 0.003	0.037± 0.003	0.037± 0.003
TV/BW%	0.222± 0.009	0.296± 0.019	0.326± 0.01	0.223± 0.006	0.261± 0.016	0.222± 0.009	0.208± 0.009	0.209± 0.006

Contd.

Table 14 (Contd.)

	Control	Common salt	Furazoli-done	Cephalexin	Cobalt chloride	Cobalt nitrite	Ascitic fluid	Liver suspension
LV/H%	66.347± 0.771	69.281± 1.357	72.061± 0.737	68.644± 1.021	64.181± 1.752	66.73 ± 0.749	68.131± 0.838	67.901± 0.832
RV/H%	14.059± 0.737	15.195± 0.442	15.066± 0.57	14.837± 0.411	17.373± 1.094	15.133± 0.5	14.882± 0.065	14.843± 0.775
Auricle/H%	19.594± 0.86	15.524± 1.005	12.873± 1.06	16.52 ± 1.009	18.446± 1.176	18.138± 0.759	16.986± 1.069	17.171± 1.145
RV/LV	0.213± 0.013	0.22 ± 0.011	0.209± 0.007	0.217± 0.006	0.276± 0.025	0.227± 0.009	0.219± 0.009	0.219± 0.013
RV/TV	0.045± 0.003	0.034± 0.004	0.056± 0.033	0.047± 0.003	0.06 ± 0.006	0.052± 0.003	0.052± 0.003	0.051± 0.003

Table 15. Average (Mean ± SE) organ weight in grams and relative organ weight (Experiment 2)

	Control	Common salt	Furazoli-done	Cephalexin	Cobalt chloride	Cobalt nitrite	Ascitic fluid	Liver suspension
Bursa	2.992± 0.477	3.17 ± 0.442	1.773± 0.19	2.303± 0.373	2.262± 0.338	2.735± 0.316	1.784± 0.326	2.188± 0.183
Spleen	2.367± 0.319	2.889± 0.208	1.764± 0.21	2.24 ± 0.177	1.802± 0.231	1.457± 0.123	1.949± 0.136	1.781± 0.247
Brain	3.22 ± 0.054	3.327± 0.068	2.513± 0.087	2.787± 0.098	2.886± 0.063	3.029± 0.133	2.918± 0.111	2.841± 0.117
L-LNG	4.67 ± 0.202	5.189± 0.336	2.984± 0.217	4.073± 0.218	4.231± 0.281	4.228± 0.275	4.349± 0.253	4.806± 0.111
R-LNG	4.957± 0.183	5.65 ± 0.242	3.111± 0.33	3.902± 0.234	4.007± 0.313	4.185± 0.196	4.239± 0.291	4.612± 0.23
Kidney	13.165± 0.863	14.121± 0.847	9.342± 0.563	10.729± 0.654	10.972± 0.42	11.98 ± 0.509	11.713± 0.759	12.939± 0.775
Liver	59.302± 1.271	64.744± 3.76	43.928± 2.547	54.188± 3.087	36.487± 1.132	36.051± 1.657	43.942± 2.207	50.92 ± 1.916
PVS+GSD	98.432± 5.16	96.554± 1.87	60.801± 3.84	99.089± 3.374	61.07 ± 3.38	64.408± 3.428	76.931± 1.875	81.602± 1.802
Intestine	82.442± 1.942	95.121± 2.598	55.188± 3.51	70.933± 2.653	56.91 ± 3.004	61.016± 2.852	55.420± 1.312	75.345± 3.516

Contd.

Table 15 (Contd.)

	Control	Common salt	Furazoli- done	Cephalexin	Cobalt chloride	Cobalt nitrite	Ascitic fluid	Liver suspension
Bursa/BW%	0.141± 0.022	0.143± 0.019	0.152± 0.0167	0.111± 0.019	0.123± 0.019	0.14 ± 0.016	0.085± 0.016	0.106± 0.009
Spleen/BW%	0.111± 0.016	0.13 ± 0.007	0.148± 0.017	0.108± 0.009	0.1 ± 0.013	0.073± 0.006	0.095± 0.009	0.088± 0.016
Brain/BW%	0.151± 0.006	0.15 ± 0.004	0.213± 0.01	0.134± 0.006	0.16 ± 0.006	0.154± 0.006	0.14 ± 0.006	0.136± 0.003
L-LNG/BW%	0.217± 0.006	0.233± 0.011	0.251± 0.013	0.195± 0.009	0.232± 0.009	0.215± 0.016	0.207± 0.009	0.231± 0.006
R-LNG/BW%	0.231± 0.009	0.256± 0.011	0.258± 0.02	0.185± 0.006	0.218± 0.009	0.213± 0.013	0.201± 0.009	0.223± 0.013
Kidney/BW%	0.609± 0.032	0.634± 0.019	0.794± 0.047	0.512± 0.025	0.617± 0.044	0.606± 0.035	0.561± 0.038	0.62 ± 0.035
Liver/BW%	2.777± 0.13	2.941± 0.215	3.967± 0.163	2.609± 0.126	2.038± 0.107	1.822± 0.076	2.106± 0.117	2.441± 0.082
PVS+GZD/BW%	4.594± 0.272	4.37 ± 0.106	5.116± 0.213	4.761± 0.193	3.355± 0.123	3.272± 0.19	3.696± 0.152	3.926± 0.13
Intestine/BW%	3.832± 0.085	4.29 ± 0.23	4.627± 0.177	3.39 ± 0.095	3.143± 0.145	3.088± 0.152	2.655± 0.082	3.331± 0.212

Table 16. ANOVA. Difference in body weight between the control and treatment groups in experiment 1 and experiment 2

Period	Source	Experiment 1		Experiment 2	
		DF	MS	DF	MS
D15	Treatment	3	180.159	7	51.964
	Error	46	2184.174	76	1649.653
D21	Treatment	3	62137.135**	7	5205.517
	Error	46	5974.198	71	4187.261
D28	Treatment	3	1193691.937**	7	199934.863**
	Error	46	11198.667	71	13920.964
D35	Treatment	3	1034476.714**	7	461774.350**
	Error	46	20057.997	70	22598.536
D42	Treatment	3	1654229.762**	7	799276.076**
	Error	46	31265.450	70	28861.894
D49	Treatment	3	2763012.270**	7	985755.832**
	Error	46	44219.591	69	40451.218
D56	Treatment	3	4250895.324**	7	36628.881**
	Error	45	65285.663	68	8974.867

** Highly significant $P<0.01$

Table 17. ANOVA. Difference in ESR, PCV, haemoglobin (Hb), serum protein (S.prot.) and serum albumin (S.alb) between the control and treatment groups (Experiment 1)

Period	Source	DF	ESR	PCV	Hb	S.prot.	S.alb.
			MS	MS	MS	MS	MS
	Treatments	3	0.671	22.685	0.107	0.148	0.010
D15	Error	46	0.372	9.423	0.302	0.274	0.045
			**	**	**	**	**
	Treatments	3	0.691	62.310	5.461	2.047	0.248
D28	Error	46	0.524	9.841	1.021	0.200	0.061
			**	**	**	**	**
	Treatments	3	3.262	49.064	11.026	2.968	0.347
D42	Error	46	0.407	5.835	1.786	0.266	0.093
			**	*	**	**	**
	Treatments	3	2.708	58.665	4.679	4.344	0.918
D56	Error	45	0.343	17.665	0.89	0.605	0.266

Table 18. ANOVA. Difference in serum globulin (S.glob), albumin-globulin ratio (A:G), serum sodium (S.sod.) and serum potassium (S.pot.) between the control and treatment groups (Experiment 1)

Period	Source	DF	S.glob.	A:G	S.sod.	S.pot.
			MS	MS	MS	MS
	Treatments	3	0.101	0.077	4.160	0.036
D15	Error	46	0.201	0.076	8.336	0.067
			**	**	**	**
	Treatments	3	1.116	0.167	160.333	123.440
D28	Error	46	0.149	0.071	21.736	1.024
			**	**	**	**
	Treatments	3	1.360	0.401	192.305	59.447
D42	Error	46	0.136	0.169	27.101	2.044
			**	**	**	**
	Treatments	3	2.070	5.990	213.599	17.502
D56	Error	45	0.388	3.931	16.247	0.941

* Significant P <0.05 ** Highly significant P<0.01

Table 19. ANOVA. Difference in heart (H); left ventricle (LV); right ventricle (RV); total ventricle (TV) and auricle (A) between the control and treatment groups (Experiment 1)

Source	DF	MS				
		H	LV	RV	TV	A
		**	**	**	**	**
Treatment	3	31.465	15.194	0.901	23.220	0.689
Error	45	1.245	0.650	0.042	0.966	0.046

Table 20. ANOVA. Difference in H/BW%; LV/BW%; RV/BW% and TV/BW% between the control and treatment groups (Experiment 1)

Source	DF	MS			
		H/BW%	LV/BW%	RV/BW%	TV/BW%
		*	**		*
Treatment	3	0.007	0.004	0.000	0.005
Error	45	0.002	0.001	0.000	0.001

Table 21. ANOVA. Difference in LV/H%; RV/H%; A/H%; RV/LV and RV/TV between the control and treatment groups (Experiment 1)

Source	DF	MS				
		LV/H%	RV/H%	A/H%	RV/LV	RV/TV
		**			*	*
Treatment	3	34.694	10.064	25.079	0.004	0.002
Error	45	7.727	4.293	11.231	0.001	0.00046

Table 22. ANOVA. Difference in bursa, spleen, brain, left lung (L-LNG) and right lung (R-LNG), between the control and treatment groups (Experiment 1)

Source	DF	MS				
		Bursa	Spleen	Brain	L-LNG	R-LNG
		**	**	**	**	**
Treatment	3	19.267	15.880	0.874	33.151	35.978
Error	45	1.914	1.055	0.119	1.869	3.501

* Significant $P<0.05$ ** Highly significant $P<0.01$

Table 23. ANOVA. Difference in kidney, liver, proventriculus + gizzard (PVS+GZD) and intestine (Int) between the control and treatment groups (Experiment 1)

Source	DF	MS			
		Kidney	Liver	PVS+GZD	Int
		**	**	**	**
Treatment	3	150.006	8279.415	9171.529	20931.911
Error	45	13.980	150.427	320.232	567.365

Table 24. ANOVA. Difference in bursa/BW%, spleen/BW%, brain/BW%, left lung/BW% (L-LNG/BW%) and right lung/BW% (R-LNG/BW%), between the control and treatment groups (Experiment 1)

Source	DF	MS				
		Bursa/ BW%	Spleen/ BW%	Brain/ BW%	L-LNG/ BW%	R-LNG/ BW%
		**	**	**	**	**
Treatment	3	0.050	0.026	0.063	0.034	0.032
Error	45	0.006	0.004	0.002	0.005	0.007

Table 25. ANOVA. Difference in kidney/BW%, liver/BW%, proventriculus + gizzard/BW% (PVS+GZD/BW%) and intestine/BW% (Int/BW%) between the control and treatment groups (Experiment 1)

Source	DF	MS			
		Kidney BW%	Liver BW%	PVS+GZD BW%	Int BW%
		**	**		**
Treatment	3	0.311	8.564	1.678	19.175
Error	45	0.023	0.341	0.787	0.997

* Significant P<0.05 ** Highly significant P<0.01

Table 26. ANOVA. Difference in ESR, PCV, haemoglobin (Hb), serum protein (S.prot.) and serum albumin (S.alb) between the control and treatment groups (Experiment 2)

Period	Source	DF	ESR	PCV	Hb	S.prot.	S.alb.
			MS	MS	MS	MS	MS
				**	**		*
	Treatments	7	0.311	14.059	2.122	0.735	0.502
D28							
	Error	71	1.187	6.745	0.805	0.406	0.194
			**	**	**	**	
	Treatments	7	2.053	71.724	7.753	2.727	0.334
D42							
	Error	69	0.531	10.744	1.164	0.083	0.293
			**	**	**	**	*
	Treatments	7	0.536	41.359	3.126	2.362	0.479
D56							
	Error	68	0.185	9.449	0.775	0.748	0.206

Table 27. ANOVA. Difference in serum globulin (S.glob), albumin-globulin ratio (A:G), serum sodium (S.sod.) and serum potassium (S.pot.) between the control and treatment groups (Experiment 2)

Period	Source	DF	S.glob.	A:G	S.sod.	S.pot.
			MS	MS	MS	MS
			**	**	**	**
	Treatments	7	0.987	2.089	86.499	6.574
D28						
	Error	71	0.227	0.366	16.930	0.311
			*	**	**	**
	Treatments	7	2.334	0.884	203.321	11.661
D42						
	Error	69	0.847	0.751	15.364	1.038
			*		**	**
	Treatments	7	0.906	0.918	420.093	35.314
D56						
	Error	68	0.335	0.498	12.574	2.666

* Significant P <0.05 ** Highly significant P<0.01

Table 28. ANOVA. Difference in heart (H); left ventricle (LV); right ventricle (RV); total ventricle (TV) and auricle (A) between the control and treatment groups (Experiment 2)

Source	DF	MS				
		H	LV	RV	TV	A
		**	**	**	**	**
Treatment	7	7.296	3.278	0.214	4.955	0.344
Error	68	1.065	0.477	0.050	0.757	0.063

Table 29. ANOVA. Difference in H/BW%; LV/BW%; RV/BW% and TV/BW% between the control and treatment groups (Experiment 2)

Source	DF	MS			
		H/BW%	LV/BW%	RV/BW%	TV/BW%
		**	**	**	**
Treatment	7	0.020	0.012	0.001	0.017
Error	68	0.002	0.001	0.00011	0.001

Table 30. ANOVA. Difference in LV/H%; RV/H%; A/H%; RV/LV and RV/TV between the control and treatment groups (Experiment 2)

Source	DF	MS				
		LV/H%	RV/H%	A/H%	RV/LV	RV/TV
		**		**	*	**
Treatment	7	50.090	9.144	38.896	0.005	0.0001
Error	68	10.532	4.682	9.971	0.002	0.00013

Table 31. ANOVA. Difference in bursa, spleen, brain, left lung (L-LNG) and right lung (R-LNG), between the control and treatment groups (Experiment 2)

Source	DF	MS				
		Bursa	Spleen	Brain	L-LNG	R-LNG
		*	**	**	**	**
Treatment	7	2.401	1.685	0.559	3.667	4.769
Error	68	1.112	0.443	0.091	0.543	0.632

* Significant $P<0.05$ ** Highly significant $P<0.01$

Table 32. ANOVA. Difference in kidney, liver, proventriculus + gizzard (PVS+GZD) and intestine (Int) between the control and treatment groups (Experiment 2)

Source	DF	MS			
		Kidney	Liver	PVS+GZD	Int
		**	**	**	**
Treatment	7	20.343	967.718	2626.945	1804.968
Error	68	4.462	40.134	107.724	88.086

Table 33. ANOVA. Difference in bursa/BW%, spleen/BW%, brain/BW%, left lung/BW% (L-LNG/BW%) and right lung/BW% (R-LNG/BW%), between the control and treatment groups (Experiment 2)

Source	DF	MS				
		Bursa/ BW%	Spleen/ BW%	Brain/ BW%	L-LNG/ BW%	R-LNG/ BW%
			**	**	*	**
Treatment	7	0.005	0.005	0.006	0.003	0.006
Error	68	0.003	0.001	0.0003	0.001	0.001

Table 34. ANOVA. Difference in kidney/BW%, liver/BW%, proventriculus + gizzard/BW% (PVS+GZD/BW%) and intestine/BW% (Int/BW%) between the control and treatment groups (Experiment 2)

Source	DF	MS			
		Kidney BW%	Liver BW%	PVS+GZD BW%	Int BW%
		**	**	**	**
Treatment	7	0.061	3.347	4.418	3.918
Error	68	0.012	0.150	0.324	0.215

* Significant P<0.05 ** Highly significant P<0.01

Fig.24 Ascites Syndrome : Chicken - Fluid accumulation and passive venous congestion. Note the oedematous head

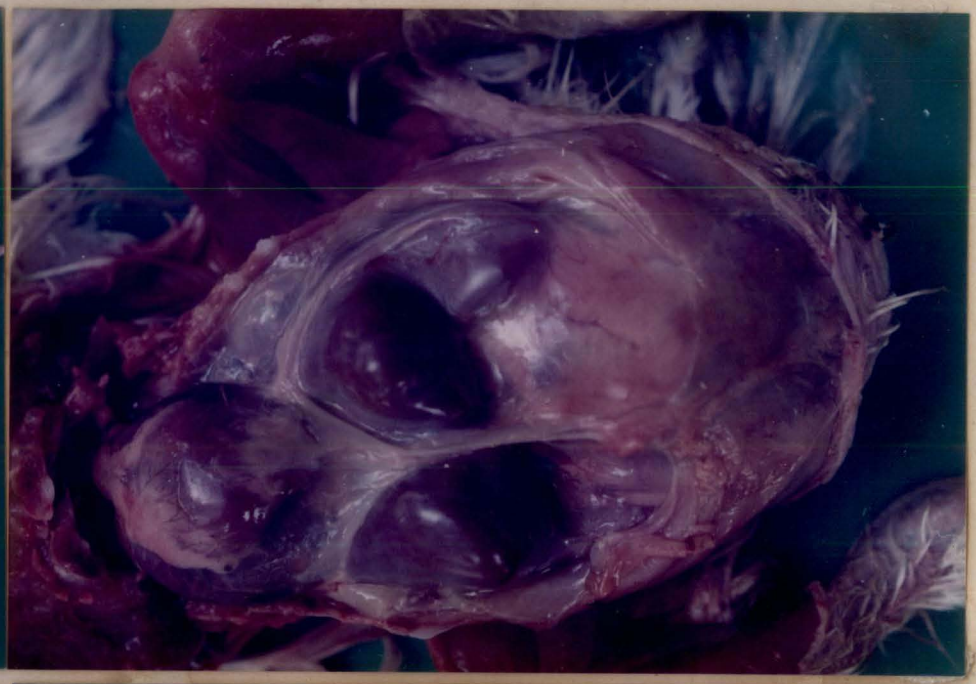
Fig.25 Ascites Syndrome : Chicken - Hydropericardium, cardiac enlargement and swollen liver with rounded borders

Fig.26 Ascites Syndrome : Frozen Chicken - The skin and peritoneum were removed to show the extent of accumulation of the straw coloured ascitic fluid. Note the congestion in the liver

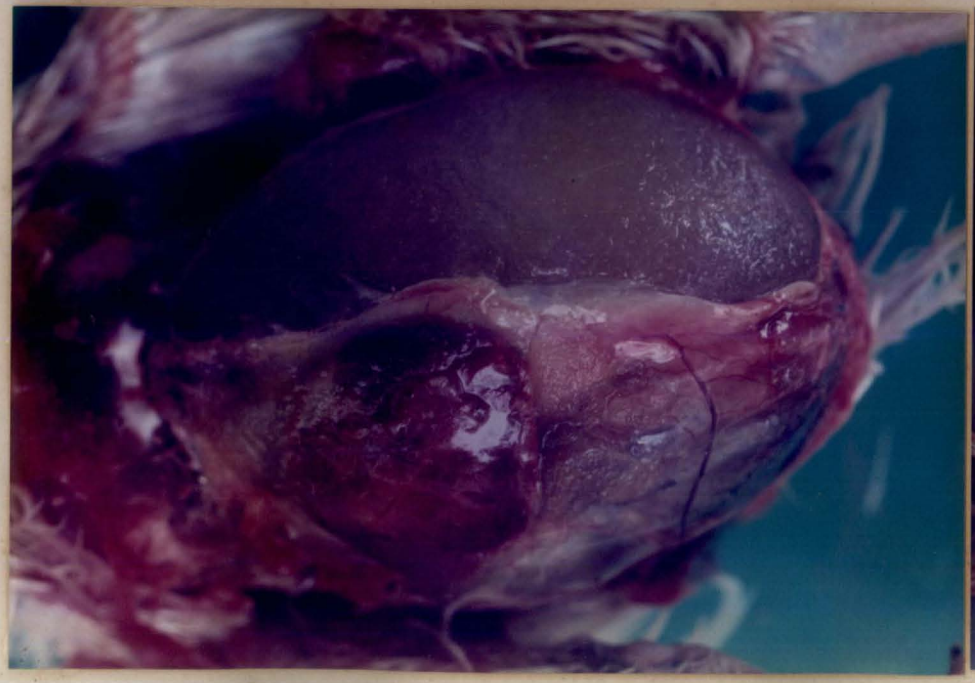
Fig.27 Ascites Syndrome : Chicken - The frozen bird has been cut to show the accumulation of ascitic fluid in the different hepatoperitoneal sacs. Congestion and dilatation of the hepatic veins are also seen

24

25



26



27

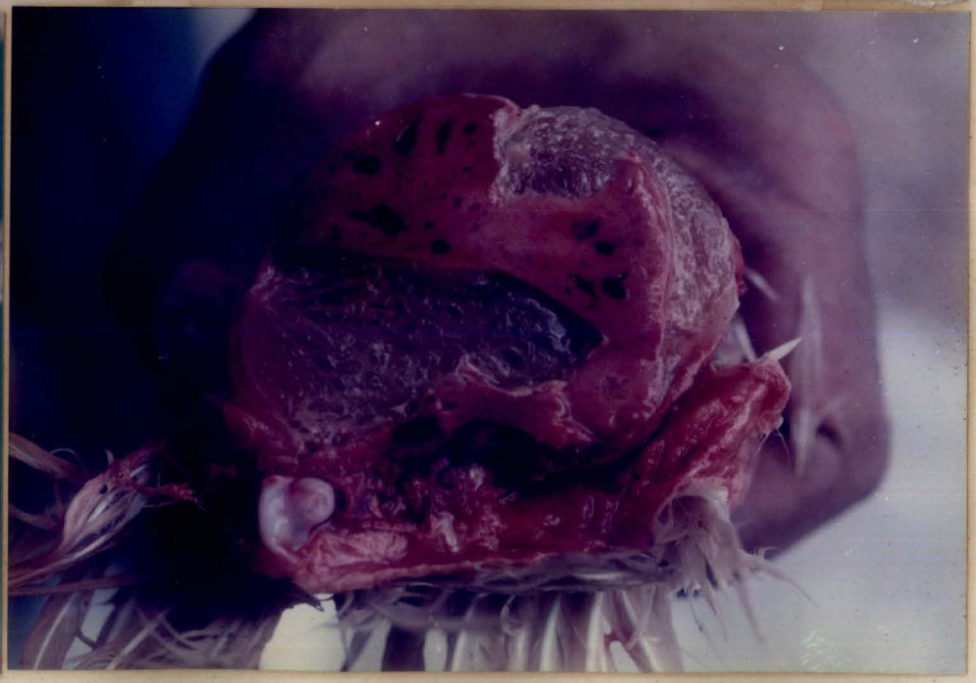


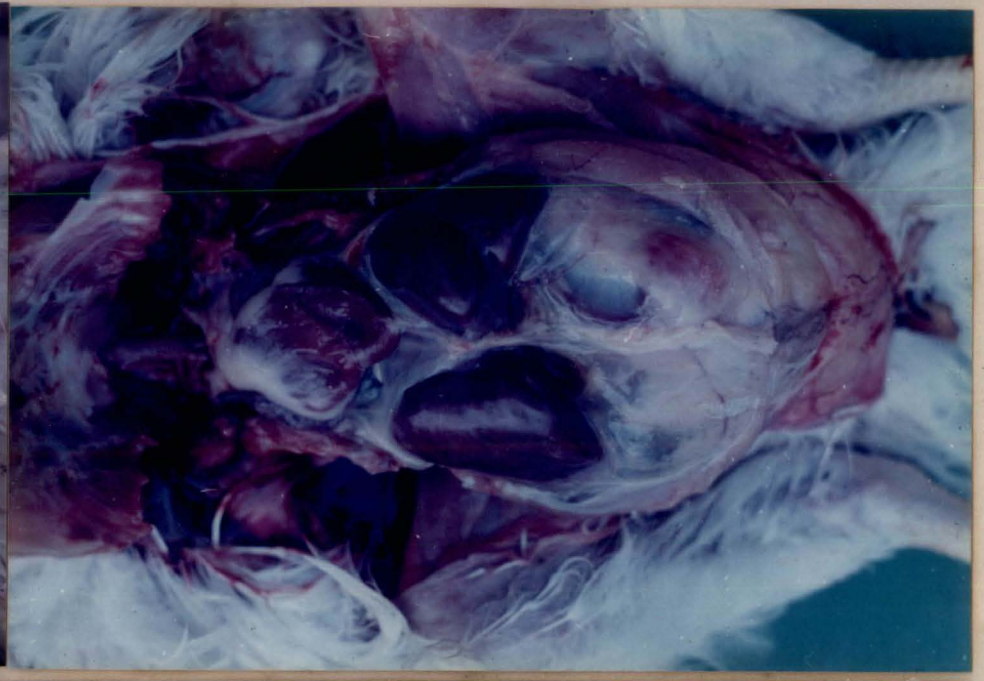
Fig.28 Ascites Syndrome induced with furazolidone (800 ppm). Note the congestion and haemorrhage in the venous plexus in between the abdomen and cloaca

Fig.29 Ascites Syndrome induced with furazolidone (800 ppm). Swollen liver with rounded borders and right sided cardiac dilatation

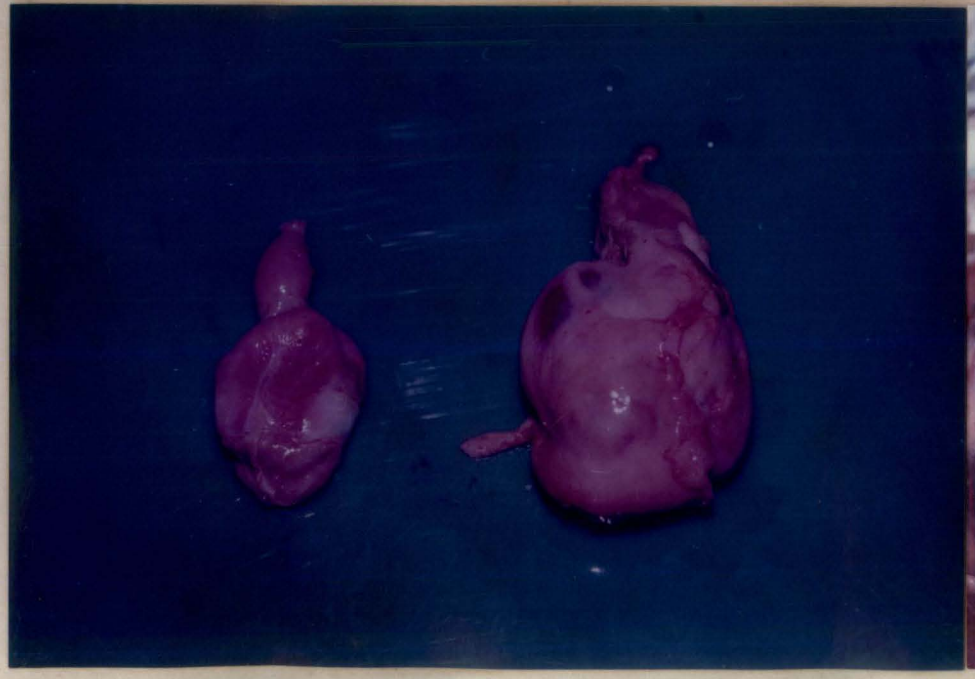
Fig.30 Proventriculus and gizzard - Monensin group (seen left) with that of a control bird (seen right). The gizzard is flabby, pliable and reduced in size

Fig.31 Ascites Syndrome induced by common salt (25,000 ppm). Ascitic fluid from the hepatoperitoneal sacs have been removed to show the congestion and irregularity on the surface of the liver, hydropericardium, presence of abdominal fat and accumulation of ascitic fluid (seen in between the intestine)

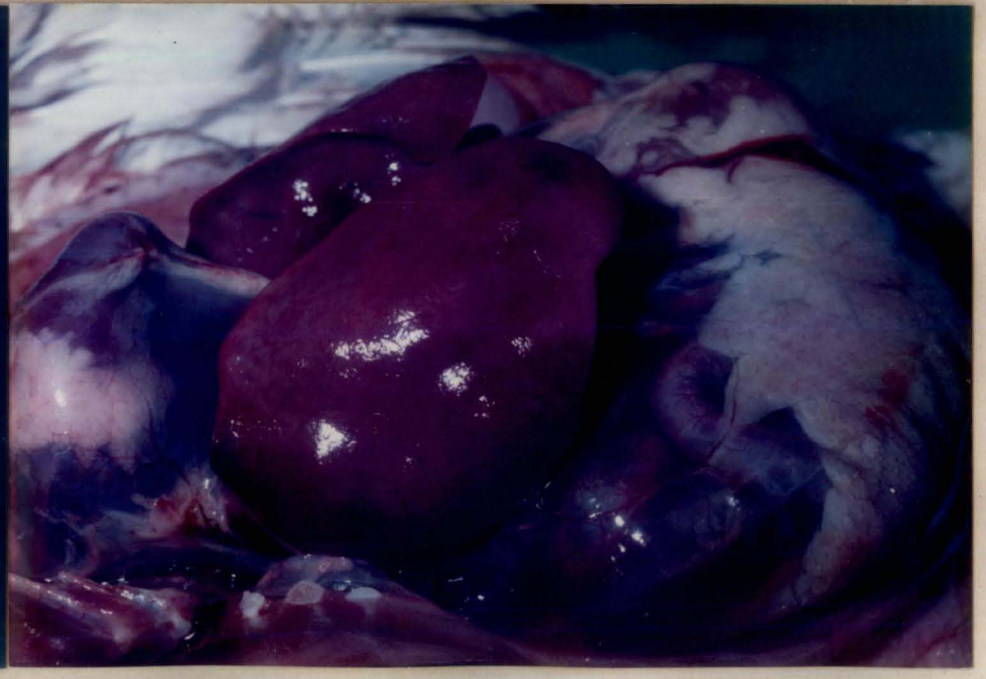
28



29



30



31

Fig.32 Nodular projections on the surface of the liver with common salt induced ascites (The reddish white material supporting the liver is cotton)

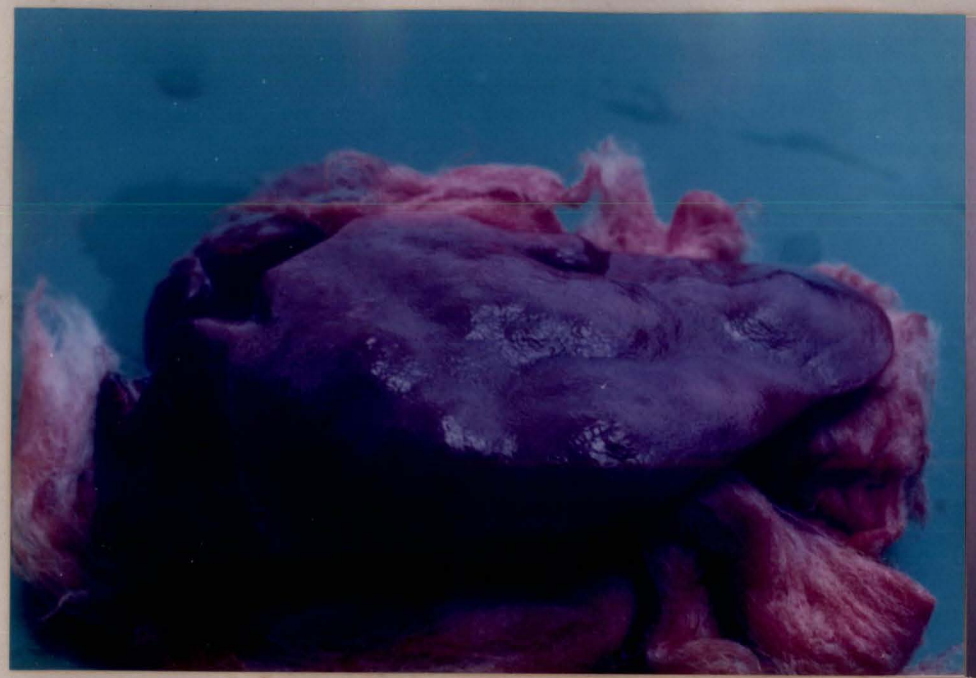
Fig.33 A formalinised specimen of intestine in common salt induced ascites syndrome to show the degree of congestion

Fig.34 Lung : Ascites Syndrome - Congestion, haemorrhage and a cartilaginous nodule (below right) are seen.

H&E x 400

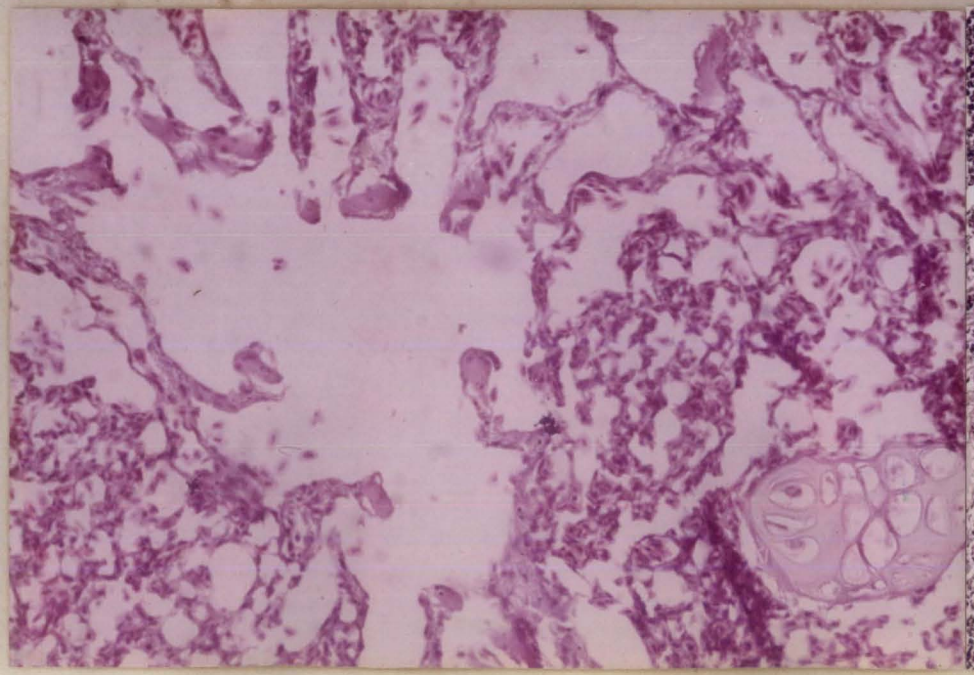
Fig.35 Lung : Ascites Syndrome - Thickening of the adventitial layer, connective tissue proliferation and oedema around the arteries. Part of an ectopic cartilaginous nodule (below right) is also seen.

Van Gieson's x 160

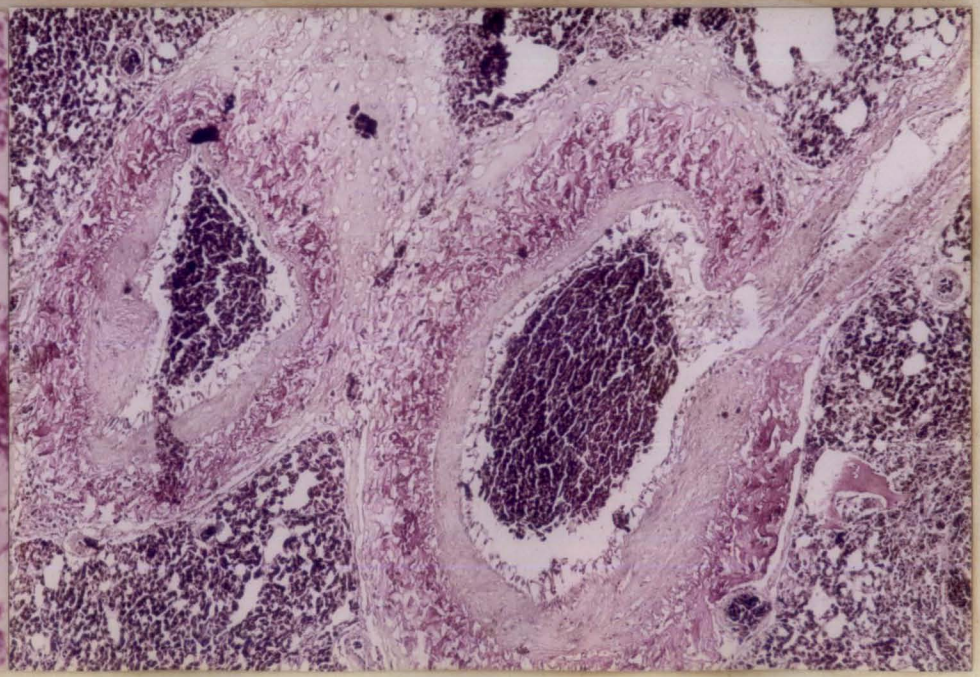


32

33



34



35

Fig.36 Lung : Ascites Syndrome - A pulmonary blood vessel occluded by a thrombus.

Van Gieson's x 400

Fig.37 Liver : Ascites Syndrome - Congestion, degeneration and dilation of sinusoids. Pyknosis of the nuclei of the hepatocytes is also seen.

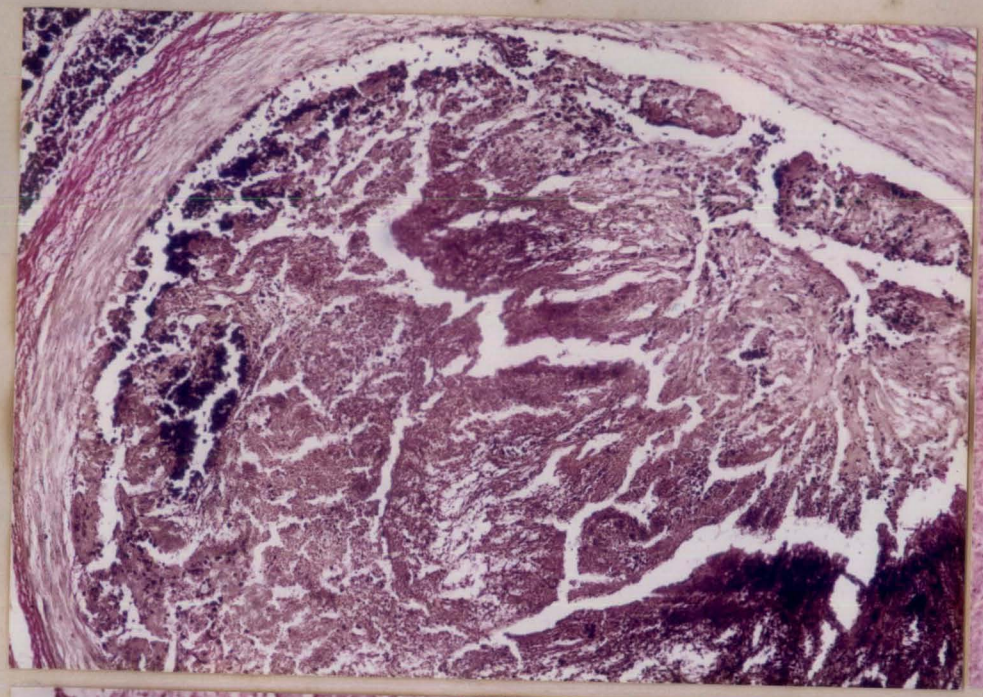
H&E x 160

Fig.38 Liver : Ascites Syndrome - Thickening of the Glisson's capsule due to connective tissue proliferation.

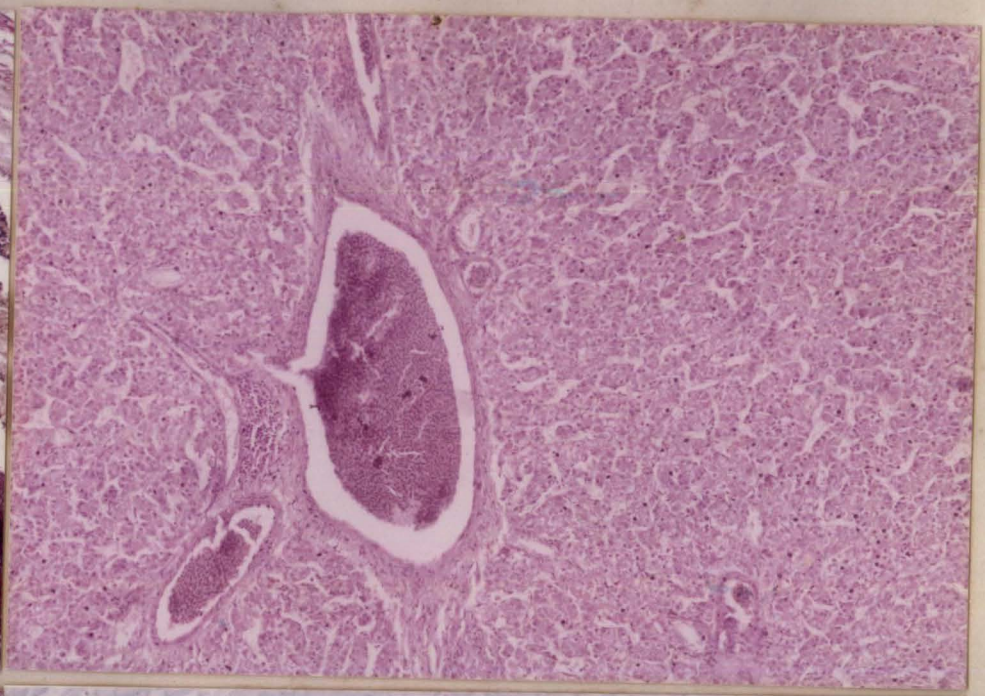
Van Gieson's x 160

Fig.39 Liver : Ascites Syndrome - Deposition of PAS positive materials in the hepatocytes. The thickening of Glisson's capsule is also evident.

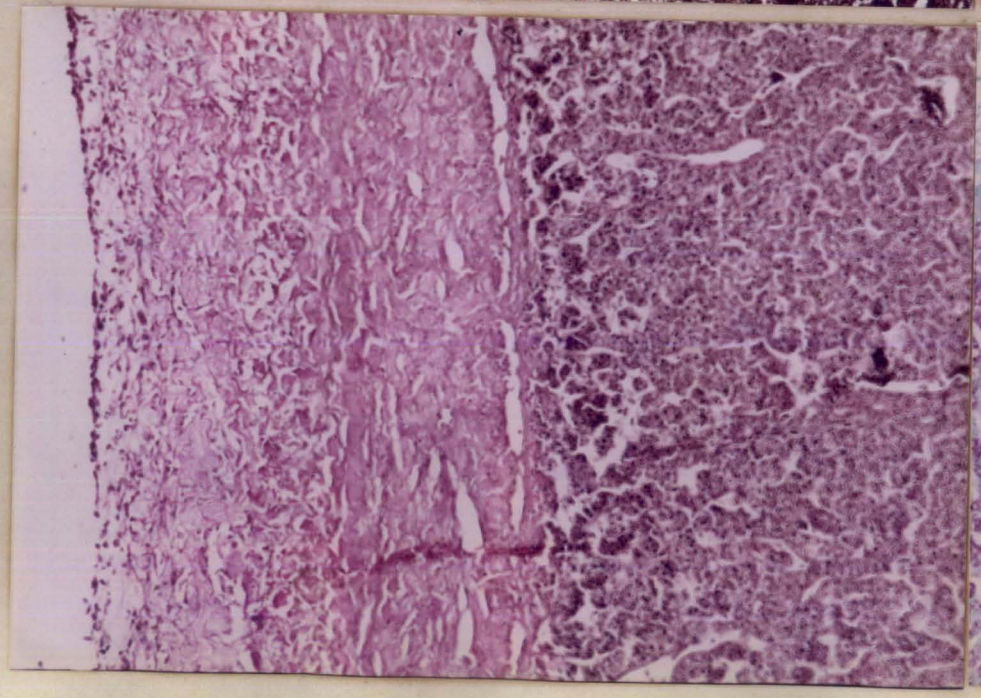
PAS x 250



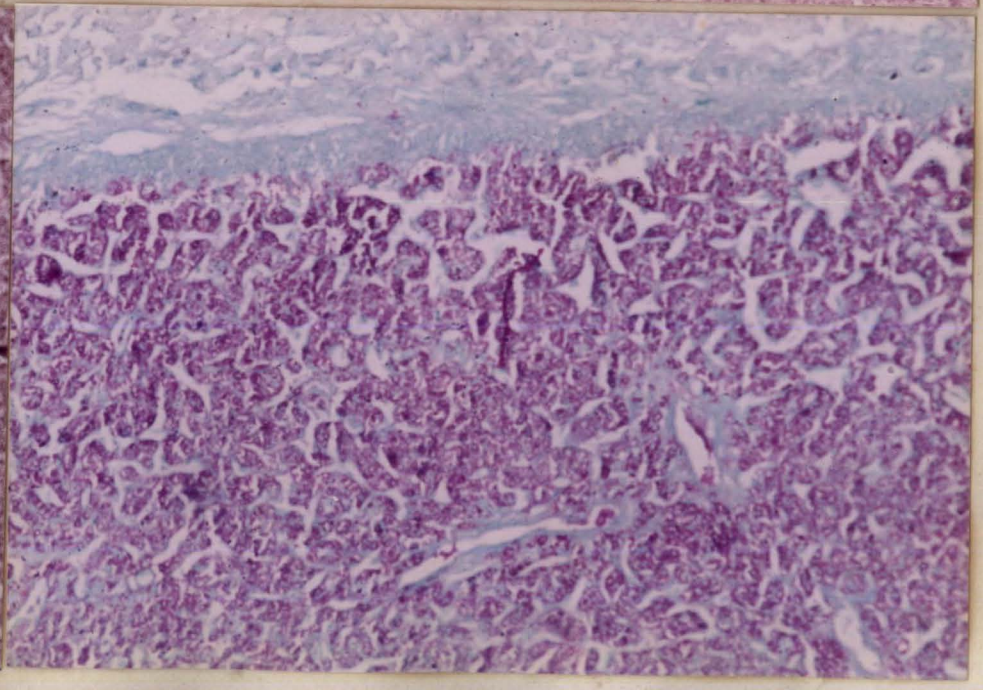
36



37



38



39

Fig.40 Cerebellum : Ascites Syndrome - Congestion in both the major blood vessels and capillaries.

H&E x 160

Fig.41 Lung : Furazolidone (800 ppm) group - Pulmonary congestion and oedema.

H&E x 250

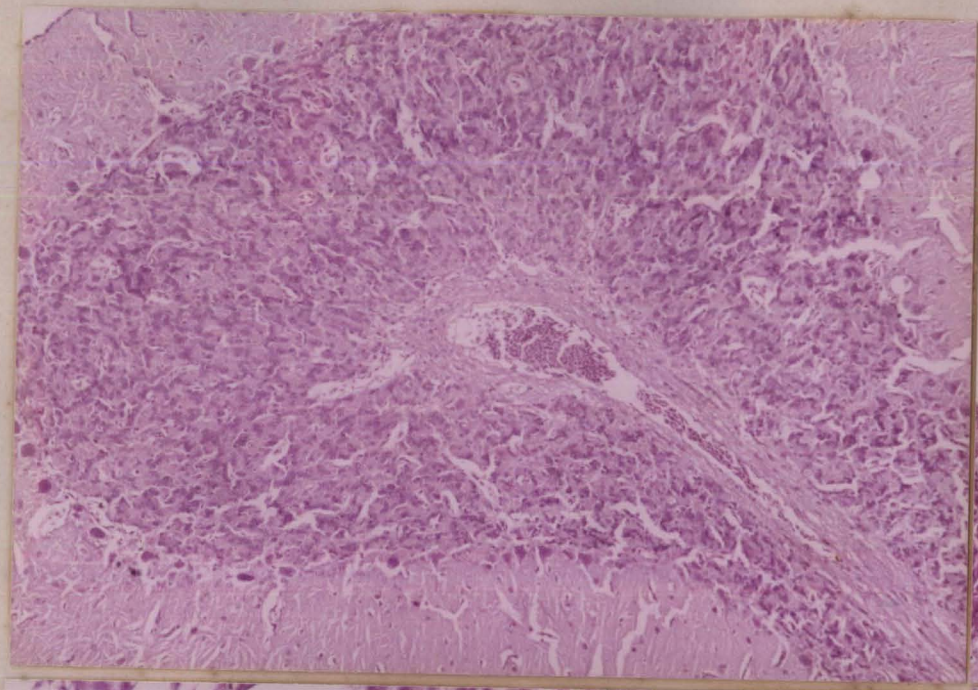
Fig.42 Lung : Ascites Syndrome in furazolidone groups - Ectopic cartilaginous nodule.

H&E x 400

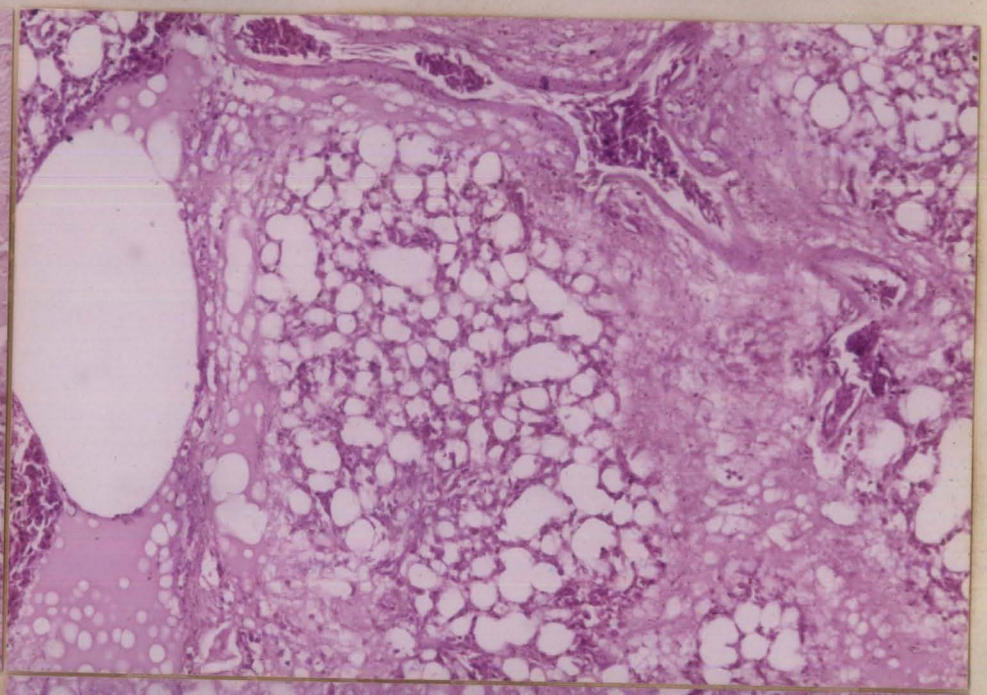
Fig.43 Kidney : Furazolidone (800 ppm) group - nephrosis.

H&E x 400

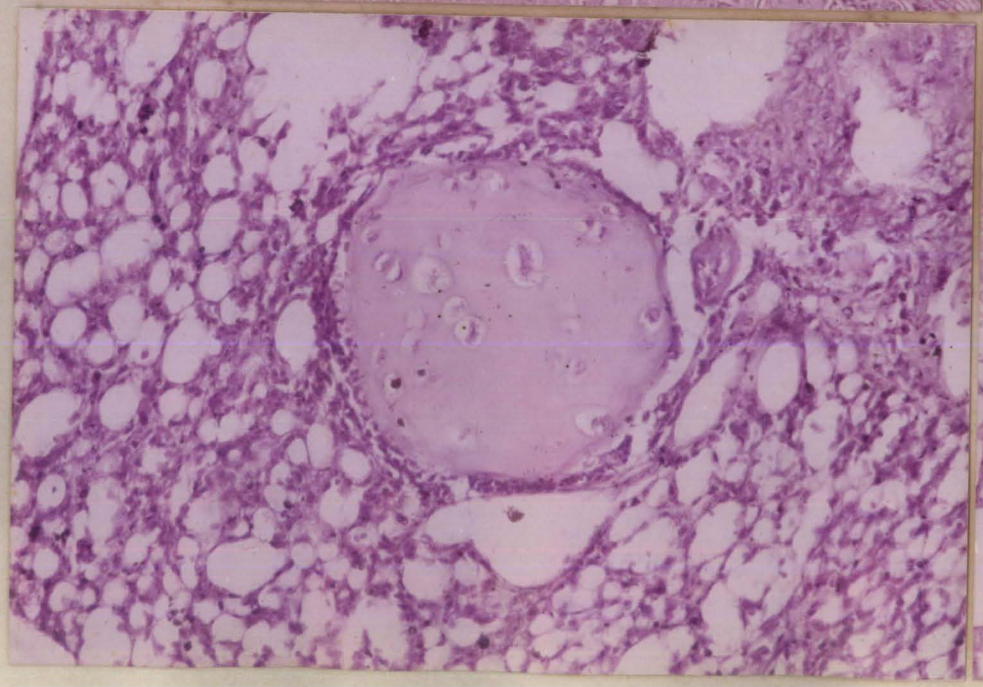
40



41



42



43

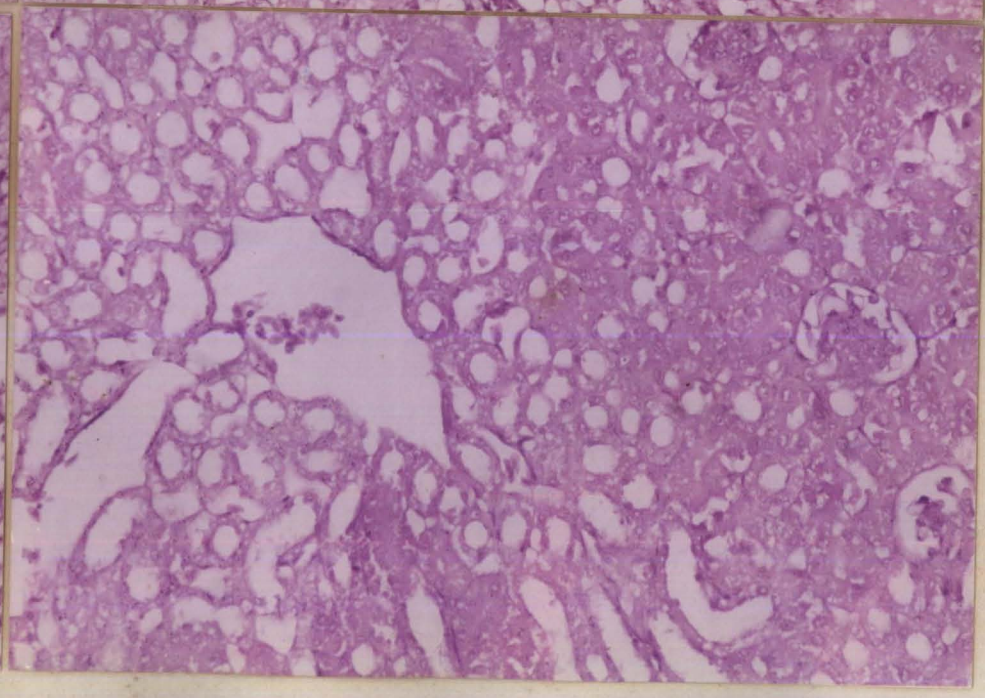


Fig.44 Kidney : Furazolidone (800 ppm) group - nephroblastoma.

H&E x 250

Fig.45 Kidney : Furazolidone (1000 ppm) group - Areas of degeneration and regeneration.

H&E x 160

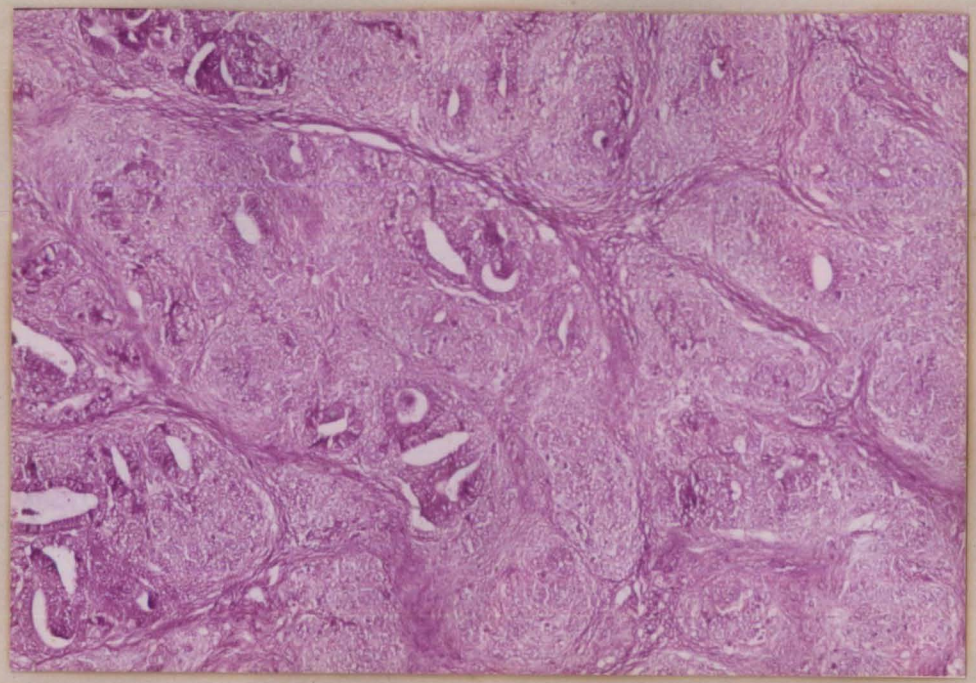
Fig.46 Lung : Pure Sodium Chloride (25,000 ppm) group - Congestion and oedema around blood vessel.

H&E x 60

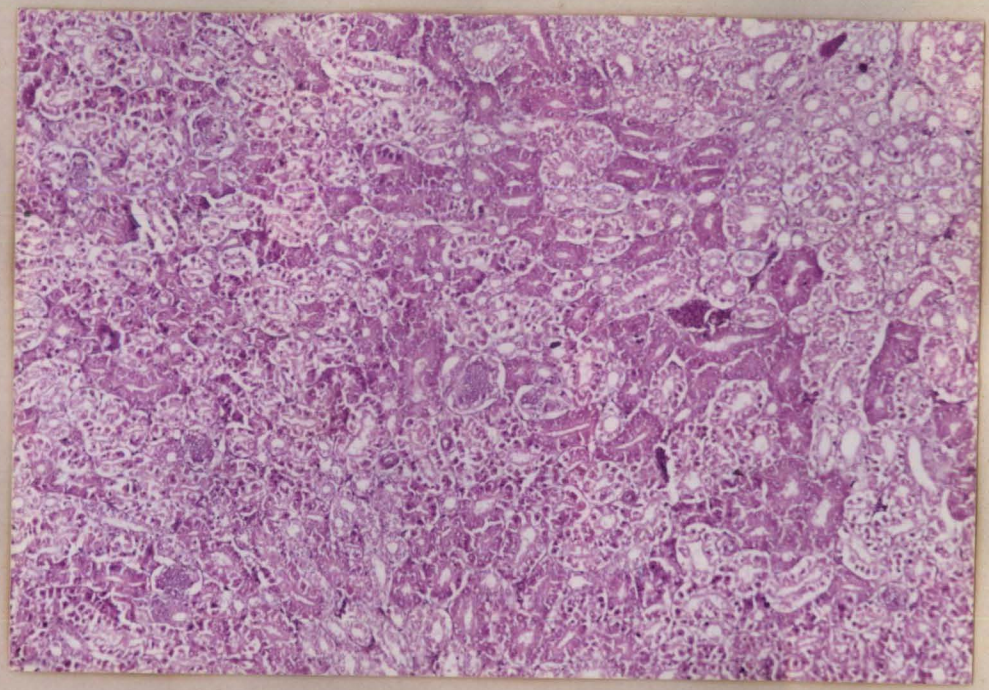
Fig.47 Lung : Pure Sodium Chloride group - Interstitial oedema and congestion.

H&E x 160

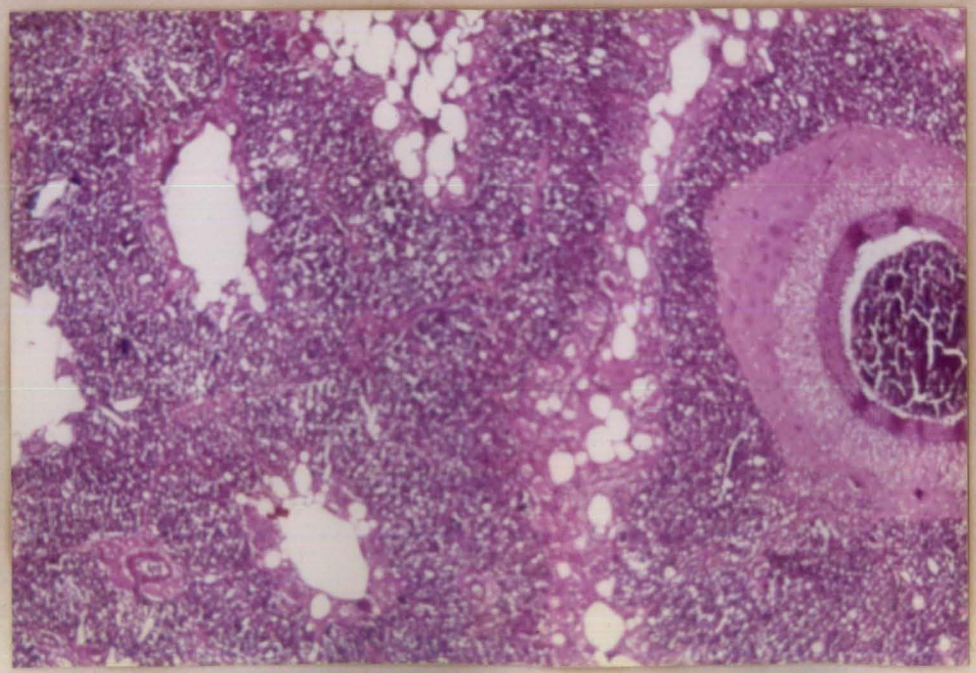
44



45



46



47

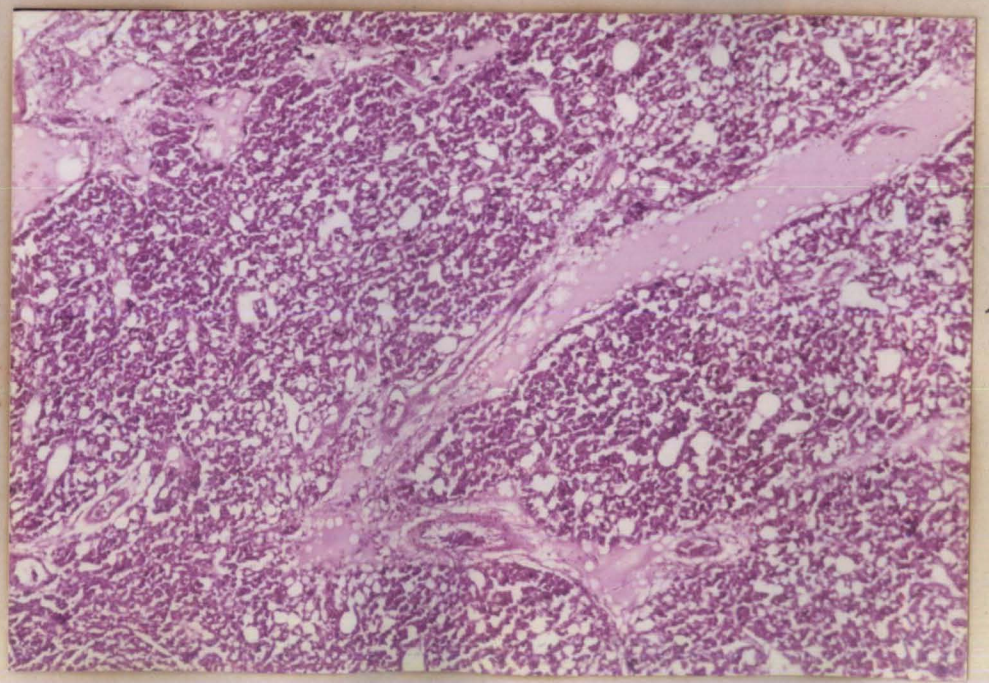


Fig.48 Liver : Pure Sodium Chloride group - Congestion, dilatation of sinusoids and loss of chord - like pattern of the hepatocytes.

H&E x 160

Fig.49 Liver : Pure Sodium Chloride group - Notice the chord-like pattern changing to acinar type.

H&E x 400

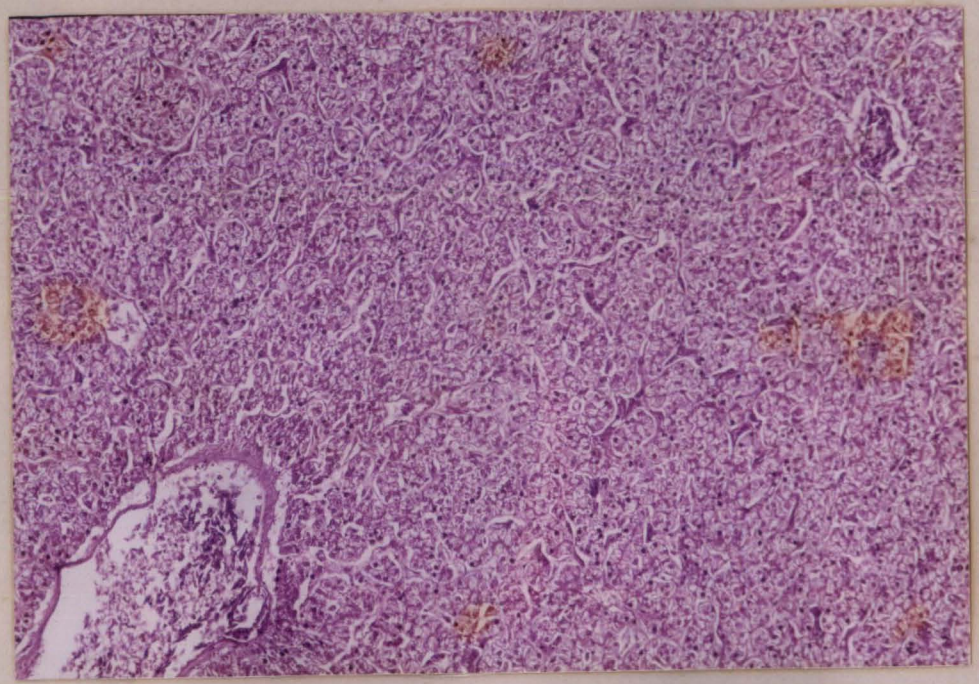
Fig.50 Liver : Monensin group - Proliferation of biliary epithelial cells. Note the necrosis of hepatocytes around the bile duct. Vacuolar degenerative changes and pyknosis are seen in the hepatocytes.

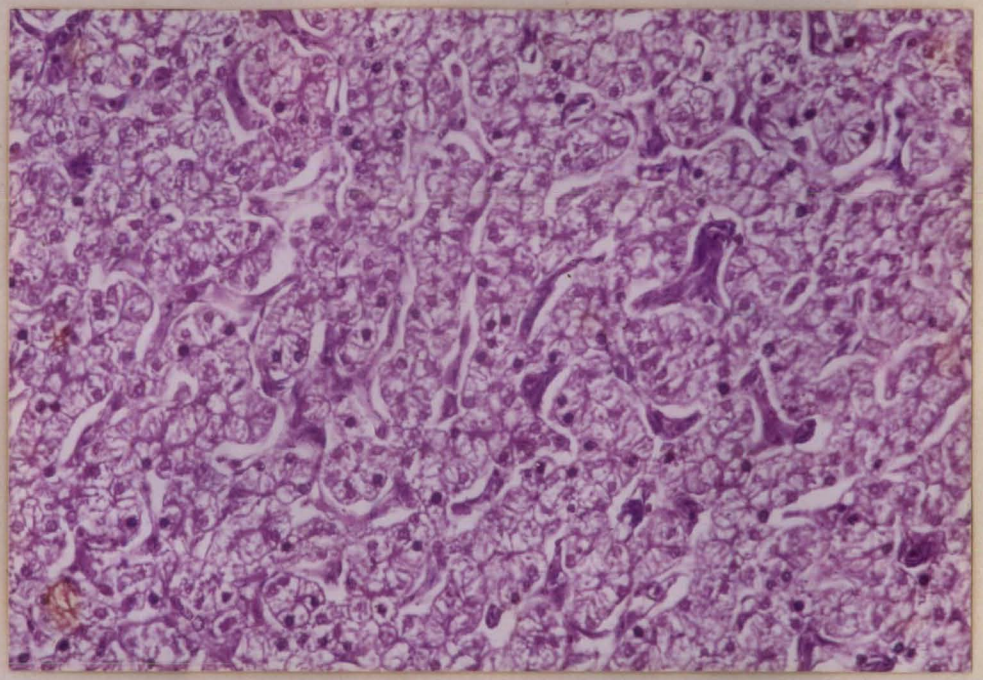
H&E x 160

Fig.51 Lung : Common salt (25,000 ppm) group - Connective tissue proliferation around the blood vessels.

Van Gieson's x 160

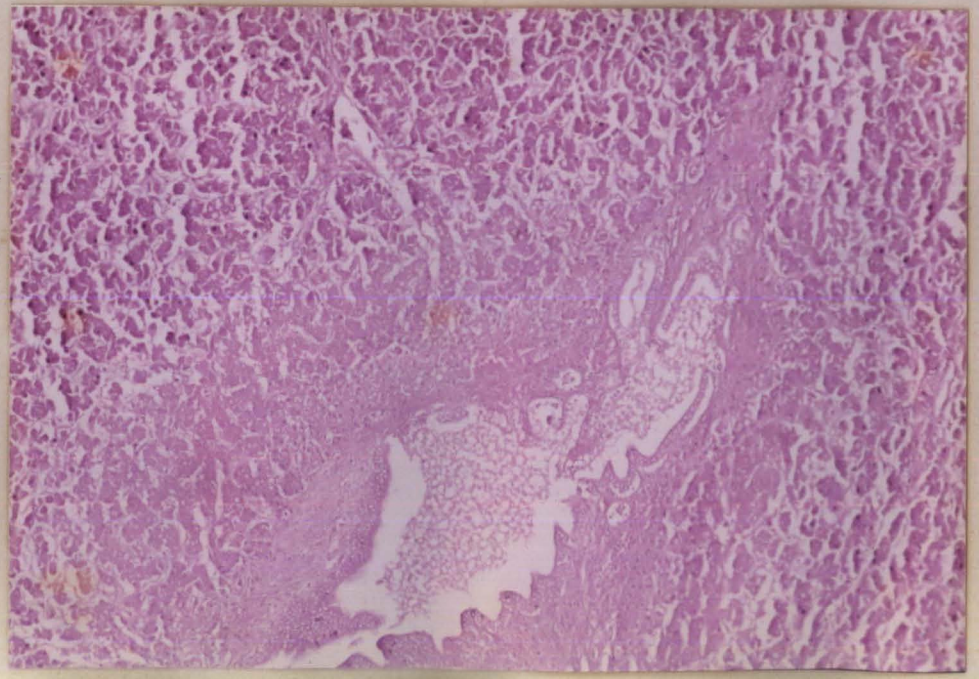
48

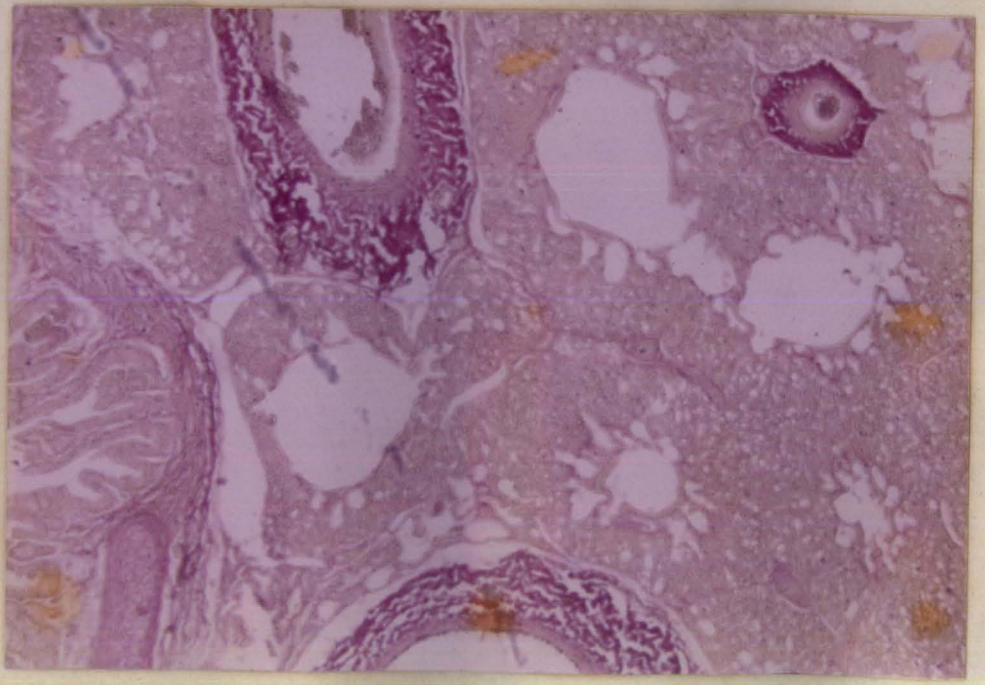




49

50





51

Fig.52 Lung : Common salt induced ascites syndrome - Ectopic cartilaginous nodule (red).

Van Gieson's x 400

Fig.53 Liver : Common salt induced ascites syndrome - Severe dilatation of the central veins.

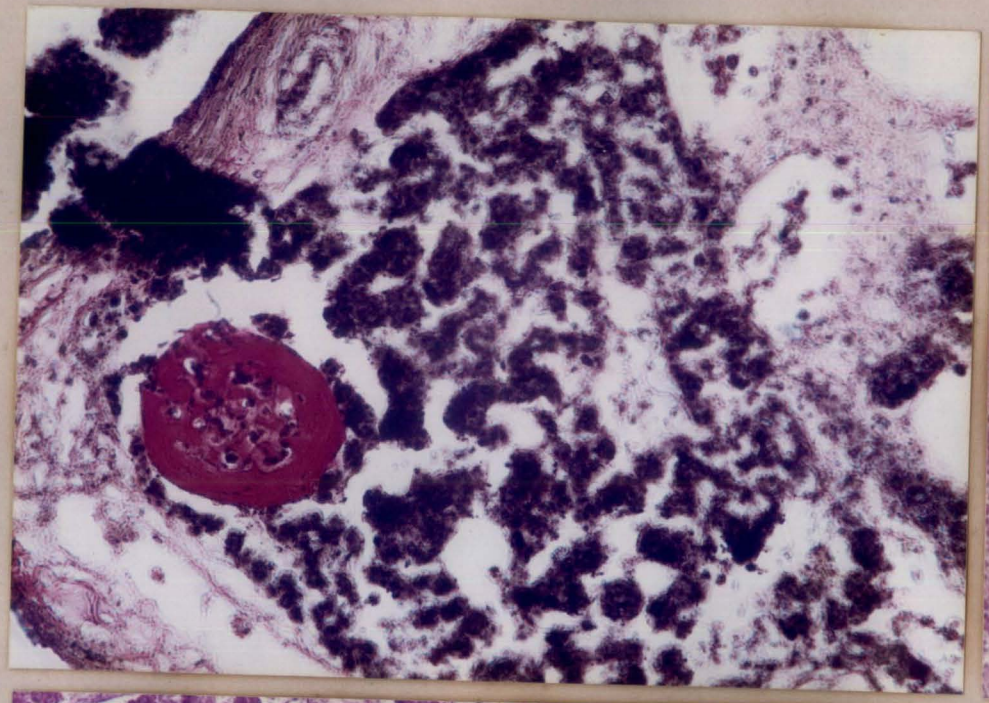
H&E x 160

Fig.54 Liver : Common salt induced ascites syndrome - Disorganisation of the hepatic tissue.

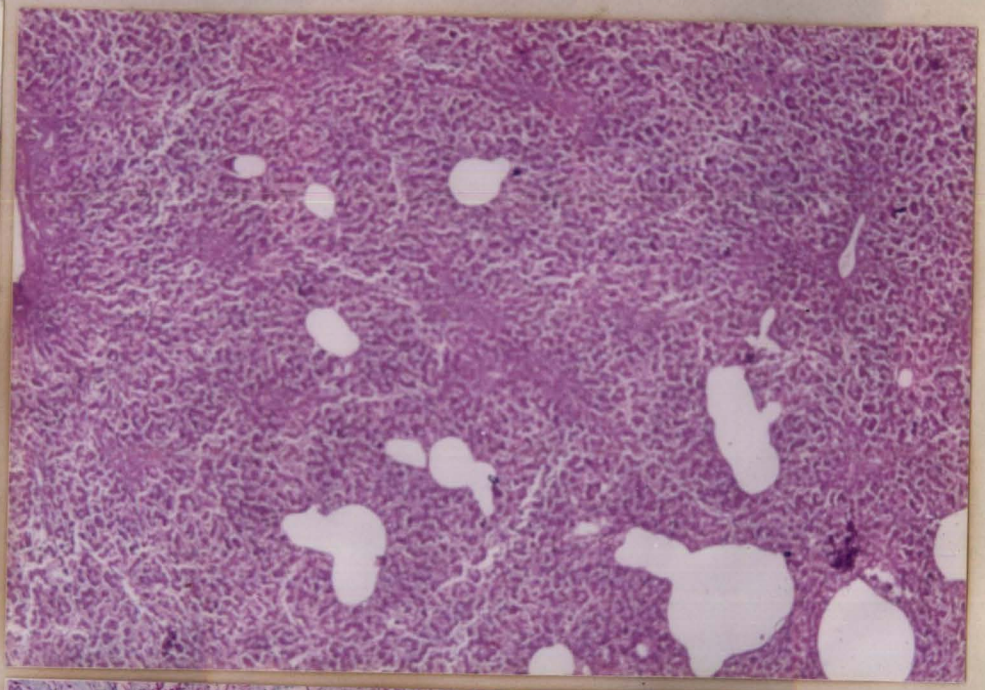
H&E x 400

Fig.55 Liver : Common salt group - Deposition of PAS positive material in the hepatocytes.

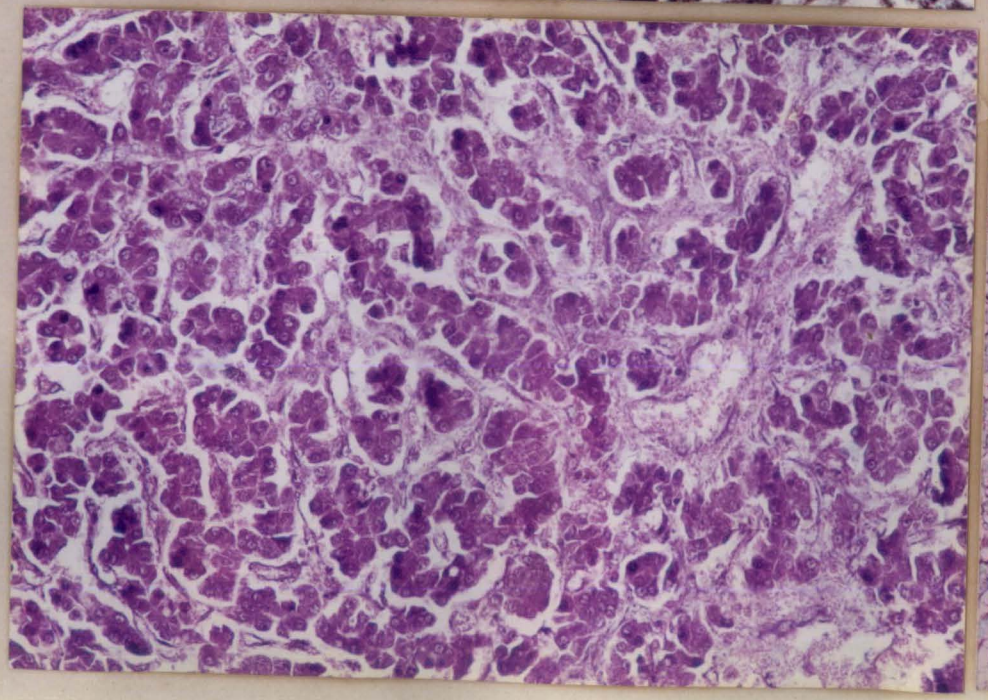
PAS x 250



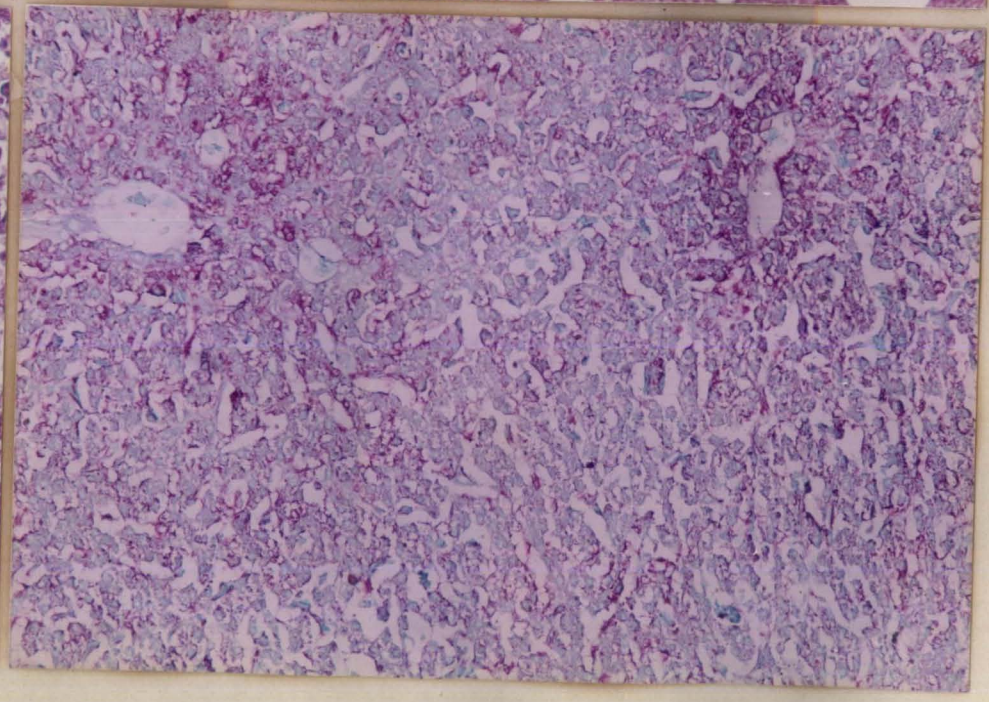
52



53



54



55

Fig.56 Spleen : Common salt induced ascites syndrome - Severe congestion.

H&E x 160

Fig.57 Kidney : Common salt induced ascites syndrome - Dilatation of the Bowman's space and thickening of the basement membrane of the glomerular tuft. Degeneration of the cells in the collecting tubules can also be appreciated.

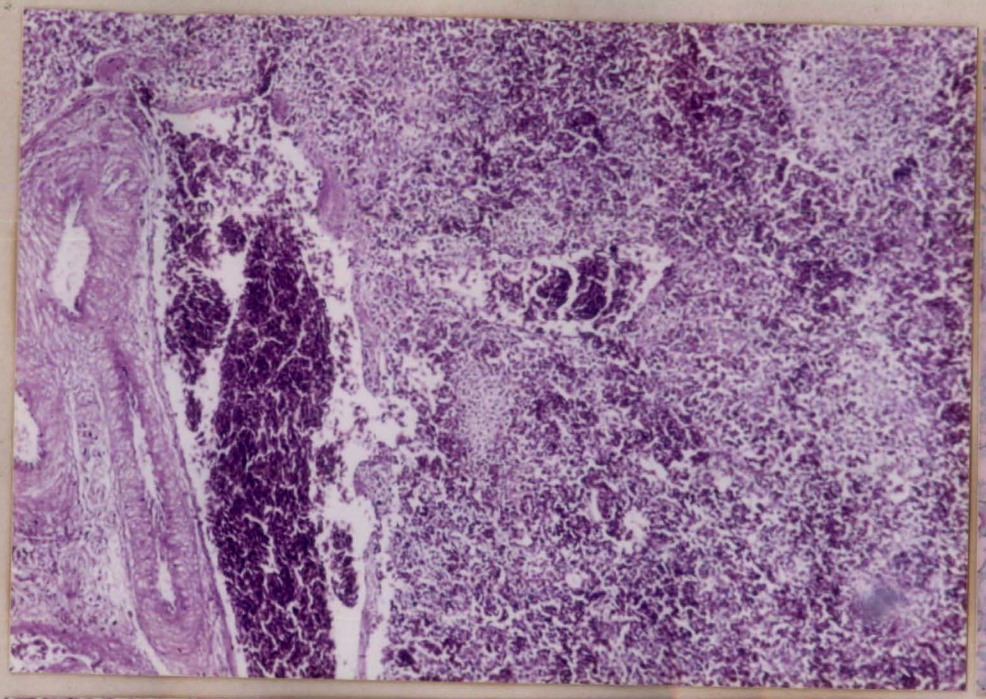
PAS x 250

Fig.58 Kidney : Cobalt chloride group. Congestion, oedema and degeneration.

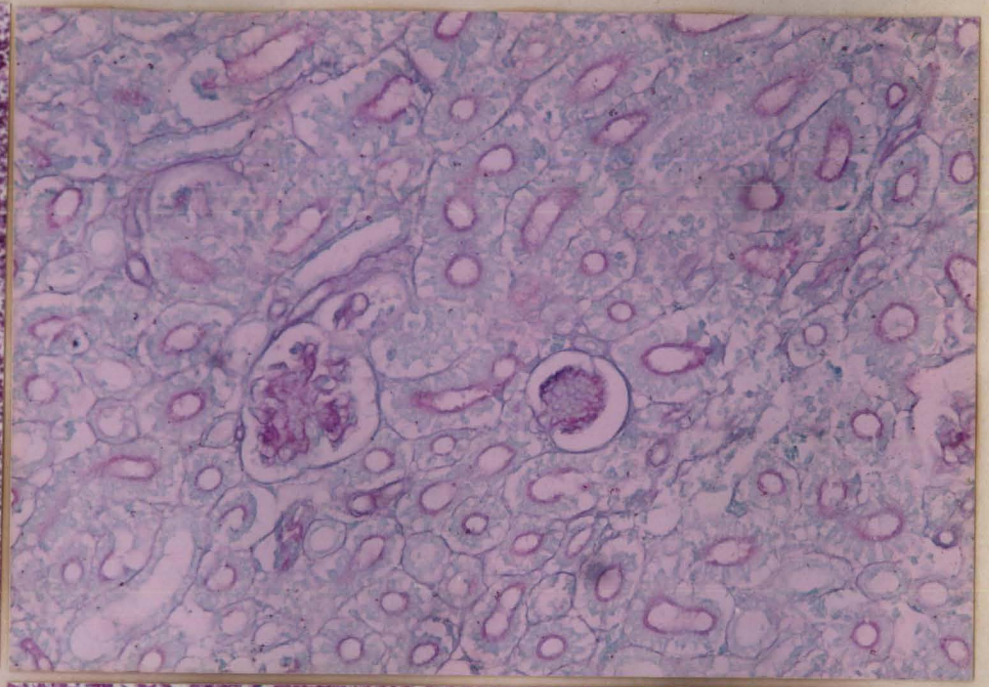
H&E x 250

Fig.59 Kidney : Cobalt nitrite group. Severe congestion.

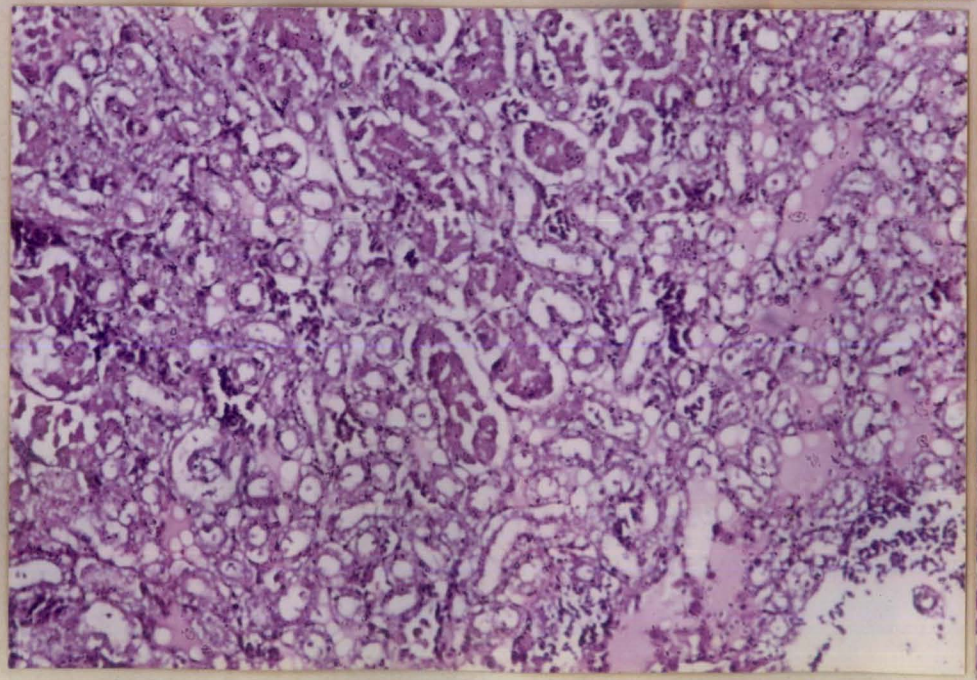
H&E x 400



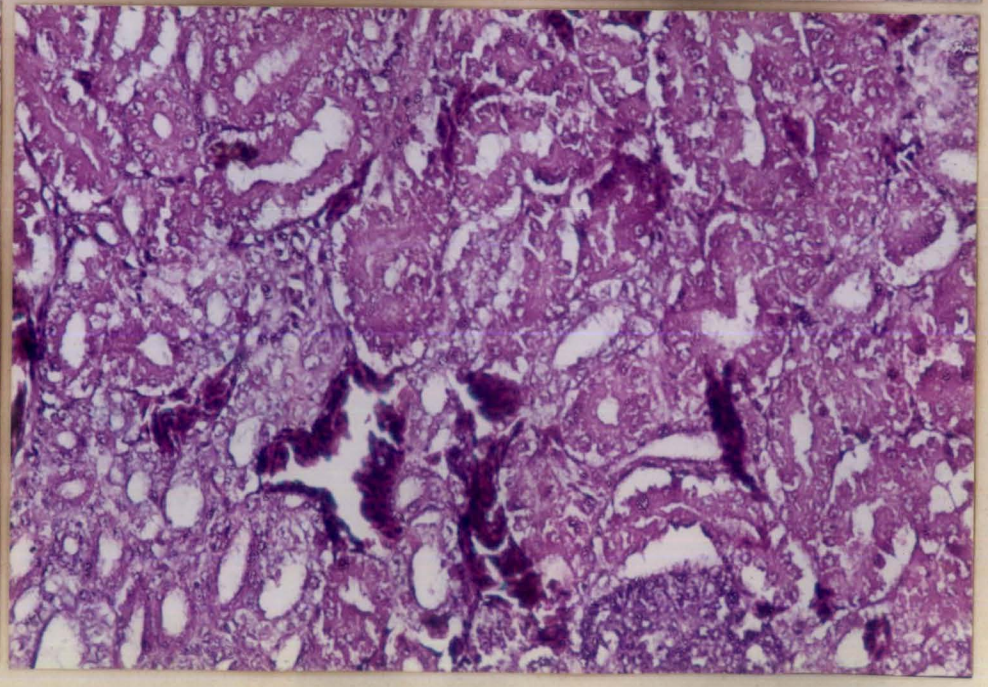
56



57



58



59

Discussion

5. DISCUSSION

Ascites syndrome (AS) is prevalent in broiler farms all over Kerala irrespective of high and low altitude areas. The incidence in the flock appears to be higher in farms located in high altitudes. Most of these farms have closed down since more power was needed to maintain the temperature and due to the prevalence of ascites syndrome. The prevalence (yearly incidence) of ascites syndrome has gradually increased from 1985 and has peaked in the year 1994. There was a drop in the prevalence from 1989 to 1990 which could be correlated to the recession in the broiler industry during that period. This is in conformity with the observations of Riddell (1991) who stated that this disease was first reported in high altitude areas and more recently in low altitudes and that the incidence appeared to be increasing.

The management practises in the farms where AS was encountered were better. This indicated that the disease happens in better managed farms where the growth rate is high and is in agreement with the observations of Walker (1994).

The case of a farmer who added salted fish and five per cent common salt in the feed is a prime example of the effect of common salt in precipitating the disease in young birds. Howell and Gumbrell (1992) also reported salt poisoning in broiler

The Na^{++} and K^{+} content in the serum as well as in the ascitic fluid were high. This might be due to Na^{++} retention as a result of high Na^{++} content in the feed. The increase in the K^{+} content which is an intracellular ion may be due to the leakage from damaged erythrocytes and other cells especially the hepatocytes. This contradicts the findings of Biswas *et al.* (1995) who got a lesser value for both Na^{++} and K^{+} content than the control birds and attributed the decreased level of serum Na^{++} to percolation leading to oedematous condition and haemoconcentration. The variation in K^{+} content was not significant in his study.

Oedematous head, ascites, hydropericardium and severe congestion of all the organs were the main gross lesions. The liver was swollen and firm to cut. In one case it was nodular. These findings are in accordance with those of Fitz-Coy and Harter-Dennis (1988) and Gowda *et al.* (1989). In one case a thrombus was noticed in the lung of a bird. Biswas *et al.* (1995) also reported such cases. They attributed this to slowing or stasis of blood due to impaired cardiac function. The histopathological lesions in the field cases are in conformity with those observed by Hall and Machicao (1968); Maxwell *et al.* (1986a); Hernandez (1987) and Wilson *et al.* (1988).

The two isolates got in the field cases were identified to be *Bacillus subtilis* and *Bacillus cereus*. These may be

contaminants or normal inhabitants of the peritoneal cavity. However, no attempt was made to study their possible pathogenesis experimentally. Perusal of available literature did not reveal isolation of any bacterial organism in ascites due to RVF.

In the present study no virus was detected on EM screening of ascitic fluid. Maxwell *et al.* (1986b) noticed virus particles in some of the EM sections and Payne *et al.* (1991) identified these to be retrovirus. However, there are no other reports of identifying virus in EM sections in broiler chickens with RVF and ascites.

The objective behind experimental studies was to check whether certain feed additives could induce AS. The first experiment to elucidate the aetiological role of sodium chloride, furazolidone and monensin groups ended up with only a single death due to AS in the furazolidone group. This prompted to take up the second experiment. In this furazolidone was administered at a higher dose and common salt was given instead of sodium chloride at the same dose rate. Since the history collected in field case study showed a majority of cases was treated with cephalexin in the chick stage, cephalexin was also administered to another group. Cobalt has been known to induce polycythaemia. Two salts of cobalt namely cobalt chloride and nitrite were given to two separate groups to study the

pathogenesis. In addition two groups were utilised for transmission studies using the ascitic fluid and liver suspension collected from field cases.

In the first experiment, the absence of ascites in the sodium chloride group on post mortem examination may be because the birds developed resistance towards sodium chloride with age. The birds were able to excrete majority of the excess sodium as they were provided *ad libitum* water to drink unlike in experiments where salt was added to drinking water. The body weight of birds in this group was lesser than the control during the first two weeks of treatment and in the last week. This is in concurrence with the findings of Bressler *et al.* (1951). The PCV and haemoglobin were higher than the control. This might be as a result of RVF. The serum protein, albumin and globulin content were lesser than the control throughout the experiment which can be attributed to liver damage and increased blood volume. Julian (1993) was of the opinion that RVF increased the blood volume and the hypoxaemia associated with RVF resulted in polycythaemia. When the increased blood volume is taken into account, this polycythaemia indicates a marked increase in RBC numbers. In sodium chloride toxicity, initially it might have caused an increase in blood volume and subsequent RVF and then polycythaemia.

Serum Na^{++} was greater than control which indicated that there was Na^{++} retention. The increased K^{+} content was however not significant.

The right ventricle to left ventricle ratio (RV:LV) and right ventricle to total ventricle ratio (RV:TV) were higher in this group which indicated that the birds had right ventricular hypertrophy and RVF. All the organs showed a significant increase in weight except the bursa. This observation pointed out that there was Na^{++} retention and consequent increase in blood volume and this had caused hypertension resulting in congestion, oedema and connective tissue proliferation. The decrease in the weight of the bursa may be due to the strain created by the treatment. The gross and histopathological examination also supported these findings and are in agreement with the findings of Scrivner (1946), Bressler *et al.* (1951), Mohanty and West (1969), Mishra *et al.* (1987) and Howell and Gumbrell (1992).

In the second experiment, at the same dose of sodium chloride (25,000 ppm), common salt was given to one group. The results were amazing even though the percentage of sodium chloride was lower in common salt. Three birds died on the 5th, 11th and 24th days of treatment. These three birds were the best in the group and exhibited respiratory distress. The birds which died on the 11th and 24th day had tense fluid filled

abdomen. Ascites, respiratory distress and diarrhoea were observed in salt toxicity by Bressler *et al.* (1951), Mishra *et al.* (1987) and Howell and Gumbrell (1992).

The common salt group had more body weight than all other groups and it was significantly greater than the control at D35 and D42. This may be due to the accumulation of ascitic fluid, excess circulating fluid volume and too much water in the gastrointestinal tract. Bressler *et al.* (1951) experimented in turkey poults with different doses of salt levels in the mash and found that addition of salt to the ration upto 1.8 per cent level had a favourable effect on the body weight. Level of 3.6 per cent salt was definitely detrimental to body weight. In this study 2.5 per cent common salt was used which is in between the experimental doses.

The PCV and haemoglobin values were generally higher as in the case of sodium chloride group but serum protein and albumin did not significantly vary with the control. Though common salt group had an initial decrease in serum globulin at D28 it picked up and at D56 it had higher value than the control. Pimental and Cook (1988) suggested that salt improved immune levels and growth at 0.25 per cent. The Na^{++} content in the serum was higher as in the case of sodium chloride group, however it had a significantly higher K^{+} content all throughout the experiment. The increase in the K^{+} content which is an intracellular ion may

be attributed to the increased fragility of the RBC which may result in the leakage of the intracellular K^+ ion on clotting. Mishra *et al.* (1987) observed that the plasma K^+level exhibited a reverse relationship with plasma sodium.

The weight and relative weight of heart, right ventricle and left ventricle were highest among all the groups. Fields *et al.* (1991) observed that sodium caused hypertension and induced left ventricular hypertrophy in human beings.

The RV:LV was higher when compared to the control but was not significant, but the RV:TV was least among all the groups. This is because the left ventricle hypertrophied to compensate the extra fluid volume and this explains why the rest of the birds survived. The RV:TV of the dead birds was higher but their values were not taken into account as it would alter the mean weights.

The weight of the spleen, lungs, liver and intestine was greater than the control due to an increase in the blood volume.

The dead birds showed sequential progressive stages of lesions. Most chronic lesions were seen in the bird which died last. Congestion was noticed in all the organs. In the first bird hydropericardium was noticed but there was no ascites. Julian (1993) observed that the broilers with lung oedema from pulmonary hypertension might die from hypoxia before ascites

developed. The lesions seen in this bird can be said to be in between that of Sudden Death Syndrome (SDS) and AS. This gives rise to the doubt whether SDS is an acute stage of AS. Hence the role of common salt in induction of SDS should be studied.

The second bird which died on the 11th day had ascites in addition to the above lesions. The liver lobules were prominent on the surface due to chronic portal hypertension. In the third bird this portal hypertension had progressed and nodular projections were seen on the surface of the liver.

Histologically congestion, oedema, haemorrhage and vacuolar degenerations were noticed in all the organs. The lungs showed ectopic cartilaginous nodules and the liver showed thickening of the Glisson's capsule. Inflammatory changes were minimal. The PAS positiveness of the hepatocytes may be due to the intracellular deposition of glycoprotein. Swayne *et al.* (1986) reported an increase in intracellular glycogen and cytoplasmic granularity in apparently unaffected turkey poults which were given 1.85 per cent of sodium chloride in the feed.

It is a matter of interest that only three birds died and the rest seven survived. It should also be noted that when pure sodium chloride was given in the first experiment, none died of ascites. This may be because the impurities in common salt may have a potentiating role rather than the effect of NaCl alone. Another reason could be the change in pH of the feed due to the

addition of common salt (Shlosberg's personal communication at the xx World's Poultry Congress).

These observations indicate that studies with common salt should be given more importance than with pure sodium chloride as this is exacting the field condition wherein we add common salt to the feed. Also in such experiments salt should be added to feed rather than to water since the former increases the body weight while the latter reduces body weight.

When furazolidone was given to birds at 800 and 1000 ppm, the birds showed nervous symptoms. Inco-ordinated gait, twisting of head and leg weakness were noticed. One bird each died of ascites. These observations are in conformity with the reports of Newberne and McEven (1957) who reported nervous symptoms in chicks with nitrofurazone toxicity and with Harwood *et al.* (1958) and Feron and Van Stratum (1966) who reported ascites in chicks fed furazolidone.

The body weight was lower than the control in the 800 ppm group and it still decreased when the dose was increased to 1000 ppm in the second group. This is in concurrence with the observations of Jensen *et al.* (1975).

The PCV, haemoglobin, serum protein, albumin and globulin were lower than the control in both the groups. This can be attributed to their lesser feed intake and liver damage.

The serum K^{+} content was lesser in both the groups. The serum Na^{++} content did not vary significantly in the 800 ppm group while it showed an initial decrease and thereafter a reversal in the 1000 ppm group. The reason for this is not clear.

While the absolute organ weights were lesser than the control, the relative organ weights were higher. This is because their body weight was lesser. In the 800 ppm group the LV to heart ratio was higher while the RV:TV was lesser than the control. In the 1000 ppm group both the LV to heart ratio and RV:TV were higher. This meant that while the birds compensated with left ventricular hypertrophy in the 800 ppm group, this compensation was not sufficient to cope up with that of the right side in the 1000 ppm group and hence the increase in RV:TV became significant. However, it should be noted that the mean PCV and haemoglobin were not higher as in the field cases of AS and the salt groups.

Moderate degree of congestion, hydropericardium and nephrosis were noticed in the birds. One bird had a tumour mass in the kidney which was identified to be nephroblastoma. Ectopic cartilaginous nodules, pulmonary congestion and oedema, loss of striation and separation of myocardial fibres were noticed. This observations are similar to the findings of Blount (1955); Klimes and Kruza (1962); Webb and Vanvleet

(1971); Reed *et al.* (1987) and Bahgat *et al.* (1990). The development of tumour in a bird may be attributed to the carcinogenic property of furazolidone.

In the first experiment when monensin sodium was given to birds at 360 ppm in feed, they exhibited reduced feed intake and weight gain. They became stunted. None of them developed ascites. Reduced feed intake and weight gain were reported by Hanrahan *et al.* (1981); Watkins *et al.* (1993); Bartov (1994) and Weisman *et al.* (1994) in monensin toxicity.

The PCV, haemoglobin, serum protein, albumin and globulin were generally lesser than the control which can be attributed to their reduced feed intake. This is in concurrence with the findings of Khan *et al.* (1994).

The serum K^{+} was higher at D28 and D42 and then reversed by D56 while the serum Na^{++} content was greater than the control. This may be due to the ionophore properties of monensin sodium.

The organ weight and RV:TV were lesser than the control. This indicated that monensin sodium might not be a potential agent in precipitating AS at this dose. All the organs were small and the gizzard musculature was weak and pliable. Focal areas of nephrosis were also noticed. Stuart (1978) observed small pale spleen, pale areas in the myocardium, haemorrhages on the heart fat, pale kidney and congested lungs in turkey fed excess of

200 ppm monensin. Hanrahan *et al.* (1981) noticed emaciation, generalised congestion, myocardial enlargement, pallor and hydropericardium with 150, 200 or 230 mg crystalline monensin sodium.

In the present study, vacuolar degenerative changes were noticed in the heart, liver and kidney. Focal necrosis of hepatocytes and mild proliferation of biliary epithelial cells were seen in the liver. Inflammatory reaction was also noticed in the lungs of few birds. Satellitosis and neuronophagia were noticed along with mild congestion and oedema in the brain. Mild lymphoid depletion was seen in the spleen and bursa. Hanrahan (1981) noted intermyofibrillar vacuolation histochemically positive for neutral fat in the myocardium and red muscle fibres.

In the second experiment cobalt was given as cobalt chloride and cobalt nitrite to two groups. The birds showed reddened skin. This might be due to polycythaemia and PH induced by cobalt. This was associated with a high PCV and haemoglobin values than the control throughout the experiment. A similar response was observed by Diaz *et al.* (1994) with cobaltous chloride in broilers. Miller *et al.* (1974) and Underwood (1975) observed cobalt induced polycythaemia in both man and animals. Both the groups showed a lesser body weight

than the control after three weeks of treatment, confirming the observations of Hill (1979) and Southern and Baker (1981).

The serum protein, albumin and globulin did not vary significantly from the control in the cobalt chloride group, while in the cobalt nitrite group the serum protein was higher at D56 and the serum albumin content though lesser at D28 picked up and was higher than the control at D56. The albumin-globulin ratio of the cobalt nitrite group was greater than the control at D28 but then onwards the difference was not significant.

The serum Na^{++} content was generally higher and the serum K^{+} was lower than the control for both the groups. The reason for this is not clear.

Though the absolute organ weight was lesser than the control, the weight of RV, RV:LV and RV:TV was greater than the control in the cobalt chloride group while only RV:TV was significantly greater than the control in the cobalt nitrite group. This is in agreement with the observations of Diaz *et al.* (1994). They were of the opinion that accidental or deliberate incorporation of excessive amounts of cobalt in chicken feed might cause ascites if the concentration was high enough. They also suggested that high dietary cobalt could be used as an experimental model to induce RVF and ascites in broiler chicken.

Moderate degree of congestion in the lungs and liver, hydropericardium, right ventricular dilatation and hypertrophy and diffuse areas of nephrosis and hepatosis were the main lesions. However, no ascites was seen on post mortem examination in both groups. Van Vleet *et al.* (1977) observed mild to moderate hydropericardium in pigs given three daily doses of cobalt as sulphate.

The cobalt chloride group showed increased number of ectopic cartilaginous nodules, congestion, haemorrhage and proliferation of connective tissue in the lungs. Degenerative changes were noticed in liver and kidney. Mononuclear infiltration in myocardium and mild oedema and congestion were noticed in the brain of few birds. The lesions were similar in the cobalt nitrite group but of lesser intensity.

Diaz *et al.* (1994) observed epicardial fibrosis in some of the birds with ascites when cobalt chloride was fed at 500 ppm. In the present study no such observations were made. They suggested that polycythaemia had a direct effect on the viscosity of blood and that the blood viscosity increased exponentially with increasing haematocrit. The absence of myocardial lesions in the cobalt-fed chicken suggested that the increased incidence of RVH, RVF and ascites observed in this group was not caused by a primary cardiotoxic effect of cobalt,

but due to higher viscosity of the blood associated with its high haemoglobin content.

When cephalexin was given to the birds no noticeable symptoms were observed. The body weight, PCV, haemoglobin, serum protein, albumin and albumin-globulin ratio were in par with the control. The serum sodium and potassium were generally lesser than the control. The organ weight and relative organ weight were lesser than the control. The RV was in fact lesser than the control and the RV:LV and RV:TV were not significant.

On post mortem examination, the birds showed mild congestion of the lungs and moderate diffuse hepatosis. Hydropericardium and dilated auricles were seen in two birds.

Histopathologic lesions included pulmonary congestion and haemorrhage, proliferation of interatrial epithelial tissue in parabronchi of the lungs, vacuolar degeneration, heterophilic infiltration and fibrin deposits between the muscle fibres of the heart, diffuse vacuolar degeneration in the liver and hypercellularity of the glomerular tuft. Mild lymphoid depletion was noticed in the spleen and bursa. Mild meningeal congestion, spongy appearance of the white matter and gliosis were seen in the brain.

Perusal of the available literature did not reveal any study relating cephalexin to AS in birds. In spite of the

presence of the lesions, the birds were apparently healthy and the RV:TV ratio was also not high when compared to the control. This meant that at this dose rate cephalexin was not a potential agent which precipitated AS during two to eight weeks. However, the lesions like hydropericardium, pulmonary congestion and haemorrhage and myocardial lesions in certain birds suggested that experiments with higher doses starting from a younger age should be tried before completely ruling out its aetiological role.

In the group injected with liver suspension none developed ascites. Their body weight, ESR and PCV did not significantly vary from the control. The group had a higher haemoglobin than the control but was significant only at D42. The serum protein, albumin and globulin did not vary significantly with the control. However on D28 the albumin-globulin ratio was higher than the control.

The serum Na^{++} content was higher than the control after the first and second week of injection and then it became lesser than the control. The serum K^{+} content also was higher after the first week of injection and then it decreased and recouped by D56.

Mild diffuse hepatosis noticed in some birds might be due to a reaction towards extraneous liver antigen.

In the group given ascitic fluid the body weight was lesser than the control at D35 and D49 and then regained weight by D56. One of the birds developed a flabby, gas filled abdomen. This might have resulted from an accidental puncture of the air sac. This bird showed mild airsacculitis on post mortem examination. This might perhaps explain the fluctuation in the body weight. Since only one control was kept for all the eight groups in experiment II, the control group was not injected with distilled water or sterile ascitic fluid/liver suspension. This can be a possible flaw in this experiment.

The ESR, PCV, Hb, serum protein, albumin, globulin and K^+ content did not show a notable change but the serum sodium was lower than the control at D42 and D56.

In both the liver suspension and ascitic fluid groups the organ weights were either lesser or did not significantly vary with the control. The RV and LV weights were lesser than the control but the RV:TV value turned significantly greater eventhough the RV:LV value was not significant. This is only because of a lower LV value and is not due to a rise in the RV weight and hence need not be taken into account. Perusal of the available literature did not reveal any transmission study in AS in broiler chicken.

From this investigation it was observed that feed additives like furazolidone, sodium chloride and cobalt could precipitate ascites syndrome. Common salt was more potent than pure sodium chloride and needs further investigation. Antibiotics like monensin sodium and cephalexin did not cause AS. The microbiological and transmission studies did not indicate a microbial etiology.

Summary

6. SUMMARY

Survey studies revealed that during the period 1985-95 there was gradual increase in the prevalence of ascites syndrome (AS). During this period, 165 cases were recorded.

Investigations carried out showed that the disease occurred more in better managed farms. Aflatoxin in the feed and salinity of well water were demonstrated to be not responsible for causing AS. However, excess of common salt in the feed was found to cause AS.

The affected birds had distended abdomen filled with fluid and difficulty in breathing. The mortality rate varied from one to thirty per cent in different farms.

The PCV and haemoglobin were very high. The Na^{++} and K^{+} content in the serum and ascitic fluid were also high. The specific gravity of the ascitic fluid indicated that it was a transudate.

Passive venous congestion and oedema were seen all over the body. Hydropericardium was noticed in most of the cases. Right ventricular dilatation and thickening, distended sinus venosus, swollen liver and meningeal congestion were the main lesions noticed. Thrombus was also encountered in the pulmonary veins.

Ectopic cartilaginous nodules in the lungs and thickening of the Glisson's capsule due to vascular connective tissue proliferation were consistently seen.

Bacillus subtilis and *Bacillus cereus* were isolated from two chicken. However, no etiological significance was attributed to them. The rest of the samples were sterile.

Electron microscopical examination of the ascitic fluid did not reveal any viral agents.

In experiment 1 the birds in the sodium chloride (99.9% pure) group initially exhibited excessive thirst, diarrhoea, hyperaemic skin and distended abdomen but by the end of the 8th week none had ascites on post mortem examination. Pulmonary congestion and oedema, epicardial congestion, hydropericardium, right ventricular thickening and diffuse hepatosis were noticed on gross examination. Severe congestion, oedema and haemorrhage were seen in the lungs. Connective tissue proliferation was evident especially around the blood vessels. Severe diffuse granular degeneration of the hepatocytes with multifocal areas of necrosis and oedema, loss of chord-like arrangement, thickening and separation of the sinusoidal endothelium were noticed in the liver. The PCV, haemoglobin, serum Na^{++} and RV:TV were high in the affected chicken.

In experiment 1 the birds in the furazolidone (800 ppm) group exhibited nervous symptoms and had lesser body weight than the control group. One bird died at the 45th day of treatment showing typical lesions of AS. The PCV, haemoglobin, serum protein, albumin, globulin and K^+, and organ weights were lesser than the control. Ectopic cartilaginous nodules in the lungs, congestion and loss of striation of myofibrils of the heart, severe diffuse granular degeneration of hepatocytes and lower nephron nephrosis were the main lesions noticed. One bird had a nephroblastoma on the left upper lobe of kidney.

The chicken in the monensin group became stunted. Their biochemical parameters, organ weights, RV:TV were lesser than the control.

The chicken in the common salt group of experiment 2 exhibited increased feed and water intake, respiratory distress, reddening of skin and distended abdomen. Their body weight, PCV, haemoglobin, serum Na^{++} and K^+ contents were higher than the control. Three birds died of AS on the 5th, 11th and 24th days of treatment. There were nodular protrusions on the surface of the liver. Congestion, oedema, haemorrhage and ectopic cartilaginous nodules were noticed in the lungs. The Glisson's capsule of the liver was thickened and there was severe congestion of the central and portal veins. There was mesangial cell proliferation and thickening of the basement membrane of

the glomerular tuft. The weight and relative organ weight of the heart, RV and LV were the highest among all the groups. The RV:LV was higher when compared to the control group. The weight of spleen, lungs, liver and intestine was higher than the control.

In the furazolidone (1000 ppm) group the chicken became stunted. They exhibited nervous symptoms and one bird died of ascites on the 53rd day of the treatment. Congestion of the lungs and heart, hydropericardium and diffuse areas of hepatosis and nephrosis were noticed on gross examination. Presence of ectopic cartilaginous nodules, pulmonary congestion and emphysema, loss of striation of the myocardial fibres, diffuse vacuolar degeneration and disorganisation of the hepatic chords, mesangial cell proliferation and gliosis, satellitosis and neuronophagia in the white matter of the brain were the main lesions. The PCV, haemoglobin, serum protein, albumin, globulin, Na^{++}, K^{+} and organ weight were lesser while the relative organ weight and RV:TV were higher than the control group.

In the cobalt chloride and nitrite groups, the birds showed a reddened skin and had a lesser body weight than the control. Moderate degree of congestion in the lungs and liver, diffuse hepatosis and hydropericardium were noticed. The PCV, haemoglobin and serum Na^{++} were higher while the serum K^{+} and

absolute organ weight were lesser than the control. The RV:TV ratio was higher than the control in both the groups. The RV:TV of cobalt chloride group was higher than the cobalt nitrite group.

The chicken in the cephalexin fed group were apparently healthy and the body weight did not differ from the control group. Though lesions like hydropericardium, pulmonary congestion and haemorrhage and myocardial lesions were seen in few of the birds, the serum Na^{+}, K^{+}, organ weight, relative organ weight and RV:TV were lesser than the control group.

The transmission studies using liver suspension and ascitic fluid collected from field cases did not reproduce AS.

References

REFERENCES

Ahmad, K., Ahamad, I., Muneer, M.A.A. and Ajmez, M. (1992). Experimental transmission of Angara disease in broiler fowls. *Studies Res. Vet. Med.* **1**: 53-55.

Akester, A.R. (1978). Microcirculation in birds. In Kaley, G. and Altura, B.M. *Microcirculation*, University Park Press, Baltimore. pp.687-735.

Albers, G. and Frankenhuis, M. (1990). Ascites, a high altitude disease in the lowlands. *Poult. (Misset)*. February/March: 24-25.

Albers, G., Barranon, Zurita and Ortiz. (1990). Correct feed restriction prevents ascites. *Poult. (Misset)*. April/May: 22-23.

Altland, P.D. (1961). Altitude tolerance of chickens and pigeons. *J. Appl. Physiol.* **16**: 141-143.

*Ashfeque, M., Muhammad, K. and Agha, B. (1983). Ascites syndrome in broiler chicks. *Pakistan Vet. J.* **3**: 190.

Bahgat, A., Nagia. and Moustafa, A. (1990). Pathological studies on furazolidone toxicosis in chicks. *Egyptian J. Comp. Pathol. clin. Pathol.* **3**: 149-158.

Bancroft, J.D. and Cook, H.C. (1984). *Manual of Histological Techniques*. Churchill Livingstone, Edinburgh, pp.18-25.

Banday, T.M. and Maqbool, M. (1992). Ascites in broilers in relation to pulmonary hypertension. *Poult. T. & T.* **2**: 7-10.

Bartov, I. (1994). Effect of growth promoters on monensin toxicity in broiler chicks. *Br. Poult. Sci.* **35**: 123-133.

Biswas, N.K., Bhowmik, M.K. and Dalapati, M.R. (1995). Ascites syndrome in broiler chickens: epizootiology and clinicohaematology. *Indian J. Vet. Pathol.* **18**: 121-124.

Blount, W.P. (1955). Nitrofurazone toxicity in ducks. *Vet. Rec.* **75**: 90.

Bressler, G.O., Gordeuk, S.Jr., Gallenbach, E.W. and Pritham, G.H. (1951). The effect of salt and carbolineum producing ascites in turkey poults. *Poult. Sci.* **30**: 738-744.

*Burton, R.R. and Smith, A.H. (1972). The effect of chronic erythrocytic polycythemia and high altitude upon plasma and blood volumes. *Proc. Soc. Exp. Biol. Med.* **140**: 920-923.

Buys, S.B. and Barnes, P. (1981). Ascites in broilers. *Vet. Rec.* **108**: 266.

*Cai, Y.N., Ou, L.C. and Smith, R.P. (1984). Severe polycythemia and hypervolemia in a rat model of chronic mountain sickness (CMS). *Federation Proc.* **43**: 905.

*Cardenas, D.M., Hernandez, A. and Osuna, O. (1985). Algunos valores hematimetricos y de proteinas totales en pollos Arbor Acres sanos y asciticos en la Sabana de Bogota. *Revista Acovez.* **29**: 42-44.

Cardona, C.J., Galey, F.D., Bickford, A.A., Charlton, B.R. and Cooper, G.L. (1993). Skeletal myopathy produced with experimental dosing of turkeys with monensin. *Avian Dis.* **37**: 107-117.

Cash, D.W.A. (1993). Attempt to reproduce knock-down syndrome in turkeys unsuccessful. *Feed Stuffs* **65**: 12.

*Coello, C.L., Paasch, L., Rosiles, R. and Casas, C. (1982). Ascites in broilers due to undetermined causes. *Proc. 31st Western Poult. Dis. Conf. and 16th Poult. Health Symp.* pp. 13-15.

Coello, C.L., Odom, T.W. and Wideman, R.F. (1985). Ascites major cause of mortality in broilers. *Poult. Dig.* **44**: 284-288.

Coello, C.L., Odom, T.W. and Bailey, C.A. (1987). Observations on the incidence of ascites in a commercial flock of broilers in Mexico. *Poult. Sci.* **66**: 84.

Cowan, S.T. 1974. *Manual of the identification of medical bacteria*, 2nd Ed. pp.22-133.

*Cowen, B.S. (1989). Sindrome dell' "Ascite aviaria" (S.A.) del pollo. *Summa*, **6**: 163-168.

Cueva, S., Sillau, H., Valenzuela, A. and Ploog, H. (1974). High altitude induced pulmonary hypertension and right heart failure in broiler chickens. *Res. Vet. Sci.* **16**: 370-374.

Diaz, G.J., Julian, R.J. and Squires, E.J. (1994). Cobalt-induced polycythaemia causing right ventricular hypertrophy and ascites in meat-type chickens. *Avian Pathol.* **23**: 91-104.

Disbrey, B.D. and Rack, J.H. (1970). *Histological Laboratory Methods*, E&S Livingston, Edinburgh. pp. 99-101.

Doll, E.R., Hull, F.E. and Insko, W.M. (1946). Toxicity of sodium chloride solution for baby chicks. *Vet. Med.* **41**: 361-363.

*Dupreez, J.H. (1985). Altitude disease in broilers in South Africa. VIII, *Int. Congress World Vet. Poult. Assoc.*, (Abst. 92).

Enkvetchakul, B., Bottje, W., Anthony, N. and Moore, R. (1993). Compromised antioxidant status associated with ascites in broilers. *Poult. Sci.* **72**: 2272-2280.

*Feron, V.J. and Van Stratum. (1966). The effect of furazolidone on broiler chickens fed rations containing amprolium or zoalene. *Tijdschr. Diergeneesk.* **9**: 571-579.

Fields, N.G., Yuan, B. and Leenan, F.H.H. (1991). Sodium-induced cardiac hypertrophy. *Circulation Res.* **68**: Suppl. 1 (Abst.) 87.

Fitz-Coy, S.H. and Harter-Dennis, J.M. (1988). Incidence of ascites in broiler and roaster chickens. *Poult. Sci.* **67**: Suppl. 1 (Abst.) 87.

Fraser, C.M. (1991). Ascites syndrome. In: Fraser, C.M. (Ed.) *The Merck Veterinary Manual*, 7th edn., pp.1628-1629 (Rahway, Merck and Co. Inc.).

Gowda, R.N.S., Satyanarayana, M.L. and Seshadri, S.J. (1989). Ascites syndrome in broilers. Cited in Scientific papers abstracts, *Natl. seminar on emerging dis. of livestock and poult.* September 1989. Madras Veterinary College, Madras - 600007.

Gowda, R.N.S., Satyanarayana, M.L., Vijayasarathi, S.K. and Seshadri, S.J. (1990). Ascites syndrome in commercial broilers. Cited in abstracts, *Natl. symp. immunol.and biomol.responses in dis. of livestock and poult.* College of Veterinary Science, Tirupathi - 517 502.

Gregory, D.G., Edwards, W.C. and Stair, E.L. (1992). A case of monensin poisoning in ostriches. *Vet. Hum. Toxicol.* **34**: 247.

Griffith, D.L.V. (1993). Intriguing tropical mysteries in poultry. *Misset World Poult.* **9**: 59-63.

Hall, S.A. and Machicao, N. (1968). Myocarditis in broiler chickens reared at high altitude. *Avian Dis.* **12**: 75-84.

Hanrahan, L.A., Corrier, D.E. and Naqa, S.A. (1981). Monensin toxicosis in broiler chickens. *Vet. Pathol.* **18**: 665-671.

*Harwood, P.D., Stung, D.I. and Wolfgang, R.W. (1958). The efficacy of furazolidone as a coccidiostat against *Eimeria tenella*, and *Eimeria necatrix*. *Proc. 2nd Natl. Symp. Nitrofurans in Agric.* Attens. Georgia, March 27-28: 107-117.

Hernandez, A. (1987). Hypoxic ascites in broilers: a review of several studies done in Columbia. *Avian Dis.* **31**: 658-661.

Hill, C.H. (1979). The effect of dietary protein levels on mineral toxicity in chicks. *J. Nutr.* **109**: 501-507.

Hoerr, F.J. (1988). Pathogenesis of ascites. *Poult. Dig.* 46, January: 8-12.

Howell, J. and Gumbrell, R.C. (1992). Salt poisoning in broiler chickens. *New Zealand Vet. J.* **40**: 85.

Huchzermeyer, F.W. (1985). Waterbelly - altitude disease. *Poult. Int.* **8**: 62-66.

Huchzermeyer, F.W., De Ruyck, A.M.C. and Van Ark, H. (1988). Broiler pulmonary hypertension syndrome. III. Commercial broiler strains differ in their susceptibility. *Onderstepoort J. Vet. Res.* **55**: 5-9.

Inchiosa, M.A. (1964). Direct, biuret determination of total protein in tissue homogenates. *J. Lab. Med.* **63**: 319-324.

Jensen, L.S., Chang, C.H. and Washburn, K.W. (1975). Differential response in cardiomyopathy in chicks and turkeys to furazolidone toxicity. *Avian Dis.* **19**: 596-602.

Julian, R.J. (1983). Ascites in broiler chickens. *55th Northeastern Conf. Avian Dis.* (Abst.) 30, (Ottawa, Canada).

Julian, R.J. (1987). The effect of increased sodium in the drinking water on right ventricular hypertrophy, right ventricular failure and ascites in broiler chickens. *Avian Pathol.* **16**: 61-71.

Julian, R.J. (1990a). Cardiovascular disease. In: Jordan, F.T.W. *Poultry Diseases*, 3rd Ed, pp. 345-353 (London, Bailliere Tindall).

Julian, R.J. (1990b). Pulmonary hypertension: a cause of right heart failure, ascites in meat-type chickens. *Feed Stuffs*. **29**: 19-22.

*Julian, R.J. (1990c). The influence of genetics on right heart failure and ascites in poultry caused by the pulmonary hypertension syndrome. *Proc. Natl. Breeders Roundtable*. May, pp.14-19. (St. Louis, Missouri, USA).

Julian, R.J. (1993). Ascites in Poultry. *Avian Pathol*. **22**: 419-454.

Julian, R.J. and Mirsalimi, S.M. (1992). Blood oxygen concentration of fast-growing and slow-growing broiler chickens, and chickens with ascites from right ventricular failure. *Avian Dis*. **36**: 730-732.

Julian, R.J. and Squires, E.J. (1994). Haematopoietic and right ventricular response to intermittent hypobaric hypoxia in meat-type chickens. *Avian Pathol*. **23**: 539-545.

Julian, R.J., Summers, J. and Wilson, J.B. (1986). Right ventricular failure and ascites in broiler chickens caused by phosphorus-deficient diets. *Avian Dis*. **30**: 453-459.

Julian, R.J., Mc Millan, I. and Quinton, M. (1989). The effect of cold and dietary energy on right ventricular hypertrophy, right ventricular failure and ascites in meat-type chickens. *Avian Pathol*. **18**: 675-684.

Julian, R.J., Friars, G.W., French, H. and Quinton, M. (1987). The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. *Avian Dis*. **31** 130-135.

Kannan, N. and Singh, B. (1994). Changes in haematological and blood gas variable in broilers at high altitude with ascites syndrome. *Indian Vet. J*. **71**: 881-884.

Khan, M.Z., Szarek, J., Markiewicz, K. and Markiewicz, E. (1993a). Effects of concurrent oral administration of toxic levels of monensin and lead on concentration of different elements in the liver of broiler chicks. *J. Vet. Med*. **40**: 466-475.

Khan, M.Z., Szarek, J., Saeed, M., Koncicki, A. and Krasnodebska, D.A. (1993b). Effects of concurrent oral administration of monensin and selenium on some haematological and biochemical parameters in broiler chickens. *J. Vet. Med.* **40**: 667-675.

*Khan, M.Z., Szarek, J., Koncicki, A., Krasnodebska, D.A. (1994). Oral administration of monensin and lead to broiler chicks effects on haematological and biochemical parameters. *Acta Vet. Hungarica.* **42**: 111-120.

Klimes, B. and Kruza, B. (1962). Toxicity of nitrofurazone for young ducklings. *Vet. Rec.* **74**: 167-168.

*Lu, Y.S., Tsai, H.J., Lee, S.H., Lee, Y.L., Cin, D.F., Dwang, M.J. and Liao, Y.K. (1992). Ascites in broiler chicks and ducklings due to right ventricular failure. *J. Chinese Soc. Vet. Sci.* **48**: 217-224.

Luna, L.G. (1968). *Manual of histologic staining methods of the Armed Forces Institute of Pathology.* 3rd Ed. Mc Graw-Hill Book Company, New York, pp.1-258

*Madej, J.A., Mazurkiewiez, M., Gawel, A., Kuryszko, J., Kotonski, B. and Sobieck, K.A. (1992). Pathomorphology and pathogenesis of the ascites hypoxia syndrome in chicks in Poland. *Medycyna Weterynaryjna.* **48**: 355-358.

*Maina, J.N. (1982). *Qualitative and quantitative observations on the lungs of Aves with comments on the lung of a species of Chiroptera: A morphological study.* Ph.D. Thesis. University of Liverpool. England.

*Martinez, L.A., Casaubon, M.T. and Navarro, R. (1991). Estudio estructural del corazon en lineas de aves resistentes y susceptibles al sindrome ascitico. *Proc. 40th Western Poult. Dis. Conference.* pp. 169-171 (Acapulo, Mexico).

Martinez, L.A., Casaubon, M.T. and Navarro, R. (1992). Structure study of the heart of chickens resistant and susceptible to ascitic syndrome. *Poult. Sci.* **71**: Supp. 1, (Abst. S91), 165.

*Martinez, H.E., Mendez, C.E., Maltos, J.F., Trenchi, H.E. and Caffarena, R.M. (1993). Ascitic syndrome of broilers in Uruguay: importance as a condemning cause in poultry slaughter houses. *Vet. Argentina* **10**: 388-395.

Maxwell, M.H. Robertson, G.W. and Spence, S. (1986a). Studies on an ascitic syndrome in young broilers. 1. Haematology and pathology. *Avian Pathol.* **15**: 511-524.

Maxwell, M.H., Robertson, G.W. and Spence, S. (1986b). Studies on an ascitic syndrome in young broilers, 2. Ultrastructure. *Avian Pathol.* **15**: 525-538.

Maxwell, M.H., Anderson, I.A. and Dick, L.A. (1988). The incidence of ectopic cartilaginous and osseous lung nodules in young broiler fowls with ascites and various other diseases. *Avian Pathol.* **17**: 487-493.

Maxwell, M.H., Dolan, T.T and Mbugua, H.C.W. (1989). An ultrastructural study of an ascitic syndrome in young broilers reared at high altitude. *Avian Pathol.* **18**: 481-494.

Maxwell, M.H., Robertson, G.W. and Mitchell, M.A. (1993). Ultrastructural demonstration of mitochondrial calcium overload in myocardial cells from broiler chickens with ascites and induced hypoxia. *Res. Vet. Sci.* **54**: 267-277.

Maxwell, M.H., Robertson, G.W. and Moseby, D. (1995). Serum troponin T values in 7-day-old hypoxia and hyperoxia treated, and 10-day old ascitic and debilitated, commercial broiler chicks. *Avian Pathol.* **24**: 333-346.

Maxwell, M.H., Spence, S., Robertson, G.W. and Mitchell, M.A. (1990). Haematological and morphological responses of broiler chicks to hypoxia. *Avian Pathol.* **19**: 23-40.

Miale, J.B. (1972). Laboratory Medicine Haematology. 4th Ed. The C.V. Mosby Company, Saint Louis, p. 1216.

Miller, M.E., Howard, O., Stohlman, F. and Flanagon, P. (1974). Mechanisms of erythropoietin production of cobaltous chloride. *Blood* **44**: 339-346.

Mirsalimi, S.M. and Julian, R.J. (1991). Reduced erythrocyte deformability as a possible contributing factor to pulmonary hypertension and ascites in broiler chickens. *Avian Dis.* **35**: 374-379.

Mirsalimi, S.M. and Julian, R.J. (1993). Saline drinking water in broiler and leghorn chicks and the effect in broilers of increasing levels and age at time of exposure. *Canadian Vet. J.* **34**: 413-417.

Mishra, L.C., Sahu, B.K., Panda, N.C. and Dehuri, P.K. (1987). Effects of excess of salt in chick ration. *Indian J. Poult. Sci.* 22: 207-211.

Mohanty, G.C. and West, J.L. (1969). Pathologic features of experimental sodium chloride poisoning in chicks. *Avian Dis.* **13**: 762-773.

Newberne. and Mc Even. (1957). Studies on drug toxicity in chicks. *Poult. Sci.* **40**: 35.

O'Brien, P.J., O'Grady, M., Lumsden, J., Holmberg, O.L., Shen, H., Weiler, J.E., Horn, R.D., Mirsalimi, S.M. and Julian, R.J. (1993). Clinical pathologic profiles of dogs and turkeys with congestive heart failure, either non induced or induced by rapid ventricular pacing and turkeys with furazolidone toxicity. *Am. J. Vet. Res.* **54**: 60-68.

Odom, T.W. (1993). Ascites syndrome: overview and update. *Poult. Dig.* **52**: 14-22.

Odom, T.W., Rosenbaum, L.M., Stolz, J.L. and Jeong, D. (1992). Experimental reduction of egg shell conductance during incubation. II. Physiological implications in a slow growing and fast growing broiler line. *Poult. Sci.* **72**: Supp.1, (Abst.14): 5.

Odom, T.W., Hargis, B.M., Coello, C.L., Arce, J. and Avila, E. (1989). Time courses changes in electro-cardiographic and hematological variables during the development of ascites in broiler chickens. *Poult. Sci.* **68**: Supp.1, (Abst.) 107.

Onderka, D.K. and Bhatnagar, R. (1982). Ultrastructural changes of sodium chloride-induced cardiomyopathy in turkey poults. *Avian Dis.* **26**: 835-841.

Owen, R.L., Wideman, R.F.Jr., Hattel, A.L. and Cowen, B.S. (1990). Use of a hypobaric chamber as a model system for investigating ascites in broilers. *Avian Dis.* **34**: 754-758.

Payne, L.N., Brown, S.R., Bumstead, N., Howes, K., Frazier, J.A. and Thouless, M.E. (1991). A novel subgroup of exogenous avian leukosis virus in chickens. *J. Gen. Virol.* **72**: 801-807.

Pimental, J.L. and Cook, M.E. Cited by Diehnelt, J. (1988). Salt improves immune levels & growth. *Poult. (Misset)*. October/November: 20-21.

Pons, W.A. and Goldblatt, L.A. (1969). Physico-chemical assay of aflatoxins. In *Aflatoxin*. Ed. Goldblatt, L.A., Academic Press, London, pp.80-96.

*Power, P., Cremin, F.M. and Flynn, A. (1984). Ascites in broilers. *Irish J. Food Sci. Technol.* **8**: 75-76.

Prajapathi, K.S. and Solanki, K.D. (1994). Etiopathological studies on ascites syndrome in broilers. *Indian Poult. Rev.* **3**: 37.

Puls, R. (1988). *Mineral Levels in Animal Health Diagnostic Data*. (Clearbrook, Sherpa International).

*Reed, W.M. and Vanvleet, J.F. (1988). Furazolidone-induced cardiomyopathy and ascites in broilers. *Proc. 37th Western Poult. Dis.* Conference. (Davis, California). pp.156-157.

Reed, W.M. and Winterfield, R.W. (1985). Ascites in White Leghorn pullets: isolation of a virus and reproduction of the disease. *Poult. Sci.* **64**: Suppl. 1, (Abst.), 167.

Reed, W.M., Vanvleet, J.F. and Wigle, W.L. (1987). Influence of sex and strain of bird on the frequency of induction of the ascites syndrome by furazolidone toxicosis in chickens. *Poult. Sci.* **66**: 164.

*Riddell, C. (1985). Ascites: an emerging problem. *Proc. 20th Natl. Meeting on Poult. Health and Condemnations*: (Ocean City, Maryland, USA). pp.92-93.

Riddell, C. (1991). Developmental, metabolic and miscellaneous disorders. In: Calnek, B.W., Barnes, H.J., Beard, C.W., Reed, W.M. and Yoder, H.W. *Disease of Poultry*, 9th Ed, pp. 827-862 (Ames, Iowa State University Press).

*Rostofer, H.H. and Rigdon, R.H. (1947). The relation of blood oxygen transport to resistance to anoxia in chicks and ducklings. *Biol. Bull.* **92**: 23-30.

*Sandino De, M.M. and Hernandez, A.V. (1985). Variacon cardiopulmonary en los valores ds hemoglobina y hematocrits durante la hiporia en pollos comerciales y criollos. *Revista de la Facultad de Medicina Veterinaria y de zootecnia*, Universidad Nacional de Colombia. **38**: 11-27.

Sarakbi, T. (1988). Broiler diseases in the Yemen. *Poult. Int.* **27**: 71-72, 7.

Schalm, C.W. (1975). *Veterinary Haematology*. Lea and Febiger, Philadelphia, 3rd Ed. pp. 39-40, 52-66.

Scheele, C.W., De Wit, W., Frankenhuis, M.T. and Vereijken, P.F.G.. (1991). Ascites in broilers. 1. Experimental factors evoking symptoms related to ascites. *Poult. Sci.* **70**: 1069-1083.

Scrivner, L.H. (1946). Experimental edema and ascites in poults. *J. Am. Vet. Med. Assoc.* **108**: 27-32.

Seyle, H. (1943). Production of nephrosclerosis in the fowl by sodium chloride. *J. Am. Vet. Med. Assoc.* **103**: 140-143.

Shlosberg, A. (1996). Personal Communication.

Shlosberg, A., Berman, E., Bendheim, U. and Plavnik, I. (1991). Controlled early feed restriction as a potential means of reducing the incidence of ascites in broilers. *Avian Dis.* **35**: 681-684.

Shlosberg, A., Pano, G., Handji, V. and Berman, E. (1992). Prophylactic and therapeutic treatment of ascites in broiler chickens. *Br. Poult. Sci.* **33**: 141-148.

Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods.* 6th Ed. The Iowa State University Press, U.S.A., pp.91-296.

Southern, L.L. and Baker, D.H. (1981). The effect of methionine or cysteine on cobalt toxicity in the chick. *Poult. Sci.* **60**: 1303-1308.

Squires, E.J. and Summers, J.D. (1993). A consideration of comparative metabolic aspects of the aetiology of sudden death syndrome and ascites in broilers. *British Vet. J.* **149**: 285-294.

Stuart, J.C. (1978). An outbreak of monensin poisoning in adult turkeys. *Vet. Rec.* **102**: 303-304.

Swayne, D.E., Shlosberg, A. and Davis, R.B. (1986). Salt poisoning in turkey poults. *Avian Dis.* **30**: 847-852.

Swire, P.W. (1980). Ascites in broilers, *Vet. Rec.* **107**: 541.

Sykes, A.H. (1960). A note on the determination of oxygen in the blood of the fowl. *Poult. Sci.* **39**: 16-18.

*Szarek, J. and Khan, M.Z. (1993). Concurrent exposure to lead, selenium or monensin effects on hepatic porphyrin levels in broiler chicken during acute toxicosis. *Scandinavian J. Lab. Anim. Sci.* **20**: 231-234.

Underwood, E.J. (1975). Cobalt. *Nutr. Rev.* **33**: 65-69.

Unverferth, D.V., Croskery, R.W., Leifer, C.V., Altschuld, R., Pipers, F.S., Thomas, J., Magorun, R.D. and Hamlin, R.L. (1983). Canine cobalt cardiomyopathy: a model for the study of heart failure. *Am. J. Vet. Res.* **44**: 989-995.

Van Vleet, J.F. and Ferrans, V.J. (1983). Congestive cardiomyopathy induced in ducklings fed graded amounts of furazolidone. *Am. J. Vet. Res.* **44**: 76-85.

Van Vleet, J.F., Rebar, A.H. and Ferrans, V.J. (1977). Acute cobalt and isoproterenol cardiotoxicity in swine. *Am. J. Vet. Res.* **38**: 991-1002.

Van Vleet, J.F., Boon, G.D. and Ferrano, V.J. (1981). Induction of lesions of selenium - vitamin E deficiency in ducklings fed silver, copper, cobalt, tellurium, cadmium or zinc. *Am. J. Vet. Res.* **42**: 1200-1217.

Vidyadaran, M.K., King, A.S. and Kassim, H. (1990). Quantitative comparisons of lung structure of adult domestic fowl and Red Jungle Fowl, with reference to broiler ascites. *Avian Pathol.* **19**: 51-58.

Walker, R. (1994). New concept in ascites physiology. *Poult. Int.* **5**: 48-51.

Watkins, K.L. and Novilla, N.N. (1994). Feed gorging and extended water restriction do not produce knock down in male turkeys fed monensin. *Poult. Sci.* **73**: 587-590.

Watkins, K.L., Novilla, M.N. and Campi, T.W. (1993). Effect of feed restriction and subsequent gorging with limited access to water on male turkeys fed graded levels of monensin. *Poult. Sci.* **72**: 677-683.

Webb, D.M. and Vanvleet, J.F. (1971). Early clinical and morphologic alternations in the pathogenesis of furazolidone-induced toxicosis in ducklings. *Am. J. Vet. Res.* **52**: 1531-1536.

Webb, D.M., Denicola, D.B. and Vleet, J.F.V. (1991). Serum chemistry alterations including creatinine kinase isoenzymes in furazolidone toxicosis in ducklings: Preliminary findings. *Avian Dis.* **35**: 662-667.

Weisman, Y., Wax, E. and Bartov, I. (1994). Monensin toxicity in two breeds of laying hens. *Avian Pathol.* **23**: 575-578.

Wilson, J.B., Julian, R.J. and Barke, I.K. (1988). Lesions of right heart failure and ascites in broiler chickens. *Avian Dis.* **32**: 246-261.

Witzel, D.A., Huff, W.E., Kubena, L.F., Harvey, R.B. and Elissalde, M.H. (1990). Ascites in growing broilers: a research model. *Poult. Sci.* **69**: 741-745.

Yersin, A.G., Huff, W.E., Kubena, L.F., Elissalde, M.H., Harvey, R.B., Witzel, D.A. and Giroir, L.E. (1992). Changes in haematological, blood gas and serum biochemical variables in broilers during exposure to simulated high altitude. *Avian Dis.* **36**: 189-196.

* Originals not consulted.

PATHOLOGY OF ASCITES SYNDROME IN BROILER CHICKEN

By

JACOB ALEXANDER

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences

KERALA AGRICULTURAL UNIVERSITY

Department of Pathology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY - THRISSUR

KERALA

1996

ABSTRACT

The post mortem reports maintained at the Department of Pathology during the period 1985-95 revealed that the prevalence of ascites syndrome (AS) was gradually on the increase. Investigations carried out on field cases showed that AS occurred in better managed farms and also in farms where the feed did not contain aflatoxin. The bacteriological study and screening of ascitic fluid in electron microscope for virus did not indicate a microbial etiology.

Two separate experiments were conducted with vencob strain of broiler chicken. In each of the experiments the feed additives were mixed in the feed and fed *ad libitum* from the second week onwards. The body weight was recorded weekly. The PCV, haemoglobin, serum protein, albumin, globulin, albumin-globulin ratio, sodium and potassium were estimated at fortnightly intervals. At the eighth week the live birds were slaughtered and the gross and histopathological lesions were evaluated. The organ weight, relative organ weight and RV:TV were also evaluated.

In the first experiment 50 chicks of two weeks of age were randomly divided into three treatment groups of 12 each. The control group consisted remaining 14 chicks. The treatment groups were given monensin sodium, pure sodium chloride (99.99%) and furazolidone in the feed at the rate of 360, 25,000 and 800 ppm respectively.

The birds in the monensin group were stunted due to less feed and water intake. The serum biochemistry, lesions, organ weight and a lesser RV:TV indicated that monensin might not cause AS.

The sodium chloride group initially exhibited excessive thirst, diarrhoea, hyperaemic skin and distended abdomen but by the end of the eighth week none had ascites on post mortem examination. The PCV, haemoglobin, serum Na^{++} and RV:TV were higher than the control.

The furazolidone group exhibited nervous symptoms and one bird died of ascites syndrome at the 45th day of treatment. The body weight, PCV, haemoglobin, serum protein, albumin, globulin, potassium and organ weight were lesser than the control group. Congestion, hydropericardium and nephrosis were noticed in the birds. Ectopic cartilaginous nodules, pulmonary congestion and oedema, loss of striation and separation of myocardial fibers and severe diffuse granular degeneration of hepatocytes were also noticed.

The second experiment consisted of 80 chicks of two weeks age randomly divided into 8 groups of 10 each. Furazolidone was administered at a higher dose (1000 ppm) and common salt was given instead of sodium chloride at the same dose rate (25,000 ppm). Cephalexin was also administered to another group at the rate of 800 ppm. Two salts of cobalt namely chloride and nitrite were given to two separate groups at 600 ppm each to elucidate its aetiological role. In addition, two groups were

utilized for transmission studies using the ascitic fluid and liver suspension collected aseptically from field cases.

The birds in the furazolidone group showed nervous symptoms, and was stunted. One bird died of ascites on the 53rd day of the treatment. The PCV, haemoglobin, serum protein, albumin, globulin, Na^{++}, K^{+} and organ weight were lesser while the relative organ weight and RV:TV were higher than the control group. These observations suggested that furazolidone was a potential agent which could cause AS.

In the common salt group three birds died of AS on the 5th, 11th and 24th days of treatment. They showed sequential progressive lesions. Increased feed and water intake, respiratory distress, and distended abdomen were noticed. Their body weight, PCV, haemoglobin, serum Na^{++} and K^{+} content were higher than the control. Congestion, oedema, haemorrhage and ectopic cartilaginous nodules were noticed in the lungs. Severe congestion of the central and portal veins and thickening of the Glisson's capsule were seen in the liver. Mesangial cell proliferation, and thickening of the basement membrane of the glomerular tuft were evident. The weight and relative weight of the heart, RV and LV were the highest among all the groups. The RV:LV was higher when compared to the control. The weight of the spleen, lungs, liver and intestine was greater than the control. These results indicated that common salt was more potent than sodium chloride in precipitating AS.

In the cobalt chloride and nitrite groups, the birds showed a reddened skin and had a lesser body weight than the control. The PCV, haemoglobin and serum Na^{++} were higher while the serum K^{+} and absolute organ weight were lesser than the control. The RV:TV ratio was higher than the control in both the groups. These suggested that excess amounts of cobalt in chicken feed might cause ascites.

When cephalexin was given to the birds no noticeable symptoms were observed. The serum Na^{++}, K^{+}, organ weight, relative organ weight and RV:TV were lesser than the control and it did not cause AS.

Transmission studies using liver suspension and ascitic fluid injection intraperitoneally did not cause ascites. A microbial etiology therefore could not be elucidated.