EVALUATION OF FURAZOLIDONE TOXICITY AND ITS AMELIORATION BY *Allium sativum* L. (GARLIC) AND ALPHA TOCOPHEROL IN BROILER CHICKEN

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By

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SEPTEMBER, 2004

CERTIFICATE

Mr. P.S. SHIVA has satisfactorily prosecuted the course of research and that the thesis entitled **"EVALUATION OF FURAZOLIDONE TOXICITY AND ITS AMELIORATION BY *Allium sativum* L. (GARLIC) AND ALPHA TOCOPHEROL IN BROILER CHICKEN"** *submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.*

Date: 08.09.04

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CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION OF FURAZOLIDONE TOXICITY AND ITS AMELIORATION BY *Allium sativum* L. (GARLIC) AND ALPHA TOCOPHEROL IN BROILER CHICKEN"** *submitted in partial fulfilment of the requirements for the degree of* ***MASTER OF VETERINARY SCIENCE*** *of the Acharya N.G.Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by* ***Mr. P.S.SHIVA*** *under my guidance and supervision. The subject of the thesis has been approved by the student's advisory committee.*

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigation has been duly acknowledged by the author of the thesis.

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CHAPTER - I

INTRODUCTION

Poultry farming in a scientific way is economized with new technologies and management practices. It has led to the inevitable use of various chemicals and drugs as feed additives for prophylactic and therapeutic purposes. Overuse of these chemical agents coupled with the genetic manipulation of the bird for easy returns, could lead to undue oxidative stress to the birds. Oxidative stress causes generation of free radicals, a common cause of pathology of birds leading to production losses.

Furazolidone, a nitrofuran antimicrobial agent is widely used in the prevention and treatment of bacterial and protozoan diseases of poultry including Salmonellosis, Coccidiosis, Staphylococcosis and *Escherichia coli* infections.

Furazolidone act by interrupting the enzymatic process of the microbial cells and at recommended doses has a low toxicity for host tissues (Biester and Schwarte, 1985). Due to a narrow margin between therapeutic and toxic concentration it becomes toxic when administered for a prolonged period or at doses higher than the therapeutic dose (Zaman *et.al.* 1995).

Furazolidone toxicity causes mutagenicity, genotoxicity and carcinogenicity. These toxic effects are due to alteration in the *in vivo* antioxidative defense mechanism (Sas, 1993) and free radical generation (Bloom and Brandt, 2001)

Oxidative damage by free radicals can be prevented by the use of antioxidants in feed. These antioxidants can be natural oils, spices, tocopherols, ascorbic acid, carotenoids or synthetic chemicals.

Garlic, one of the oldest of all cultivated plants contains more than 100 biologically active principles and useful secondary metabolites. These include unique lipid soluble organosulfur components, water soluble organosulfur compounds and flavonoids, notably alliin, allicin, vinyldithiins, S-allyl cysteine (SAC), S-allyl mercaptocysteine (SAMC) and selenium (Borek, 2001).

The medicinal use of garlic have a long history and its uses as a remedy for heart disease, tumours and headaches are documented in the Egyptian Codex Ebers dating from 1550BC. Garlic is mentioned in the Bible and has been a traditional treatment in many countries notably the North East,

China and India (Block, 1985). Recent studies validated many of the medicinal properties attributed to garlic. Their exploitable properties include antioxidant (Helen *et al,*1999) chemical scavenging (Nakagawa *et al,*1988) and anticarcinogenic (Sheen *et al,*1998) activities.

Vitamin E or α-tocopherol, a standard antioxidant known for its antioxidant property protects the unsaturated bonds of phospholipids present in the cell membrane against free radical damage (Tappel, 1972; Infers and Sies, 1988).

Since the reports on the use of Garlic as antioxidant feed supplement in poultry is scanty, the present study was designed and conducted with the following objectives:

1. To study the oxidative damage of Furazolidone in broiler chicken.
2. To evaluate *Allium sativum L.* (Garlic) against Furazolidone toxicity.
3. To evaluate Alpha-tocopherol against Furazolidone toxicity.
4. To study the efficiency of Alpha-tocopherol, Garlic and STRESSEAZE (an Ayurvedic Veterinary product) in alleviating Furazolidone induced oxidative stress in broiler chicken.

Chapter II

REVIEW OF LITERATURE

CHAPTER - II

REVIEW OF LITERATURE

2.1 FURAZOLIDONE

Furazolidone, [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone] a nitrofuran derivative has been used for more than forty years in the treatment of certain bacterial and protozoal infections in man and animals. Although more than 3500 nitrofurans are synthesized, only a few have been used in chemotherapy eg., Furazolidone, Nitrofurazone, Nitrofurantoin, Furaltadone, Nifuraldezone (Ali, 1983). It remains an important commonly used drug in veterinary medicine, especially in poultry (Ali, 1999).

2.1.1 Mechanism of action of nitrofurans

Nitrofurans are bacteriostatic and act by blocking oxidative decarboxylation of pyruvate to acetyl co-enzyme A, depriving susceptible organisms of vital energy pathways (Adams, 2001).

The likely mode of action of nifurtimox, a nitrofuran derivative is through the production of activated forms of oxygen. It is reduced to nitro anion radical in the presence of pyridine nucleotides which then reacts with

oxygen to produce superoxide and regeneration of nifurtimox. The activated oxygen molecules exert their toxic effects on the parasite (Adams, 2001).

2.1.2 Therapeutic uses

Furazolidone is potentially used in the local treatment of infections of udder, uterus and skin (Prescott and Baggott, 1993).

Furazolidone has been administered to chickens, turkeys and swine for control of various digestive tract infections like bacterial enteritis, dysentery, giardiasis and coccidiosis (Booth and McDonald, 1982).

For chicken, the therapeutic concentration of furazolidone in feed is 0.04% for 10 days (Brander *et al.*, 1982).

Furazolidone @ 4.4 mg/kg 3 times daily orally, cured experimental Salmonella diarrhoea in a group of foals. In swine, furazolidone and other nitrofurans have been extensively used in the prevention and treatment of colibacillosis and salmonellosis.

2.1.3 Toxicity of Furazolidone

2.1.3.1 General toxicity

Radchuck (1965) reported signs of toxicity in chickens and hens 4-6 hours after oral administration of 300 mg/kg body weight. The birds became inapparent, drowsy and unaware of the surrounding environment. These signs

were followed by paralysis and death. The LD_{50} of the drug was found to be 380 mg/kg, and 500 mg/kg was lethal to all the birds studied.

Furazolidone at a concentration of 400 ppm inhibited normal growth of young laying chicken with depressed haematocytologic function and egg weight (Booth and McDonald, 1982).

Stryczek (1971) reported that the toxic signs of furazolidone (0.02% w/w in the feed, 14 days) in geese consisted of nervous signs, viz., wobbly gait, inability to stand up and tendency to fall down. Post mortem findings were haemorrhages in the serous membranes, digestive tract and heart and excess of fluid in body cavities to the extent of deforming the birds.

Accidental administration of furazolidone in feed over 3000 ppm in pigs caused nervous signs (Borland, 1979).

A reduction in packed cell volume and haemoglobin concentration was found in chickens given furazolidone at a feed level of 0.04% (w/w) for 3 weeks (Ehlhardt *et al.*, 1975). However, anaemia was not found in chickens given the drug 0.04% (w/w) in the feed for 10 days (Ali, 1981).

Furazolidone when given to chicken, duckling and turkey poults at a dose of 0.04% w/w in the feed for 10 days was found to produce anorexia and growth reduction (Ali and Barlet, 1982).

Furazolidone when fed to ducklings at concentrations over 250 mg/kg feed, induced toxicosis characterized by high mortality, inappetence and

failure to gain weight, signs of CNS dysfunction, cardiac dilatation, cystic testicular dilatation and eventually congestive heart failure (Webb and Van Vleet, 1991).

Chicken (Zaman *et al.*, 1995) and Japanese quails (Arbid *et al.*, 1990) when given furazolidone (400-1000 mg/kg feed) in their feed for 3-4 weeks, showed anorexia, decreased body weight, ascites, leg weakness and nervous derangements (such as convulsions and torticollis). Treated birds showed hepatotoxicity, anaemia and decreased plasma protein concentration.

High oral doses of furazolidone as nitrofurantoin caused central nervous effects in animals and lower doses in calves and dogs caused haemorrhagic diathesis with thrombocytopenia, anaemia, leukocytopenia and prolonged bleeding times. Anorexia, nausea and vomiting occur with high doses in all species (Prescott and Baggot, 1993).

O'Brien *et al.* (1993) reported that furazolidone decreased serum cholesterol, total protein, albumin and globulin concentrations and attributed the hypocholesterolemia to decreased feed intake.

Sidorov and Luders (1971) and Sidorov (1973) reported that furazolidone (0.04%) w/w for 7 days or 0.01% w/w for 21 days) exerted a non-specific stress in chicken.

2.1.3.2 Furazolidone and free radical generation

Furazolidone when given a single oral dose of 75, 150 of 300 mg/kg body weight, decreased reduced glutathione (GSH) and ascorbic acid concentration and an increased lipid peroxidation in the tissue of rats. Concomitant treatment with dimethyl sulphoxide, a free radical scavenger ameliorated the effect of Furazolidone on GSH (Ali, 1992).

The ability of furazolidone to enhance free radical reactions when incubated with turkey cardiac or hepatic membranes was determined by Stroo and Schaffer (1989). The study revealed that superoxide dismutase and catalase attenuated the furazolidone induced stimulation of oxygen consumption, indicating that the drug had promoted the formation of superoxides and hydrogen peroxide. Lipid peroxidation was stimulated in liver cells.

In the chicken, furazolidone 300 mg/kg per day for 3 days reduced the blood glutathione peroxidase, glutathione reductase, malonaldehyde (MDA), α-tocopherol, selenium, iron, CAT and GSH and a transient increase in lipid peroxidation concentration was noted (Sas, 1993).

Furazolidone has been shown to be an inhibitor of MAO activity in the tissues of rats, chicken, turkey and duck. The gut flora was probably the site of furazolidone transformation into an active MAO inhibitor in poultry (Ali, 1983). MAO occurs in highest concentrations in the liver, stomach, kidney and

intestine. MAO metabolize catecholamines to inactive deaminated metabolites. (Murray *et al.*, 2002). By virtue of its MAO inhibitory action, furazolidone increased the concentration of catecholamines in the gut and other tissues (Ali, 1999).

Furazolidone (0.04% w/w, 10 days) increased the plasma corticosterone level in chicken (Ali, 1983). This effect was attributed to the stimulation of biosynthesis of corticosterone in the adrenal gland (Ali, 1999).

Anuradha (2003) reported that furazolidone @ 150 mg/kg body weight produced oxidative stress and elevation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) with hypoproteinemia, hypoalbuminemia and hypocholesterolemia.

2.1.3.3 Effect on Liver

In the turkey, Staley *et al.* (1978) reported that by feeding furazolidone (0.05% for 4 weeks) there was a disordered hepatic metabolism, as judged by a decreased aspartate transaminase activity in serum. Simpson *et al.* (1979) showed that furazolidone treatment (0.05% w/w for 42 days) in turkeys resulted in hypotension and decreased protein concentration and trypsin inhibitory factor in the plasma.

Webb *et al.* (1991) found an elevated serum AST activity and hyperbilirubinaemia in the furazolidone treated birds.

Zaman *et al.* (1995) reported a decrease in total protein and globulin concentration when furazolidone (400 mg/kg feed) was fed for four weeks to broiler chicken.

Khan *et al.* (1995) fed furazolidone in the feed to broiler chicken (400, 800 and 1000 mg/kg feed) for four weeks and reported that livers of all the intoxicated birds had congestion, haemorrhage and enlargement. Microscopic changes mainly comprised of dilated central veins and cytoplasmic vacuolation of hepatocytes.

2.1.3.4 Effect on heart

Furazolidone at a dose of 700 ppm, added to the feed of turkey poults at 2 weeks of age induced a cardiomyopathy similar to spontaneous round heart disease (Czarnecki *et al.*, 1975). The birds were found to show an increase in the activity of lactate dehydrogenase glutamic oxaloacetic transaminase, and creatine phosphokinase (CPK) and hypoproteinaemia (Czarnecki *et al.*, 1981).

Mc Callum *et al.* (1989) studied the effect of furazolidone (0.5 and 2 µg/ml of perfusate) on isolated heart of 3 week old broiler chicken; furazolidone at both concentrations, caused detrimental changes in myocardial vascular resistance (two-fold increase) and lactate dehydrogenase release (six-fold increase).

In the ducking, Webb and Van Vleet (1991) fed a ration containing 700 mg of furazolidone / kg of feed for 27 days and observed cardiac chamber dilatation with thinning of myocardium and pericardial effusion.

Furazolidone when incubated with cardiac sarcosomes increased epinephrine oxidation in a concentration dependant manner (Stroo and Schaffer, 1989).

Lax and Kukolich (1992) explained the biochemical basis of furazolidone induced cardiomyopathy using electron paramagnetic resonance (EPR) spectroscopy and showed that furazolidone was reduced to corresponding nitro radical by ascorbate and hypoxanthine and the glutathione (GSH) prevented the formation of this anionic radical.

Biventricular cardiac dilatation and thinning of ventricular walls were observed in broiler chicken fed with furazolidone at 400 or 800 mg/kg feed for 30 days (Islam *et al.*, 1995, Khan *et al.*, 1995).

2.2 ANTIOXIDANTS

Cells have antioxidant defences to protect against free radicals and other ROS. These include the enzymes SOD, which dismutases superoxide; catalase and glutathione peroxidase which destroy toxic peroxides and small molecules including glutathione. External sources of antioxidant nutrients that are essential for antioxidant protection include antioxidant vitamin C,

vitamin E, Vitamin A, Provitamin A, the mineral selenium, a component of selenium dependent glutathione peroxidase (Borek, 1993) and zinc which is a cofactor for about 200 human enzymes including cytoplasmic antioxidant Cu-Zn SOD (Knight, 2000).

2.2.1 Vitamin E (α-Tocopherol)

Vitamin E is the most widely distributed antioxidant in nature, being found in both plant and animal kingdoms. There are atleast eight structural isomers of tocopherol. Among these, α-tocopherol is the best known isomer and possess the most antioxidant activity (Burton *et al.*, 1982).

Vit. E by virtue of its unique molecular structure of phytyl side chain, enables specific physico-chemical interactions with arachidonyl residues of membrane phospholipds which are more prone to attack by free radicals (Maggio *et al.*, 1977).

Because of the lipophilic property of the tocopherol molecule, Vit. E is the major free radical chain terminator in the lipophilic environment eg. plasma lipoproteins. Intracellularly, Vit. E is associated with lipid - rich membranes such as mitochondria and endoplasmic reticulum. Thus the antioxidant action of tocopherol is expected to be highly effective in protecting against membrane lipid peroxidation by reacting with lipid peroxyl

and alkoxyl radicals (Infers and Sies, 1988, Ross and Moldens, 1991 and Murray *et al.*, 2002).

Zhang *et al.* (1998) reported that Vit. E acted as a potent antioxidant in the metabolism of free radicals by inhibiting lipid peroxidation and repairing free radical induced damage of biological membranes. Vit. E reduced the oxidative damage and altered cell cycle which was induced by hydroxy radicals in cultured human oral epithelial cells (Royack *et al.*, 2000).

Ognjanovic *et al.* (2003) in their study on cadmium induced toxicity in rats observed oxidative damage which was manifested as an increase in the activity of erythrocytic antioxidant enzymes (SOD, GSH-Px, GSH-R and Catalase) and lipid peroxidation. Vitamin E exhibited a protective role against the oxidative stress and maintained the enzyme activities on par with the control group.

Jayasree *et al.* (2003) reported modulation of antioxidant scavenging enzyme and an improved body weight gain with Vitamin E supplementation in deltamethrin induced toxicity in broiler chicken. Vitamin E prevented tissue damage and serum enzymes AST and ALT were within the physiological limits.

Herbal source

Dietary supplementation of antioxidants, particularly natural compounds from medicinal plants having antioxidant and immunomodulatory activities have potential in handling the excess generation of free radicals.

A list of plants having antioxidant properties is given in Table 1.

Table 1 : Antioxidant properties of some medicinal plants

Plant / compound	Antioxidant properties	Reference
Aloe vera (leaf extract)	Glutathione peroxidase activity	Sabeh *et al.* 1993
Allium sativum (oil)	Decreased lipid peroxidation	Helen *et al.* 1999
Andrographis paniculata	Decreased lipid peroxidation	Guo *et al.*1995
Azadiractha indica (seed extract - antioxidant principle)	Decreased lipoxygenase activity	Selvam *et al.* 1995
Curcuma longa (antioxidant protein)	Inhibited lipid peroxidation, protects SH and Ca^{2+},ATPase	Selvam *et al.*, (1995)
Emblica officinalis (ascorbic acid conjugated to gallic acid)	Decreased lipid peroxidation	Bhattacharya *et al.*, 1999
Glycyrrhiza glabra (glabridin)	Antioxidant effects	Belinky *et al.*, 1998
Hemidesmus indicus	Inhibited lipid peroxidation, increases SOD	Alam and Gomes, 1998
Tinospora cordifolia (root)	Increased levels of glutathione and Vit. E Inhibited lipid peroxidation	Mathew and Kuttan, 1997
Withania somnifera	Protected glutathione and enzymes	al-Hindawi *et al.*, 1992

2.2.2 Garlic (*Allium sativum* L.)

2.2.2.1 Medicinal Properties

Garlic is one of the oldest of all cultivated plants. It possessed antimicrobial, antioxidant, antithrombotic, antitumour, hypolipidemic, hypoglycemic, and antiarthritic properties (Devasagayam and Sainis, 2002).

Allicin, one of the sulphur compounds of garlic possesses antioxidant and antimutagenic properties (Rabinkov *et al.*, 1998). Odourless cloves of garlic contain alliin, a sulphur containing amino acid derivative, which is also known as S-allyl-cysteine sulphoxide (SACS). Alliin is converted to allicin by the enzyme allinase, which is activated when garlic is cut or crushed. Allicin, a volatile compound gives garlic its pungent smell (Block, 1985).

Thattee *et al.* (2000) studied the effect of allicin on apoptosis and observed that allicin arrested proliferation of cancer cells.

Prem Kumar *et al.* (2000) reported that supplementation of garlic powder (1%) in the feed reduced the serum and meat cholesterol, and improved feed efficiency in broiler chicken.

Lomar *et al.* (2002) reported that fresh garlic extract had a great effect than garlic powder extract against candida infection.

Khalid *et al.* (1995) found that administration of garlic provided protection against hyperlipidemic and thrombus formation in experimentally induced cholesterol atherosclerosis in chicken.

Ohaeri (2001) investigated the effect of garlic oil on the concentration of various enzymes in the serum and tissue of streptozotocin induced diabetic rats and found a reduction in the serum alanine and aspartate transferases.

Bhushan *et al.* (1979) reported that raw garlic caused a decrease in the blood cholesterol concentration even if it is within normal limits in human subjects.

Bordia (1981) studied the effect of garlic on blood cholesterol concentration in humans and reported a 6-9% reduction in total cholesterol, 11% reduction in LDL cholesterol and 17% reduction in triglyceride concentration.

Liu and Yeh (2000) used primary rat hepatocytes and tested the inhibitory potential on cholesterogenesis of organsulphur compounds derived from garlic and found that S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) of garlic were equally potent in inhibiting cholesterol synthesis.

Sendl *et al.* (1992) using a modified liver homogenate model demonstrated that garlic reduced the serum cholesterol concentration primarily by inhibiting cholesterol synthesis if taken in sufficient amount and that this

effect arised from a mixture of multiple compounds from the sulfur - containing class of thiosulfinates, ajoenes and dithiines. Qureshi *et al.* (1983) evaluated the inhibition of cholesterol and fatty acid biosynthesis by polar fractions of garlic in 5 week old male broiler chicken. A dose related decrease in the activities of HMG-CoA reductase, cholesterol 7 alpha hydroxylase and fatty acid synthetase was observed resulting in decreased serum total cholesterol and low density lipoprotein concentration. The most effective dose for the decrease was 0.54% methanol soluble fraction and 1.2% water soluble fraction equivalent to 6% of the fresh garlic.

Fritz *et al.* (1995) fed broiler chicken with 1% garlic and selected herbs at 2 levels to observe their influence on performance and meat quality. Performance of broilers after 8 weeks feeding period was similar. The best sensoric evaluation was obtained by the meat from garlic-fed groups. The meat was superior than control and herbal supplemented groups with respect to smell, palatability, juiciness and tenderness. The abdominal fat percentage was lower in garlic-fed groups.

2.2.2.2 Antioxidant properties

Garlic oil and extract have been shown to provide protection against lipid peroxidation and increased concentration of glutathione (Helen *et al.*, 1999; Upadhyay, 1997).

In the mice, Nakagawa *et al.* (1988) found that the constituents of the garlic extract SAC and SAMC, protected the liver cells from the liver toxins paracetamol and carbon tetrachloride which induced acute hepatitis by enhancing the activity of glutathione, and acted as chemical scavengers.

Rajasree and Rajamohan (1998) reported that administration of water soluble protein of garlic (500 mg/kg b.wt./ day) to alcohol fed rats showed increased antiperoxide activity and decreased activity of glutathione peroxidase and glutathione-S-transferase compared to a standard drug gugulipid (50 mg/kg body weight / day).

Using an *in vitro* system of endothelial cells exposed to oxidant copper ions. Ide and Lau (1997) showed that aged garlic extract and S-allyl cysteine scavenged ROS, inhibited the oxidation of LDL and inhibited endothelial cell injury by oxidized LDL.

Sumioka *et al.* (1998) reported that S-allyl mercaptocysteine (SAMC), a water soluble organosulfur compound in ethanol extract of garlic, suppressed the plasma ALT activity and increased the hepatic lipid peroxidation, in acetaminophen - induced liver injury.

The effect on glutathione reductase activities of feeding garlic oil to white albino rats maintained on high sucrose and alcohol diets was studied by Adoga (1986). The increased activity of GSH-R in liver, kidney, and serum of

sucrose and alcohol fed rats were restored to near normal concentration by garlic oil.

2.3.3 Stresseaze

Several herbal products have been reported to modulate the antioxidant defense system. Stresseaze is one of its kind and is a polyherbal Veterinary product from Indian Herbs Research and Supply Company Limited., Saharanpur, Uttar Pradesh.

Composition

Each 10 g contains, extracts of

Gramya	-	3.00 g
Varahkarni	-	3.00 g
Vrishya	-	3.00 g
Sahkar	-	0.30 g
Excipients	-	q.s

It is reported to have antistress, adaptogenic and antioxidant properties (Chatterjee, 1996).

Wheeler and Wilson (1998) reported that Stresseaze was effective in alleviating the parturition stress in sows and improved the immune status of the sows as well as piglets.

Chatterjee (1994) reported that administration of Stresseaze helps in maintaining the level of antioxidant substances within physiological limits in laboratory animals.

Stresseaze effectively scavenged superoxide and hydroxyl radicals and possesses antioxidant and strong antiperoxide activity (Chatterjee, 1996).

Stresseaze treated birds showed an increase of the order of 10% in live weight gain and a decrease of 6% in FCR (Wheeler, 1994).

Narasimhan (1996) reported the restoration of glutathione level by Stresseaze which was decreased in the event of radical mediated damage by carbon tetrachloride intoxication.

Chapter III

MATERIAL AND METHODS

CHAPTER - III

MATERIAL AND METHODS

3.1 BIRDS

Hundred day-old male broiler chicken of VenCobb strain procured from M/s V.S.N. Hatcheries Pvt. Ltd., Chittoor were used in the experiment.

The experiment was conducted at the Department of Pharmacology & Toxicology and the Department of Livestock Production and Management, College of Veterinary Science, Tirupati. The experimental protocol was approved by the institutional animal ethical committee.

3.2 GARLIC

Bulbs of medium sized garlic of good quality were procured from the local market, Tirupati.

3.3 DRUGS AND CHEMICALS

a. Furazolidone B.P (99.29% assay) was a kind gift from Vet. India Pharmaceuticals Ltd., Hyderabad.

b. Vit. E (dl-α-Tocopherol 50% premix, E.Merck Chemicals, Germany).

c. Stresseaze, an Ayurvedic Veterinary Medicine from Indian Herbs Research and Supply Co. Ltd., Saharanpur, Uttar Pradesh.

Composition : Each 10 g contains extracts of :

Gramya	:	3.00 g
Varahkarni	:	3.00 g
Vrishya	:	3.00 g
Sahkar	:	0.30 g
Excipeints	:	q.s.

3.4 FEED

The feed was formulated using the following ingredients.

Feed composition : kg of ingredients / 100 kg of feed.

Ingredients	Starter ration	Finisher ration
Maize	57	62
Soyabean meal	30	25
Fish meal	10	10
Mineral mixture	3	3
Hyblend (AB_2D_3K)	20 G	20 G
Biovital Vet.	25 G	25 G
Coccidiostat	40 G	40 G

Chicken were fed the starter feed upto 4 weeks of age and later with finisher feed.

3.5 ADMINISTRATION OF DRUGS

Garlic was finely chopped daily and added fresh in the feed. Furazolidone and Vitamin E were mixed in the feed. Stresseaze was dissolved in drinking water and administered to the birds.

3.6 EXPERIMENTAL DESIGN

Hundred day-old male broiler chicken were weighed, identified individually and randomly assigned to ten groups of ten each. They were maintained to laboratory conditions for a period of two weeks and the following treatments were given from day 15 to 42.

Group 1	:	Reference feed
Group 2	:	Furazolidone @ 400 ppm in feed.
Group 3	:	Garlic @ 1% in feed.
Group 4	:	Garlic @ 2% in feed
Group 5	:	Vitamin E @ 300 ppm in feed.
Group 6	:	Stresseaze @ 0.5 g / 100 birds in drinking water (day 15 to 28) and @ 1.0 g / 100 birds in drinking water (day 29 to 42).
Group 7	:	Furazolidone @ 400 ppm in feed+Garlic @ 1% in feed.
Group 8	:	Furazolidone @ 400 pm in feed + Garlic @ 2% in feed.

Group 9 : Furazolidone @ 400 ppm in feed + Stresseaze @ 0.5 g / 100 birds (day 15 to 28) and @ 1.0 g / 100 birds (day 29 to 42) in drinking water.

Group 10 : Furazolidone @ 400 ppm in feed + Vitamin E @ 300 ppm in feed.

During the experimental period, growth rate, feed efficiency and general health of the birds were monitored, and the blood was collected for biochemical analysis on day 28 and 42.

At the end of the experimental period, liver, heart, kidney and intestine were collected from each group in 10 percent formaldehyde solution for histopathological analysis. Liver samples were collected in ice-cold normal saline for lipid peroxidation test.

3.7 FEED CONVERSION RATION (FCR)

FCR was calculated to determine the feed efficiency using the formula

$$FCR = \frac{\text{Total feed consumed (kg)}}{\text{Total body weight gained (kg)}}$$

3.8 CLINICAL OBSERVATIONS

Bird groups were observed daily for feed intake, alertness and for the presence of any signs of furazolidone toxicity.

3.9 RBC ENZYME PROFILE

3.9.1 Assay of Glutathione peroxidase (GSH-Px) activity (Paglia and Valentine, 1967)

Materials

Phosphate buffer (pH 7.4)

0.2 mM NADPH

50 mM Reduced glutathione

0.25 mM H_2O_2

Method

Sl.No.	Reagent	Test tube (ml)
1.	Phosphate buffer	2.0
2.	Haemolysate	0.1
3.	Reduced glutathione	0.1
4.	H_2O_2	0.1

Tubes were incubated at 25°C for 15 minutes following which 0.1 ml NADPH was added. Then enzyme activity was monitored at 60 sec. interval for 5 min at 320 nm.

Calculation

Erythrocytic glutathione peroxidase activity = $\Delta A_{320} \times 3781$ units/ml

Where, Δ_{320} is the change in absorbance per minute at 320 nm.

3.9.2 Assay of Glutathione Reductase (GSH-R) activity (Spectrophotometric method - Raghuramulu, 1983)

Materials :

Haemolysate

Phosphate buffer

0.1 M Sodium bicarbonate

50 mM GSSG solution

250 µ M FAD

4 mM NADPH

80 mM EDTA

Method :

Sl.No.	Reagent	Test tube (ml)
1.	Phosphate buffer	2.0
2.	Haemolysate	0.1
3.	50 mM GSSG	0.1
4.	250 µM FAD	0.1
5.	80 mM EDTA	0.5

Tubes were incubated at 37°C for 15 min, following which 0.1ml NADPH solution was added. Then enzyme activity was monitored at 60 sec. interval for 5 minutes at 340 nm.

Calculation

Erythrocyte glutathione reductase activity

$$= \Delta A_{340} \times 3781 \text{ units /ml}$$

Where, ΔA_{340} is the change in absorbance per minute at 340 nm.

3.9.3 Assay of erythrocytic catalase activity (UV Spectrophotometric analysis - Britton and Machlly, 1956)

Materials :

1. Haemolysate
2. 0.01 M phosphate buffer (pH 7.0)
3. 1 : 500 H_2O_2 in phosphate buffer

Method :

To 1 ml 1 : 500 H_2O_2 in a test tube, 1 ml haemolysate sample was added, mixed properly and the enzyme activity was monitored at 30 second intervals for 5 minutes at 280 nm wave length.

Calculation :

$$\text{Velocity constant (K)} = \frac{2.3}{t_2 - t_1} \log \frac{X_1}{X_2} \text{ sec}^{-1}$$

Where t_1, and t_2 are the times corresponding to a pair of readings of the absorbance X_1 and X_2

X_1 = A280 at t_1

X_2 = A280 at t_2

Specific activity of catalase (K_1) = K/e $mole^{-1}$ sec^{-1}

Where,

e = avian erythrocyte catalase molarity = 2.5×10^{-2} moles

3.10 Assay of blood glutathione (GSH) concentration (Patterson and Lazarow, 1956)

Materials

1 M sulfosalicylic acid

1% starch solution

0.001 N potassium iodate

5% potassium iodide

Blood sample

Method

(a) Preparation of haemolysate and precipitation of protein

Blood was haemolysed by adding distilled water in 1:8 ratio, shaken gently and allowed the mixture to stand for 5-10 minutes. Protein was precipitated by slowly adding 1 ml 1 M sulfosalicylic acid to 1 ml haemolysate. The extract was obtained by removing the precipitated protein

by centrifuging and then decanting the extract or by filtering through dry filter paper.

(b) Preparation for titration

Two millilitre aliquot of sulfosalicylic acid extract was placed in a test tube. To this 0.5 ml 4% sulfosalicylic acid, 0.5 ml 5% potassium iodide and 1 drop 1% starch solution were added.

(c) Titration of sample

The test tube containing the sample was placed against a white background in a water bath containing ice and water. A microburette was used to add 0.001 N potassium iodate to the sample. End point was confirmed on the development of sharp persistent blue colour.

Calculation :

$$\text{GSH concentration (mg/100 ml)} = \frac{500 \times \text{volume of 0.001 N Potassium iodate used}}{3.26}$$

3.11 LIPID PEROXIDATION

Determination of 2-Thiobarbituric acid value (TBA) (Wittee *et al.*, 1970)

Ten gram liver was blended with 25 ml of extracting solution stored at 4°C containing 20 per cent trichloroacetic acid in 2 M orthophosphoric acid.

The resulting solution was transferred quantitatively into a 50 ml volumetric flask and made upto 50 ml with distilled water and mixed thoroughly by shaking. A 25 ml portion was filtered through Whatman No.1 filter paper 5 ml of the filtrate was transferred to a test tube followed by 5 ml 2-thiobarbituric acid (0.005 M in distilled water). The tube was stoppered and the solution was mixed by inversion and kept in dark for 12-15 hours at room temperature. The resulting colour was measured at 530 nm in a spectrophotometer.

The TBA value used to express the result of the extraction method was calculated by multiplying the absorbance by the K value (5.2) for extraction.

3.12 SERUM BIOCHEMICAL PROFILE

The serum was separated from the clotted blood collected on day 28 and 42 and used for the estimation of the following biochemical parameters.

1. Aspartate aminotransferase (AST)
2. Alanine aminotransferase (ALT)
3. Total proteins and albumin
4. Total cholesterol

The commercially available diagnostic kits (Span Diagnostic Ltd., Surat) were employed for the estimation of all biochemical parameters.

3.13 HISTOPATHOLOGICAL STUDIES

A detailed post mortem examination of the birds sacrificed from each group was conducted at the end of the experiment. The gross pathological changes if any, were noted. Tissue pieces of heart, liver, kidney and intestine were collected and fixed in 10% formalin. The fixed tissues were processed and stained with H & E stain (Singh and Sulochana, 1997).

3.14 STATISTICAL ANALYSIS

The data was subjected to two-way analysis of variance (ANOVA) as per the method by Snedecor and Cochran (1994).

Chapter IV

RESULTS

CHAPTER - IV

RESULTS

4.1 PRODUCTION PARAMETERS

4.1.1 Body weight gain

The body weight gain (g) of different groups of birds is presented in Table 2 and Fig. 1. There was a significant ($P < 0.05$) decrease in body weight in group 2 (663.42 ± 24.32 g) compared to group 1 (787.40 ± 20.75 g) at the end of 4th week. The body weight gain of other groups did not differ significantly ($P > 0.05$) among themselves and from group 1. At the end of 6th week, a significant ($P<0.05$) decrease in the body weight gain of group 2 (1000.42 ± 49.75 g) was noticed compared to group 1 (1433.40 ± 52.82 g). The corresponding body weight gain of groups 7, 8, 9 and 10 were 1251.50 ± 53.36, 1324.00 ± 49.69, 1344.58 ± 50.81 and 1274.65 ± 53.52 g.

4.1.2 The feed consumption

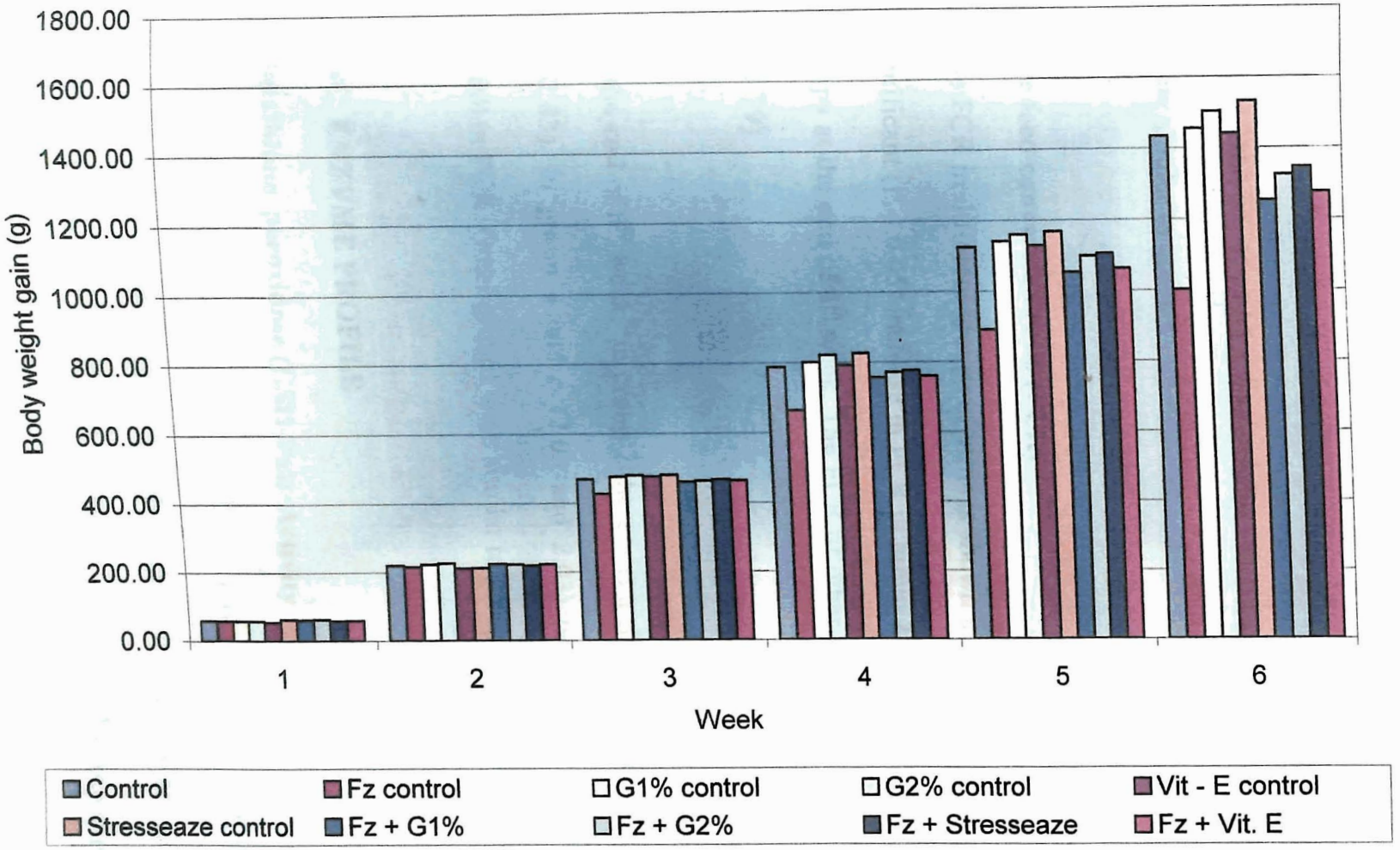
The feed consumption (g) of the birds is given in Table 3 and Fig. 2. There was significantly ($P < 0.05$) lower feed intake in birds of group 2 (1365.82 g) compared to group 1 (14082.02 g) at the end of 4th week. The feed consumption by birds of other groups were on par with group 1.

Table 2 : Body weight gain (g) of different groups of broiler chicken

	Group	Age (weeks)					
		0-1	0-2	0-3	0-4	0-5	0-6
1	Control	59.52^{a} ± 3.42	220.40^{a} ± 6.72	468.15^{a} ± 10.52	787.40^{a} ± 20.75	1124.90^{a} ± 43.74	1433.40^{a} ± 52.82
2	Fz control	58.55^{a} ± 2.78	215.55^{a} ± 6.15	425.64^{a} ± 11.38	663.42^{b} ± 24.32	890.44^{b} ± 45.62	1000.42^{b} ± 49.75
3	G1% control	58.50^{a} ± 2.96	223.50^{a} ± 7.33	475.50^{a} ± 11.13	801.00^{a} ± 19.56	1140.50^{a} ± 39.84	1454.00^{a} ± 55.89
4	G2% control	58.80^{a} ± 3.17	226.80^{a} ± 7.48	478.80^{a} ± 10.96	820.80^{a} ± 23.86	1158.80^{a} ± 46.15	1501.80^{a} ± 51.32
5	Vit. E control	54.90^{a} ± 3.59	211.00^{a} ± 6.59	474.98^{a} ± 12.59	791.70^{a} ± 24.67	1128.65^{a} ± 42.39	1440.65^{a} ± 54.67
6	Stresseaze control	63.50^{a} ± 2.82	212.50^{a} ± 8.56	479.50^{a} ± 11.73	826.00^{a} ± 24.93	1168.00^{a} ± 41.91	1530.50^{a} ± 54.18
7	Fz + G1%	61.65^{a} ± 2.76	224.50^{a} ± 7.39	459.50^{a} ± 10.89	755.20^{a} ± 21.82	1052.50^{a} ± 41.15	1251.50^{a} ± 53.36
8	Fz + G2%	63.05^{a} ± 3.05	222.85^{a} ± 6.86	463.00^{a} ± 10.74	770.84^{a} ± 19.94	1098.00^{a} ± 45.87	1324.00^{a} ± 49.69
9	Fz + Stresseaze	58.44^{a} ± 2.71	218.64^{a} ± 7.94	465.72^{a} ± 11.83	775.58^{a} ± 24.82	1104.45^{a} ± 40.56	1344.58^{a} ± 50.81
10	Fz + Vit. E	59.35^{a} ± 3.15	222.66^{a} ± 6.79	463.26^{a} ± 9.88	759.31^{a} ± 21.63	1062.23^{a} ± 39.82	1274.65^{a} ± 53.57

n = 10
Two way ANOVA
The values with different superscripts are statistically different ($P<0.05$)

Fig. 1 : Body weight gain (g) of different groups of broiler chicken



A significant ($P<0.05$) reduction in feed consumption of group 2 birds was observed at the end of 5^{th} and 6^{th} week also, when compared to group 1 birds. The 5^{th} and 6^{th} week values of group 2 and group 1 birds were 2053.27; 2833.77 and 2203.02; 3213.02 g respectively. The feed consumption of other groups were similar to group 1.

4.1.3 The feed conversion ratio (FCR)

The FCR in different groups of birds is shown in Table 4, Fig. 3. There was a significant ($P < 0.05$) increase in FCR in group 2 (2.06) compared to all other groups at the end of 4^{th} week. The FCR of other groups were similar to group 1 (1.79).

At the end of 6^{th} week, in group 2 there was a significant ($P<0.05$) rise in FCR (2.83). In group 4 and 6 (2.03 and 2.03) there was a significant ($P<0.05$) fall in FCR. Other groups were similar to group 1 (2.24) ($P > 0.05$).

4.2 RBC ENZYME PROFILE

4.2.1 Glutathione peroxidase (GSH-Px): Activity

The GSH-Px activity (units/ml) in different groups is given in Table 5 and Fig. 4. There was a significant ($P < 0.05$) increase in GSH-Px activity in group 2 (97.42 ± 2.68 and 138.63 ± 2.86) in 4^{th} and 6^{th} weeks respectively

Table 3 : *The feed consumption (g) in different groups of broiler chicken*

	Group	Age (Weeks)					
		0-1	**0-2**	**0-3**	**0-4**	**0-5**	**0-6**
1	Control	102.52[a]	372.52[a]	798.02[a]	1408.02[a]	2203.02[a]	3213.02[a]
2	Fz control	108.32[a]	372.32[a]	762.32[a]	1365.82[b]	2053.27[b]	2833.77[b]
3	G1% control	117.00[a]	387.00[a]	817.00[a]	1422.50[a]	2207.50[a]	3132.50[a]
4	G2% control	117.00[a]	377.00[a]	803.50[a]	1393.50[a]	2161.50[a]	3041.50[a]
5	Vit. E control	101.00[a]	371.00[a]	801.00[a]	1391.00[a]	2150.00[a]	3040.00[a]
6	Stresseaze control	117.00[a]	388.50[a]	818.50[a]	1408.50[a]	2173.00[a]	3103.24[a]
7	Fz + G1%	115.50[a]	389.00[a]	797.50[a]	1403.00[a]	2193.50[a]	3028.12[a]
8	Fz + G2%	117.28[a]	392.70[a]	812.95[a]	1411.45[a]	2197.81[a]	3040.29[a]
9	Fz + Stresseaze	104.45[a]	380.95[a]	804.37[a]	1399.71[a]	2178.27[a]	3021.81[a]
10	Fz + Vit. E	114.35[a]	388.55[a]	801.00[a]	1391.00[a]	2140.00[a]	2955.50[a]

Two way ANOVA

The values with different superscripts are statistically different ($P<0.05$)

Table 4 : Feed conversion ratio (FCR) in different groups of broiler chicken

	Group	Age (Weeks)					
		1	2	3	4	5	6
1	Control	1.72[a]	1.69[a]	1.70[a]	1.79[a]	1.96[a]	2.24[a]
2	Fz control	1.85[a]	1.73[a]	1.79[a]	2.06[b]	2.31[b]	2.83[b]
3	G1% control	2.00[a]	1.73[a]	1.72[a]	1.78[a]	1.94[a]	2.15[a]
4	G2% control	1.99[a]	1.66[a]	1.68[a]	1.70[a]	1.87[a]	2.03[c]
5	Vit. E control	1.84[a]	1.76[a]	1.69[a]	1.76[a]	1.90[a]	2.11[a]
6	Stresseaze control	1.84[a]	1.83[a]	1.71[a]	1.71[a]	1.86[a]	2.03[c]
7	Fz + G1%	1.87[a]	1.73[a]	1.74[a]	1.86[a]	2.08[a]	2.42[a]
8	Fz + G2%	1.86[a]	1.76[a]	1.76[a]	1.83[a]	2.00[a]	2.30[a]
9	Fz + Stresseaze	1.79[a]	1.74[a]	1.73[a]	1.80[a]	1.97[a]	2.25[a]
10	Fz + Vit. E	1.93[a]	1.75[a]	1.73[a]	1.83[a]	2.01[a]	2.32[a]

Two way ANOVA
The values with different superscripts are statistically different ($P<0.05$)

Fig. 2 : The feed consumption (g) in different groups of broiler chicken

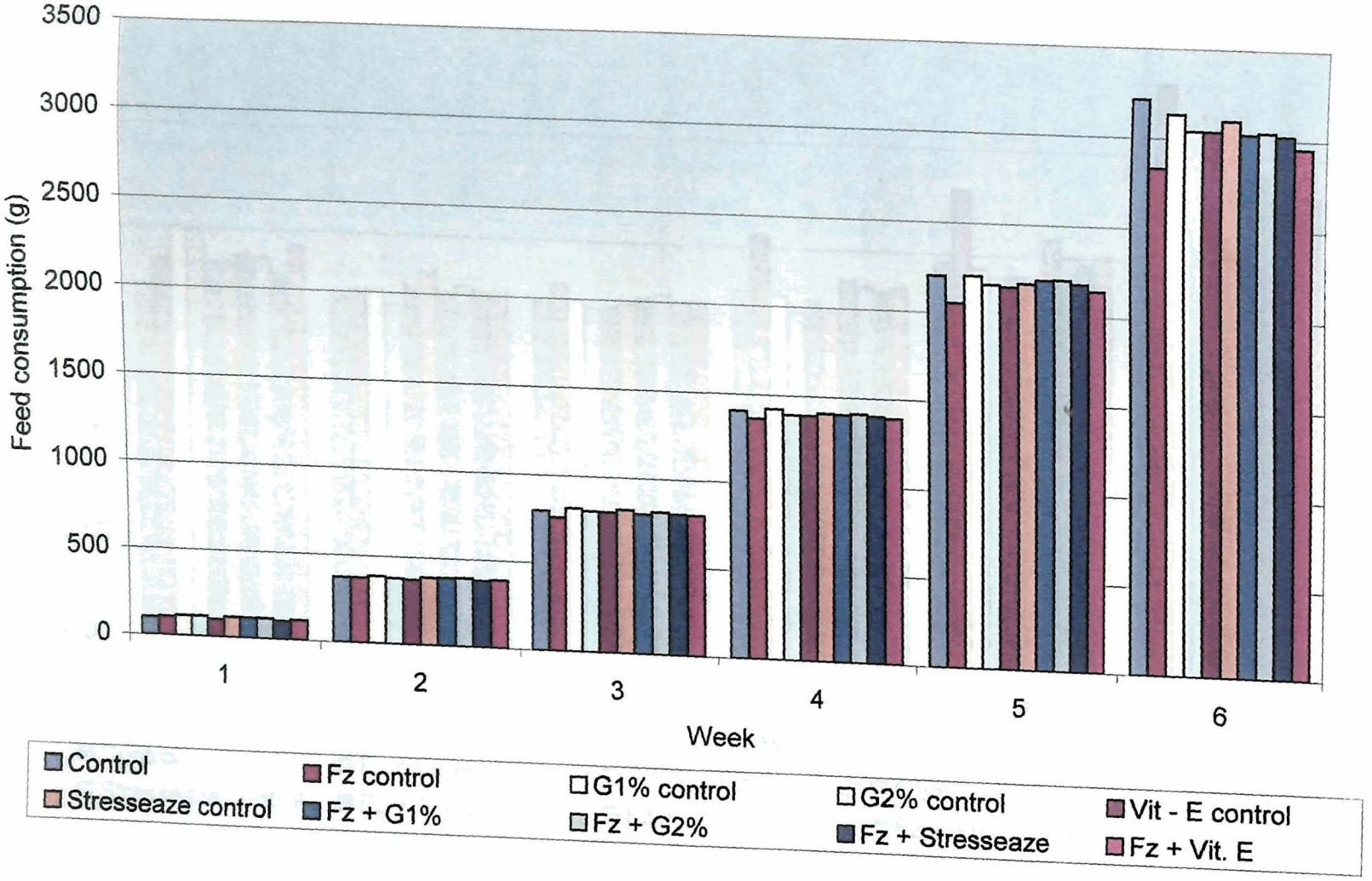
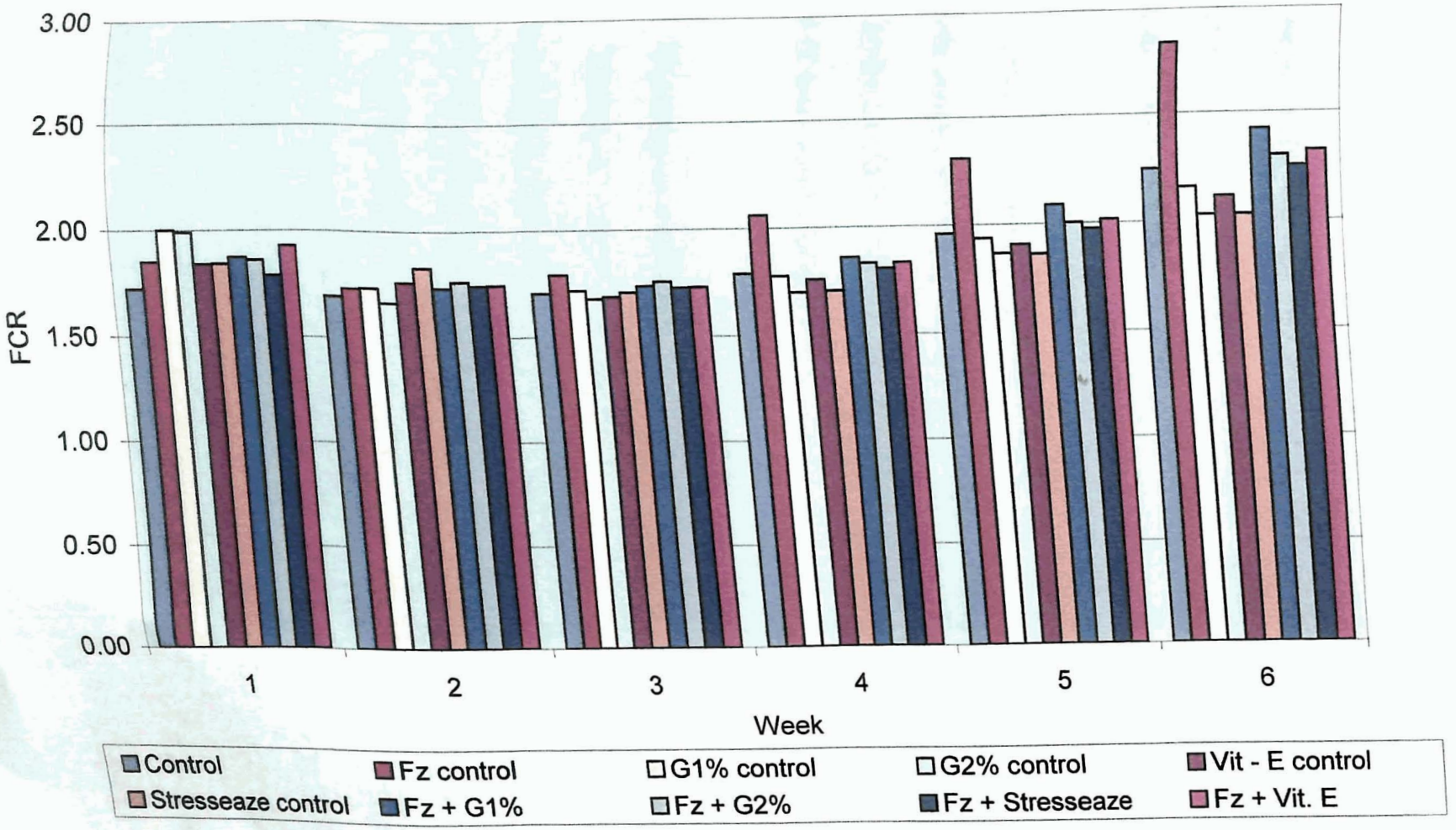


Fig. 3 : Feed conversion ratio (FCR) in different groups of broiler chicken



compared to other groups. The GSH-Px values in groups 7, 8, 9 and 10 at the end of 4th week were 93.15 ± 2.78, 89.62 ± 2.84, 87.44 ± 2.65 and 89.78 ± 2.94 respectively. Though they showed a slight increase in GSH-Px activity than group I (86.56 ± 2.97), they did not differ significantly ($P > 0.05$) except in group 7 (93.15 ± 2.78).

Group 7 at week 6 (95.96 ± 2.68) showed a significant increase in GSH-Px activity ($P<0.05$) than group 1 (87.99 ± 2.98), but significantly lower than group 2 (138.63 ± 2.86). The activity of GSH-Px enzyme in groups 7, 8, 9 and 10 were similar to group 1.

4.2.2 Glutathione reductase (GSH-R) activity

There was a significant increase ($P < 0.05$) in GSH-R activity (units / ml) in group 2 in both 4th and 6th weeks compared to other groups as shown in Table 5, Fig. 5. The GSH-R values were 76.25 ± 1.32 and 103.03 ± 1.32 at 4th and 6th weeks respectively. The 7th group showed a significant rise in GSH-R during the 4th week (51.74 ± 1.34) compared to group 1 (40.30 ± 1.50) but was significantly lower ($P < 0.05$) compared to group 2 (76.25 ± 1.23). However the enzyme GSH-R activity in group 7 at week 6 (43.65 ± 1.23) did not differ significantly ($P > 0.05$) compared to group 1 (41.15 ± 1.48). The enzyme GSH-R activity values of other treatment groups were similar to group 1.

4.2.3 Catalase

The catalase activity (moles / sec.) in different groups at the end of 4th and 6th week is given in Table 6, Fig. 6. An increase in catalase activity in group 2 at the end of both 4th and 6th weeks was noticed compared to group 1. The catalase value in groups 2 and 1 were 4.81 ± 0.26, 5.32 ± 0.26 and 3.63 ± 0.34, 3.68 ± 0.24 at the end of 4th and 6th weeks respectively.

The groups 7, 8, 9 and 10 though showed a slight increase in catalase activity did not differ significantly ($P > 0.05$) compared to group 1 at the end of both 4th and 6th weeks.

4.3 GLUTATHIONE (GSH)

The RBC glutathione content (mg/100 ml) of various groups is given in Table 6 and Fig.7. At the end of the 4th week, in group 2, had a significantly ($P< 0.05$) lower glutathione content (8.53 ± 0.58) compared to control (10.72 ± 0.60) and other groups. Group 6 had a higher glutathione content (12.24 ± 0.42), compared to control (10.72 ± 0.60).

During 6th week, group 2 GSH value (5.58 ± 0.46) was significantly ($P<0.05$) lower compared to other groups. Group 6 had recorded a higher

value (13.28 ± 0.40) compared to other groups. The values in groups 7, 8, 9 and 10 did not differ significantly ($P > 0.05$) compared to control group.

4.4 LIPID PEROXIDATION

The TBA values of liver in different groups at the end of 6th week is given in Table 7, Fig. 8. Group 2 showed a significantly ($P < 0.05$) higher TBA value (1.01 ± 0.06) compared to other groups. Group 7 had shown a significant rise in TBA value (0.50 ± 0.04) compared to group 1 (0.42 ± 0.03) but was significantly lower ($P < 0.05$) compared to group 2. The values in groups 8 (0.46 ± 0.06), 9 (0.44 ± 0.03) and 10 (0.47 ± 0.05) were similar to the control group (0.42 ± 0.03).

4.5 SERUM PARAMETERS

4.5.1 Aspartate aminotransferase (AST) concentration

Serum AST(unit/ml) activities of different groups recorded at the end of 4th and 6th weeks is given in Table 8, Fig. 9.

A significant ($P < 0.05$) rise in the AST activity was seen in group 2 (139.41 ± 3.18 and 145.09 ± 2.96), compared to control group (122.75 ± 3.28 and 124.77 ± 3.25) at the end of 4th and 6th weeks respectively. However no significant difference ($P > 0.05$) was noted among the control group and

Table 5 : Glutathione peroxidase (GSH-Px) and Glutathione reductase activities (units /ml) in different groups of broiler chicken

	Group	Glutathione peroxidase (GSH-Px) (units/ml)		Glutathione reductase activity (units /ml)	
		4th week	6th week	4th week	6th week
1.	Control	$86.56^{aA} \pm 2.97$	$87.99^{aA} \pm 2.89$	$40.30^{aA} \pm 1.48$	$41.15^{aA} \pm 1.48$
2.	Fz control	$97.42^{bA} \pm 2.68$	$138.63^{bA} \pm 2.86$	$76.25^{bA} \pm 1.32$	$103.03^{bA} \pm 1.32$
3.	G1% control	$84.49^{aA} \pm 2.35$	$85.22^{aA} \pm 2.45$	$39.52^{aA} \pm 1.46$	$40.14^{aA} \pm 1.16$
4.	G2% control	$83.99^{aA} \pm 1.98$	$84.12^{aA} \pm 2.92$	$37.48^{aA} \pm 1.35$	$38.74^{aA} \pm 1.35$
5.	Vit - E control	$84.27^{aA} \pm 2.46$	$84.65^{aA} \pm 1.96$	$39.83^{aA} \pm 1.23$	$40.21^{aA} \pm 1.24$
6.	Stresseaze control	$81.81^{aA} \pm 2.50$	$83.92^{aA} \pm 2.34$	$37.89^{aA} \pm 1.48$	$38.72^{aA} \pm 1.19$
7.	Fz + G1%	$93.15^{cA} \pm 2.78$	$95.96^{cA} \pm 2.68$	$51.74^{cA} \pm 1.34$	$43.65^{aA} \pm 1.23$
8.	Fz + G2%	$89.62^{aA} \pm 2.84$	$91.54^{aA} \pm 2.54$	$42.42^{aA} \pm 1.26$	$42.52^{aA} \pm 1.15$
9.	Fz + Stresseaze	$87.44^{aA} \pm 2.65$	$89.16^{aA} \pm 1.94$	$41.81^{aA} \pm 1.45$	$40.40^{aA} \pm 1.36$
10.	Fz + Vit. E	$89.78^{aA} \pm 2.94$	$92.38^{aA} \pm 2.34$	$43.11^{aA} \pm 1.38$	$42.90^{aB} \pm 1.44$

Two way ANOVA
Values are mean ± SE n = 6
The values with different superscripts are statistically different ($P < 0.05$)
Capital alphabets - Horizontal comparison (within group)

Table 6 : The catalase activity (moles / sec) and glutathione (GSH) content (mg/100ml) in different groups of broiler chicken

	Group	Catalase activity (moles / sec)		Glutathione (GSH) content (mg/100ml)	
		4th week	6th week	4th week	6th week
1.	Control	$3.63^{aA} \pm 0.34$	$3.68^{aA} \pm 0.24$	$10.72^{aA} \pm 0.60$	$11.63^{aA} \pm 0.44$
2.	Fz control	$4.81^{bA} \pm 0.26$	$5.32^{bA} \pm 0.26$	$8.53^{bA} \pm 0.58$	$5.58^{bB} \pm 0.46$
3.	G1% control	$3.62^{aA} \pm 0.32$	$3.51^{aA} \pm 0.20$	$11.44^{aA} \pm 0.36$	$12.23^{aA} \pm 0.38$
4.	G2% control	$3.57^{aA} \pm 0.29$	$3.49^{aA} \pm 0.19$	$11.58^{aA} \pm 0.49$	$13.09^{cB} \pm 0.42$
5.	Vit - E control	$3.60^{aA} \pm 0.33$	$34.7^{aA} \pm 0.28$	$11.46^{aA} \pm 0.57$	$12.54^{aA} \pm 0.35$
6.	Stresseaze control	$3.56^{aA} \pm 0.28$	$3.46^{aA} \pm 0.31$	$12.24^{cA} \pm 0.42$	$13.28^{cA} \pm 0.40$
7.	Fz + G1%	$4.12^{aA} \pm 0.34$	$4.21^{aA} \pm 0.36$	$9.72^{aA} \pm 0.53$	$10.80^{aB} \pm 0.43$
8.	Fz + G2%	$3.73^{aA} \pm 0.26$	$3.99^{aA} \pm 0.25$	$9.94^{aA} \pm 0.39$	$11.05^{aB} \pm 0.34$
9.	Fz + Stresseaze	$3.7^{aA} \pm 0.31$	$3.95^{aA} \pm 0.33$	$10.03^{aA} \pm 0.48$	$10.88^{aA} \pm 0.36$
10.	Fz + Vit. E	$3.74^{aA} \pm 0.28$	$4.10^{aA} \pm 0.28$	$9.78^{aA} \pm 0.56$	$10.76^{aA} \pm 0.43$

Two way ANOVA
Values are mean ± SE n = 6
The values with different superscripts are statistically different ($P < 0.05$)
Capital alphabets - Horizontal comparison (within group)

Table 7 : Thiobarbituric acid (TBA) values of liver in different groups of broiler chicken

	Group	6th week
1.	Control	$0.42^{a} \pm 0.03$
2.	Fz control	$1.01^{b} \pm 0.06$
3.	G1% control	$0.41^{a} \pm 0.03$
4.	G2% control	$0.39^{a} \pm 0.09$
5.	Vit - E control	$0.41^{a} \pm 0.04$
6.	Stresseaze control	$0.41^{a} \pm 0.07$
7.	Fz + G1%	$0.50^{c} \pm 0.04$
8.	Fz + G2%	$0.46^{a} \pm 0.06$
9.	Fz + Stresseaze	$0.44^{a} \pm 0.03$
10.	Fz + Vit. E	$0.47^{a} \pm 0.05$

Two way ANOVA
Values are mean ± SE n = 6
The values with different superscripts are statistically different ($P < 0.05$)
Capital alphabets - Horizontal comparison (within group)

Fig. 4 : Glutathione peroxidase (GSH-Px) activity (units/ml) in different groups of broiler chicken

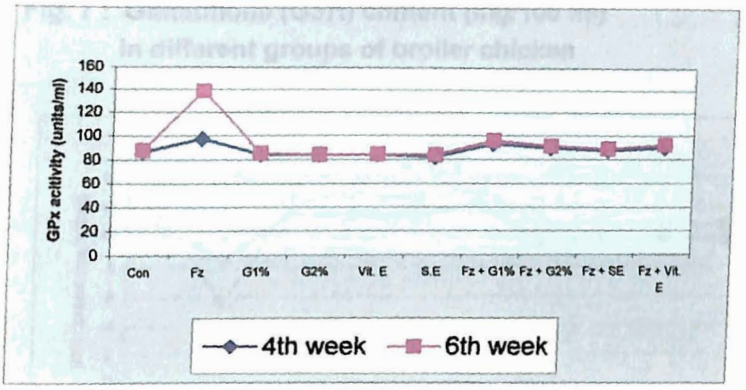


Fig. 5 : Glutathione reductase (GSH-R) activity (units /ml) in different groups of broiler chicken

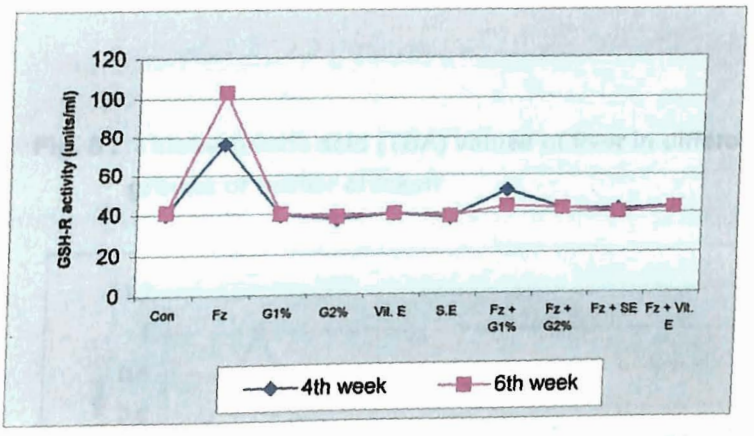


Fig. 6 : Catalase activity (moles / sec) in different groups of broiler chicken

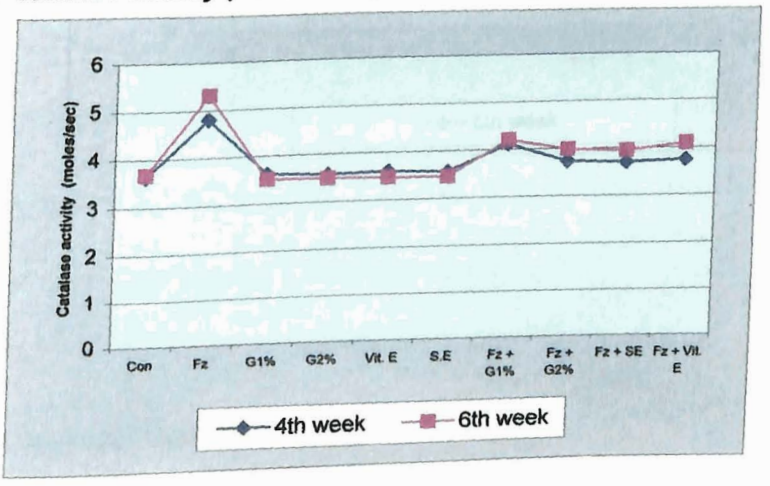


Fig. 7 : Glutathione (GSH) content (mg/100 ml) in different groups of broiler chicken

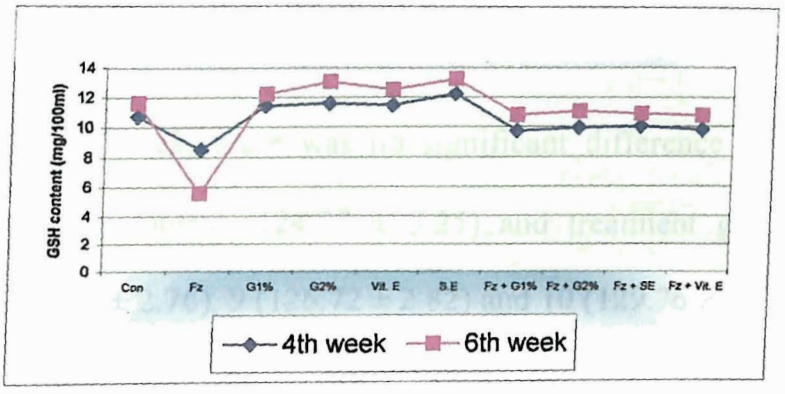
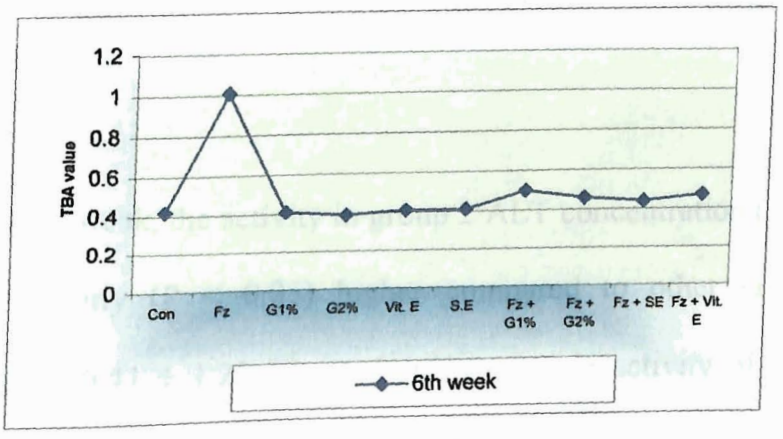


Fig. 8 : Thiobarbituric acid (TBA) values of liver in different groups of broiler chicken



groups 7 (128.08 ± 2.73), 8 (125.08 ± 2.56), 9 (122.91 ± 2.68) and 10 (126.02 ± 2.39) at the end of 4th week.

At the end of 6th week, there was no significant difference in AST concentration among the control (124.77 ± 3.25) and treatment groups 7 (130.76 ±2.58), 8 (128.74 ± 2.76), 9 (126.72 ± 2.82) and 10 (129.76 ± 2.62).

4.5.2 Alanine amino transferase (ALT) concentration

At 4th week, serum ALT activity (units/ml) in group 2 (18.92 ± 1.46) showed a significant increase ($P < 0.05$) compared to other groups (Table 8, Fig. 10).

At the end of 6th week, the activity in group 2 ALT concentration (25.42 ± 1.28) was significantly ($P < 0.05$) higher compared to other groups. However the group 7 (0.41 ± 1.23) showed an increase in activity of ALT compared to groups, 8 (18.44 ± 1.28), 9 (17.76 ± 1.35) and 10 (18.72 ± 1.32) but significantly ($P < 0.05$) lower compared to group 2.

4.5.3 Total cholesterol concentration

The total cholesterol concentration (mg/dl) in various groups is given in Table 9 and Fig. 12.

At the end of 4th week, in groups 3 (149.02 ± 2.32), 4 (141.05 ± 2.18), 6 (160.83 ± 2.76), 7 (162.92 ± 2.58), 8 (159.06 ± 2.19), 9 (161.30 ± 2.78) and 10 (160.45 ± 2.56), total cholesterol concentration was significantly ($P < 0.05$) decreased compared to group 1 (172.40 ± 2.68). Group 1 cholesterol concentration did not differ significantly ($P > 0.05$) in group 2 (165.51 ± 2.74) and 5 (164.08 ± 2.82 mg/dl).

At 6th week, a significant ($P < 0.05$) decrease in cholesterol content in group 2 (161.10 ± 2.72) compared to group 1 (174.03 ± 2.35). The total cholesterol concentration in groups 3 (165.15 ± 2.46), 4 (156.79 ± 2.5) and 6 (164.39 ± 2.49) groups 7 (162.34 ± 2.38), 8 (162.12 ± 2.42), 9 (163.47 ± 2.53) and 10 (164.88 ± 2.39 mg/dl).

4.5.4 Total protein concentration

The serum total protein concentration (g/dl) in various groups is given in Table 10 and Fig. 11.

In Group 2, total protein concentration was (3.21 ± 0.36 mg/dl) was significantly ($P < 0.05$) lower compared to other groups at the end of the 4th week. There was an increase in the total protein concentration in group 2

Table 8 : Aspartate amino transferase (AST) and Alanine amino transferase (ALT) activities (units/ml) in different groups of broiler chicken

	Group	AST		ALT	
		4th week	6th week	4th week	6th week
1.	Control	$122.75^{aA} \pm 3.28$	$124.77^{aA} \pm 3.25$	$15.30^{aA} \pm 1.60$	$17.42^{aA} \pm 1.31$
2.	Fz control	$139.41^{bA} \pm 3.18$	$145.09^{bA} \pm 2.96$	$18.92^{bA} \pm 1.46$	$25.42^{bB} \pm 1.28$
3.	G1% control	$121.75^{aA} \pm 2.76$	$122.09^{aA} \pm 2.63$	$14.84^{aA} \pm 1.58$	$16.43^{aA} \pm 1.42$
4.	G2% control	$120.80^{aA} \pm 2.64$	$119.42^{aA} \pm 2.78$	$13.92^{aA} \pm 1.38$	$14.76^{aA} \pm 1.39$
5.	Vit - E control	$121.26^{aA} \pm 2.82$	$121.76^{aA} \pm 2.53$	$15.27^{aA} \pm 1.54$	$15.42^{aA} \pm 1.25$
6.	Stresseaze control	$119.75^{aA} \pm 2.50$	$118.09^{cA} \pm 2.64$	$12.92^{aA} \pm 1.42$	$14.47^{cA} \pm 1.36$
7.	Fz + G1%	$128.08^{aA} \pm 2.73$	$130.76^{aA} \pm 2.58$	$17.59^{aA} \pm 1.49$	$20.41^{dA} \pm 1.23$
8.	Fz + G2%	$125.08^{aA} \pm 2.56$	$128.74^{aA} \pm 2.76$	$16.82^{aA} \pm 1.36$	$18.44^{aA} \pm 1.28$
9.	Fz + Stresseaze	$122.91^{aA} \pm 2.68$	$126.72^{aA} \pm 2.82$	$16.5^{9aA} \pm 1.53$	$17.76^{aA} \pm 1.35$
10.	Fz + Vit. E	$126.02^{aA} \pm 2.39$	$129.76^{aA} \pm 2.62$	$16.64^{aA} \pm 1.49$	$18.72^{aA} \pm 1.32$

Two way ANOVA
Values are mean ± SE n = 6
The values with different superscripts are statistically different ($P < 0.05$)
Capital alphabets - Horizontal comparison (within group)

Table 9 : Total cholesterol concentration (mg/dl) in different groups of broiler chicken

	Group	Total cholesterol	
		4th week	6th week
1.	Control	$172.40^{aA} \pm 2.68$	$174.03^{aA} \pm 2.35$
2.	Fz control	$165.51^{abA} \pm 2.74$	$161.10^{bA} \pm 2.72$
3.	G1% control	$149.02^{cA} \pm 2.32$	$165.15^{bB} \pm 2.46$
4.	G2% control	$141.05^{cA} \pm 2.18$	$156.79^{bB} \pm 2.35$
5.	Vit - E control	$164.08^{aA} \pm 2.82$	$169.81^{aA} \pm 2.56$
6.	Stresseaze control	$162.92^{bA} \pm 2.58$	$164.39^{ba} \pm 2.49$
7.	Fz + G1%	$162.92^{bA} \pm 2.58$	$162.34^{bA} \pm 2.38$
8.	Fz + G2%	$159.06^{aA} \pm 2.19$	$162.12^{bA} \pm 2.42$
9.	Fz + Stresseaze	$161.30^{bA} \pm 2.48$	$163.47^{bA} \pm 2.53$
10.	Fz + Vit. E	$160.45^{bA} \pm 2.56$	$164.88^{bA} \pm 2.93$

Two way ANOVA
Values are mean ± SE n = 6
The values with different superscripts are statistically different ($P < 0.05$)
Capital alphabets - Horizontal comparison (within group)

Fig. 9 : Aspartate amino transferase (AST) activity (units/ ml) in different groups of broiler chicken

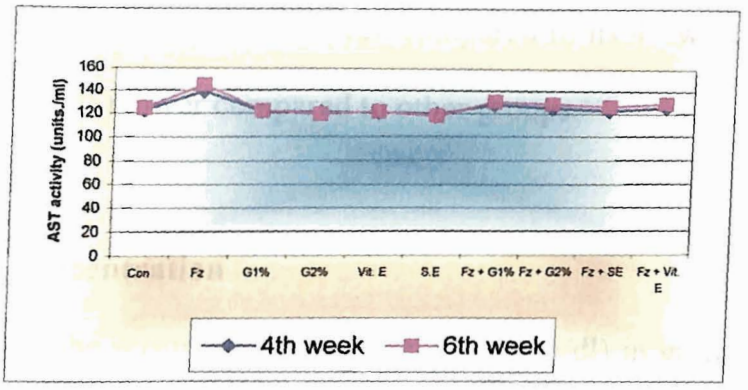


Fig. 10 : Alanine amino transferase (ALT) activity (units/ml) in different groups of broiler chicken

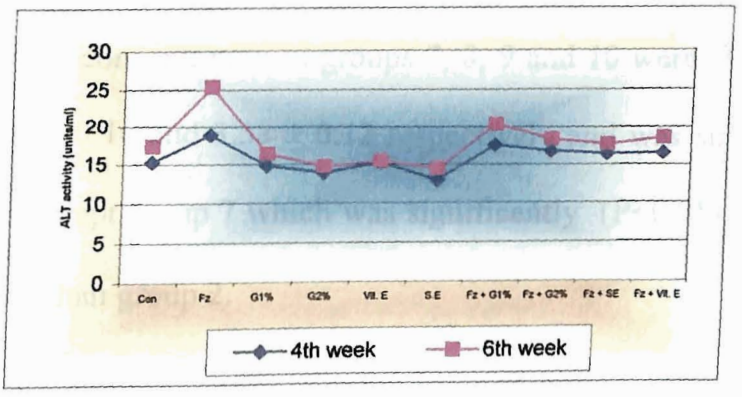
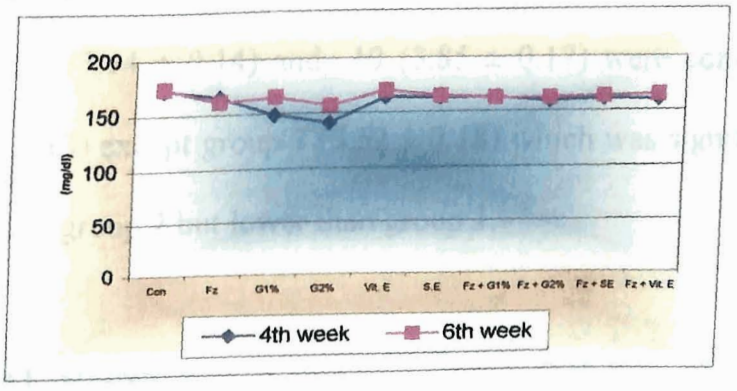


Fig. 11 : Total cholesterol concentration (mg/dl) in different groups of broiler chicken



(4.04 ± 0.34 mg/dl) at the end of 6th week compared to its 4th week value, but significantly (P < 0.05) lower compared to other groups.

4.5.5 Albumin concentration

At 4th week, the serum albumin concentration (g/dl) in group 2 (1.98 ± 0.16 showed a significant (P 0.05) decrease compared to other groups.

The albumin concentration in groups 7, 8, 9 and 10 were 3.21 ± 0.13, 3.41 ± 0.18, 3.47 ± 0.16 and 3.38 ± 0.12 respectively and was similar to the value of group 1 except group 7 which was significantly (P<0.05) lower than group 1 but higher than group 2.

The 6th week albumin concentration value in group 2 (2.54 ± 0.11) was significantly (P<0.05) lower compared to other groups. The values in groups 8 (3.89 ± 0.11), 9 (3.94 ± 0.14) and 10 (3.85 ± 0.17) were comparable to group 1 (4.04 ± 0.13) except group 7 (3.52 ± 0.18) which was significantly (P < 0.05) higher than group 2 but lower than group 1.

4.6 CLINICAL SIGNS

Group 2 birds showed a mild reduction in feed and water consumption in the 3rd and 4th week compared to the control birds. These intensified further

Table 10 : *Total proteins (g/100 ml) and Albumin (g/100ml) contents in different groups of broiler chicken*

	Group	Total proteins		Albumin	
		4th week	6th week	4th week	6th week
1.	Control	$5.08^{aA} \pm 0.32$	$5.63^{aA} \pm 0.36$	$3.59^{aA} \pm 0.11$	$4.04^{aA} \pm 0.13$
2.	Fz control	$3.21^{bA} \pm 0.36$	$4.04^{bB} \pm 0.34$	$1.98^{bA} \pm 0.16$	$2.54^{bB} \pm 0.11$
3.	G1% control	$5.15^{aA} \pm 0.29$	$5.65^{aA} \pm 0.28$	$3.61^{aA} \pm 0.18$	$3.97^{aB} \pm 0.14$
4.	G2% control	$5.22^{aA} \pm 0.34$	$5.72^{aA} \pm 0.38$	$3.67^{aA} \pm 0.21$	$4.03^{aB} \pm 0.16$
5.	Vit - E control	$5.17^{aA} \pm 0.28$	$5.68^{aA} \pm 0.73$	$3.64^{aA} \pm 0.14$	$3.98^{aB} \pm 0.13$
6.	Stresseaze control	$5.26^{aA} \pm 0.39$	$5.76^{aA} \pm 0.36$	$3.72^{aA} \pm 0.10$	$4.12^{aB} \pm 0.12$
7.	Fz + G1%	$4.78^{aA} \pm 0.24$	$5.12^{aA} \pm 0.41$	$3.21^{cA} \pm 0.13$	$3.52^{cB} \pm 0.18$
8.	Fz + G2%	$4.91^{aA} \pm 0.37$	$5.28^{aA} \pm 0.29$	$3.41^{aA} \pm 0.18$	$3.89^{aB} \pm 0.11$
9.	Fz + Stresseaze	$4.98^{aA} \pm 0.42$	$5.32^{aA} \pm 0.30$	$3.47^{aA} \pm 0.16$	$3.94^{aB} \pm 0.14$
10.	Fz + Vit. E	$4.86^{aA} \pm 0.39$	$5.24^{aA} \pm 0.41$	$3.38^{aA} \pm 0.12$	$3.85^{aB} \pm 0.17$

Two way ANOVA
Values are mean ± SE n = 6
The values with different superscripts are statistically different ($P < 0.05$)
Capital alphabets - Horizontal comparison (within group)

Fig. 12 : Total proteins (g/100 ml) in different groups of broiler chicken

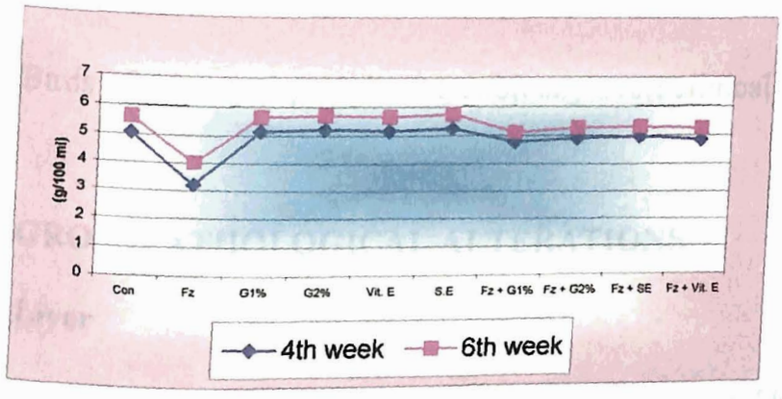
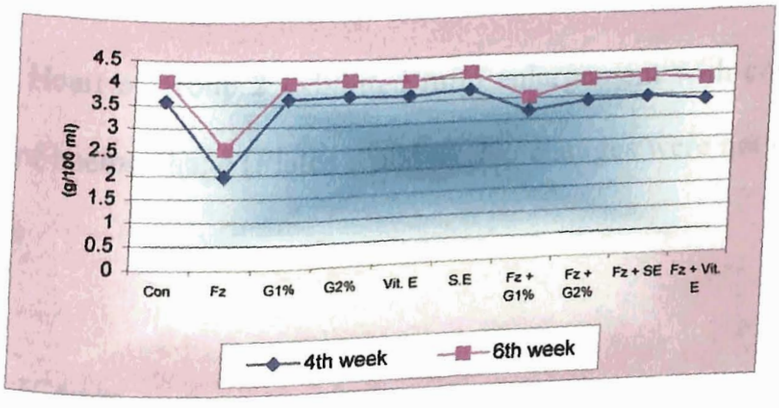


Fig. 13 : Albumin (g/100 ml) content in different groups of broiler chicken



during 5th and 6th week. There was lethargy and depression among the birds. However no mortality was observed during the experimental period.

Birds of other groups did not exhibit any overt clinical signs.

4.7 GROSS PATHOLOGICAL ALTERATIONS

4.7.1 Liver

In group 2, the liver showed mild enlargement paleness and fatty changes (Plate 1). Patchy areas of haemorrhage were noticed in few cases (Plate 2). Livers of other groups did not show such changes.

4.7.2 Heart

Heart of group 2 exhibited mild enlargement with congestion and few areas of haemorrhage (Plates 3 and 4). No changes were not observed in other groups.

4.7.3 Kidney

In group 2, kidneys exhibited mild congestion. Kidneys of other groups were normal.

4.7.4 Intestines

Mild catarrhal changes were noticed in the intestines of group 2. Remaining groups did not show any appreciable gross pathological signs.

4.8 HISTOPATHOLOGY

4.8.1 Liver

The histopathological studies of livers in group 2 revealed degenerative changes with mild sinusoidal haemorrhages (Plate 5). Nodular infiltration of mononuclear cells (Plate 6) was noticed consistently in group 2.

In other groups the changes were not specific.

4.8.2 Heart

Microscopically, the hearts of group 2 revealed focal areas of haemorrhages and mild infiltration of inflammatory cells in between cardiac muscle fibres and sarcolytic changes (Plates 7 and 8).

Remaining groups did not reveal any lesions of pathological significance.

4.8.3 Kidney

On microscopic examination, kidneys of group 2 revealed focal nodular infiltration of inflammatory cells (Plate 9). mild intertubular haemorrhages

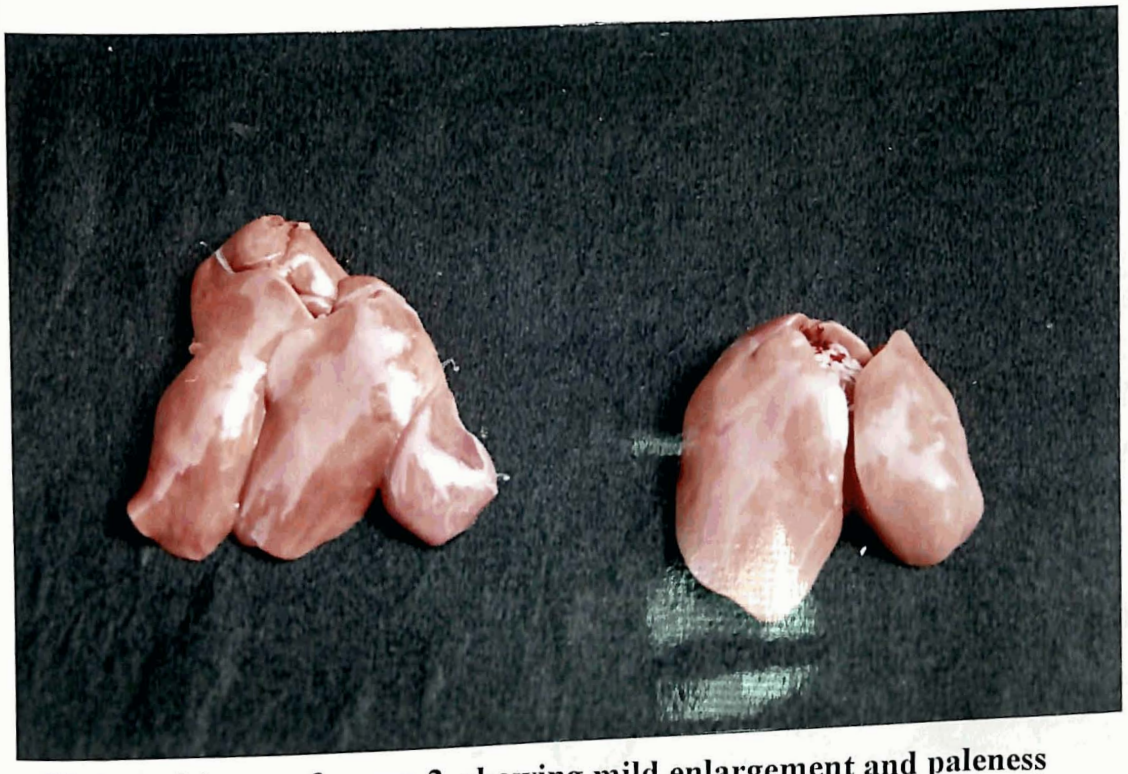


Plate 1. Livers of group 2, showing mild enlargement and paleness

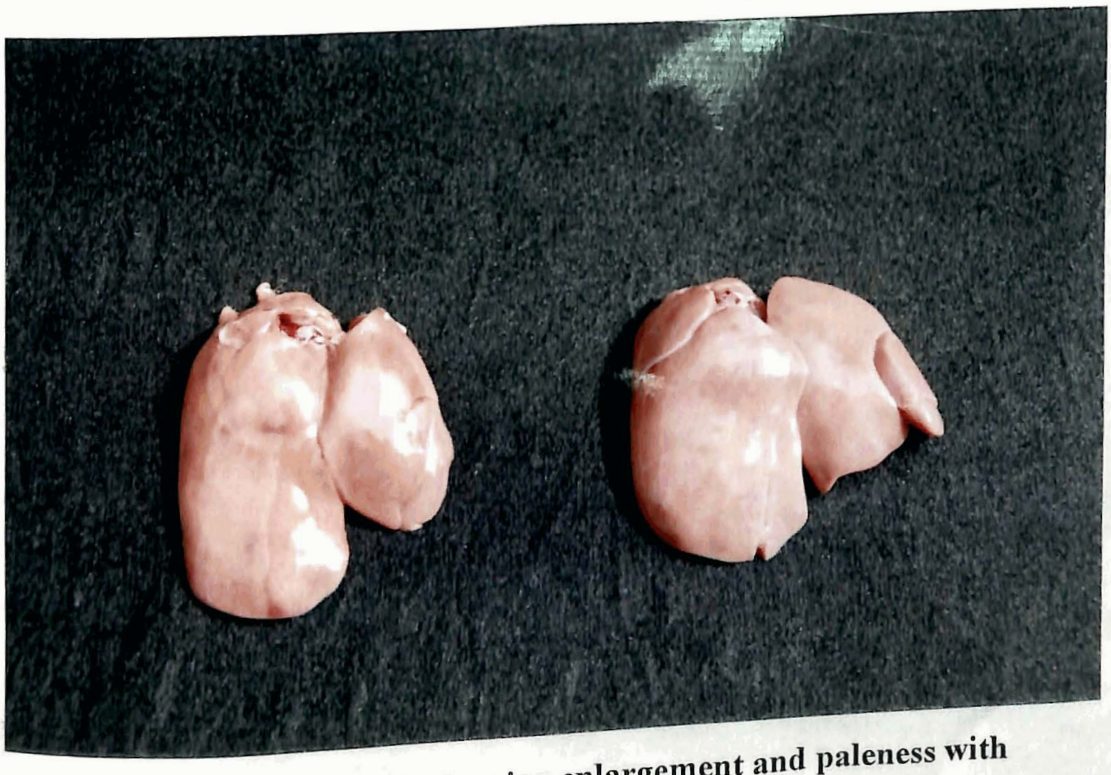


Plate 2.Livers of group 2, showing enlargement and paleness with ecchymoses

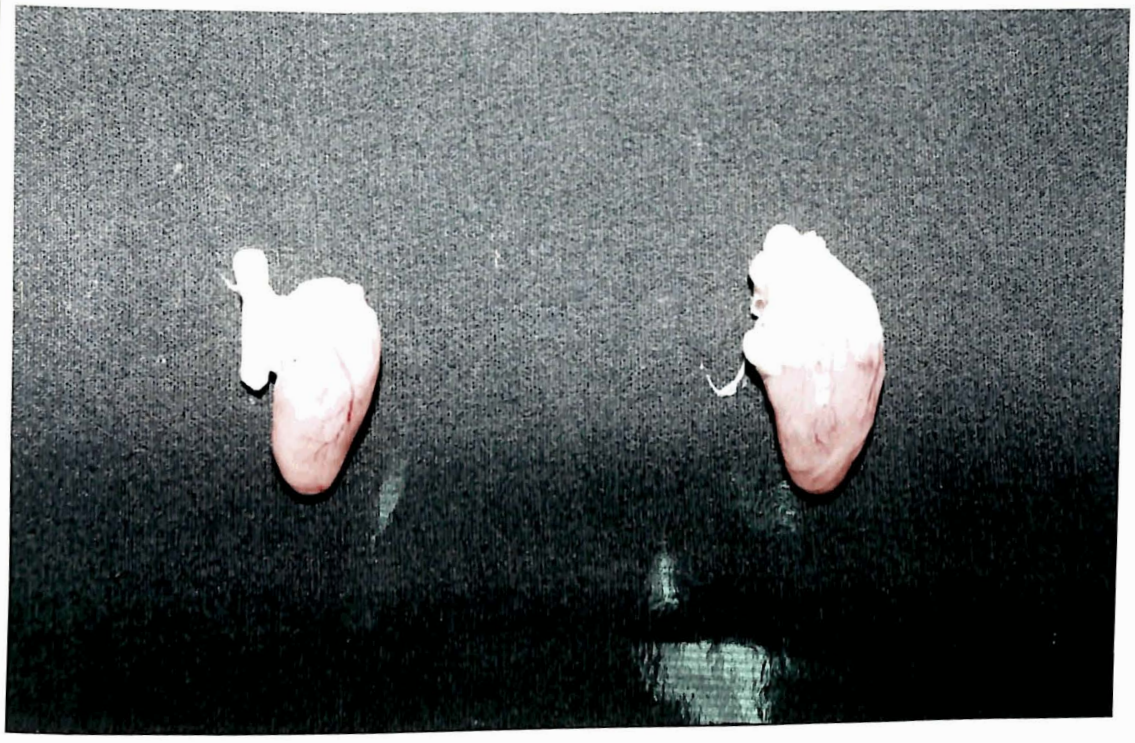


Plate 3.Hearts of group 2 showing mild enlargement, with mild enlargement of blood vessels and areas of haemorrhage

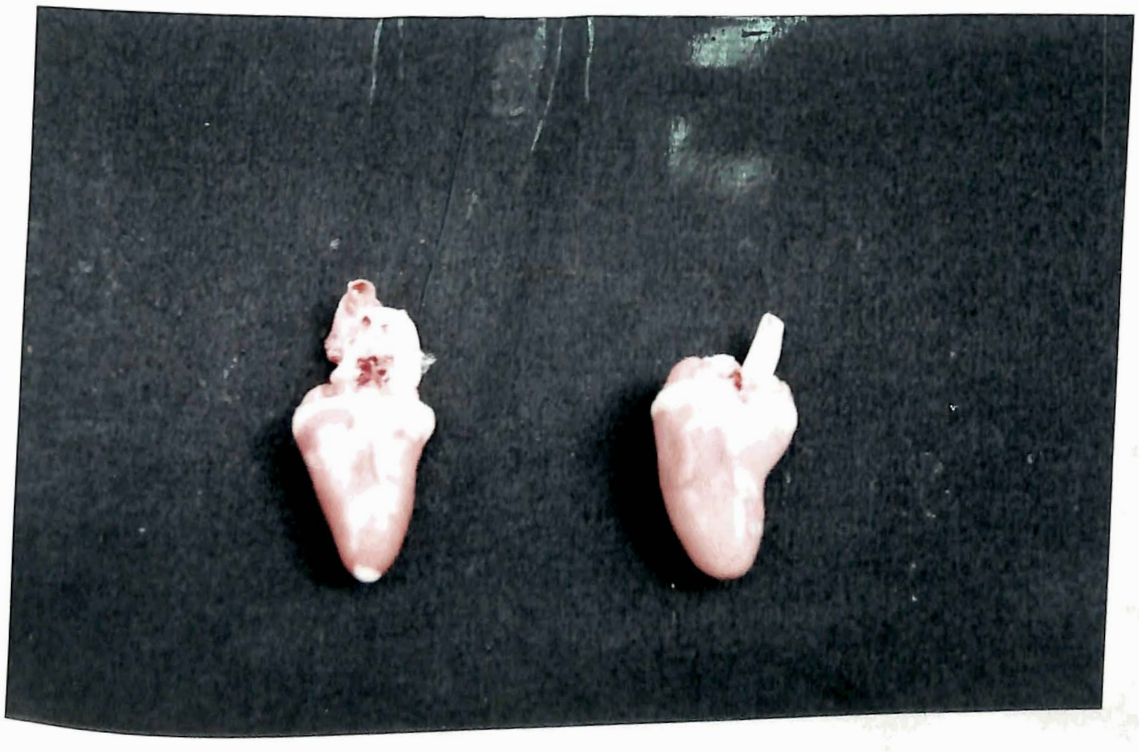


Plate 4.Hearts of group 2 showing mild enlargement

with hyperplasia of glomeruli and degenerative changes in tubular epithelium (Plate10). Kidneys of other groups did not show any lesions.

4.8.4 Intestines

Intestines from group 2 revealed mild catarrhal changes with desquamation of superficial lining epithelium (Plate 11).

Such lesions were not observed in the remaining groups.

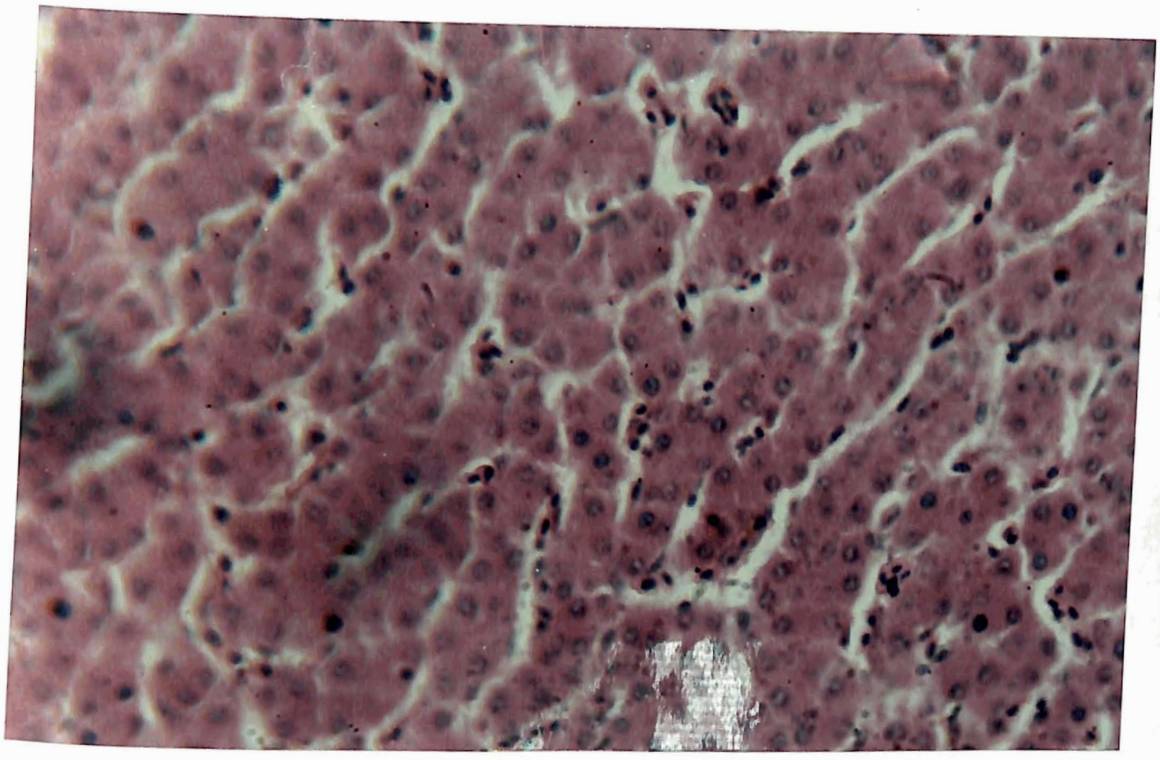


Plate 5.Liver section of group 2 showing mild sinusoidal haemorrhages H & E 280x

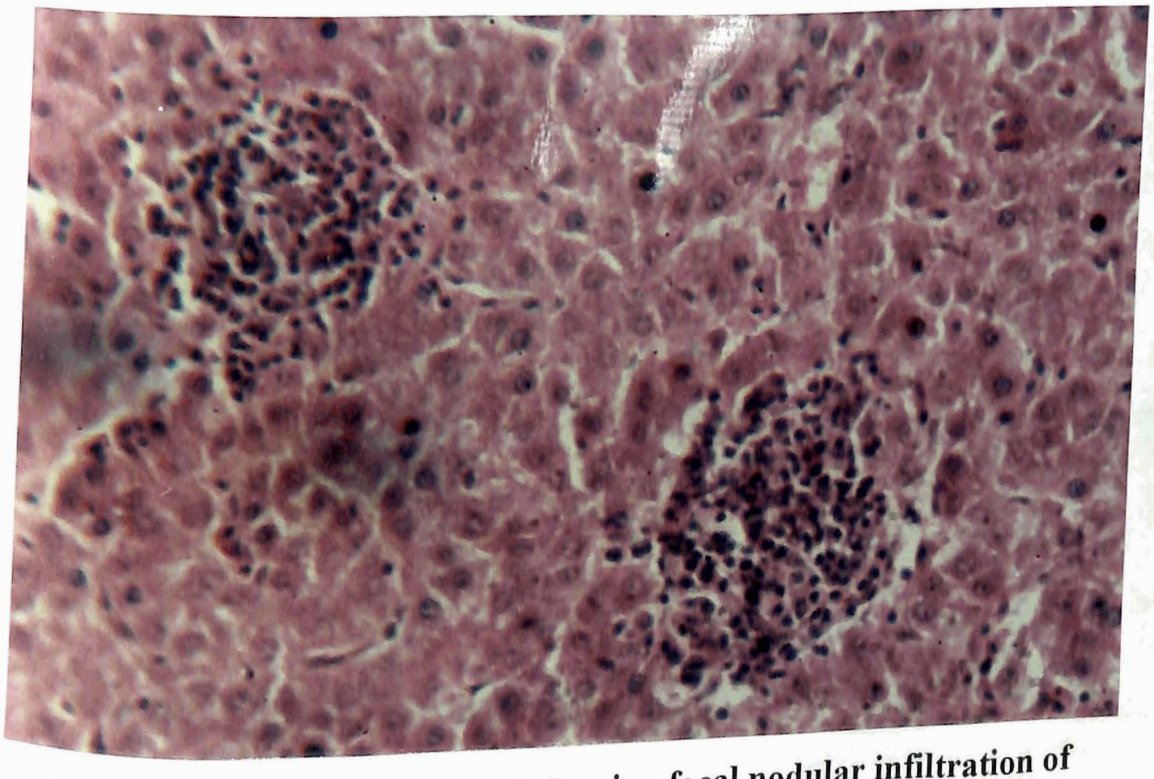


Plate 6.Liver section of group 2 showing focal nodular infiltration of inflammatory cells H & E 280x

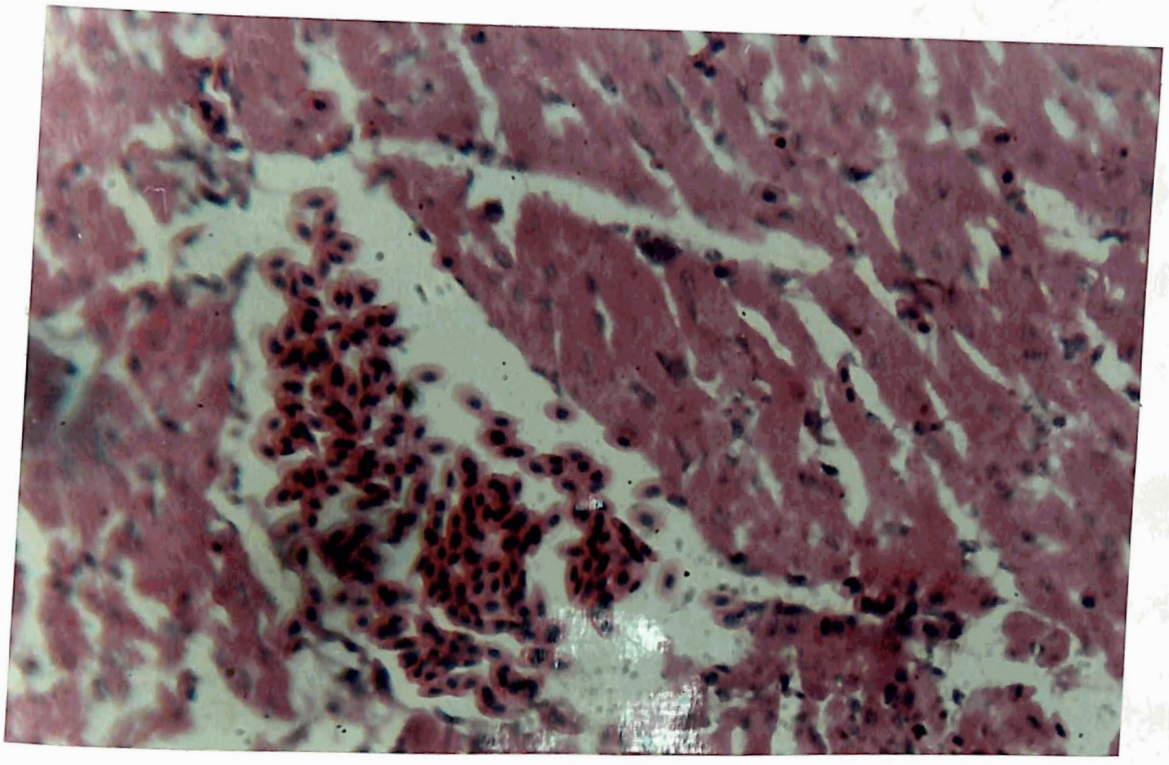


Plate 7.Heart section of group 2, showing focal areas of haemorrhages and sarcolytic changes of muscle fibres H & E 280x

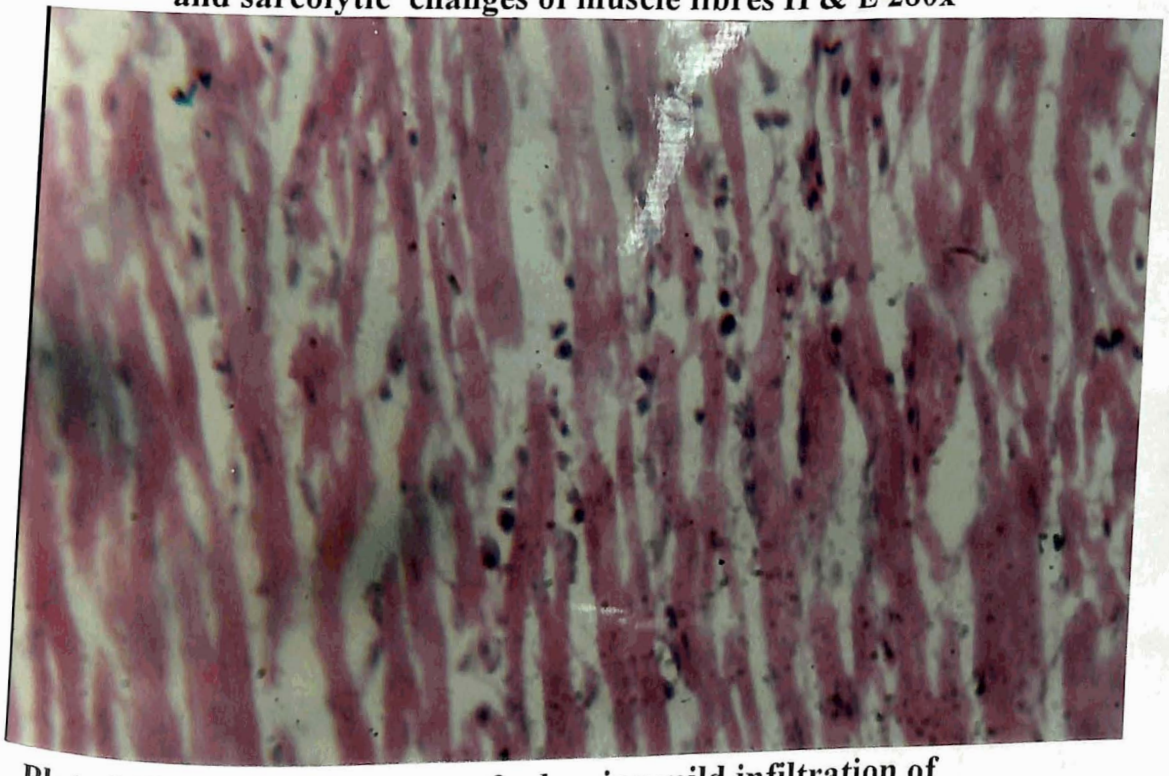


Plate 8.Heart section of group 2, showing mild infiltration of inflammatory cells in between cardiac muscle fibres and sarcolytic changes H & E 70x

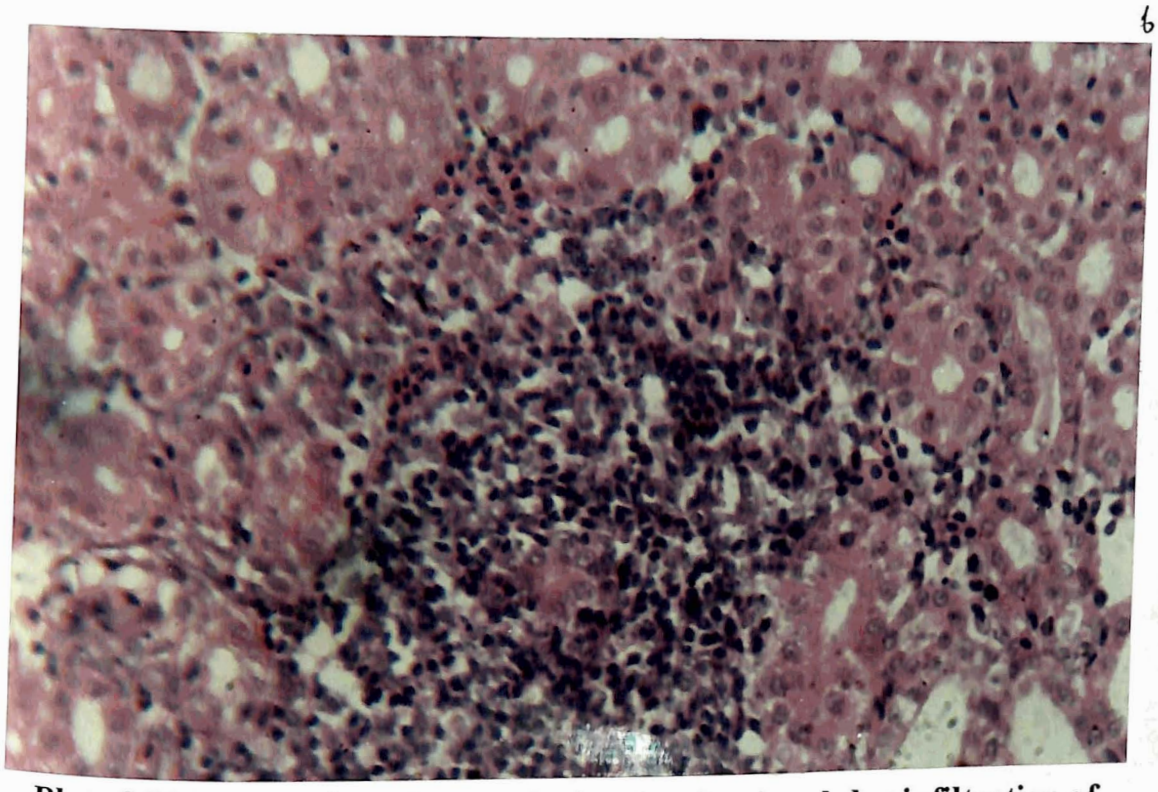


Plate 9.Kidney section of group 2, showing focal nodular infiltration of inflammatory cells in between tubules H & E 280x

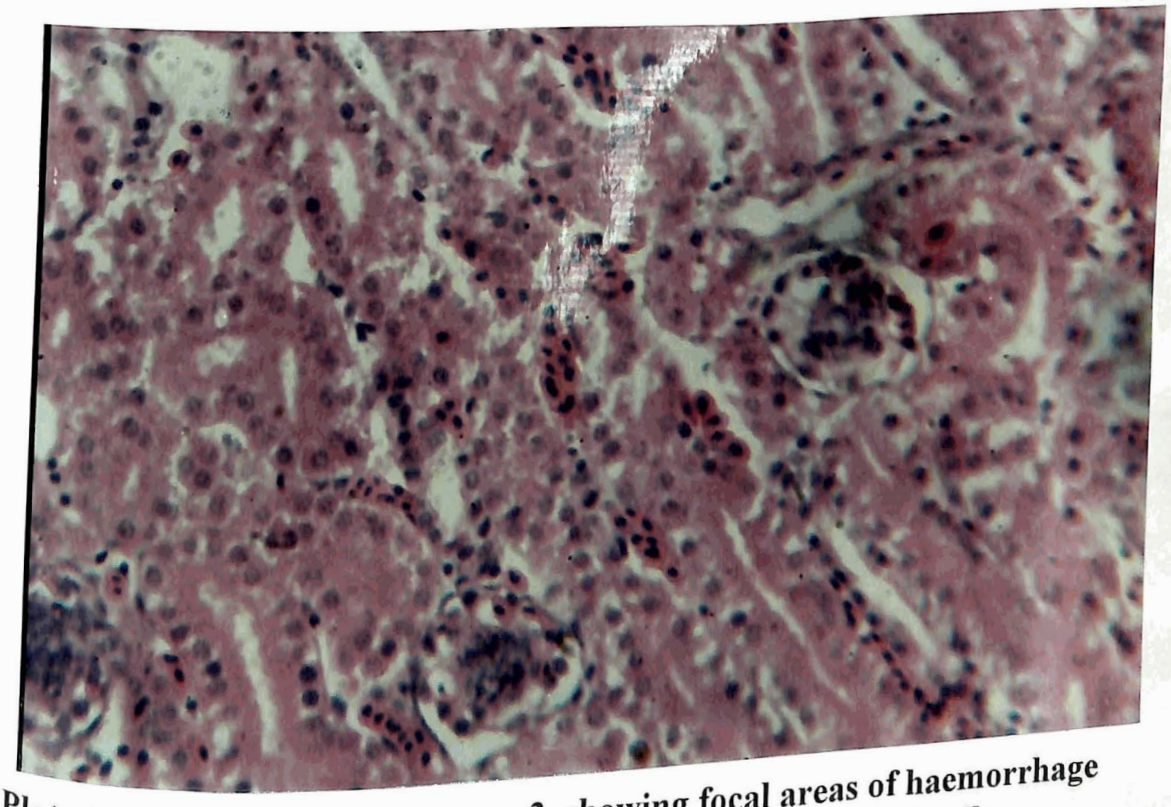


Plate 10. Kidney section of group 2, showing focal areas of haemorrhage and mild degenerative changes in tubular epithelial cells H & E 280x

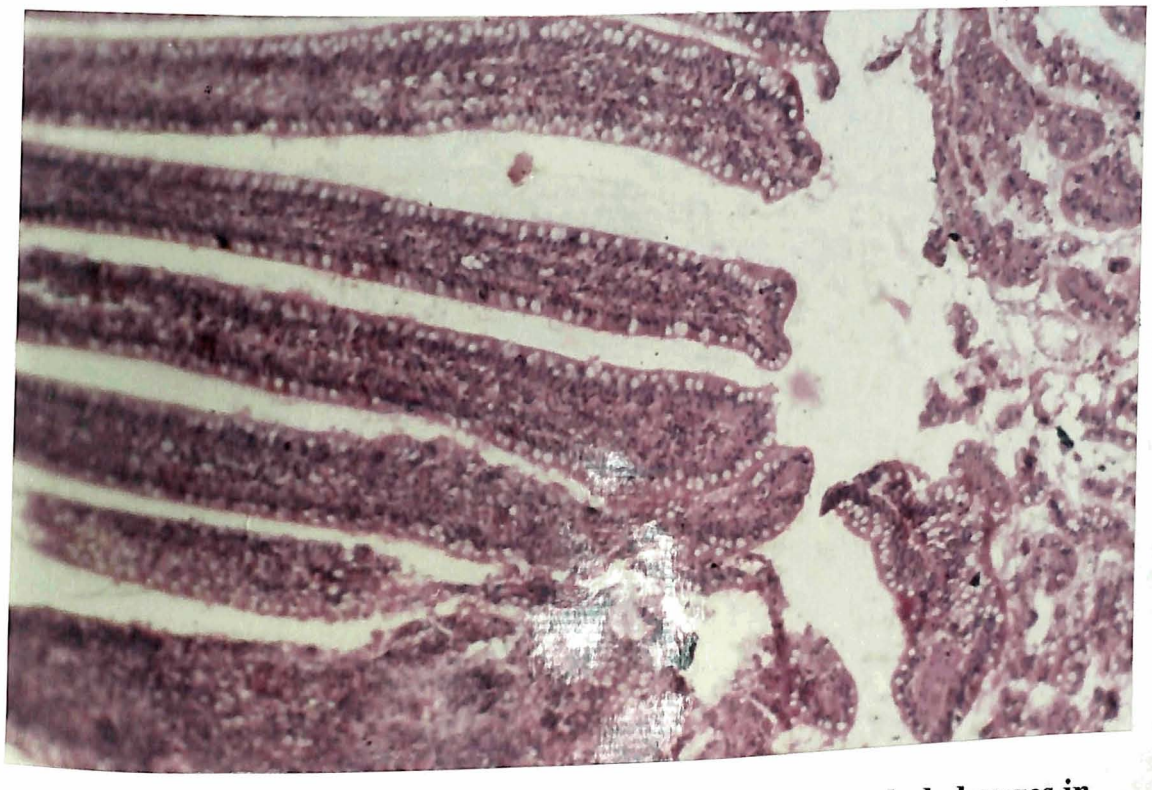


Plate 11. Intestine section of group 2, showing mild catarrhal changes in the mucosa and desquamation of superficial lining epithelium H & E 70x

Chapter V

DISCUSSION

Chapter V

DISCUSSION

CHAPTER - V

DISCUSSION

The present investigation was undertaken to study the adverse effect of furazolidone, @ 400 mg/kg in broiler chicken by monitoring the performance, biochemical parameters including erythrocyte antioxidant enzyme profile, serum ALT, AST, total protein, albumin and total cholesterol concentration and the extent of lipid peroxidation of liver. Attempts were made to study the protective effect of garlic, α-tocopherol and stresseaze in the event of oxidative damage by furazolidone.

5.1 EFFECT ON GROWTH PERFORMANCE

In the present study, the FCR in group 2 birds was significantly higher ($P < 0.05$) compared to other groups, which could be related to the low feed consumption and low body weight gain in birds of group 2 compared to birds of control and other treatment groups. The decreased growth performance in group 2 birds might be due to anorectic property of furazolidone coupled with less efficient utilization of the consumed feed (Ali and Barlet, 1982; Czarnecki, 1984; Arbid *et al.*, 1990; Webb and Van Vleet, 1991 and Zaman *et al.*, 1995).

Garlic 2% control and Stresseaze control groups showed a better feed efficiency than other groups.

The groups treated with garlic, Vitamin E and stresseaze plus furazolidone performed similar to control group. Similar increase in feed efficiency was observed when birds were fed 1% garlic in feed which yielded best sensoric evaluaton and low abdominal fat percentage (Fritz *et al.*, 1995, Premkumar *et al.*, 2000), Vitamin E + deltamethrin (Jayasree *et al.*, 2003), and stresseaze (Wheeler, 1994).

5.2 EFFECT ON ERYTHROCYTE ANTIOXIDANT DEFENSE SYSTEM:

The GSH-Px, GSH-R and catalase concentration were significantly higher ($P < 0.05$) and glutathione content was significantly lower ($P < 0.05$) in group 2 birds compared to control group birds. This might be the result of oxidative stress induced by furazolidone through the mediation of free radicals (Ali, 1992). There exists a balance between the production of free radicals and their scavenging by the antioxidant defense mechanism of the body within the physiological limits (Bandyopadhyay *et al.*, 1999). The altered antioxidant enzyme activity could be due to enhanced free radical oxidative damage induced by furazolidone hence depriving the antioxidant defenses.

The GSH-Px, GSH-R and catalase concentration in treatment groups 7, 8, 9 and 10 were similar to control group. However group 7 had slightly higher GSH-Px concentration than group 1, because of partial protective effect of garlic 1%, but significantly lower than group 2.

In the garlic treated groups, GSH-Px activity was normal rather than increased in group 2. Garlic might have prevented free radical production and/ or increased the conversion of oxidized glutathione back to reduced glutathione which was the essential substrate for chemical scavenging. This is in agreement with the findings of Rajasree and Raja Mohan (1998) and Upadhyay (1997).

The increased GSH-R concentration in group 7 at the end of 4^{th} week was brought to normal by the end of 6^{th} week. Diallyl disulphide, one of the active principles of garlic might have interacted in an exchange reaction with the enzyme GSH-R which contain thiol groups (Adoga, 1986).

Induction of protective antioxidant enzyme systems after exposure to sublethal doses of free radicals or ROS (Rana *et al.*, 2002) had been brought to the normal concentration by the scavenging activity of garlic which might

have provided protection against the oxidative stress. The present findings are in agreement with that of Nakagawa *et al.* (1988).

Vitamin E prevented the alteration in the antioxidant enzyme system (Ognjanovic *et al.*, 2003) by protecting the unsaturated bonds of phospholipids present in the cell membrane (Infers and Sies, 1988) against free radical damage. This was substantiated by Jayasree *et al.*, (2003) who reported similar results in deltamethrin induced toxicity.

In stresseaze supplemented groups, the concentration of antioxidant enzymes and glutathione were kept under physiological limits which might be due to the powerful superoxide anion and hydroxyl radical scavenging action of stresseaze (Narasimhan, 1996).

5.3 EFFECT ON LIPID PEROXIDATION

Lipid peroxidation arising from the reaction of free radicals with lipids is an important feature of cellular injury. It is brought about by the interaction between free radicals of diverse origin and unsaturated fatty acids in lipids which result in a series of alterations and the consequent degeneration of cell membranes (Yu, 1994).

The increased TBA values in the liver of group 2 birds account for the increased lipid peroxidation brought about by Furazolidone (Stroo and Schaffer, 1989 ; Ali, 1992; Sas 1993). Groups 7, 8, 9 and 10 had recorded values significantly lower than group 2. This could be due to the protective effect of garlic, vitamin E and stresseaze.

In the present study, garlic decreased the lipid peroxidation in a dose dependant manner and the decreased lipid peroxidation observed in garlic supplemented birds might be due to their rich sulfur containing compound in the form of cysteine derivatives (SAC and SAMC). Sulfoxides, thiols and sulfides of garlic can trap electrons from other systems, thus preventing superoxide formation to a certain extent and scavenging many free radicals (Klanns, 1983).

The low TBA values in groups 5 and 10 might be due to the protection of unsaturated phospholipids present in the cell membrane by Vitamin E against free radical damage (Tappel, 1972; Infers and Sies, 1988).

The significant reduction in TBA values of stresseaze groups might be due to their ROS scavenging property. This is in agreement with the findings of Chatterjee (1996).

5.4 EFFECT ON SERUM BIOCHEMICAL PROFILE

The serum enzymes AST and ALT serve as biomarker indices of the extent of tissue damage, especially liver and heart (Kaneko *et al.*, 1997). The increased serum AST and ALT concentration in group 2 birds indicate the damage of liver and heart which could be correlated with the hypoproteinemia and hypoalbuminemia and with the degenerative changes observed in the histopathology of the liver, heart and kidney of group 2 birds. The changes were in conformity with the studies of Staley *et al.* (1978), Czarnecki *et al.* (1981), Zaman *et al.* (1995) and Anuradha (2003).

The elevated concentration of the enzymes AST and ALT might have resulted from hypoxic cellular damage and leakage (Webb *et al.*, 1991). The hypoproteinemia and hypoalbuminemia might be the result of impaired production by the liver and increased excretion by the kidneys and can be correlated with the increased lipid peroxidation of liver.

The S-allyl cysteine (SAC) and S-allyl mercapto cysteine (SAMC) fractions of garlic could prevent superoxide formation and scavenge many free radicals (Klanns, 1983). The damage of the organs were then prevented and the activities of the enzymes, total protein and albumin concentration were in the normal range in the garlic treated groups. Similar findings were reported by Ohaeri (2001) and Sumioka *et al.* (1998).

Furazolidone control birds recorded a significant decrease in serum total cholesterol concentration compared to control birds at the end of 6th week and this might be due to decreased hepatic synthesis attributable to decreased feed intake (O'Brien *et al.*, 1993; Anuradha, 2003).

The garlic control groups had hypochloesterolaemic action and was in accordance with Khalid *et al.* (1995) and Premkumar *et al.* (2000) in poultry. The polar fractions of garlic could cause a dose related decrease in the enzymes involved in cholesterol and fatty acid biosynthesis viz., HMG CoA reductase, cholesterol 7 alpha hydroxylase and fatty acid synthetase and thus account for hypocholesterolemia in the garlic-treated groups (Qureshi *et al.*, 1983). Similar findings were observed by Bhushan *et al.*, (1979), Bordia (1981), Sendl *et al.* (1992) and Liu and Yeh (2000).

Though furazolidone control birds had recorded a low cholesterol level, the higher feed efficiency and normal picture of histopathology in garlic-fed groups (groups 7 & 8) confirmed the positive effects.

The Vitamin E and stresseaze-treated birds also showed low cholesterol level which were corroborative with the results of Jayasree *et al.* (2003) and Geetha (1993).

Vitamin E and Stresseaze by their antioxidant properties averted the ill effects of the free radicals and protected the organs from damage which was evident from the normal values of the enzyme activities, total proteins and albumin concentration and the histopathology of liver, heart and kidney of the respective treatment groups. The findings were in accordance with Zang *et al.*, (1988) who reported the protective and repairing capacity of vitamin E in free radical induced damage of biological membranes.

The furazolidone induced changes in serum biochemical enzymes and protein contents were prevented in group 9 birds which might be due to the antioxidant property of Stresseaze offering protection against tissue damage including liver parenchymal cells (Geetha, 1993 and Kamath, 1994).

5.5 CLINICAL SIGNS

Group 2 birds exhibited reduction in feed and water intake and was reflected in poor feed efficiency. Similar findings were recorded by Webb and Van Vleet (1991) and Zaman *et al.* (1995). The anorexigenic and growth retarding potential of furazolidone might be the reason for this set back (Ali and Barlet, 1982).

5.6 EFFECT ON GROSS AND HISTOPATHOLOGICAL ALTERATIONS

In the present study pathological lesions of degenerative type were observed in liver, heart, kidney and intestines in group 2 birds. The degenerative changes and tissue damage support the results of serum AST, ALT, total proteins and albumin concentration. Specimens and sections from other groups did not show any deviations.

The structural integrity of groups 7, 8, 9 and 10 reveal the antioxidant and protective role of garlic (Sumioka *et al.*, 1998), vitamin E (Jayasree *et al.*, 2003) and Stresseaze (Chatterjee, 1996).

The present study emphasized the oxidative damage of furazolidone when the therapeutic dose was prolonged beyond the recommended duration. Garlic, Vitamin E and Stresseaze offered protection against the free radical insult.

In view of the above facts, it is recommended to include the antioxidant and hypocholesteremic garlic which is relatively economical and easily available at farmer's doorstep, in the poultry feed to manage the oxidative stress in general and furazolidone toxicity in particular.

Chapter VI

SUMMARY

CHAPTER - VI

SUMMARY

Furazolidone, a nitrofuran antimicrobial was fed to birds @ 400 mg/kg feed and studied for its toxic effects. Garlic, Vitamin E and Stresseaze (a Veterinary Ayurvedic product) were evaluated for their therapeutic efficacy and antioxidant properties against furazolidone induced toxicity. A total of one hundred male VenCobb broiler chicken were randomly divided into ten groups, consisting of ten each. Group I served as control, groups 2, 3, 4, 5 and 6 were furazolidone, garlic 1%, garlic 2%, vitamin E and Stresseaze control groups respectively, while groups 7, 8, 9 and 10 were treated with Fz + garlic 1%, Fz + garlic 2%, Fz + Stresseaze and Fz + Vit. E respectively. The treatments to all groups were started simultaneously on day 15 and continued upto 42 days.

The experiment was carried out for six weeks and body weights and feed consumption for each group were recorded at weekly intervals. During the experimental period, the birds were observed for overt clinical signs if any and blood and serum were collected at the end of 4^{th} and 6^{th} week to assess erythrocyte antioxidant and serum biochemical profiles. At the end of 6^{th} week, birds were sacrificed and livers were collected to estimate the extent of

lipid peroxidation, and liver, heart, kidney and intestine samples were collected for histopathological study.

Body weight gains, feed consumption and feed efficiency were significantly ($P < 0.05$) reduced in group 2 when compared to other groups. The anorexigenic effect of furazolidone might be responsible for these effects. However, other groups (group 3, 4, 5, 6, 7, 8, 9 and 10) were not affected. It could be attributed to the protective effects of garlic, Vitamin E and Stresseaze against furazolidone adverse effects.

The erythrocyte antoxidant enzymes levels of (GSH-Px, GSH-R and catalase) were increased and glutathione concentration decreased in group 2 birds, probably due to the oxidative stress imposed by furazolidone. Garlic, vitamin E and Stresseaze have offered protection by their free radical scavenging action. However, garlic 2% offered better protection than garlic 1%.

A significant ($P < 0.05$) increase in TBA values of liver in group 2 birds might be the result of increased lipid peroxidation due to the production of free radicals by furazolidone. Garlic 2%, vitamin E and Stresseaze scavenged the free radicals and offered better protection.

The serum AST and ALT activities showed a significant ($P < 0.05$) increase in group 2 whereas the enzyme activities of other groups were in the range of control group except group 7. The elevation of these enzymes would have occurred from toxic cellular changes in organs especially liver and heart. Garlic, vitamin E and stresseaze offered cellular protection against furazolidone toxicity.

Hypoproteinemia and hypoalbuminemia were observed in group 2. The degenerative changes in liver and kidney as evident from histopathology might be the cause for the decreased condition. Garlic, vitamin E and stresseze prevented the tissue damage and their values were in the range of the control group.

Hypocholesterolemia observed in garlic groups might be due to the property of garlic, acting as a weak HMG Co-A reductase inhibitor. The decreased cholesterol concentration in group 2 in conjunction with production parameters might be due to inanition and poor feed efficiency. Vitamin E and stresseaze were able to decrease the total cholesterol concentration.

Clinically furazolidone induced stress among the birds of group 2 was reflected as reduced feed and water intake, depression and lethargy.

Gross observation on necropsy of group 2 showed mild hypertrophy with focal areas of haemorrhage in liver and heart. There was congestion in kidneys and mild catarrhal changes in intestines. Other groups did not reveal any lesions.

Histopathology of liver, heart, kidney and intestine of group 2 revealed pathological degenerative changes. No change was observed in other groups.

The present study elucidates the oxidative stress produced by furazolidone toxicity and the ability of garlic, vitamin E and Stresseaze in ameliorating the toxic effects. Garlic 2% accomplished better results than garlic 1%. The protection offered by garlic 2%, vitamin E and Stresseaze were comparable.

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APPENDICES

APPENDIX

Reagents or solutions

(a) Phosphate buffer (0.1 M)

Solution X : Dissolved 2.722 g of potassium dihydrogen Phosphate in 200 ml distilled water (0.1 M)

Solution Y : Dissolved 1.741 g of dipotassium hydrogen Phosphate in 100ml of distilled water (0.1 M)

Poured 100 ml of solution Y into a beaker and titrated against solution x to a desired pH. Stored the buffer at 4°C.

(b) Frozen RBC

Red blood cells were separated from EDTA added blood, washed with 0.9% saline, diluted with an equal volume of water and frozen at -20°C overnight.

(c) Haemolysate

RBC (frozen) : 200 µl

0.1 M phosphate buffer: 3 ml

(pH 7.4)

mixed well and centrifuged

(d) 50 mM oxidized glutathione (GSSG) solution

Oxidized glutathione : 155 mg (Mol. wt. 612.64)

Distilled water : 50 ml

0.8 M NaOH : 60 µl

Mixed well and placed in an ice bath. Prepared fresh daily.

(e) **50 mM reduced glutathione**

Reduced glutathione : 153 mg (mol. wt. 307.32)

Distilled water : 10 ml

Mixed well and placed in an ice bath.

(f) **250 µM Flavin adenine dinucleotide (FAD)**

FAD (disodium salt) : 1.0 mg (mol. wt. 829.52)

Distilled water : 5 ml

(g) **4 mM NADPH**

NADPH : 33.2 mg (mol. wt. 833.4)

1% Sodium bicarbonate : 10 ml

(h) **0.2 mM NADPH :**

NADPH : 1.67 mg

Distilled water : 10 ml

mixed well and placed in an ice bath.

(i) 80 mM EDTA

Dipotassium EDTA : 3.2 mg (mol. wt. 404.47)

Distilled water : 100 ml

(j) **0.1 M Sodium bicarbonate :**

Sodium bicarbonate : 8.4 g (Mol. wt : 106.00)

Distilled water : 1 cc

Stored at 4°C and prepared fresh for every 14 days

(k) **1:500 Hydrogen peroxide :**

Hydrogen peroxide : 1 ml

0.1 M phosphate buffer : 500 ml

(pH 7)

(l) **1 M Sulfosalicylic acid solution**

Sulfosalicylic acid : 25.4 g (mol. wt. 254.22)

Distilled water : 100 ml

(m) **4% Sulfosalicylic acid solution**

1 M Sulfosalicylic acid solution: 46 ml

Distilled water : 250 ml

(n) **1% starch solution**

Starch solution : 1 g

Distilled water : 10 ml

Made it into a paste and slowly added it to 90 ml boiling water.

(o) **0.005 N Potassium iodate solution**

Potassium iodate : 0.1783 g (Mol. wt. 214.00)

Distilled water : 1 litre

(p) 5% Potassium iodide

Potassium iodide	:	5 g
Distilled water	:	100 ml

(q) Serum preservative

Thiomersal	:	10 g
Sterile distilled water	:	100 ml

Stored at 4°C