DETOXIFICATION OF CITRININ AND FUMONISIN IN FEED AND ITS EFFECT ON SERUM BIOCHEMICAL PROFILE IN BROILERS

By

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CERTIFICATE

DR. ASHOK KUMAR DEVARASETTI has satisfactorily prosecuted the course of research and that the thesis entitled ‘‘**DETOXIFICATION OF CITRININ AND FUMONISIN IN FEED AND ITS EFFECT ON SERUM BIOCHEMICAL PROFILE IN BROILERS**’’ 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 there of has not been previously submitted by him for a degree of any University.

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

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DECLARATION

I, **DR. ASHOK KUMAR DEVARASETTI,** hereby declare that the thesis entitled **"DETOXIFICATION OF CITRININ AND FUMONISIN IN FEED AND ITS EFFECT ON SERUM BIOCHEMICAL PROFILE IN BROILERS"** submitted to **Sri**

Venkateswara Veterinary University for the Degree of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part there of has not been published earlier in any manner.

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ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
μg	:	Microgram(s)
μg/g	:	Microgram per gram
μl	:	Microlitre(s)
μM	:	Micromolar
A/G ratio	:	Albumin globulin ratio
ALP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
ANOVA	:	Analysis of variance
AOAC	:	Association of Analytical Chemists
ALP	:	Alkaline phosphatase
AST	:	Aspartate aminotransferase
BUN	:	Blood Urea Nitrogen
Bursa	:	Bursa of Fabricius
bw	:	Body weight
Ca^{2+}	:	Calcium divalent cation
CD	:	Critical Difference
CK	:	Creatinine kinase
dl	:	Deciliter (s)

EDTA	:	Ethylene diamine tetraacetic acid
Eg	:	*Exampli gratica* / Example
ELA	:	Enzyme linked Immunoassay
ELISA	:	Enzyme Linked Immuno Sorbent Assay
FCR	:	Feed conversion ratio
Fig	:	Figure (s)
Fig.	:	Figure
FB_1	:	Fumonisin B_1
FB_2	:	Fumonisin B_2
FB_3	:	Fumonisin B_3
FB_4	:	Fumonisin B_4
g	:	Gram (s)
GGT	:	Gamma Glutamyl Transferase
GIT	:	Gastro Intestinal Tract
HCL	:	Hydro chloric acid
HE	:	Haematoxylin and Eosin
Hb	:	Hemoglobin
HPLC	:	High Performance Liquid Chromatography
hr	:	Hour
H_2SO_4	:	Sulphuric acid

IBD	:	Infectious bursal disease
IU	:	International unit(s)
KA units	:	King Angstrom units
Kg	:	Kilogram(s)
L	:	Liter(s)
LD_{50}	:	Lethal dose 50
LLE	:	Liquid-Liquid Extraction
M	:	Molar
mg	:	Milligram(s)
mg/kg	:	Milligram per kilogram
mM	:	Millimolar
mg%	:	Milligram percentage
MNC	:	Mono nuclear cells
mm	:	Millimeter
min	:	Minute(s)
ml	:	Milliliter(s)
Mol. wt.	:	Molecular weight
MSS	:	Mean sum of square
MTCC	:	Institute of Microbial Technology, Chandighar
N	:	Normal
Na_2EDTA	:	DiSodium Ethylene diamine tetraacetic

		acid
NaOH	:	Sodium Hydroxide
ng	:	Nanogram (s)
NPN	:	Non Protein Nitrogen
NS	:	Not significant
^{o}C	:	Degree(s) centigrade (celsius)
^{o}F	:	Degree(s) Fahrenheit
OH^{-}	:	Hydroxyl radical
pH	:	- Log hydrogen ion concentration
ppb	:	Parts per billion
ppm	:	Parts per million
psi	:	Pounds per square inch
RBC	:	Red blood cell (corpuscle)
RD	:	Ranikhet Disease
RNA	:	Ribonucleic acid
rpm	:	Revolutions per minute
SE	:	Standard Error
Sec	:	Second(s)
SGOT	:	Serum Glutamate Oxaloacetate Transaminase
SAX-Column	:	Strong anionic exchange column
Tab	:	Table
TLC	:	Thin Layer Chromatography

Units/L	:	Units per liter
UV	:	Ultra violet
V/V	:	Volume/volume
Wk(s)	:	Week(s)
$\overline{x}$	:	Arithmetic mean

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

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ABSTRACT

Citrinin and fumonisin toxins were produced from *Pencillium citrinum* and *Fusarium moniliforme* respectively and both were quantified using Thin Layer Chromatography (TLC) method. The toxins citrinin @ 50 ppm and fumonisin @ 10 ppm were mixed in broiler feed to attain required concentrations.

Four diets for broilers were prepared diet 1 – basal diet (control), diet 2 – basal diet + citrinin (50 ppm) + fumonisin (10 ppm), diet 3 – basal diet + citrinin (50 ppm) + fumonisin (10 ppm) + activated charcoal (0.4%) and diet 4 – basal diet + citrinin (50 ppm) + fumonisin (10 ppm) + activated charcoal (0.4%) + lyophilized yeast culture (0.1%). Each diet was fed to a group of day old broiler chicks with four replicates having eight birds in each replicate in a completely randomized design for six weeks.

The weekly body weights and weekly body weight gains were significantly ($P < 0.01$) lower on diet 2 containing citrinin (50 ppm) + fumonisin (10 ppm). The weekly body weights and weekly body weight gains increased gradually on diet 3 containing activated charcoal. Further significant ($P < 0.01$) improvement was recorded on diet 4 containing activated charcoal and yeast culture. Feed consumption was significantly higher ($P < 0.01$) on basal diet compared to other test diets. Feed conversion ratio was the highest on diet 2 when compared to other test diets.

On diet 2 containing citrinin and fumonisin, the biochemical profile revealed elevated serum enzyme activities of AST, ALT, ALP, GGT indicating liver toxicity. These studies also recorded decreased levels of serum proteins, albumin, glucose, cholesterol, triglycerides, calcium, uric acid and increased levels of serum bilirubin. Increased serum creatinine and BUN recorded indicates toxicity to kidney. These studies on liver and kidney function tests indicate possible damage of these organs in the chicks fed on diet 2. Further, supporting the biochemical study, degenerative changes and central venous congestion in liver and marked degenerative changes in kidney and lymphoid depletion in bursa of Fabricius, disruption of cardiac muscle fibres, disruption of intestinal villi were observed on histopathology in birds fed with citrinin and fumonisin.

The birds on diet 3 containing citrinin, fumonisin and activated charcoal showed significant ($P < 0.01$) improvement in body weight gains and feed consumption. Efficiency of feed utilization on diet 3 was comparable to that of diet 1. The serum enzymes like AST, ALT, ALP and GGT were markedly improved and serum creatinine, BUN, calcium, serum uric acid were moderately improved when compared to diet 2. There was mild improvement in serum proteins, cholesterol, glucose and triglyceride levels compared to diet 2. All other biochemical parameters and histopathological findings showed improvement compared to toxin fed group (diet 2). This indicates that activated charcoal (0.4 %) had partial amelioration on toxic effects of citrinin and fumonisin. The birds fed on diet 4 recorded the serum enzymes like AST, ALT, ALP and GGT in the liver nearer to the control group (diet 1). All the biochemical parameters and histopathological findings were also nearer to that of the control group and within the normal range indicating that activated charcoal and lyophilized yeast culture had a complementary effect in ameliorating the toxic effect of citrinin and fumonisin.

These results indicate that activated charcoal (0.4 %) could adsorb and ameliorate the toxins (citrinin and fumonisin) to some extent and activated charcoal (0.4 %) and lyophilized yeast culture (0.1 %) showed complementary effect in amelioration of the toxic effect caused by citrinin and fumonisin.

CHAPTER I

INTRODUCTION

Mycotoxins are a diverse family of secondary metabolites produced by various genera of fungi. Mycotoxins cause a serious hazard to livestock health, productivity and to human health. It is estimated that approximately 25% of all food commodities produced on earth are contaminated by mycotoxins (Harris., 1998).

The fungi that produce mycotoxins can contaminate these commodities during production, processing, transport and storage. The economic impact of mycotoxins are at all levels of production, marketing and utilization. The ease and frequency with which mycotoxins contaminate agricultural commodities, the chronic exposure to contaminated feeds, can mean the difference between profit and loss for the poultry industries. (Jones *et al*., 1982; Nichols., 1983; Hamiliton., 1984).

The poultry industry has an important role in the Indian agricultural economy. It is facing heavy economic losses due to many health hazards caused by mycotoxins in poultry feed. Mycotoxicosis is the toxic syndrome resulting from the intake of mould contaminated feed, which has toxic metabolites of moulds called mycotoxins. Moulds are many but not all are pathogenic. Only some species of *Aspergillus*,

Pencillum, *Fusarium* etc. elaborate the potent toxic metabolites like Aflatoxin, Citrinin, Fusariotoxin etc. The moulds grow on any stored feed, the highest incidence being on corn, maize, wheat bran and poultry feed. The optimal conditions needed for toxin production by moulds are > 12 % moisture content of feeds, 85 % relative humidity and 25 - 30^0 C temperature. The mould growth and toxin production also depends on the type of substrate, duration of exposure, maturity and storage conditions of grains (Raja Rajeswari and Lakshmanachar., 1991).

Mycotoxicosis is of public health importance since these toxins and their metabolites are present in poultry products of, which birds are fed with contaminated feed. The moulds in feeds which produce mycotoxins, utilize the nutrients in feed ingredients and make them unavailable to poultry and animals. Further after feeding, these mycotoxins also modify many physiological processes in animals, birds and it will even interfere with the endocrine system resulting in disease and in low productivity of animals and poultry. Among the harmful mycotoxins identified, aflatoxin, T_2 toxin and citrinin account for major share of the productivity losses (Raja Rajeswari and Lakshmanachar., 1991).

The moulds producing citrinin and fumonisin are primarily *Pencillium citrinum* and *Fusarium moniliforme*, respectively. Citrinin is nephrotoxic and causes increased water intake and excess urine excretion indicating that this toxin acts directly on the kidneys to alter several tubular transport processes. (Ames *et al*., 1976; Nelson *et al*.,1980; Hnatow and Wideman., 1985; Gurunath Reddy., 2004; Priyadarshini., 2005). Fumonisin toxicity in broiler birds was characterized by decreased body weight gain, black sticky diarrhoea, hepato-toxicity, gastro-intestinal toxicity and altered serum parameters (Marijanovic *et al*., 1991; Brown *et aI*., 1992; Piramanayagam *et al*., 2003;

Sateesh *et al*., 2004 and Jayasri., 2006).

In the field conditions, poultry rations contaminated with multiple mycotoxins are commonly seen which may have interactive effect on the health of birds resulting in economic loss to farmers. So, to over come the economic losses caused by these toxins, scientists attempted many strategies like physical, chemical and biological treatments to reduce the effect of toxins and to detoxify them. Even though several methods have come up, many are not economical. The adsorptive materials that bind the mycotoxins and immobilize them in the gastro-intestinal tract reducing the bio-availability of toxin have been recently used. The studies on detoxification and combined effect of citrinin and fumonisin are few.

Hence, an attempt was made to estimate the effect of citrinin and fumonisin in contaminated broiler feed and to test the efficiency of activated charcoal (0.4 %) and lyophilized yeast culture (0.1 %) to adsorb the toxins with the following objectives :

1. To study the effect of adsorbents in amelioration of combined toxic effects of citrinin and fumonisin on the performance of broilers.
2. To study the effect of adsorbents on the combined effect of citrinin and fumonisin on serum biochemical profile and histopathology of vital organs in broilers.

3. CHAPTER II

4. REVIEW OF LITERATURE

5.

6. 2.1 CHEMISTRY OF CITRININ AND FUMONISIN

7. 2.1.1 History

8. Citrinin was first isolated as a pure compound from a culture of *Penicillium citrinum* by Hetherington and Raistrick (1931). Later, yellowish colored rice, imported from Thailand to Japan in 1951 was found to be contaminated with *Penicillium citrinum* and subsequent investigations showed that isolates of this fungus produced citrinin.

9. Fumonisins are a group of recently discovered secondary metabolites produced by *Fusarium moniliforme* (Gelderblom et al., 1988). *Fusarium moniliforme* is a species of fungi that may frequently contaminate the grains and may produce mycotoxins known as fumonisins. These fumonisins include fumonisin B_1, B_2, B_3 and B_4; among these fumonisin B_1 (FB_1) is the major metabolite (Gelderblom et al., 1992).

10. Citrinin has been found as a natural contaminant of rice in Japan (Saito *et al.,* 1971) wheat, barley and oats in Canada (Scott *et al.,* 1972) and barley and oats in Denmark (Krogh *et al*., 1973).

11. *Fusarium moniliforme* is one the most prevalent fungi on maize, other grains and agricultural commodities in the United States and throughout the world (Marasas et al., 1988).

12.

13. Fumonisin is linked to the death of horses in USA during 1980's which was due to contaminated corn(Jayasri 2006). The fumonisins were identified by a South African group led by Marasas (Bezuidenhout *et al*., 1988 and Gelderblom *et al*., 1988) during investigation of cause of "Leukoencephalomalacia" in horses.
14. Fumonisins were first isolated from cultures of the fungus *F.moniliforme.* Also, other *Fusarium* sps. have been found to be producers of fumonisins include *F.proliferatum* (Ross *et al*., 1990) and *F.nygamai* (Thiel *et al*., 1992).
15. Citrinin was found as a natural contaminant in cereals, decaying tomato fruits and country-cured ham (Mislivec and Tuite., 1970; Neely *et al.,* 1972; Harwig and Chen., 1974; Wu *et al*., 1974 and Harwig *et al.,* 1989).
16. *Penicillium citrinum* was found among moulds isolated from till plant (*Seasum indicum)* in India and its capacity to produce citrinin was demonstrated by Reddy and Reddy (1983).
17. Citrinin was reported to be a food contaminant in tribal areas of Medak district in Andhra Pradesh by Reddy *et al.* (1986). Citrinin was identified in compounded feed and various feed ingredients used in poultry rations in Namakkal area (Tamil Nadu) by Ahamad and Vairamuthu (2000).
18. In spite of the worldwide natural occurrence of fumonisin in maize, which is a major dietary ingredient, no natural disease out break in poultry attributable to fumonisin has yet been reported. During October 1995, unseasonal rains caused by a cyclone resulted in heavy damage to the maize and sorghum crop in the deccan plateau region of South India. Consumption of rain-damaged mouldy sorghum and maize resulted in widespread of fumonisin toxicity in poultry which was reported by Prathapkumar *et al*. (1997).

19. **2.1.2 Incidence**

20. Punam Jeswal and Jeswal (1990) reported that out of 124 cattle feed samples tested from different parts of Bihar, 45 samples were contaminated with aflatoxin, citrinin, ochratoxin and zearalenine in varying amounts.

21. Corn based food and feeds from different countries have been shown to contain FB_1 at levels 0.05 to 5 ppm. Less than one third of samples tested were positive for presence of fumonisin (Jayasri, 2006).

22. Out of 42 feed samples from poultry farms in Punjab 26 and 6 samples showed positive for aflatoxin and citrinin respectively (Raina and Singh, 1991).

23. Pittet *et al*. (1992) reported contamination of 36.7 % of 120 corn-based samples at 0.05 – 0.75 ppm of FB_1 in Switzerland.

24. Ahamad and Vairamuthu (2000) reported that out of 63 samples of compounded feed analyzed, 28 samples (44.4 %) were positive for citrinin and contamination was 12.69 % for citrinin alone.

25. Cheng *et al*. (2002) reported 41 % incidence of FB_1 in corn based feeds of Taiwan occurring at a concentration of 5 ppm of total fumonisins.

26. Sundaram *et al*. (1999) reported that 171 (12.2 %) out of 1405 compounded feed samples, were positive for citrinin and all these samples were co-contaminated with aflatoxins.

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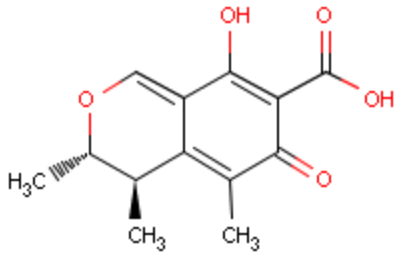
29. 2.1.3 Structure of citrinin



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32. Structure of citrinin

33. The structure of citrinin and de-carboxy citrinin was proposed by Brown *et al.*(1989). Citrinin (3R trans) -4, 6-dihydro -8hydroxy 3, 4,

5-tri methyl -6-oxo -3H-2-benzo pyran 7-carboxylic acid, $C_{13}H_{14}O_5$. Molecular weight 250, forms lemon-yellow needles (melting point 175^{o} C) when crystallized from absolute ethanol or benzene -cyclohexane. It is soluble in hot ethanol, ethyl acetate, benzene, acetone and chloroform and insoluble in water (Spector *et al*., 1957 and Ciegler *et al.*, 1977).

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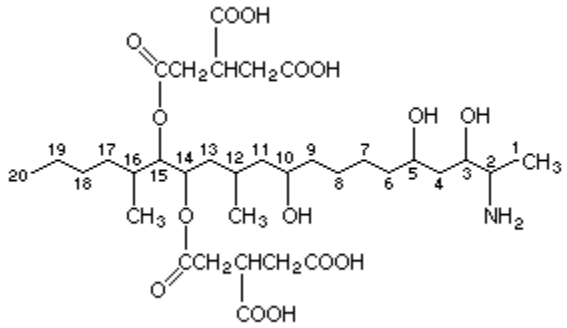
41. 2.1.4 Structure of fumonisin



42. Structure of fumonisin

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44. The fumonisins are a group of mycotoxins which have a 2-amino-12,16-dimethyl polyhydroxyeicosane back bone esterified with propane-1,2,3-tricarbolic acid side chains on C-14 and C-15. Fumonisins are strongly polar compounds and FB_1 is a stable

compound that persists through normal food processing procedures. The structural similarity of fumonisin B_1 with sphingosine, a complex amino alcohol is said to be important in elucidation of the biological effect.

45. **2.1.5 Estimation of citrinin**

46. In most determinations and assays of citrinin in feed samples Thin Layer Chromatography (TLC), Rapid Fluorescence Liquid Chromatography, Indirect and Direct Enzyme Immunoassay and High Performance Liquid Chromatography were employed.

47. Thin layer chromatography was used by Ramaswamy *et al*. (1979) for the determinations of citrinin in feed. Citrinin extracted from feed with methanol and water was made alkaline with 10% sodium carbonate. The aqueous solution was filtered and extracted with chloroform. Aqueous layer was acidified with 2N HCL and then extracted with chloroform. Chloroform extract was concentrated and spotted on thin layer chromatographic plate developed in chloroform-acetone -ethanol -water (60+40+10+1). Citrinin viewed under UV light after the visual or flurodensitometric quantitation used for estimation.

48. Citrinin can be detected on TLC plates and measured by flouro densitometry (Roberts and Mora 1979). Silica gel plates prepared as a slurry with aqueous 0.05 M Na_2 EDTA, spread at 0.5 mm, activated at 105°C for 1 hr and developed in acetic acid -benzene (5+95). Limit of detection was l0 ng citrinin /zone.

49. Simple, systematic analytical method for multiple mycotoxins including citrinin was developed by Quiko Takeda *et al.* (1979). Mycotoxins were extracted with 20% H_2S0_4-4% KCl - acetonitrile (2+20+178), defatted with isooctane and

transferred to chloroform. Chloroform extract was cleaned up by silica gel column chromatography and eluted by benzene - acetone - acetic acid (75+20+5). Each fraction was analyzed by thin layer chromatography for final determination.

50. Rapid Fluorescence Liquid Chromatographic procedure was developed for citrinin in corn by David *et al.* (1999). Ground corn was extracted with methylene chloride and 0.5N phosphoric acid.

51.

The extract was added to liquid -liquid extraction (LLE) column containing a diatomaceous-earth adsorbent, previously impregnated with sodium bicarbonate solution. After drying, the column was eluted with methanol -water (4+1) and aliquots were taken for quantification by reversed -phase -liquid chromatography with fluorescence detection.

52. David *et al.* (1999) estimated the citrinin in barley by Indirect and Direct Enzyme Immunoassay by the raised polyclonal antibodies against the citrinin in rabbits after immunization with citrinin conjugated to hemocyanin. The antibodies were used in a competitive indirect enzyme immunoassay .

53. 2.1.6 Estimation of fumonisin

54. Larry G.Rice et al. (1995) studied the Liquid Chromatographic method for the determination of fumonisin in corn and poultry feeds by using extraction solvents like acetonitrile – water (50 + 50 v/v) or methanol – water (75 +25 v/v) or 100 % water. The acetonitrile – water solvent gave higher extraction efficiencies and faster extraction times than other solvents. Extraction was followed by C_{18} solid-phase extraction column clean-up. Fumonisin B_1 was measured by precolumn

derivatization with o-pthalaldehyde followed by isocratic separation on a C_{18} reversed-phase column with mobile phase of 50 mM potassium dihydrogen phosphate (pH 3.3) – acetonitrile (60 +40).

55. Schnieder *et al.* (1995) developed a competitive direct dipstick immunoassay and enzyme linked immuno assy (ELA) for fumonisin detection.

56. 2.2 EFFECT OF CITRININ AND FUMONISIN ON POULTRY PERFORMNCE

57. Ames *et al.* (1976) fed citrinin to laying hens at 50 and 250 μg/g of diet for 3 weeks and could not find any effect on body weight, feed consumption, egg production, egg weight or egg shell quality. In another experiment, Ames *et al.*, (1976) fed broiler chicks with 62.5, 125, 250 and 500 μg citrinin per g of diet for 3 weeks and recorded depression of body weight and severe decrease in feed consumption at 500 μg level.

58. Weibking *et al*. (1993) observed a significant reduction in body weight gains and feed intake in broiler chicken treated with 450 ppm and 552 ppm of fumonisin B_1 for a duration of 3 weeks.

59. Roberts and Mora (1978) fed broiler chicks with 0, 33, 65, 130 and 260 ppm citrinin for 6 weeks and recorded increased water consumption, diarrhoea and dehydrated appearance in chicks received 130 and 260 ppm citrinin.

60. Broiler chicks fed *Fusarium* contaminated culture material have been shown to exhibit increased liver, kidney, and proventriculus weights, and increased mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, increased relative organ

weights, alterations in serum constituents and enzyme activities (Weibking *et al.,* 1993; Brown *et al.,* 1992; Ledoux *et al.,* 1992 and Kubena *et al*., 1997).

61. When *Penicillium citrinum* contaminated corn was incorporated in diet, broiler chicks showed very significant growth depression, poor bone development and exudative diathesis. During the second week 50% of chicks died, 85 % of chicks died within 5 weeks. The survived chicks were stunted, emaciated with ruffled feathers and lacked comb development (Roberts and Mora, 1979).
62. *F.moniliforme* contaminated culture material with 100 mg/Kg of fumonisin B_1 in feed suggested that the fumonisins are toxic to chicken and turkeys (Ledoux *et al.,* 1992 and Weibking *et al.,* 1993).
63. Fumonisin B_1 has been known to cause leukoencehephalomalacia in horses (Marasas *et al.,* 1988; Kellerman *et al.,* 1990 and Wilson *et al.,* 1990), pulmonary edema and hydrothorax in swine (Harrison *et al.,* 1990; Ross *et al.,* 1990; and Colvin *et al.,*1993), hepatotoxic and carcinogenic effects in rats (Gelderblom *et al.,* 1991) and acute hepatic and renal toxicity with significant mortality was reported in fumonisin toxicosis in lambs (Edrington *et al.,* 1995). Fumonisin has also been associated with human oesophageal cancer (Syndenham *et al.,* 1990 and Marasas *et al.,* 1988).
64. Ingestion of grains contaminated by *P.citrinum* caused kidney damage in rats (Krogh *et al.,* 1973 and Nagai *et al.,* 1957), and dogs (Carlton *et al.,* 1974). Liver changes have been observed in rats (Sakai *et al.,* 1995), rabbits (Ramadoss and Shanmugasundaram, 1973) and chicks (Damodaran and shanmugasundaram, 1971).

65. Dietary citrinin caused kidney and liver enlargement (Ames *et al.,* 1976) and alterations in morphology of the intestinal villi in broiler chickens (Witlock *et al.,* 1977). Forgacs *et al.* (1962) observed that *P.citrinum* contaminated corn caused a hemorrhagic syndrome in chicken.

66. Uma *et al*. (1995) recorded significant decrease in body weight gain and food consumption in birds fed on diet containing 125 and 250 ppm citrinin from day old to 6 th week. Leukopenia, more specially lymphopenia was induced by the higher doses level during the later stages. Lesions were found mainly in the kidney, liver and intestines. Kidney lesions indicated that citrinin is a potent nephrotoxin.

67. In poultry, fumonisin is associated with reduced performance, loss of weight gain, increased organ weights, hepatic necrosis and altered serum biochemical profile and enzyme activities (Brown *et al*., 1992; Ledoux *et al*., 1992; Weibking *et al*., 1993 and Kubena *et al*., 1997).

68. Espada *et al*. (1994) reported a 55 % reduction in body weight gain of broiler chicken receiving 30 ppm of FB_1 for 8 days. Henry and Wyatt (1994) observed no adverse effect on weight gain upon feeding of 80 ppm of dietary fumonisin to broiler chicken. Ledoux *et al*. (1992) reported 13 % reduction in body weight gain in fumonisin toxicity in broilers.

69. Citrinin in rats, causes enlarged kidneys, and in rabbits it alters liver metabolism (Ramadoss and Shanmugasundaram, 1973). Recently Carlton *et al*.(1974), investigated citrinin toxicosis in beagle dogs, observed increased blood urea nitrogen, glucosuria,

proteinuria, increased urinary activities of lactic dehydrogenase, glutamic oxalacitic transaminase and isocitric dehydrogenase and pronounced renal lesion. Similar effects have been observed in swine (Krogh *et al*., 1973).

70. Kubena *et al*. (1997) observed that 18-20% reduction in body weight in FB_1 treated broilers in a 3 week experimental trail and the efficiency of feed utilization was reported to be adversely affected. Kubena *et al*. (1999) reported no significant differences in over all body weights in a chronic study with laying hens receiving 100 to 200 ppm of FB_1 for 28 days.
71. Henry *et al.,* (2000) reported that dietary fumonisin at 80 ppm or less concentrations couldn't adversely effect the body weight gain, feed efficiency and water consumption of broilers in 21 days of exposure.

72. 2.3 EFFECT OF CITRININ AND FUMONISIN ON SERUM BIOCHEMICAL PROFILE:

73. Henry *et al.* (2000) reported that serum total protein, globulin, uric acid, alkaline phosphatase and lactic dehydrogenase were not altered in birds fed with 80 mg/Kg of FB_1. But calcium, potassium, sodium, chloride concentrations, SGOT : AST ratio, sphinganine : sphingosine ratio and free sphinganine levels of chicks fed 80 mg/Kg were significantly higher.
74. Espada *et al.,* (1994) observed that triglycerides, uric acid, alkaline phosphatase activity decreased, but gamma glutamyl transferase, aspartate aminotransferse, lactic dehydrogenase, creatine kinase, and cholesterol were increased in young chicks fed with 10 mg/Kg of FB_1 .
75. Broiler chicks (0-3 weeks) fed with citrinin at 62. 5, 125, 250 and 500 µg/g of diet recorded unaltered serum protein, glucose, cholesterol, uric acid, calcium and

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78. phosphorus. However, the serum sodium concentrations were elevated significantly at 125 and 250 µg level (Ames *et al.* 1976).

79. Kubena *et al.,* (1997) observed that serum concentrations of total protein, albumin, aspartate aminotransferase and gamma glutamyltransferase were significantly increased in chicks fed with diet containing FB_1.

80. Sateesh *et al*. (2004) observed that total proteins, AST, ALT and ALP values were significantly elevated and serum albumins were significantly reduced in chicks fed with FB_1.

81. Manning *et al.* (1985) fed citrinin 300 µg/g and ochratoxin A 3 µg/g individually or in combination to broilers from day of hatch to 3 weeks of age. They observed elevated serum total protein, albumin, globulin, cholesterol, calcium and reduced alkaline phosphatase in citrinin fed birds.

82. Espada *et al*. (1997) reported that decreased prothrombin time, increased plasma fibrinogen and increased antithrombin III activity. They also observed decreased serum albumin concentration and increased serum globulins in young chicks fed with *F. moniliforme* culture material containing FB_1.

83. Piramanayagam and Titus George (2001) reported that fumonisin at different doses of 80, 160, 320 ppm resulted increased serum total protein, cholesterol, creatinine and decreased albumin.

84. Broilers fed with citrinin at 125 and 250 ppm by Uma *et al*. (1994) recorded that gradual increase of uric acid upto 6^{th} week. She suggested that higher doses or

85. further exposure to toxin may cause considerable damage to kidneys and liver which affected the serum uric acid levels significantly.

86. Fumonisin upto 50 ppm did not alter serum glucose, phosphorus, uric acid, cholesterol, albumin, total protein, globin, potassium and chloride levels but elevated serum sphinganine : sphingosine ratio and decreased serum calcium (Broomhead *et al.,* 2002).

87. Weibking *et al*. (1993) reported that no alteration of glucose, total protein, albumin due to fumonisin consumption upto 525 ppm but he reported that elevation of free sphinganine, sphinganine : sphingosine ratio in serum.

88. 2.4 EFFECT OF CITRININ AND FUMONISIN ON VITAL ORGANS:

89. 2.4.1 Gross lesions

90. 2.4.1.1 Liver : Ahamad and Vairamuthu (2001) studied the individual and combined effects of citrinin (150 ppm) and aflatoxin (0.5 ppm) in broilers. They noticed hepatomegaly, congestion and discoloration of liver with gall bladder distension.

91. Kubena *et al*. (1997) observed increased relative weights of liver in fumonisin treated broilers.

92. Brown *et al*. (1992) observed increase in liver weight in broilers due to consumption of dietary FB_1 at 300 ppm.

93. Weibking *et al*. (1993) reported increased liver weight in fumonisin treatment of broilers at levels of 375 ppm to 525 ppm.

94. Espada *et al*. (1994) observed decrease of liver weight in broilers treated with 300 ppm of dietary fumonisin.

95. Pratapkumar *et al*. (1997) noticed enlarged, friable and pale livers in fumonisin treated cockerels at 8.5 ppm levels.

96. Henry *et al*. (2000) could not observe significant difference in relative weight of liver in birds treated with 20,40,80ppm of dietary fumonisin.

97. Broomhead *et al*. (2002) reported no significant difference in relative weight of liver in broilers treated with 50 ppm of dietary FB_1.

98. Gurunath reddy (2004) observed pale and enlarged liver in broilers fed with 50 ppm of citrinin for 6 weeks.

99. Sateesh *et al*. (2005) reported that livers were pale and enlarged in broiler chicken fed with fumonisin contaminated feed at 400 ppm level.

100. Priyadarshini (2005) reported that the broiler chicks fed on diet containing 25 ppm of citrinin and 1 ppm of aflatoxin showed pale and enlarged liver with round borders.

101. **2.4.1.2 Kidney:** Roberts and Mora (1978) fed chicks with 250 ppm of citrinin toxin showed anaplastic changes in kidney.

102. Increased kidney weights in fumonisin treated birds were reported in broilers (Kubena *et al*., 1997). Weibking *et al*. (1993) also observed increased kidney weight in fumonisin treated birds at 325 to 525 ppm.

103. Uma and Vikram Reddy (1995) observed congestion, enlargement and mottling of kidneys in broiler chicks fed with 250 ppm of citrinin.

104. Piramanayagam and Titus (2002 b) reported increase in kidney weight in broilers fed with 160 ppm of dietary fumonisin. Kidney weights were found to be non-significantly altered due to fumonisin consumption of broilers at 20, 25, 50, 80 and 300 ppm (Broomhead *et al*., 2002; Brown *et al*., 1992 and Henry *et al*., 2000).

105. Gurunath Reddy (2004) reported swollen and haemorragic kidney when broilers fed with 50 ppm of citrinin for 6 weeks.

106. Sateesh *et al*. (2005) reported that kidneys were pale and enlarged in broiler chicken fed with fumonisin contaminated feed at 400 ppm level.

107. Priyadarshini (2005) reported that broiler chicks fed on diets containing 1 ppm of aflatoxin and 25 ppm of citrinin for 6 weeks showed moderately enlarged kidneys with occasional hemorrhages and all the lobes of the kidneys were uniformly enlarged grossly.

108. **2.4.1.3 Spleen :** Gurunath Reddy (2004) observed spleenomegaly when broilers fed with 50 ppm of citrinin for 6 weeks.

109. Kubena *et al*. (1997) observed increased spleen weight when broilers fed with fumonisin at 300 ppm level. Decrease in absolute weight of spleen was recorded in fumonisin toxicity of broilers at doses of 300 ppm (Espada *et al*., 1994).

110. Priyadarshini (2005) reported that the broiler chicks fed on diets containing 1 ppm of aflatoxin and 25 ppm of citrinin showed moderately congested spleen with occasional mottling appearance.

111. Henry *et al*. (2000); Broomhead *et al*. (2002) and Brown *et al.* (1992) reported that the spleen weights were not altered when the broilers fed with fumonisin ranging from 20-300 ppm (20 ,25, 40, 50, 80 and 300).

112. 2.4.1.4 Bursa: Marijianovic *et al*. (1991) reported decrease in the weight of Bursa of Fabricius due to fumonisin toxic effect in broilers.

113. Gurunath Reddy (2004) observed reduced size of Bursa of Fabricius when broilers fed with 50 ppm for 6 weeks.

114. Espada *et al.* (1994) observed decrease in weight of Bursa in broilers due to fumonisin consumption at 300 ppm. But there was no significant change in relative weight of Bursa of Fabricius in birds fed with FB_1 upto 50 ppm (Broomhead *et al*. 2002).

115. Priyadarshini (2005) reported at Bursa of Fabricius of the broiler chicks fed on diets containing 1 ppm of aflatoxin and 25 ppm of citrinin for 6 weeks showed hyperemic changes with mild enlargement, atrophy in few birds and Bursal folds showed edematous appearance.

116. Sateesh *et al*. (2005) reported that Bursa of Fabricius were shrunken in broiler chicks fed with FB_1 at 400 ppm level.

117. 2.4.1.5 Heart : Ledoux *et al*. (1996) observed ascites, hydropercardium, cardiomagaly and myocardial paleness in chicken, ducks and turkey poults fed with moniliformin and FB_1 toxins contaminated ration.

118. Broomhead *et al*. (2002) observed no significant change in heart weight when broilers fed with fumonisin at 50 ppm level.

119. **2.4.1.6 Intestines :** Marijanovic *et al*. (1991) reported catarrhal exudates, slight erythema of duodenum in white leg horn chicken fed with fusarium moniliforme at 0.05, 5 and 25% levels contaminated feed.

120. Brown *et al*. (1992) observed catarrhal changes in the intestines when broilers fed with fumonisin at 300 ppm level. Similar findings were observed by Piramanayagam *et al*. (2003)

121. Witlock *et al*. (1977) fed the broiler chickens with rations containing 250 µg of citrinin/g feed for 3 weeks and observed significant changes in the midgut.

122. Sateesh *et al*. (2005) reported catarrhal exudates in intestines in broiler chicks fed with FB_1 at 400 ppm level.

123. Roberts and Mora *et al*. (1978) reported that broiler chicks fed with citrinin levels of 0, 33, 65, 130 and 260 ppm for 6 weeks exhibited lesions like hemorrhagic jejunum.

124. **2.4.2 Microscopic lesions**

125. **2.4.2.1 Liver :** Roberts and Mora (1978) fed broiler chicks with rations containing 0, 33, 65, 135 and 260 ppm citrinin for six weeks and observed lymphocytic infiltration and lymphoid aggregates in hepatocytes.

126. Uma and Vikram Reddy (1995) observed slight to moderate congestion with dilatation of the central veins and sinusoids in the liver, focal kupffer cell proliferation and mononuclear cell infiltration in the portal triad in 125 ppm citrinin fed birds, while 250 ppm citrinin fed birds had lymphoid aggregates in the portal triads.

127. Brown *et al*. (1992) reported hepatic multifocal necrosis, biliary hyperplasia in fumonisin toxicity of broiler.

128. Ahamad and Vairamuthu (2001) observed diffuse mild sinusoidal dilatation with engorgement of blood vessels, vascular degeneration of hepatocytes with multi focal mononuclear cells and heterophilic infiltration in livers when broilers were fed with 150 ppm of citrinin.

129. Primanayagam and Titus George (2002 b) observed increased kupffer cell activity with focal areas of mononuclear cell infiltration and fatty changes in liver of birds affected with dietary fumonisin at 80 ppm.

130. Gurunath Reddy (2004) reported focal areas of lymphoid aggregates with bile duct hyperplasia, central vein congestion and moderately dilated sinusoidal spaces in the liver when broilers fed with 50 ppm of citrinin for 6 weeks.

131. Priyadarshini (2005) reported central vein congestion with focal lymphoid aggregates. She also reported the bile duct hyperplasia with sinusoidal dilatation and round cell infiltration in broilers fed with 1 ppm of aflatoxin and 25 ppm of citrinin for 6 weeks.

132. Jayasri (2006) reported hydropic degeneration with marked fatty changes and dilated sinusoidal spaces. She also observed the bile duct hyperplasia with congestion of central vein and focal areas of lymphoid aggregates in the liver of broilers fed with 1 ppm of aflatoxin and 10 ppm fumonisin for 6 weeks.

133. **2.4.2.2 Kidney:** Roberts and Mora (1978) fed broiler chicks with rations containing 0, 33, 65, 135 and 260 ppm of citrinin for six weeks and kidneys had lymphocytic infiltration.

134. Mehdi *et al.*(1981) administered a single LD_{50} dose (95 mg/kg) of citrinin to broiler chicks and recorded most prominent changes in the kidney viz., degeneration and necrosis of the tubular epithelial cells of both proximal and distal convoluted tubules.

135. Piramanayagam and TitusGeorge (2002 b) reported congestion of kidneys with tubular epithelial degeneration and vacuolar changes in proximal convoluted tubules of fumonisin fed (80 ppm) broiler chicken.

136. In ducklings, diet containing 500 mg of citrinin per kg feed caused chronic interstitial nephritis mostly at the medullary region. While the diet containing 250 mg of citrinin per kg feed caused nephropathy characterized by degeneration, necrosis and mineralization of tubular epithelial cells at cortical and medullary region (Mehdi *et al*., 1984).

137. Brown *et al*. (1985) and Manning *et al*. (1986) fed layer chicks with 300 µg of citrinin daily for 0 - 21 days and observed intra nuclear membrane bound inclusions in the epithelium of the proximal convoluted tubules. They also observed elongated tortuous and ring shaped mitochondria and increase in the size and number of peroxisomes and secondary lysosomes. Some proximal convoluted tubular cells had cytoplasmic aggregates of smooth endoplasmic reticulum. Birds fed on citrinin from 0 to 7 days had found similar lesions but milder changes.

138. Sateesh *et al*. (2005) reported hemorrhagic and nephritic changes in kidneys, cystic dilatation of the renal tubules with albuminous casts, glomerular shrinkage and hypercellularity of the glomerular tufts when broilers fed with 200 ppm of fumonisin, but at 400 ppm of fumonisin the kidney revealed intestinal nephritis more prominent characterized by mononuclear cell infilteration causing pressure atrophy of the renal tubules.

139. Uma and Vikram Reddy (1995) observed interstitial haemorrhage in kidneys and degeneration in the tubular epithelium, endothelial hyperplasia in few glomeruli in 125 ppm citrinin fed birds. Hypercellularity and filling up of bowmans capsule were observed in 250 ppm citrinin fed birds.

140. Ahamad and Vairamuthu (2001) observed multifocal mononuclear cells (MNC) infiltration and diffuse inter tubular fibroblast proliferation in the proximal convoluted tubules, hyper cellularity of glomeruli in kidneys, when broilers fed with 150 ppm of citrinin.

141. Gurunath Reddy (2004) observed that the kidneys of birds fed with 50 ppm citrinin were swollen with haemorrhages. He also recorded degenerative changes like focal lymphoid aggregates, inter tubular haemorrhages, marked congestion and degenerative changes in tubules with disrupted architecture.

142. Priyadarshini (2005) reported that the kidney of the broiler chicks fed on diets containing 1 ppm aflatoxin and 25 ppm of citrinin revealed marked inter tubular congestion, hemorrhages and marked degenerative changes with some tubules showing casts.

143. Jayasri (2006) observed that kidneys of broilers fed with 1 ppm aflatoxin and 10 ppm fumonisin for 6 weeks had the marked congestion, degenerative changes in the tubules, marked inter tubular hemorrhages, focal areas of round cell infiltration and disrupted architecture.

144. **2.4.2.3 Spleen:** Ahamad and Vairamuthu (2001) reported that spleen showed mild to moderate lymphoid depletion and degenetation of lymphocytes in broilers fed with 150 ppm of citrinin.

145. Gurunath Reddy (2004) observed that the spleen of broilers fed with 50 ppm of citrinin were swollen and depletion of germinal centers with focal areas of congestion. He also reported mild congestion of trabecular arteries and reticulo endothelial cell hyperplasia.

146. Priyadarshini (2005) reported that the kidney of the broiler chicks fed on diets containing 1 ppm aflatoxin and 25 ppm of citrinin revealed moderate congestion of trabecular arteries and moderately depleted germinal centers.

147. **2.4.2.4 Bursa:** Mehdi *et al.* (1981) fed 95 mg/kg of citrinin to broilers and observed lymphoid necrosis and depletion of lymphocytes in the bursa of Fabricius.

148. Piramanayagam and Titus George (2002 b) reported elevated mucosal activity in fumonisin fed at 40-80 ppm in broilers. He observed follicular cysts, lack of differentiation between cortex and medulla and depletion of lymphoid cells at 160 ppm of FB_1 in broiler chicks.

149. Ahamad and Vairamuthu (2001) observed diffuse lymphoid aggregation with degeneration of lymphocytes and bursal epithelial hyperplasia when broilers fed with 150 ppm of citrinin.

150. Broomhead *et al*. (2002) could not observe any lesions in bursa of Fabricius of birds treated with fumonisin (50 ppm).

151. Gurunath Reddy (2004) observed that the broilers fed with 50 ppm of citrinin showed severe depletion of lymphoid follicles and cystic spaces in epithelium of follicles. He also observed the inter follicular edema and few follicles showing voculations were observed in bursa of Fabricius.

152. Sateesh *et al*. (2005) observed mild to moderate thickening of the inter follicular tissue with lymphocytolytic activity of the follicular tissue in broilers fed with 200 ppm of fumonisin.

153. Priyadarshini (2005) reported that the bursa of Fabricius of broiler chicks fed on diets containing 1 ppm aflatoxin and 25 ppm of citrinin showed cystic spaces in the follicles and marked inter follicular hemorrhages in addition to the cystic spaces.

154. 2.4.2.5 Heart: Piramanayagam (1998) reported mild edema, focal degenerative changes in cardiac muscle fibres, pericarditis, generalized loss of cross striations and fragmentation of myocardial fibres in broiler chicks fed with different levels of toxin i.e., 80, 160, 320 ppm FB_1 / Kg feed.

155. Uma and Vikram Reddy (1995) observed loss of cross striations, sarcolysis and focal areas of hyalinization. He also found mononuclear cell infiltration and haemorrhages between the cardiac muscle fibres when birds fed with 250 ppm citrinin.

156. Ledoux *et al*. (1996) reported multifocal to generalized loss of striations and thinning of cardiomyocytes in day old turkey when fed with 475 mg FB_1 / Kg feed.

157. Brown *et al*. (1992) reported small foci of acute myocardial necrosis in day old broilers fed with 300 mg FB_1 / Kg feed for 2 weeks.

158. Ahamad and Vairamuthu (2001) observed myocardial degeneration with mono nuclear cell infiltration in the heart when birds fed with 150 ppm citrinin for 4 weeks.

159. Sateesh *et al*. (2005) reported that the heart of the broilers fed with 200 ppm of fumonisin for 6 weeks showed congestion with edema of the cardiac muscle fibers and fragmentation of the myocardial fibers with loss of cross striations.

160. Gurunath Reddy (2004) observed that the heart of broilers fed with 50 ppm of citrinin showed mild disruption of myocardial fibres and mild inter tubular haemorrhages with disrupted myocardial fibrils.

161. 2.4.2.6 Intestines : Witlock *et al.* (1977) observed 'herring bone' like pattern, continuous villar ridges which were oriented around the gut lumen and the fusion of the villi in the convoluted ribbon forms when broilers fed with 500 µg of citrinin/g feed for 3 weeks.

162. Brown *et al.* (1992) reported intestinal goblet cell hyperplasia in fumonisin treated broilers.

163. Broomhead et al. (2002) observed no significant changes in gastro intestinal track of broilers fed with 50 ppm of fumonisin.

164. Uma and Vikram Reddy (1995) observed focal desquamation of the lining of the epithelium, mild catarrhal change with infiltration of mononuclear cells in the lamina propria and mucosa of small intestine in birds received 250 ppm citrinin.

165. Piramanayagam *et al*. (2003) noticed catarrhal changes in intestines, increased mucous cell activity and mononuclear cell infilteration when birds treated with fumonisin.

166. Ahamad and Vairamuthu (2001) observed mild to moderate goblet cell hyperactivity of enterocytes in intestines of birds fed with 150 ppm citrinin for 4 weeks.

167. Sateesh *et al*. (2005) observed mild to moderate catarrhal changes characterized by increased goblet cell activity, desqumation of mucosal epithelium along with massive mono nuclear cell infiltration (MNC) of intestines in broilers fed with 200 ppm of fumonisin.

168. Gurunath reddy (2004) reported catarrhal changes, disrupted villi, mild fibroblastic proliferation between the villi when birds fed with 50 ppm of citrinin for 6 weeks.

CHAPTER III

MATERIALS AND METHODS

3.1 TOXIN PRODUCTION

3.1.1 Production of citrinin

Citrinin was produced by growing *Penicillium citrinum* (MTCC-2547) on maize flakes using the method of Nelson *et al.* (1980). Maize flakes were taken separately into conical flasks and moisture was adjusted to 40% by adding triple glass distilled water and soaked for 4 hrs. Then mouths of the flasks were plugged with non-adsorbant cotton and sealed with aluminum foil.

They were autoclaved at 15 Psi (pressure per square inch), 121^{o}C for 15 minutes and then cooled. Then the maize was inoculated with spore suspension *P. citrinum* (about 10^{6} spores/ml maintained on Czapak's agar) and incubated at room temperature (28 –32^{o} C) for 21 days.

These flasks were hand shaken vigorously for two times a day to avoid clump formation. After 48 hrs, the moulds growth appeared by white spot, which turned into green after 3-4 days and black to yellow colour at the end of 21 days. On 21st day the mouldy maize was autoclaved at 15 Psi, 121^{0} C for 15 minutes to kill the spores. The mouldy solid maize flakes were dried overnight at 50^{o} C in hot air oven, powdered and stored in refrigerator at 4^{O}C.

3.1.2 Production of fumonisin

Fumonisin was produced by growing *Fusarium moniliforme* MTCC 156 culture on maize. The moisture was made to 35% by adding water and soaked over night. Then they were autoclaved (15 Psi, 121^{o}C, 20 min).

The maize grains were inoculated with normal saline suspension of 7 day old *Fusarium moniliforme* culture on potato sucrose agar. The maize, after inoculation, was incubated for 5 weeks at 25^{o}C. The fungus was killed by incubating the culture with chloroform: acetone (1:1) mixture overnight. Then the maize grains were dried in hot air oven at 40^{o}C for 48 hr. Dried maize were powdered and stored in dark place.

3.2 ESTIMATION OF TOXINS

3.2.1 Estimation of citrinin

The citrinin from mouldy maize was estimated by Thin Layer Chromatography according to modified Mario O. Topia method:

3.2.1.1 Reagents

1. Acetonitrile
2. Hexane
3. Chloroform
4. Sodium sulphate anhydrous
5. 10% oxalic acid (10 gm oxalic acid dissolved in methanol to make 100 ml solution)
6. Acetone
7. Ethylacetate
8. 5 N HCL
9. 4% KCL
10. Citrinin standard (5 μg/ml dissolved in chloroform)

3.2.1.2 Procedure

About 10g of ground maize samples, blended at high speed for 3 min with 36 ml of Acetonitrile, 4ml of 4% KCL and 0.8ml of 5 N HCL

↓

Filtered the extract through (Whatman No.1) filter paper

↓

20 ml filtrate was transferred into a 250 ml separating funnel, 20 ml of water and 20 ml of hexane were added and shaken well. The hexane layer was discarded.

Lower layer collected and 20 ml of hexane added, shaken well and collected the lower layer

The resulting acetonitrile phase extracted with two 10 ml portions of chloroform.

Chloroform layer collected and dried over anhydrous sodium sulphate (5 g)

Chloroform layer evaporated to dryness at 50° C in hot air oven.

The residue dissolved in 0.2 ml chloroform.

2, 5, 10, 15, 20 µl of Chloroform residues spotted on 10% oxalic acid dipped activated TLC plate with the standard spots (2,5,10, 15,20 µl)

The plate was developed using toluene-ethyl acetate - formic acid (5:4:1) in equilibrated chamber.

The plates were dried and viewed under long wave length and spots were identified. The intensity of the fluorescent spots of the samples was compared with that of standard spots (citrinin appeared as lemon yellow)

The citrinin content was calculated by the formula

$$\text{Citrinin (Ppb)} = \frac{S \times C \times D}{T \times E} \times 1000$$

Where

S=Standard volume which matches with test volume in fluorescence intensity

C= Concentration of standard (5 μg/ml),

D= Dilution factor.

T= Test volume which matches with standard volume in fluorescence intensity.

E= Effective weight of the sample= 4.9 gm for 10 gm of samples

3.2.2 Estimation of fumonisin

The concentration of fumonisin in culture was estimated by TLC according to Pratapkumar (1997)

3.2.2.1 Reagents

1) Methanol : water (3:1)
2) 1 M NaOH
3) 1 M HCL
4) 0.5% acetic acid in methanol

5) 0.5% p-anisaldehyde in methanol: acetic acid: sulphuric acid (85:10:5)

6) Methanol : 4% Aqueous KCL (3:2)

7) Standard: Fumonisin standard in methanol 1 µg/ml.

3.2.2.2 Extraction

- 50 ml of methanol: water was added to 5 g ground sample and shaken at 350 rpm for one hour.
- Filtered the contents through Whatman No.4 filter paper and made up the volume of filtrate to 100ml.
- The pH of the filtrate was adjusted to 5.8 using 1 M NaOH /1 M HCL.

3.2.2.3 Cleanup

- The filtrate was purified by passing through a strong anion exchange 'SAX" column (SYSAX, 500-6).
- The SAX column was first equilibrated with 3 ml of methanol followed by 5 ml methanol : water (3:1). The flow rate was adjusted to 2 ml/min.
- 10 ml of the filtrate was applied to the 'SAX' column.
- Then the column was washed with 8 ml of methanol: water (3:1) followed by 5 ml methanol.
- Then the toxin was eluted from the column by washing the column with 20 ml of 0.5% acetic acid in methanol. The flow rate was adjusted at 1 ml/mt.
- The eluant was evaporated to dryness under a flow of nitrogen stream at 60^{o}C.
- The residue was redissolved in methanol and dried again.

3.2.2.4 Quantification

- The dried residue was dissolved in acetonitrile : water (1:1) and spotted on reverse phase TLC plate along with standard.
- The TLC plate was developed in methanol : 4% acqueous KCL (3:2) in glass tank.
- The developed plate was air dried at room temperature and sprayed with p-anisaldehyde spray.
- Then the plate was heated at 120^{o}C for 10 min.
- Fumonisin B_1 appeared as reddish purple spot.

Fumonisin B_1 content was calculated using the formula

$$\text{Fumonisin (ppm)} = \frac{S \times Y \times V}{Z \times W}$$

Where,

S = Volume in µ1 of standard spot comparable to that of 'Z' 'µ1' of sample spot

Z = Volume in 'µ1' of sample spot comparable to that of standard spot.

V= Volume in 'µ1' of dissolved residue before spotting.

Y = Concentration of *Fumonisin* B1 standard.

W= Weight of the sample

3.3 EXPERIMENTAL STUDIES

3.3.1 Experimental chicks

One hundred and twenty eight day old male broiler chicks were randomly divided in to 4 groups with 4 replicates of 8 birds in each group.

3.3.2 Experimental design

The experimental design was completely randomized design with four groups of chicks fed with the following experimental diets.

Diet 1 - Basal diet (control group)

Diet 2 - Basal diet + 50 ppm citrinin + 10 ppm fumonisin.

Diet 3 - Basal diet +50 ppm citrinin + 10 ppm fumonisin + 0.4% activated charocoal.

Diet 4 - Basal diet + 50 ppm citrinin + 10 ppm fumonisin + 0.4 % activated charcoal + 0.1% lyophilized yeast culture.

3.3.3 Housing and management

The experimental birds were identified with wing bands affixed to the wings of chicks. The birds of all groups were reared under uniform standard conditions provided in battery brooders throughout the period of study. Brooding was done for 3 weeks using incandescent bulbs. Feed and water were provided *ad libitum*. Feeding was done in linear trough feeders. Water was supplied through the system of nipple drinkers.

3.3.4 Experimental diets and feeding

Broiler starter ration was given from 0-3 weeks and finisher ration was given from 4-6 weeks. The ingredient composition of the basal ration is given in Table 1. Required quantities of toxins and adsorbents were added to the basal diet to prepare different experimental rations.

Tab : 1 Ingredient composition of basal diet

Ingredients	Starter ration	Finisher ration
Maize	55 Kg	60 Kg
Soybean	40 Kg	35 Kg
Shell grit	1 Kg	1 Kg
DCP	1 Kg	1 Kg
Oil	2 Kg	2 Kg
Salt	0.3 Kg	0.3 Kg
Trace minerals	0.15Kg	0.15 Kg
Choline chloride	0.10 Kg	0.10 Kg
Coccidiostat	0.05 Kg	0.05 Kg
Vit B12	0.020 Kg	0.02 Kg

Vit A, D3, K	0.020 Kg	0.02 Kg
Vit B mix	0.025 Kg	0.025 Kg
Lysine	0.1 Kg	0.1 Kg
Methionine	0.25 Kg	0.25 kg

3.3.5 Medication and Vaccination

1) 1st day – B-Complex vitamin.
2) 2 nd day – Thiamutin
3) 3,4,5 th day – Antibiotic treatment (Enrofloxacin)
4) 7th day – RDF1 intraocular
5) 14th day – IBD – Intermediate strain – intraocular
6) 21st day – RD booster –Lasota – intraocular
7) 28th day – IBD booster – Intermediate strain – intraocular.

3.3.6 Performance

Weekly feed consumption was recorded replicate-wise in all the experimental groups and feed consumption was expressed per bird. The chicks were weighed individually every week using electronic balance. The mortality was recorded. The dead birds were thoroughly examined by conducting postmortem examination.

3.3.7 Serum biochemical profile

Blood was collected from four birds in each treatment from wing vein on 14^{th} , 28^{th} and 42^{nd} days. Serum was separated and was stored at $-20^{o}C$ for subsequent analysis.

The individual samples were analysed for:

- Glucose (O-Toluidene method),
- Total proteins (Biuret method),
- Albumin (BCD dye binding method),
- Cholesterol (Wybenga and Pileggi's method),
- Serum uric acid (Phosphotungstic acid method),
- Calcium (O-cresolphalein complexone method),
- Triglycerides (GPO method),
- Creatinine (Alkaline picrate method),
- Asparatate transaminase (AST, Reitman and Frenkel method) and
- Alkaline phosphatase (Kind and Kings method) were estimated using kits supplied by M/s Qualigens Fine Chemicals.
- Gamma glutamyl transferase (GGT) was estimated with the kits supplied by M/s Chema diagnostica by kinetic colorimetric method.

3.3.8 Effect on vital organs

At the end of the experiment (6 weeks), 4 representative birds from each group were slaughtered, the vital organs (liver, kidney, spleen, bursa of Fabricius, heart, and intestine) were collected and stored in 10% neutral buffered formalin for further tissue processing and histopathological studies.

The sections were stained with routine haematoxylin and eosin staining. Gross morphological changes in various organs were also observed and recorded.

3.4 STATISTICAL ANALYSIS

The experimental data were analysed as per the procedures of Snedecor and Cochran (1994).

CHAPTER IV

RESULTS

Citrinin and fumonisin were produced on maize grits. Both were quantified and incorporated in the poultry broiler ration, fumonisin @ 10 ppm and citrinin @ 50 ppm. Activated charcoal and Yeast culture were used as adsorbents. The results are cited below:

4.1 TOXIN PRODUCTION

4.1.1 Citrinin

Pure culture of *Penicillium citrinum* (MTCC-2547) was inoculated on already prepared Czapak's agar slants (Plate 1). After 21 days, sterilized distilled water was added and spore suspension was prepared. The maize grits were used as substrate for *Penicillium citrinum,* then the spore suspension was inoculated and was regularly observed for changes in external appearance. Mould growth started as white shaggy appearance after 2 days. Which later turned to green and finally black to yellow at the end of 21 days (Plate 2). Afterwards the mouldy maize grains were autoclaved to kill the spores and then dried overnight in hot air oven at 50^0C. Dried maize grains were powdered and stored.

4.1.2 Fumonisin

Fumonisin was produced by growing *Fusarium moniliforme* MTCC 156 culture on maize. The maize grains were inoculated with normal saline suspension of 7 day old *Fusarium moniliforme* culture on potato sucrose agar (Plate 3). The maize, after inoculation, was incubated for 5 weeks at 25^0C

(Plate 4). The fungus was killed by incubating the culture with chloroform: acetone (1:1) mixture overnight. Then the maize grains were dried in hot air oven at 40^0C for 48 hr. Dried maize were powdered and stored in dark

4.2 BROILER PERFORMANCE

4.2.1 Weekly body weights

The mean values of the weekly body weights are given in Tab 2 and Fig 3. The overall mean body weights of broiler chicks for different diets1,2,3 and 4 were 750.08, 612.26, 667.29 and 693.64 g

respectively. The mean weekly body weights from 1-6 weeks were 42.19, 112.96, 235.50, 440.78, 828.93, 1283.14 and 1822.24 g respectively.

A significant decrease ($P < 0.01$) in body weights were found in chicks fed with Diet : 2.

4.2.2 Body weight gains

The mean value of the weekly body weight gains are given in Tab 3 and Fig 4. The mean body weight gains of broiler chicks for different diets 1, 2, 3 and 4 were 326.05, 266.91, 290.78 and 302.95 g, respectively. 46 *ın weekly body weight gains from 1-6 weeks were 70.76, 122.54, 205.28, 388.14, 454.21 and 539.10g respectively.*

A significant decrease ($P < 0.01$) in body weight gains was found in chicks fed with toxin (Diet 2).

4.2.3 Feed Consumption

The means of weekly feed consumption are presented in Tab 4 and Fig 5 The mean values over different periods from 1-6 weeks, were 403.37, 1050.84, 1772.43, 2760.53, 3205.59 and 2808.06 g, respectively. The mean values of feed consumption of different treatment groups were 2131.22, 1910.22, 1961.68 and 1997.41 g, respectively for group 1, 2, 3 and 4.

Feed consumption on diet 1 remained significantly ($P < 0.01$) higher compared to all other diets.

4.2.4 Feed conversion ratio

The mean values of feed conversion ratios are given in Tab 5 and Fig 6 The mean feed conversion ratios over different periods were 1.42, 2.14, 2.16, 1.78, 1.76 and 1.30 respectively for 1-6 weeks. The mean feed conversion ratios on diets 1, 2, 3 and 4 were 1.70, 1.86, 1.76 and 1.73 respectively.

The feed conversion ratios were significantly ($P < 0.01$) higher in toxic control (diet-2), as compared to diet 4 and control (diet 1).

4.3 SERUM BIOCHEMICAL PROFILE

4.3.1 Serum proteins

4.3.1.1 Serum Total Proteins: The mean serum total protein values pertaining to different periods and different treatments are presented in Tab 6 and Fig 7. The mean serum total protein values for different diets 1, 2, 3 and 4 were 4.27, 2.36, 2.84 and 3.59 g % respectively. The mean serum total protein values at different periods of 14, 28, 42 days were 2.77, 3.52 and 3.50 g % respectively.

The birds fed on diet 2 had significantly ($P < 0.01$) lowest serum total protein levels. A significant increase in Serum total Protein value observed at 28^{th} and 42^{nd} days of collection.

4.3.1.2 Serum Albumin: The mean serum albumin values are presented in Tab 7 and Fig 8. The mean serum albumin concentrations in chicks on diet 1, 2, 3 and 4 were 2.09, 0.96, 1.32 and 1.81 g %, respectively. The mean serum albumin values at different periods of 14, 28 and 42 days were 1.48, 1.65 and 1.50 g % respectively.

The birds on diet 2 recorded lowest serum albumin concentration.

4.3.1.3 Serum Globulin: The mean serum globulin values are given in Tab 8 and Fig 9. The mean serum globulin levels at different diets 1, 2, 3 and 4 were 2.18, 1.39, 1.52 and 1.78 g % respectively. The mean serum glo

centrations at

different periods of collection at 14, 28 and 42 days were 1.29, 1.86 and 2.00 g % respectively.

The birds fed on diet 2 had lower serum globulin concentration. A significant increase serum globulins were recorded at different periods of collection.

4.3.1.4 Serum A/G Ratio: The results of A/G ratio were given in Tab 9 and Fig 10. The mean serum A/G ratios affected by different treatment diets 1, 2, 3 and 4 were 1.15, 0.74, 0.98 and 1.12 respectively. The mean serum A/G ratios at 14, 28, 42 days were 1.24, 0.93 and 0.83 respectively.

A lowest A/G ratio was observed in diet 2 birds. The serum A/G values were decreased gradually from 14 to 42 days.

4.3.2 Serum Glucose

The mean serum glucose concentrations are given in Tab 10 and Fig 11. The serum glucose concentrations on different diets 1, 2, 3 and 4 were 162.54, 115.96, 133.70 and 144.80 mg %, respectively. The mean serum glucose values over different periods were 115.48, 136.13 and 166.14 mg % at 14, 28 and 42 days, respectively.

The birds on diet 2 had significant ($P < 0.01$) lowest serum glucose concentrations. A significant ($P < 0.01$) increase in serum glucose values over different periods was observed from 14 to 42 days.

4.3.3 Serum Cholesterol

The serum cholesterol concentration in different treatments and periods are given in Tab 11 and Fig 12.

The mean serum cholesterol concentrations on different diets 1, 2, 3 and 4 were 179.52, 87.45, 106.34 and 115.61 mg % respectively. The mean serum cholesterol values at different periods of 14, 28 and 42 days were 125.32, 116.96 and 124.41 mg %, respectively.

The birds fed on diet 2 had significant ($P < 0.01$) lowest serum Cholesterol levels.

4.3.4 Serum Triglycerides

The serum triglycerides levels recorded in the experiment were presented in Tab 12 and Fig 13. The mean serum triglycerides levels for different experimental diets 1, 2, 3 and 4 were 112.20, 94.08, 98.96 and 103.47 mg % respectively.

The mean serum triglyceride levels at different periods were 91.99, 104.07 and 110.47 mg % at 14, 28 and 42 days respectively.

The birds on diet 2 recorded significantly ($P < 0.01$) lowest serum triglycerides. A significant ($P < 0.01$) increased levels of serum Triglycerides were recorded at different periods from 14 to 42 days.

4.3.5 Serum Bilirubin

The mean serum bilirubin values are presented in Tab 13 and Fig 14. The mean serum bilirubin values as affected by different experimental rations on diets 1, 2, 3 and 4 were 2.70, 3.05, 2.79 and 2.66 mg %, respectively. The mean serum bilirubin concentrations affected by different periods were 2.37, 2.95 and 3.09 mg %, respectively.

The birds fed on diet 2 had highest ($P < 0.01$) serum bilirubin levels. A significant ($P < 0.01$) increase in serum bilirubin was observed from 14 to 28 and 42 days.

4.3.6 Serum uric acid

The results of serum uric acid concentration are presented in Tab 14 and Fig 15. The mean serum uric acid concentration in broilers fed on diets 1, 2, 3 and 4 were 6.12, 4.86, 5.31 and 5.51 mg %, respectively. The

serum uric acid concentrations at different periods were 5.36, 5.26 and 5.74 mg %, at 14, 28 and 42 days, respectively. The birds fed on diet 2 had significa[illegible] (P < 0.01) lowest Serum uric acid concentration. The serum uric acid levels were increased from 14 to [illegible] of collection.

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4.3.7 Serum blood urea nitrogen (BUN)

The mean BUN values are depicted in Tab 15 and Fig 16. The mean BUN values of birds on diets 1, 2, 3 and 4 were 6.34, 7.03, 6.50 and 6.40 mg %, respectively. The mean BUN values at 14, 28 and 42 days were 6.53, 6.57 and 6.60 mg %, respectively.

The BUN levels were significantly increased ($P < 0.01$) in birds fed on diet 2. Gradually, significantly increased BUN values over different periods was observed from 14 to 42 days.

4.3.8 Serum creatinine

The Serum creatinine values are presented in Tab 16 and Fig 17. The Serum creatinine concentrations levels as affected by different diets 1, 2, 3 and 4 were 0.71, 0.77, 0.75 and 0.70 mg % respectively. The mean Serum creatinine concentrations at 14, 28 and 42 days were 0.68, 0.75 and 0.76 mg % respectively.

The birds on diet 2 had significantly highest Serum creatinine concentrations. A significant ($P < 0.01$) increase in Serum creatinine values over different periods was observed from 14 to 42 days.

4.3.9 Serum calcium

The results pertaining to Serum calcium concentrations were given in Tab 17 and Fig 18. The mean Serum calcium levels of birds on diets 1, 2, 3 and 4 were 1, 7.98 and 8.38 mg %, respectively. The mean Serum calcium levels at different periods of 1 d 42 days were 8.08, 7.31 and 8.71 mg %, respectively.

Serum calcium levels were significantly ($P < 0.01$) lowest in birds fed on diet 2 . A significant ($P < 0.01$) increase in Serum calcium was observed from 14 to 42 days.

4.4 SERUM ENZYMES

4.4.1 Serum aspartate aminotransferase (AST)

The results of AST values are given in Tab 18 and Fig 19. The mean AST levels in birds on different diets 1, 2, 3 and 4 were 58.11, 65.60, 60.74 and 57.63 units/ml respectively. The mean AST levels at different periods were 54.72, 59.70 and 67.14 units/ml at 14, 28 and 42 days, respectively.

Birds on diet 2 had significant ($P < 0.01$) highest AST levels. A significant ($P < 0.01$) increased serum AST levels were observed at different periods from 14 to 42 days.

4.4.2 Serum alanine aminotransferase (ALT)

The mean ALT values are presented in the Tab 19 and Fig 20. The mean ALT values in birds fed on diets 1, 2, 3 and 4 were 17.75, 21.88, 17.80 and 15.30 units/ml respectively. The mean ALT values at 14, 28 and 42 days were 16.43, 17.10 and 21.01 units/ml, respectively.

Serum ALT levels were Significant (P< 0.01) higher in birds fed on diet 2. A significant increased ALT values were observed from 14 and 28 days to 42 day.

4.4.3 Serum alkaline phosphatase (ALP)

The mean Serum alkaline phosphatase values are depicted in Tab 20 and Fig 21. Birds on diets 1, 2, 3 and 4 recorded Serum alkaline phosphatase values of 24.61, 42.21, 29.65 and 28.91 KA units, respectively. The mean Serum alkaline phosphatase values at 14, 28 and 42 days were 20.50, 37.52 and 36.45 KA units, respectively.

The birds on diet 2 had significantly highest (P < 0.01) alkaline phosphatase levels. A significant (P < 0.01) increase in alkaline phosphatase was observed from 14 to 28 and 42 days.

4.4.4 Serum gamma glutamyl transferase (GGT)

The mean GGT levels in serum are presented in Tab 21 and Fig 22.The mean serum GGT levels as affected by different experimental diets 1, 2, 3 and 4 were 10.30, 20.72, 13.42 and 7.61 units/L, respectively. The mean serum GGT levels at 14, 28 and 42 days were 8.88, 12.48 and 17.67 units/L, respectively.

The birds on diet 2 had significant ($P < 0.01$) highest GGT concentrations. A significant ($P < 0.01$) increase in GGT values over different periods was observed from 14 to 42 days.

4.5 PATHOLOGY

4.5.1 Gross Pathology

4.5.1.1 Liver: The livers from diet 2 birds were pale and enlarged with rounded borders. The livers of diet 3 and diet 4 were moderatly pale with slight enlargement.

4.5.1.2 Kidney: The kidneys of birds from diet 2 were moderately enlarged with occasional hemorrhages. All the lobes of the kidneys were uniformly enlarged. Mild to moderate enlargement of kidney with mild hyperemic changes were observed in diet 3 and diet 4.

4.5.1.3 Spleen: Spleens from diet 2 birds were moderately congested with occasional mottling. Spleens from diet 3 and diet 4 birds showed mild congestion. The birds from diet 1 did not reveal any lesions of significance.

4.5.1.4 Bursa of Fabricius: Bursa of Fabricius from diet 2 birds showed hyperemic changes with mild enlargement. On sectioning bursal folds showed edematous appearance. Very mild hyperemic changes were seen in diet 3 birds and no apparent lesions of pathological significance were seen in diet 4 fed birds.

4.5.1.5 Heart: The birds fed on diet 2 showed congestion of coronary vessels. The birds fed on diet 3 showed mild congestion.

4.5.1.6 Intestines: The intestines of birds from diet 2 recorded mild congestion.

4.5.2 Histopathology

4.5.2.1 Liver: Microscopically the livers from diet 2 birds showed mild to moderate central vein congestion with paracentral infiltration by lymphocytes (Plate 5) and focal areas of lymphoid aggregates (Plate 6). Some sections showed bile duct hyperplasia and dilatation of sinusoidal spaces (Plate 7).

Liver sections from diet 3 birds showed mild central vein congestion with bile duct hyperplasia (Plate 8) and very mild congestion of sinusoidal spaces (Plate 9).

Liver sections from diet 4 birds showed tubular rearrangement of hepatic cells indicating regeneration (Plate 10) with very mild central vein congestion.

4.5.2.2 Kidney: The Kidney sections from diet 2 birds revealed inter tubular congestion and hemorrhages (Plate 12) with degenerative changes in tubules. Few sections showed focal lymphoid aggregates (Plate 11).

Kidney sections from diet 3 birds showed lymphoid aggregates with inter tubular congestion (Plate 14) and mild degenerative changes in tubular epithelium. Few tubules showed casts in the lumen.

In diet 4 fed bird's kidney sections showed, inter tubular congestion and hemorrhages with very mild degenerative changes in tubular epithelium.

4.5.2.3 Spleen: The sections of spleen from b 89 et 2 showed mild depletion of germinal centers (Plate 15) with mild congestion of trabecular a

Microscopically the spleen from diet 3 birds sh ild depletion of germinal centers (Plate 16). Spleen sections from diet 4 birds showed very mild sub-capsular hemorrhages.

4.5.2.4 Bursa of Fabricius: Sections of Bursa of Fabricius from diet 2 birds revealed, follicles with cystic spaces (Plate 18) and in some areas cystic spaces were seen in follicular epithelium. Marked depletion of lymphoid follicles (Plate 19) and few follicles showing moderate depletion were observed.

The sections of Bursa of Fabricius from birds fed on diet 3 showed mild depletion of follicles and some follicles showed washed out appearance (Plate 20). Some sections showed inter follicular fibrosis.

The sections of Bursa of Fabricius from birds fed on diet 4 showed very mild depletion and fibrosis between inter follicular spaces with few follicles showing tiny cystic spaces.

4.5.2.5 Heart: Microscopic picture of heart from birds fed on diet 2 recorded mild inter fibrillar hemorrhages (Plate 21) with disruption of cardiac muscle fibers.

The heart sections from diet 3 fed birds showed lymphocytic infiltration between cardiac muscle fibers and some fibers showing myocardial disruptio

t sections of diet 4 fed birds showed very mild hemorrhages between cardiac muscle fibers.

How ever diet 1 fed birds (control group) showed no gross and microscopic lesions of pathological significance.

4.5.2.6 Intestines: The intestine sections from diet 2 fed birds showed disruption of villus epithelium (Plate 22) and some areas showing sub-mucosal hemorrhages.

Microscopic picture of intestines from diet 3 fed birds showed mild sub-mucosal hemorrhage between villi. While birds fed on diet 4 did not revealed any microscopic lesions of pathological significance as like that of birds fed on diet 1 (control group).

CHAPTER V

DISCUSSION

Citrinin @ 50 ppm and fumonisin @ 10 ppm were mixed in feed and this contaminated feed treated with activated charcoal (0.4%) and / or lyophilized yeast culture (0.1 %) and the efficacy of these as adsorbents in reducing the toxicity of citrinin and fumonisin in broiler diets and effect on broiler performance, serum biochemical profile and histopathology of vital organs in broilers are discussed in this chapter.

5.1 BROILER PERFORMANCE

5.1.1 Weekly body weights

The body weights of birds fed on diet 2 containing toxins were significantly ($P < 0.01$) lower compared to those on control diet and diet 3 and 4 containing activated charcoal and lyophilized yeast culture (Tab 2 and Fig 3). The decrease in body weights might be due to decreased feed intake and altered metabolism due to citrinin and fumonisin.

The body weights of the birds fed on diet 3 containing citrinin, fumonisin and activated charcoal were significantly higher than those on diet 2 indicating that addition of activated charcoal gave protection in terms of body weights. This indicates that activated charcoal could adsorb both the toxins. The body weights of birds fed on diet 4 were significantly improved compared to those on diet 2 and reached next to the levels of control diet. The improvement in diet 4 fed birds may be due to the supplementation of enzymes by lyophilized yeast culture that increase the feed utilization (Day *et al*., 1987).

The body weights increased significantly ($P < 0.01$) upto the end of experimental period.

5.1.2 Body weight gains

The body weight gain was significantly ($P < 0.01$) decreased when birds were fed on diet 2 (Tab 3 and Fig 4). The depression in growth upon feeding citrinin individually was reported by many researchers (Ames et al.*, 1976; Roberts and Mora, 1979; Uma and Vikram Reddy, 1995 and Gurunath Reddy, 2004). Similar findings were reported with fumonisin (Weibking* et al.*, 1993; Brown* et al.*, 1992; Ledoux* et al.*, 1992; Espada* et al.*, 1994 and Piramanayagam* et al.*, 2002 a). The decreased body weight gain might be due to decreased feed intake and altered metabolism due to toxicosis.*

Though the body weight gains of the birds on diet 3 were significantly ($P < 0.01$) lower compared to diet 1, the body weight gain recorded on diet 4 was nearer to diet 1.

This implies that depression of body weights caused by citrinin and fumonisin was partially ameliorated by activated charcoal and further ameliorative effect was recorded by combination of activated charcoal and lyophilized yeast culture. The improvement in diet 4 may be due to the supplementation of enzymes by yeast culture, that increases feed utilization (Day et al.*, 1987).*

The body weight gains increased significantly ($P < 0.01$) upto the end of experimental period.

5.1.3 Feed consumption

The mean feed consumption on diet 2 containing citrinin and fumonisin was significantly least ($P < 0.01$) compared to all the diets (Tab 4 and Fig 5) and it may be because of stress due to toxin in diet. This trend was observed by Ames et al. *(1976), Uma and Vikram Reddy (1995) and Gurunath Reddy (2004) in broilers fed with citrinin and Weibking* et al. *(1993), Brown* et al. *(1992), Ledoux* et al. *(1992) in broilers fed with fumonisin individually.*

The feed intake was significantly ($P < 0.01$) higher in diet 3 compared to diet 2. Feed consumption was further improved on diet 4. This indicates that activated charcoal could ameliorate the effect of toxins partially. Further improvement was observed on diet 4 containing activated charcoal and lyophilized yeast culture indicating that yeast culture had a complementary effect as feed consumption significantly ($P < 0.01$) increased.

The feed consumption of the birds increased significantly ($P < 0.01$) upto the end of experimental period.

5.1.4 Feed conversion ratio (FCR)

The mean FCR was highest (P < 0.01) on diet 2 containing citrinin and fumonisin (Tab 5 and Fig 6). Similar findings were observed by Uma and Vikram Reddy (1995) and Gurunath Reddy (2004) with citrinin and Jayasri (2006) with fumonisin individually. Though the FCR on diet 3 and diet 4 were higher than on diet 1, the values of diet 4 were nearer to that of diet 1. It indicates that activated charcoal at 0.4 % level partially ameliorated the toxic effect of citrinin and fumonisin on FCR and activated charcoal (0.4 %) with lyophilized yeast culture (0.1 %) could ameliorate the effect of toxins to a greater extent.

5.2 SERUM BIOCHEMICAL PROFILE

5.2.1 Serum proteins

5.2.1.1 Total proteins: *The serum total protein concentrations differed significantly (P < 0.01) among experimental diets (Tab 6 and Fig 7). The birds fed on diet 2 containing citrinin and fumonisin toxins recorded significantly lower serum total protein concentration. Similar observations were made by Gurunath Reddy (2004) in broilers fed with citrinin and Kubena* et al. *(1999); Sateesh* et al. *(2004) and Jayasri (2006) in broilers fed with fumonisin. The lowest levels of total proteins in diet 2 fed birds might be due to liver damage, the main site for protein synthesis. This reduction in levels of serum total proteins was an indication of impaired protein metabolism and hepatocellular damage due to fumonisin (Jayasri., 2006).*

The birds fed on diet 3 had significantly ($P < 0.01$) higher total proteins than those fed on diet 2 indicating partial amelioration of toxicosis by activated charcoal.

The birds fed on diet 4 had significantly higher total protein concentration compared to those on diet 2 and 3 indicating that activated charcoal and yeast culture had complementary effect on ameliorating the combined toxicosis of citrinin and fumonisin. Though the total protein levels in diet 4 were significantly lower than in control birds, the values were well within the normal range.

Supplementation of enzymes by yeast culture, a protein rich source, may be the reason for improvement in the serum total protein levels in birds fed on diet 4 (Day *et al.,* 1987). The serum total protein levels at 14 day differed significantly ($P < 0.01$) with values at 28^{th} and 42^{nd} days of collection. Serum protein levels increased with the age of birds.

5.2.1.2 Serum albumin: The serum albumin levels were lowest ($P < 0.01$) on diet 2 fed birds (Tab 7 and Fig 8). Similarly decrease in serum albumin levels was observed by Espada *et al*. (1997); Kubena *et al*. (1999); Piramanayagam *et al*. (2001) and Sateesh *et al*. (2004) in broilers fed with fumonisin. Decreased serum albumin level on diet 2 fed birds may be attributed to inhibitory effect of fumonisin on protein synthesis and damage to the liver caused by the toxins.

The serum albumin levels on diet 3 fed birds were significantly higher than on diet 2 fed birds. This might be attributed to the partial ameliorative effect of activated charcoal. The serum albumin values on diet 4 were significantly ($P<0.01$) higher than on diet 2 and diet 3 fed birds indicating complementary effect of yeast culture and activated charcoal in ameliorating the combined toxicosis of citrinin and fumonisin. It indicates that both activated charcoal (0.4 %) and lyophilized yeast culture (0.1 %) have ameliorated more effectively than the activated charcoal (0.4 %) individually.

5.2.1.3 Serum globulins: The serum globulin levels in birds on diet 2 were significantly ($P < 0.01$) reduced (Tab 8 and Fig 9) on all test diets. It may be attributed to the effect of fumonisin on globulin synthesis (Jayasri., 2006).

The mean serum globulin levels on diet 3 and diet 4 fed birds were increased gradually.

Increased serum globulin values on diet 3 indicate that activated charcoal has overcome the inhibitory effect of citrinin and fumonisin on globulin synthesis partially. Further increased values of globulins on diet 4 fed birds imply that both activated charcoal and lyophilized yeast culture had complementary effect in adsorbing the toxins when compared to activated charcoal alone.

The serum globulin levels significantly ($P < 0.01$) increased with the age of birds, which is within normal range.

5.2.1.4 Serum A/G ratio: *Birds fed on diet 2 having citrinin and fumonisin recorded lowest A:G ratio (Tab 9 and Fig 10).*

The albumin globulin ratio in the birds fed on diet 3 and diet 4 were improved and were within the normal range. Albumin/Globulin ratio decreased with age of birds.

5.2.2 Serum glucose

The serum glucose concentrations were significantly (P< 0.01) different among the diets (Tab 10 and Fig 11). Birds fed on diet 2 have significantly lower serum glucose concentrations. Hypoglycemia was seen on diet 2 birds in the present experiment may be attributed to the effect of citrinin (Gurunath Reddy 2004). Since no significant change in serum glucose concentrations was observed in fumonisin toxicity (Henry *et al*., 2000 and Weibking *et al*., 1993) in broilers.

Significantly increased concentration of serum glucose in birds fed on diet 3 when compare to birds fed on diet 2 might be due to ameliorative effect of activated charcoal on glucose concentration but significantly lower levels compared to diet 1 indicate that activated charcoal has partial ameliorative effect on glucose concentration affected by citrinin and fumonisin. Improvement in glucose concentration was seen in birds fed on diet 4 might be due to the presence of yeast culture in the diet.

The serum glucose levels differed significantly (P< 0.01) at different periods of collection. The serum glucose concentrations increased with age of the birds. However, the serum glucose levels were within the normal range throughout the experiment.

5.2.3 Serum cholesterol

The cholesterol concentrations were significantly (P < 0.01) reduced on diet 2 fed birds (Tab 11 and Fig 12). Similar observations were reported by Ames *et al*. (1976) and Gurunath Reddy (2004) in broilers fed with citrinin. Weibking *et al*. (1993) observed significant decrease in serum cholesterol in fumonisin culture material in broilers.

Though there was significant (P < 0.01) difference between diet 2 and diet 3 fed birds. The mean serum concentration of cholesterol on diet 3 fed birds was increased, indicating that inclusion of activated charcoal (0.4 %) could ameliorate the effect of citrinin and fumonisin toxins.

The serum cholesterol concentration on diet 4 fed birds was next to that on diet 1 fed birds implying that combination of activated charcoal and lyophilized yeast culture ameliorate the effect of citrinin and fumonisin on serum cholesterol.

5.2.4 Serum triglycerides

The serum triglyceride levels were significantly ($P < 0.01$) decreased on diet 2 fed birds (Tab 12 and Fig 13). Reduced serum triglyceride biosynthesis was reported by Endo and Kuroda, (1976) in broilers fed with citrinin. Espada *et al*. (1994) also observed a depression in serum triglycerides levels in broilers treated with 10 ppm of FB_1 for 8 days.

The triglyceride levels in diet 3 fed birds were significantly ($P < 0.01$) higher than on diet 2, but not comparable to control values indicating partial ameliorative action of activated charcoal. The triglyceride levels in birds on diet 4 were significantly ($P < 0.01$) higher than on diet 2 and diet 3 indicating that activated charcoal and yeast culture had complementary effect in ameliorating the combined toxicosis of citrinin and fumonisin.

However, combination of yeast culture and activated charcoal could efficiently ameliorate the depressant effect of combined toxicosis of citrinin and fumonisin on serum triglycerides.

The serum triglyceride levels differed significantly ($P < 0.01$) with the period of collection. These values were in accordance with the age of the birds.

5.2.5 Serum bilirubin

The serum bilirubin concentrations of birds on diet 2 containing citrinin and fumonisin were significantly ($P < 0.01$) higher than birds on control diet (Tab 13 and Fig 14). It may be due to impaired liver function which might be due to hepatic damage caused by fumonisin as confirmed by Marijanovic *et al*. (1991); Brown *et al.* (1992); Piramanayagam *et al*. (2001); Sateesh *et al*. (2004) and Jayasri (2006).

The serum bilirubin levels of birds on diet 3 were lower than on diet 2 fed birds indicating that activated charcoal partially ameliorated the effect of citrinin and fumonisin on serum bilirubin. The values of serum bilirubin levels of birds on diet 4 were nearer to that values on diet 1 fed birds (control group) indicating that activated charcoal and lyophilized yeast culture protected liver damage caused by citrinin and fumonisin.

5.2.6 Serum uric acid

Lower uric acid levels were observed in birds fed on diet 2 (Tab 14 and Fig 15). It may be due to the damage to liver and impaired utilization of protein (Abo Norag *et al.,* 1995). Ames *et al*. (1976), Uma and Vikram Reddy (1995), Gurunath Reddy (2004) and Priyadarshini (2005) reported that serum uric acid levels did not differ significantly in broilers fed with citrinin but Espada *et al*. (1994) reported decreased serum uric acid levels in broilers fed with fumonisin.

The serum concentrations of uric acid of birds on diet 3 were higher than in birds fed on diet 2 indicating that activated charcoal was effective in providing moderate protection against toxic effect. The serum concentrations of uric acid of birds on diet 4 were significantly higher when compared to the birds on diet 2 indicating that activated charcoal and yeast culture had complementary effect on maintaining uric acid level in the serum.

5.2.7 Blood urea nitrogen (BUN)

The blood urea nitrogen levels differed significantly ($P < 0.01$) among different diets (Tab 15 and Fig 16). Elevated BUN levels were observed by Roberts and Mora (1978), Uma and Vikram Reddy (1995), Gurunath Reddy (2004) and Priyadarshini (2005) in broilers fed with citrinin. Elevation of BUN levels were also observed with fumonisin by Kubena *et al*. (1997) in broilers.

The BUN levels in birds fed on diet 2 were significantly higher than test diets 2 and 3 indicating the damage of kidney resulting in decrease in excretion of NPN compounds such as uric acid, creatinine and urea. The kidney damage due to citrinin was reported by Roberts *et al*. (1978) and Gurunath Reddy (2004).

The BUN levels were significantly ($P < 0.01$) decreased in birds fed on diet 3 compared to diet 2. This indicates that activated charcoal could over come the effect of citrinin and fumonisin partially. The BUN levels in

birds on diet 4 were lower than on diet 2, diet 3 and nearly equal to diet 1 fed birds (control group) indicating that activated charcoal in addition with lyophilized yeast culture could ameliorate the effect of toxins.

The blood urea nitrogen levels increased gradually with age of the birds and differed significantly (P < 0.01) between periods.

5.2.8 Serum creatinine

The serum creatinine levels were significantly (P < 0.01) elevated in birds on diet 2 (Tab 16 and Fig 17). The higher serum creatinine levels recorded in birds on diet 2 may be due to kidney damage caused by citrinin (Gurunath Reddy., 2004 and Priyadarshini., 2005). The elevated serum creatinine levels were also observed by Espada *et al*. (1994) and Piramanayagam *et al*. (2001) in fumonisin toxicosis in broilers.

The serum creatinine levels in birds on diet 3 were less than those of the birds on diet 2 indicating that activated charcoal could protect the kidneys from damage caused by citrinin and fumonisin. However, the serum creatinine levels on diet 4 were nearer to diet 1 fed birds (control group) indicating that activated charcoal and yeast culture had complementary effect on ameliorating the toxic effects of citrinin and fumonisin.

The serum creatinine levels increased significantly ($P < 0.01$) on diet: 2 birds but came to normal on birds fed with diet: 4.

5.2.9 Serum calcium

The serum calcium concentrations were lowest ($P < 0.01$) in birds on diet 2 (Tab 17 and Fig 18). The reduced serum calcium levels in citrinin toxicity was observed by Glahn *et al*. (1989). Decreased serum calcium levels was observed by Broomhead *et al*. (2002) in fumonisin toxicity in birds. Decreased calcium levels may be due to altered renal, intestine and parathyroid regulation of calcium (Glahn *et al.,* 1989). It also may be due to alteration in vitamin D_3 metabolism which regulated calcium metabolism in the body.

The serum calcium levels were significantly improved in birds on diet 3 indicating that activated charcoal has ameliorated the toxic effects of citrinin and fumonisin.

The serum calcium levels on diet 4 were significantly higher ($P < 0.01$) than on diet 3 indicating that activated charcoal and yeast culture have complementary effect in ameliorating the toxic effects.

The serum calcium levels decrease significantly ($P < 0.01$) in birds on diet 2 and calcium levels in birds on diets 1, 3 and 4 were in the normal range. The serum calcium levels differ significantly ($P < 0.01$) with period of collection.

5.3 SERUM ENZYMES

5.3.1 Aspartate aminotransferase (AST):

The mean serum AST levels differed significantly ($P < 0.01$) between birds on diet 2 and diets 1, 3 and 4. The birds fed on diet 2 recorded significantly highest levels of serum AST (Tab 18 and Fig 19). Elevated serum AST levels were observed in fumonisin toxicity (Espada *et al*., 1994; Sateesh *et al*., 2004; Kubena *et al*., 1997; and Piramanayagam *et al*., 2001).

Continuous administration of toxins might have caused severe hepatocellular damage leading to increased cellular permeability resulting in the leakage of enzyme into circulation.

In diet 3 fed birds, serum AST levels were significantly reduced when compared to diet 2 indicating that activated charcoal has partial amelioration on combined toxicosis of citrinin and fumonisin.

The serum AST levels on diet 4 fed birds were nearer when compared to the values on diet 1 fed birds (control group) indicating that activated charcoal and yeast culture can combat the toxic effects of citrinin and fumonisin.

5.3.2 Alanine aminotransferase (ALT):

The mean serum ALT levels differed significantly ($P < 0.01$) with different diets (Tab 19 and Fig 20). The serum ALT levels were highest in birds fed on diet 2. Liver has the greatest ALT specific activity and hence increased levels of serum ALT indicating hepatocellular injury. The elevated levels of ALT may be attributed to damage caused by citrinin and fumonisin in hepatic tissue as evidenced by histopathology of liver(Plates 5,6 and 7). Increased levels of ALT were observed in albino rats fed on citrinin by Thirunavakkarsu *et al*. (2001) and fumonisin by Piramanayagam *et al.* (2001) and Sateesh *et al*. (2004).

The serum ALT activity on diet 3 was reduced significantly ($P < 0.01$) when compared to that on diet 2 fed birds suggesting the protective action of activated charcoal against the hepatocellular damage.

The birds on diet 4 had significantly ($P < 0.01$) reduced serum ALT levels, but within the normal range indicating that activated charcoal and yeast culture had a complementary effect in reducing the ALT activity.

The serum ALT levels increased significantly ($P < 0.01$) as the age of birds advances to 42 days.

5.3.3 Alkaline phosphatase (ALP)

The highest ALP activity was observed in birds on diet 2 affected with citrinin and fumonisin (Tab 20 and Fig 21). Similar findings were reported by Sateesh *et al*. (2004).

Serum alkaline phosphatase activity was significantly (P< 0.01) reduced in birds fed on diet 3 compared to those birds on diet 2, but not to comparable level of control diet birds indicating partial protection by activated charcoal from combined toxicity of citrinin and fumonisin.

The birds fed on diet 4 had reduced alkaline phosphatase activity more than diet 3. This indicate that activated charcoal and yeast culture put together had better amelioration against combined toxicity of citrinin and fumonisin with regards to ALP activity in broilers.

5.3.4 Gamma glutamyl transferase (GGT)

The serum GGT concentrations differ significantly (P < 0.01) among the test diets (Tab 21 and Fig 22). The highest activity of GGT was observed in birds fed on diet 2.

GGT is a sensitive indicator of liver disease, viz., liver inflammation, a space occupying lesion, or obstruction of the biliary tract. Kubena *et al.* (1997) and Espada *et al*. (1993) observed elevated GGT in fumonisin toxicity of broilers. The liver damage (Plates 5, 6 and 7) recorded in this experiment confirms the above view.

The birds on diet 3 have significantly reduced GGT activity compared to those on diet 2 indicating the protective effect provided to liver by activated charcoal.

The birds fed on diet 4 have significantly ($P < 0.01$) reduced serum GGT values less even than diet 3, but these values were within the normal range, indicating that toxicity of citrinin and fumonisin was more effectively ameliorated by activated charcoal and lyophilized yeast culture than activated charcoal alone.

The serum GGT concentrations were significantly ($P < 0.01$) different among the diets and periods of collection.

5.4 HISTOPATHOLOGY OF VITAL ORGANS

5.4.1 Liver

The birds fed on diet 1 did not reveal any lesions of pathological significance. The livers of birds fed on diet 2 showed pale and enlarged livers with rounded borders. These results were in accordance with the findings of Roberts and Mora (1978), Ahamad and Vairamuthu (2001) and Gurunath Reddy (2004) in citrinin toxicity of broilers. Similar findings were observed by Sateesh *et al*. (2005) and Jayasri (2006) in fumonisin toxicity.

Microscopically, the livers of birds fed on diet 2 showed mild to moderate central vein congestion and paracentral infiltration by lymphocytes (Plate 5) with focal areas of lymphoid aggregates (Plate 6). Some sections showed bile duct hyperplasia and dilatation of sinusoidal spaces (Plate 7). These results were in agreement with the reports of Roberts and Mora (1978), Uma and Vikram Reddy (1995), Ahamad and

Vairamuthu (2001), Gurunath Reddy (2004), and Priyadarshini (2005) in birds with citrinin toxicity. In fumonisin toxicosis, similar findings were reported by Brown *et al*. (1992), Piramanayagam *et al*. (2002 b) and Jayasri (2006).

Liver sections from diet 3 fed birds showed mild central vein congestion with bile duct hyperplasia (Plate 8) and very mild congestion of sinusoidal spaces (Plate 9) indicating that activated charcoal could over come the effect of citrinin and fumonisin to some extent. Similar findings were reported by Priyadarshini (2005) in citrinin toxicity in broilers and Jayasri (2006) in fumonisin toxicity in broilers.

Liver sections from birds fed on diet 4 showed tubular rearrangement of hepatic cells indicating regeneration (Plate 10) with very mild central venous congestion. Which indicated that activated charcoal and lyophilized yeast culture had ameliorated the effect of citrinin and fumonisin on liver to a very greater extent. This was in accordance with the findings of Gurunath Reddy (2004) in broilers fed with citrinin and Jayasri (2006) in broilers fed with fumonisin.

5.4.2 Kidney

The prominent gross lesions observed in diet 2 were moderately enlarged kidneys with occasional hemorrhages. Mild to moderate enlargement of kidney with mild hyperemic changes were observed in diet 3 and diet 4. The results were in agreement with Uma and Vimram Reddy (1995) and Gurunath Reddy (2004) in

broilers fed with citrinin. Kubena *et al.* (1997), Weibking *et al*. (1993) and Jayasri (2006) in broilers fed with fumonisin.

Histopathologically the kidney section on diet 2 revealed that inter tubular congestion and focal lymphoid aggregates (Plate 11) and inter tubular hemorrhages (Plate 12) with degenerative changes in tubules (Plate 13). Few sections showed focal lymphoid aggregates (Plate 11). Similar findings were observed in citrinin toxicity in broilers by Roberts and Mora (1978), Mehdi *et al*. (1981), Uma and Vikram Reddy (1995) and Gurunath Reddy (2004).

Kidney sections from diet 3 birds showed lymphoid aggregates (Plate 14) with mild degenerative changes in tubular epithelium and few tubules showing casts in the lumen, indicating the birds fed on diet containing activated charcoal had ameliorative effect on citrinin and fumonisin.

In diet 4 fed bird's kidney sections showed inter tubular congestion with very mild degenerative changes in tubular epithelium indicating the beneficial effect of activated charcoal and yeast culture against combined toxicity of citrinin and fumonisin when compared to activated charcoal alone. Similar findings were observed in citrinin toxicity in broilers by Uma and Vikram Reddy (1995), Gurunath Reddy (2004) and Priyadarshini (2005).

5.4.3 Spleen

The sections of spleen from birds fed on diet 2 showed mild depletion of germinal centers (Plate 15) and mild congestion of trabecular arteries. This is in accordance with Roberts and Mora (1978), Mehdi *et al*. (1981) and Gurunath Reddy (2004) in broilers fed with citrinin.

Microscopically the spleen from diet 3 birds showed very mild depletion of germinal centers (Plate 16) indicating that activated charcoal was partially effective in alleviating the effects of citrinin and fumonisin.

Very mild depletion of germinal centers and sub-capsular hemorrhages (Plate 17) in spleen of birds on diet 4 indicated that activated charcoal and lyophilized yeast culture reduced the toxic effect of citrinin and fumonisin on spleen to a greater extent when compared to activated charcoal alone. This results are in accordance with Uma and Vikram Reddy (1995), Gurunath Reddy (2004) and Priyadarshini (2005) in broilers fed with citrinin alone.

5.4.4 Bursa of Fabricius

Microscopic sections of the Bursa of Fabricius from diet 2 fed birds revealed that few follicles showing cystic spaces (Plate 18) and cystic spaces in the follicular epithelium were seen in some areas. Marked depletion of lymphoid follicles (Plate 19) were seen. Similar observations were noticed in broilers fed with citrinin by Mehdi *et al*. (1981), Ahamad and Vairamuthu (2001), Uma and Vikram Reddy (1995),

Gurunath Reddy (2004) and Priyadarshini (2005). Similar findings were reported in broilers fed with fumonisin by Piramanayagam *et al*. (2002 b), Sateesh *et al*., (2005) and Jayasri (2006).

The sections of Bursa of Fabricius from birds fed on diet 3 showed marked depletion of follicles and few follicles showing washed out appearance (Plate 20), indicating that activated charcoal had partial ameliorative effect on citrinin and fumonisin.

The microscopic sections of Bursa of Fabricius from diet 4 fed birds showed very mild depletion of follicles and few follicles showing tiny cystic spaces, indicating that activated charcoal and lyophilized yeast culture could protecting the Bursa of Fabricius from citrinin and fumonisin.

5.4.5 Heart

Microscopic picture of heart from birds fed on diet 2 recorded that mild inter fibrillar hemorrhages and marked disruption of cardiac muscle fibers (Plate 21). This is in accordance with results of Ahamad and Vairamuthu (2001) and Gurunath Reddy (2004) in citrinin toxic birds. Similar findings were reported in fumonisin toxic birds by Ledoux *et al*. (1996), Brown *et al*. (1992), and Sateesh *et al*. (2005).

The heart sections from diet 3 fed birds showed lymphocytic infiltration between cardiac muscle fibers with myocardial disruption, indicating that activated charcoal could partially ameliorate the combined toxic effect of citrinin and fumonisin.

Microscopic sections of heart from diet 4 fed birds showed very mild hemorrhages between cardiac muscle fibers with very few fibers showing disrupted cardiac muscle fibers. It was clearly indicating that activated charcoal and lyophilized yeast culture could ameliorate the toxic effects with a greater extent. The above results are in accordance with Gurunath Reddy (2004) and Priyadarshini (2005) in broilers fed with citrinin. Similar findings were reported in fumonisin toxic birds, by Jayasri (2006).

5.4.6 Intestines

The microscopic sections of intestines from diet 2 fed birds revealed that disruption of villus epithelium (Plate 22) and some areas showing sub-mucosal hemorrhages. The results were in agreement with the report of Uma and Vikram Reddy (1995) and Gurunath Reddy (2004) in citrinin toxicity. Similar findings were noticed in fumonisin toxic bird by Brown *et al*. (1992), Piramanayagam *et al*. (2002 b), Sateesh *et al*. (2005) and Jayasri (2006).

Microscopic picture of intestines from diet 3 fed birds showed mild sub-mucosal hemorrhage between villi and few villi showing disruption, indicating that activated charcoal could partially ameliorate the toxic effects of citrinin and fumonisin.

While microscopic sections of intestines from diet 4 fed birds did not revealed any microscopic lesions of pathological significance as like that of birds fed on diet 1 (control group), indicating that the activated charcoal and lyophilized yeast culture could completely ameliorate the toxic effects of citrinin and fumonisin.

The results of this study indicate that combination of citrinin (50 ppm) and fumonisin (10 ppm) showed deleterious effect on growth, biochemical profile and marked to moderate damage to vital organs. Activated charcoal (0.4%) showed only partial ameliorative effect on combined toxicity of citrinin and fumonisin in broilers. Combination of activated charcoal (0.4%) and yeast culture (0.1%) showed better ameliorative effect due to complementary effect than activated charcoal alone on combined toxicity of citrinin and fumonisin.

CHAPTER VI

SUMMARY

Citrinin and fumonisin are frequent contaminants of feed ingredients, which are highly hepatotoxic and nephrotoxic agents that adversely affect the metabolism, depress the body growth and feed consumption, resulting in poor performance of the broilers. Hence, in the present study, an attempt was made to study the combined effect of dietary citrinin and fumonisin on serum biochemical profile in broilers and their amelioration by using activated charcoal and/or lyophilized yeast culture as adsorbents. Four experimental diets were prepared: diet: 1 – basal diet, diet: 2 – basal diet + citrinin (50 ppm) + fumonisin (10 ppm), diet: 3 – basal diet + citrinin (50 ppm) + fumonisin (10 ppm) + activated charcoal (0.4 %) and diet: 4 – basal diet + citrinin (50 ppm) + fumonisin (10 ppm) + activated charcoal (0.4 %) + lyophilized yeast culture (0.1 %). These diets were tested on 128 broiler chicks. They were randomly distributed into 4 dietary treatments of four replicates in each treatment. Each replicate included 8 birds. The chicks were allotted to different experimental diets at random in a completely randomised design. The effect of citrinin and fumonisin on growth, feed consumption, FCR, serum biochemical profile and vital

organs in broilers and the amelioration of the toxic effects by the two adsorbents, activated charcoal and lyophilized yeast culture, were studied.

Citrinin and fumonisin were produced from cultures of *Pencillium citrinum* and *Fusarium moniliforme*, respectively on selected medium. The amount of citrinin and fumonisin in the pure culture material were measured by TLC. The culture material of citrinin and fumonisin were mixed with poultry diet, levels being maintained at the rate of 50 ppm and 10 ppm, respectively (diet 2). The toxin treated diet was added with activated charcoal (0.4 %) in diet 3 and with activated charcoal (0.4 %) and lyophilized yeast culture (0.1 %) in diet 4.

The weekly body weight gains and feed consumption revealed that feeding of citrinin and fumonisin reduce the body weight gains and feed consumption. A slight increase in body weight gain and feed consumption was observed in birds fed on diet 3 due to adsorption of toxins by activated charcoal. The chicks fed on diet 4 containing citrinin, fumonisin, activated charcoal and lyophilized yeast culture showed improvement in body weight gain and feed consumption due to growth promoting activities of lyophilized yeast culture and complementary effect on adsorbent capacity of activated charcoal.

The feed conversion ratio on diet 2 containing citrinin and fumonisin were high compared to other diets due to toxicity stress. The FCR on diet 3 decreased compared to diet 2 indicating the feed conversion efficiency was improved by activated charcoal. The FCR on diet 4 was significantly lower but numerically nearer to that on diet 1 indicating that activated charcoal and lyophilized yeast culture could ameliorate the effect of citrinin and fumonisin to a greater extent.

A significantly decreased serum total proteins and albumins in citrinin and fumonisin fed chicks indicated the impaired protein synthesis. There was improvement in the concentration of serum total proteins, albumins on diet 3 containing citrinin and fumonisin and activated charcoal. The diet 4 containing activated charcoal and lyophilized yeast culture alleviated the depressant effect of citrinin and fumonisin on serum proteins to a greater extent.

Hypoglycaemia and hypocholesterolemia were observed in diet 2 and diet 3. But the chicks fed on diet 4 showed glucose and cholesterol levels nearer to that of the control diet indicating the efficiency of activated charcoal and lyophilized yeast culture in diet. The serum bilirubin levels were increased in diet 2 birds. They were also brought to normal with the inclusion of activated charcoal and lyophilized yeast culture in diet 4.

Serum uric acid values decreased on diet 2 due to hepatotoxicity were partially improved by activated charcoal. Serum uric acid levels further improved by inclusion of activated charcoal and lyophilized yeast culture. The increase of serum creatinine and blood urea nitrogen due to toxicity of citrinin and fumonisin were partially alleviated by inclusion of activated charcoal. Serum creatinine and blood urea nitrogen levels were nearer to normal in birds on diet 4 indicating that activated charcoal and lyophilized yeast culture could completely ameliorate the effects of toxins (citrinin and fumonisin).

Decrease in serum calcium levels were observed in citrinin and fumonisin containing diet due to alteration in calcium metabolism in kidney and intestine. The effects were partially alleviated in birds on diet 3 indicating that activated charcoal was not effective in protecting

the effects of citrinin and fumonisin toxicity. In birds on diet 4 the calcium levels were within the normal range indicating that activated charcoal and lyophilized yeast culture ameliorated the effects of citrinin and fumonisin.

Serum enzymes like AST, ALT and ALP activities were increased in diet 2 indicating severe hepatocellular damage. Very high levels of GGT observed in livers of broilers fed on citrinin and fumonisin diet, further confirming liver damage caused by diet 2. The serum activity of all these enzymes in birds fed on diet 3 were decreased than on diet 2 indicating that activated charcoal partially protected liver from damage caused by citrinin and fumonisin. The serum enzyme activities in birds on diet 4 were well within the normal range and were nearer to control diet indicating that activated charcoal and lyophilized yeast culture could ameliorate the toxicity of citrinin and fumonisin to a larger extent.

The pathological studies at the end of the experimental period, showed livers with pale, enlarged and rounded borders. Kidneys were enlarged and hemorrhagic. Moderately enlarged and hyperaemic bursa of Fabricius was seen in birds fed on diet 2. The lesions were moderate in diet 3 birds and very mild in diet 4 fed birds.

The histopathology studies revealed central vein congestion with focal lymphoid aggregates and bile duct hyperplasia in the liver. Degenerative changes with inter tubular congestion and hemorrhages were seen in kidney. Cystic spaces in bursa of Fabricius and intertrabecular arteries in spleen were seen in birds fed on diet 2. In the birds on diet 3 the lesions were of moderate degree and in birds on diet 4 the lesions were very mild.

Based on the above observations, it is concluded that activated charcoal could partially bind to citrinin and fumonisin and thus reduced the bio-availability of the toxins whereas activated charcoal and lyophilized yeast culture efficiently ameliorated and protected the chicks from citrinin and fumonisin at the experimental level studied.

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Table 6: Mean values of Serum total protein (g%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	3.14 ± 0.04	4.41 ± 0.36	5.27 ± 0.31	**4.27^{a} ± 0.30**
2	2.25 ± 0.11	2.98 ± 0.19	1.85 ± 0.15	**2.36^{d} ± 0.16**
3	2.80 ± 0.13	3.31 ± 0.09	2.43 ± 0.01	**2.84^{c} ± 0.12**
4	2.92 ± 0.24	3.41 ± 0.11	4.45 ± 0.10	**3.59^{b} ± 0.21**
Overall Mean ± SE	**2.77^{b} ± 0.10**	**3.52^{a} ± 0.16**	**3.50^{a} ± 0.38**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum total protein

Source of variation	DF	MSS	F
Periods	2	2.89	27.51**
Diets	3	8.47	80.57**
Periods x Diets	6	2.07	19.76**
Error	36	0.10	
Total	47	47.46	

** Significant (P < 0.01) C.D periods: 0.31 C.D diets: 0.36

Table 7: Mean values of Serum albumin (g%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	1.89 ± 0.16	2.24 ± 0.07	2.15 ± 0.02	**2.09[a] ± 0.06**
2	1.04 ± 0.08	1.21 ± 0.05	0.64 ± 0.05	**0.96[d] ± 0.08**
3	1.39 ± 0.09	1.27 ± 0.04	1.31 ± 0.17	**1.32[c] ± 0.05**
4	1.62 ± 0.01	1.91 ± 0.04	1.90 ± 0.03	**1.81[b] ± 0.04**
Overall Mean ± SE	**1.48[b] ± 0.09**	**1.65[a] ± 0.11**	**1.50[b] ± 0.16**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per Treatment.

Analysis of variance on Serum albumins

Source of variation	DF	MSS	F

Periods	2	0.14	6.42**
Diets	3	3.02	132.27**
Periods x Diets	6	0.15	6.59**
Error	36	0.02	
Total	47	11.11	

** Significant (P < 0.01) C.D periods: 0.14 C.D diets: 0.16

Table 8: Mean values of Serum globulin (g%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall
	14	28	42	Mean ± SE
1	1.25 ± 0.19	2.17 ± 0.37	3.12 ± 0.28	**2.18[a] ± 0.27**
2	1.21 ± 0.19	1.76 ± 0.16	1.21 ± 0.20	**1.39[b] ± 0.12**
3	1.41 ± 0.12	2.04 ± 0.10	1.12 ± 0.17	**1.52[b] ± 0.13**
4	1.30 ± 0.25	1.50 ± 0.07	2.55 ± 0.07	**1.78[b] ± 0.01**

Overall Mean ± SE	**1.29^{b} ± 0.08**	**1.86^{a} ± 0.10**	**2.00^{a} ± 0.24**	

a-b Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum globulins

Source of variation	**DF**	**MSS**	**F**
Periods	2	2.26	18.09**
Diets	3	1.43	11.47**
Periods x Diets	6	1.44	11.52**
Error	36	0.12	
Total	47	22.02	

****** Significant (P < 0.01) C.D periods: 0.34 C.D diets: 0.39

Table 9: Mean values of Serum A/G Ratio as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	1.64 ± 0.36	1.12 ± 0.23	0.70 ± 0.06	**1.15[a] ± 0.17**
2	0.95 ± 0.25	0.69 ± 0.04	0.59 ± 0.17	**0.74[a] ± 0.09**
3	1.01 ± 0.12	0.62 ± 0.04	1.31 ± 0.42	**0.98[a] ± 0.15**
4	1.36 ± 0.27	1.27 ± 0.04	0.74 ± 0.01	**1.12[a] ± 0.11**
Overall Mean ± SE	**1.24[a] ± 0.13**	**0.93[a] ± 0.08**	**0.83[a] ± 0.11**	

a Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum A/G Ratio

Source of variation	DF	MSS	F
Periods	2	0.71	5.04*
Diets	3	0.42	2.96*
Periods x Diets	6	0.41	2.89*
Error	36	0.14	
Total	47	10.28	

****** Significant (P < 0.01) C.D periods: 0.36 C.D diets: 0.41

Table 19: Mean values of Serum ALT (units/ml) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	16.12 ± 0.63	17.49 ± 0.65	19.65 ± 1.32	**17.75^{b} ± 0.63**
2	19.56 ± 1.76	21.56 ± 0.28	24.52 ± 1.37	**21.88^{a} ± 0.88**
3	17.62 ± 0.59	16.18 ± 1.78	19.61 ± 1.50	**17.80^{b} ± 0.79**
4	12.45 ± 0.50	13.16 ± 0.52	20.29 ± 0.79	**15.30^{c} ± 1.15**
Overall Mean ± SE	**16.43^{b} ± 0.80**	**17.10^{b} ± 0.89**	**21.01^{a} ± 0.74**	

a-c Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum ALT

Source of variation	DF	MSS	F

Periods	2	98.07	26.84**
Diets	3	89.21	24.41**
Periods x Diets	6	8.84	2.42*
Error	36	3.65	
Total	47	648.44	

****** Significant (P < 0.01) C.D periods: 1.83 C.D diets: 2.12

Table 18: Mean values of Serum AST (units/ml) as affected by different experimental diets and periods of collection

Diet	**Periods (Days)**			**Overall Mean ± SE**
	14	**28**	**42**	
1	51.55 ± 1.68	57.62 ± 1.68	65.18 ± 0.94	**58.11[b] ± 1.89**
2	58.74 ± 0.66	62.45 ± 2.19	75.62 ± 1.73	**65.60[a] ± 2.41**

3	53.50 ± 1.39	60.62 ± 1.02	68.12 ± 0.95	**60.74^{b} ± 1.95**
4	55.12 ± 0.94	58.13 ± 0.88	59.65 ± 1.82	**57.63^{b} ± 0.85**
Overall Mean ± SE	**54.72^{c} ± 0.86**	**59.70^{b} ± 0.80**	**67.14^{a} ± 1.63**	

a-c Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum AST

Source of variation	**DF**	**MSS**	**F**
Periods	2	624.66	105.27**
Diets	3	159.92	26.95**
Periods x Diets	6	37.28	6.28**
Error	36	5.93	
Total	47	2166.45	

****** Significant (P < 0.01) C.D periods: 2.34 C.D diets: 2.70

Table 11: Mean values of Serum total cholesterol (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	160.18 ± 1.09	183.57 ± 1.40	194.82 ± 0.65	**179.52^{a} ± 4.57**
2	101.32 ± 1.71	85.85 ± 1.25	75.18 ± 0.74	**87.45^{d} ± 3.43**
3	116.72 ± 1.80	95.18 ± 1.67	107.14 ± 1.59	**106.34^{c} ± 2.88**
4	123.09 ± 1.33	103.24 ± 1.41	120.51 ± 2.26	**115.61^{b} ± 2.89**
Overall Mean ± SE	**125.32^{a} ± 5.79**	**116.96^{b} ± 10.40**	**124.41^{a} ± 11.71**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum total cholesterol

Source of variation	DF	MSS	F
Periods	2	336.75	51.45**
Diets	3	19152.18	2926.16**
Periods x Diets	6	844.97	129.09**
Error	36	6.54	
Total	47	63435.50	

****** Significant (P < 0.01) C.D periods: 2.46 C.D diets: 2.84

Table 12: Mean values of Serum triglycerides (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	100.16 ± 1.94	110.23 ± 0.44	126.20 ± 1.66	**112.20^{a} ± 3.44**
2	82.12 ± 1.45	98.46 ± 1.83	98.65 ± 0.74	**94.08^{d} ± 2.10**
3	91.23 ± 0.54	102.12 ± 1.16	103.55 ± 0.76	**98.96^{c} ± 1.78**
4	91.46 ± 1.12	105.48 ± 1.61	113.48 ± 0.98	**103.47^{b} ± 2.92**
Overall Mean ± SE	**91.99^{c} ± 1.53**	**104.07^{b} ± 1.28**	**110.47^{a} ± 2.84**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum triglycerides

Source of variation	DF	MSS	F
Periods	2	1408.59	283.34**
Diets	3	711.87	143.19**
Periods x Diets	6	66.76	13.42**
Error	36	4.97	

Total	47	5532.25	

** Significant (P < 0.01) C.D periods: 2.14 C.D diets: 2.47

Table 10: Mean values of Serum glucose (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall
	14	28	42	Mean ± SE
1	138.24 ± 4.51	153.74 ± 4.25	195.65 ± 4.11	**162.54^{a} ± 7.90**
2	95.12 ± 3.38	113.06 ± 7.71	139.70 ± 4.22	**115.96^{d} ± 6.31**
3	109.40 ± 6.78	135.62 ± 5.37	156.08 ± 8.28	**133.70^{c} ± 6.84**
4	119.16 ± 5.51	142.11 ± 5.10	173.12 ± 6.17	**144.80^{b} ± 7.45**
Overall Mean ± SE	**115.48^{c} ± 4.67**	**136.13^{b} ± 4.57**	**166.14^{a} ± 6.02**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum glucose

Source of variation	DF	MSS	F
Periods	2	10382.06	108.58**
Diets	3	4587.18	47.97**
Periods x Diets	6	94.36	0.98 NS
Error	36	95.61	
Total	47	38534.00	

****** Significant (P < 0.01) C.D periods: 9.40 C.D diets: 10.85

Table 13: Mean values of Serum bilirubin (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	2.25 ± 0.14	2.85 ± 0.21	3.01 ± 0.10	**2.70^{b} ± 0.12**
2	2.67 ± 0.17	3.14 ± 0.03	3.36 ± 0.05	**3.05^{a} ± 0.10**
3	2.45 ± 0.08	2.93 ± 0.11	3.01 ± 0.06	**2.79^{b} ± 0.08**
4	2.12 ± 0.07	2.89 ± 0.05	2.98 ± 0.22	**2.66^{b} ± 0.13**
Overall Mean ± SE	**2.37^{b} ± 0.07**	**2.95^{a} ± 0.05**	**3.09^{a} ± 0.06**	

a-b Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum bilirubin

Source of variation	DF	MSS	F
Periods	2	2.32	46.91**
Diets	3	0.37	7.58**
Periods x Diets	6	0.02	0.53 NS
Error	36	0.04	
Total	47	7.70	

****** Significant (P < 0.01) C.D periods: 0.21 C.D diets: 0.24

Table 15: Mean values of Serum BUN (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	6.28 ± 0.05	6.35 ± 0.30	6.41 ± 0.11	**6.34^{c} ± 0.09**

2	6.98 ± 0.24	7.02 ± 0.20	7.10 ± 0.29	**7.03^{a} ± 0.11**
3	6.52 ± 0.15	6.51 ± 0.11	6.49 ± 0.13	**6.50^{b} ± 0.06**
4	6.36 ± 0.67	6.42 ± 0.29	6.43 ± 0.17	**6.40^{c} ± 0.20**
Overall Mean ± SE	**6.53^{c} ± 0.16**	**6.57^{b} ± 0.12**	**6.60^{a} ± 0.10**	

a-c Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum BUN

Source of variation	**DF**	**MSS**	**F**
Periods	2	0.02	0.09 **
Diets	3	1.18	5.14**
Periods x Diets	6	0.00	0.02 **
Error	36	0.23	
Total	47	11.92	

****** Significant at 1% CD periods: 0.03 C.D diets: 0.10

Table 16: Mean values of Serum creatinine (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	0.62 ± 0.06	0.74 ± 0.04	0.79 ± 0.02	**0.71^{c} ± 0.03**
2	0.71 ± 004	0.79 ± 0.02	0.81 ± 0.03	**0.77^{a} ± 0.02**
3	0.74 ± 0.05	0.77 ± 0.04	0.75 ± 0.04	**0.75^{b} ± 0.02**
4	0.68 ± 0.03	0.72 ± 0.03	0.71 ± 0.02	**0.70^{d} ± 0.01**
Overall Mean ± SE	**0.68^{c} ± 0.02**	**0.75^{b} ± 0.01**	**0.76^{a} ± 0.01**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per

treatment.

Analysis of variance on Serum creatinine

Source of variation	DF	MSS	F
Periods	2	0.02	5.39**
Diets	3	0.01	2.20 **
Periods x Diets	6	0.00	1.01 **
Error	36	0.00	
Total	47	0.31	

****** Significant (P < 0.01) C.D periods: 0.06 C.D diets: 0.01

Table 21: Mean values of Serum GGT (units/litre) as affected by different

experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	6.32 ± 0.41	9.69 ± 0.95	14.90 ± 0.75	**10.30^{c} ± 1.16**
2	12.39 ± 0.36	20.36 ± 1.00	29.42 ± 1.66	**20.72^{a} ± 2.25**
3	9.92 ± 0.17	11.98 ± 0.35	18.36 ± 1.20	**13.42^{b} ± 1.18**
4	6.92 ± 0.05	7.92 ± 0.28	8.01 ± 0.06	**7.61^{d} ± 0.17**
Overall Mean ± SE	**8.88^{c} ± 0.66**	**12.48^{b} ± 1.30**	**17.67^{a} ± 2.10**	

a-d Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum GGT

Source of variation	DF	MSS	F
Periods	2	312.05	172.39**
Diets	3	384.31	212.31**

Periods x Diets	6	44.00	24.31**
Error	36	1.81	
Total	47	2106.25	

****** Significant (P < 0.01) C.D periods: 1.29 C.D diets: 1.49

Table 20: Mean values of Serum ALP (KA units) as affected by different experimental diets and periods of collection

Diet	**Periods (Days)**			**Overall Mean ± SE**
	14	**28**	**42**	
1	19.56 ± 1.11	25.45 ± 1.52	28.82 ± 0.59	**24.61^{c} ± 1.32**
2	21.67 ± 2.211	47.12 ± 1.27	57.85 ± 2.59	**42.21^{a} ± 4.88**
3	19.71 ± 1.58	38.26 ± 2.46	30.98 ± 1.37	**29.650^{b} ± 2.56**
4	21.08 ± 1.75	37.52 ± 0.85	28.15 ± 3.60	**28.91^{b} ± 2.39**

Overall Mean ± SE	**20.50^{b} ± 0.72**	**37.52^{a} ± 0.85**	**36.45^{a} ± 3.43**	

a-c Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum ALP

Source of variation	DF	MSS	F
Periods	2	1412.19	127.09**
Diets	3	688.98	62.00**
Periods x Diets	6	226.09	20.34**
Error	36	11.11	-
Total	47	6647.92	-

****** Significant (P < 0.01) C.D periods: 3.20 C.D diets: 3.70

Table 17: Mean values of Serum calcium (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	8.33 ± 0.14	8.62 ± 0.37	9.95 ± 0.34	**8.96^{a} ± 0.26**
2	7.24 ± 0.48	5.91 ± 0.50	7.30 ± 0.12	**6.81^{b} ± 0.27**
3	8.36 ± 0.34	7.12 ± 0.16	8.46 ± 0.55	**7.98^{a} ± 0.26**
4	8.41 ± 0.08	7.61 ± 0.09	9.13 ± 0.02	**8.38^{a} ± 0.19**
Overall Mean ± SE	**8.08^{b} ± 0.18**	**7.31^{c} ± 0.29**	**8.71^{a} ± 0.29**	

a-c Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum calcium

Source of variation	DF	MSS	F
Periods	2	7.79	24.96**
Diets	3	9.88	31.65**
Periods x Diets	6	0.72	2.31 NS
Error	36	0.31	-
Total	47	60.85	-

****** Significant (P < 0.01) C.D periods: 0.53 C.D diets: 0.62

Table 14: Mean values of Serum uric acid (mg%) as affected by different experimental diets and periods of collection

Diet	Periods (Days)			Overall Mean ± SE
	14	28	42	
1	5.42 ± 0.30	6.01 ± 0.29	6.95 ± 0.42	**6.12[a] ± 0.25**
2	5.60 ± 0.37	4.35 ± 0.23	4.65 ± 0.22	**4.86[c] ± 0.21**
3	5.09 ± 0.14	5.30 ± 0.35	5.54 ± 0.31	**5.31[b] ± 0.14**

4	5.35 ± 0.22	5.37 ± 0.31	5.83 ± 0.46	**5.51^{b} ± 0.17**
Overall Mean ± SE	**5.36^{b} ± 0.12**	**5.26^{c} ± 0.19**	**5.74^{a} ± 0.26**	

a-b Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Serum uric acid

Source of variation	**DF**	**MSS**	**F**
Periods	2	1.02	3.39*
Diets	3	3.29	10.86**
Periods x Diets	6	1.17	3.88**
Error	36	0.30	-
Total	47	29.92	-

****** Significant (P < 0.01) C.D periods: 0.20 C.D diets: 0.47

Table 3: Mean values of Weekly body weight gains (g) in broilers as affected by different experimental diets and periods.

Period weeks	Experimental diets				Overall mean ± S.E.
	1	2	3	4	
1	76.18 ± 1.09	63.84 ±1.15	69.65 ± 3.77	73.37 ± 1.28	**70.76^{f} ± 1.12**
2	143.84 ± 5.55	109.84 ± 4.32	116.84 ± 6.14	119.65 ± 5.86	**122.54^{e} ± 2.92**
3	239.28 ± 8.06	169.03 ± 11.35	202.68 ± 8.28	210.12 ± 7.73	**205.28^{d} ± 4.91**
4	425.21 ± 13.15	347.46 ± 12.42	380.68 ± 10.07	399.18 ± 12.56	**388.14^{c} ± 6.42**
5	475.46 ± 14.11	432.84 ± 12.03	444.68 ± 20.04	463.84 ± 25.50	**454.21^{b} ± 9.24**
6	596.31 ± 38.27	478.43 ± 49.38	530.12 ± 32.29	551.53 ± 30.55	**539.10^{a} ± 19.09**
Over all mean ± S. E.	**326.05^{a} ± 15.28**	**266.91^{c} ± 14.53**	**290.78^{bc} ± 14.03**	**302.95^{ab} ± 14.72**	

a-f Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Weekly body weight gains (g)

Source of variation	DF	MSS	F
Diets	3	4650563.00	432.42**
Periods	5	116672.00	10.84**
Diets x Periods	15	8085.36	0.75**
Error	744	10754.51	

Total	767	31725480.00	

**Significant (P<0.01) C.D periods: 33.39 C.D diets: 27.26

Table 4: Mean values of Weekly feed consumption (g) in broilers as affected by different experimental diets and periods.

Period weeks	Experimental diets				Overall mean ± S.E.
	1	2	3	4	
1	416.37 ± 6.63	393.12 ± 8.22	397.50 ± 7.11	406.50 ± 15.58	**403.37^{e} ± 4.82**
2	1220.37 ± 8.63	952.87 ± 11.46	1005.12 ± 29.06	1025.00 ±29.72	**1050.84^{d} ± 20.99**
3	2023.37 ± 59.25	1508.62 ± 27.19	1750.37 ± 28.13	1807.37 ± 34.08	**1772.43^{c} ± 37.87**
4	2864.87 ± 85.23	2756.75 ± 56.70	2690.50 ± 22.63	2730.00 ± 11.97	**2760.53^{b} ± 26.59**
5	3254.62 ± 56.48	3221.87 ± 26.95	3128.50 ± 36.82	3217.37 ± 61.79	**3205.59^{a} ± 23.16**
6	3007.75 ± 76.77	2628.12 ± 57.49	2798.12 ± 48.76	2798.25 ± 34.89	**2808.06^{b} ± 35.43**
Over all mean ± S. E.	**2131.22^{a} ± 153.10**	**1910.22^{d} ± 152.02**	**1961.68^{c} ± 147.83**	**1997.41^{b} ± 150.14**	

a-e Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on feed consumption

Source of variation	DF	MSS	F
Diets	3	39595020.00	3265.58**
Periods	5	428060.46	35.30**
Diets x Periods	15	60320.70	4.97**
Error	168	12124.95	
Total	191	202201088.00	

**Significant (P<0.01) C.D periods: 70.91 C.D diets: 0.36

Table 5: Mean values of Weekly feed conversion ratio in broilers as affected by different experimental diets and periods.

Period weeks	Experimental diets				Overall mean ± S.E.
	1	2	3	4	

1	1.36 ± 0.03	1.54 ± 0.07	1.42 ± 0.04	1.38 ± 0.09	**1.42^{c} ± 0.03**
2	2.12 ± 0.09	2.16 ± 0.10	2.15 ± 0.11	2.15 ± 0.06	**2.14^{a} ± 0.04**
3	2.11 ± 0.09	2.23 ± 0.08	2.15 ± 0.10	2.15 ± 0.07	**2.16^{a} ± 0.04**
4	1.68 ± 0.05	1.98 ± 0.06	1.76 ± 0.07	1.71± 0.14	**1.78^{b} ± 0.04**
5	1.71 ± 0.09	1.86 ± 0.07	1.75 ± 0.07	1.72 ± 0.07	**1.76^{b} ± 0.03**
6	1.26 ± 0.08	1.37 ± 0.12	1.32 ± 0.14	1.26 ± 0.13	**1.30^{c} ± 0.05**
Over all mean ± S. E.	**1.70^{b} ± 0.05**	**1.86^{a} ± 0.05**	**1.76^{ab} ± 0.05**	**1.73^{b} ± 0.06**	

a-c Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on feed conversion ratio

Source of variation	DF	MSS	F
Diets	3	4.03	67.23**
Periods	5	0.21	3.52*
Diets x Periods	15	0.01	0.23 NS
Error	168	0.05	

Total	191	31.06	

**Significant (P<0.01) C.D periods: 0.15 C.D diets: 0.12

Table 2: Mean values of Weekly body weights (g) in broilers as affected by different experimental diets and periods.

Period weeks	Experimental diets				Overall mean ± S.E.
	1	2	3	4	
1	42.03 ± 0.58	41.56 ±0.46	42.37± 0.42	42.81 ± 0.34	**42.19^{g} ± 1.12**
2	118.21 ± 0.96	105.40 ± 1.02	112.03 ± 3.86	116.18 ± 1.26	**122.96^{f} ± 2.92**
3	262.06 ± 5.63	215.25 ± 4.24	228.87 ± 4.53	235.84 ±5.83	**235.50^{e} ± 4.91**
4	501.34 ± 8.76	384.28 ± 10.52	431.56 ± 8.63	445.96 ± 6.52	**440.78^{d} ± 6.42**
5	926.56 ± 7.92	731.75 ± 4.89	812.25 ± 10.35	845.15 ± 10.49	**828.93^{c} ± 9.24**
6	1402.03 ±11.63	1164.59 ± 11.01	1256.93 ± 19.13	1309.00 ± 23.39	**1283.14^{b} ± 19.09**
7	1998.34 ± 36.72	1643.03 ± 50.24	1787.06 ± 27.56	1860.53 ± 26.32	**1822.24^{a} ± 21.16**

Over all mean ± S. E.	**750.08[a] ± 45. 83**	**612.26[d] ± 38.11**	**667.29[c] ± 40.97**	**693.64[b] ± 42.70**		

a-g Means with in rows and columns with no common superscripts differ significantly.

Values represent the X ± Standard error of four replicates of 8 broilers each per treatment.

Analysis of variance on Weekly body weight gains (g)

Source of variation	DF	MSS	F
Diets	3	57040948.00	7119.16**
Periods	6	735036.93	91.73**
Diets x Periods	18	96373.84	12.02**
Error	868	8012.31	
Total	895	353140224.00	

**Significant (P<0.01) C.D periods: 28.82 C.D diets: 21.78

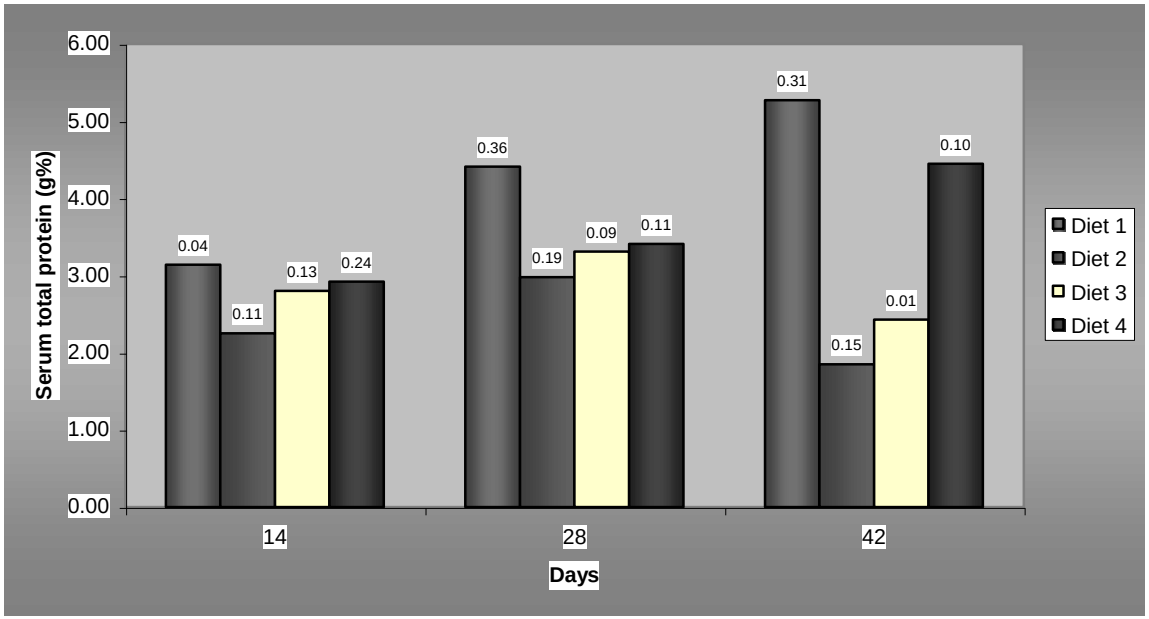


Fig:7 Mean values of Serum total protein (g%) in broilers as affected by different experimental diets and periods of collection.

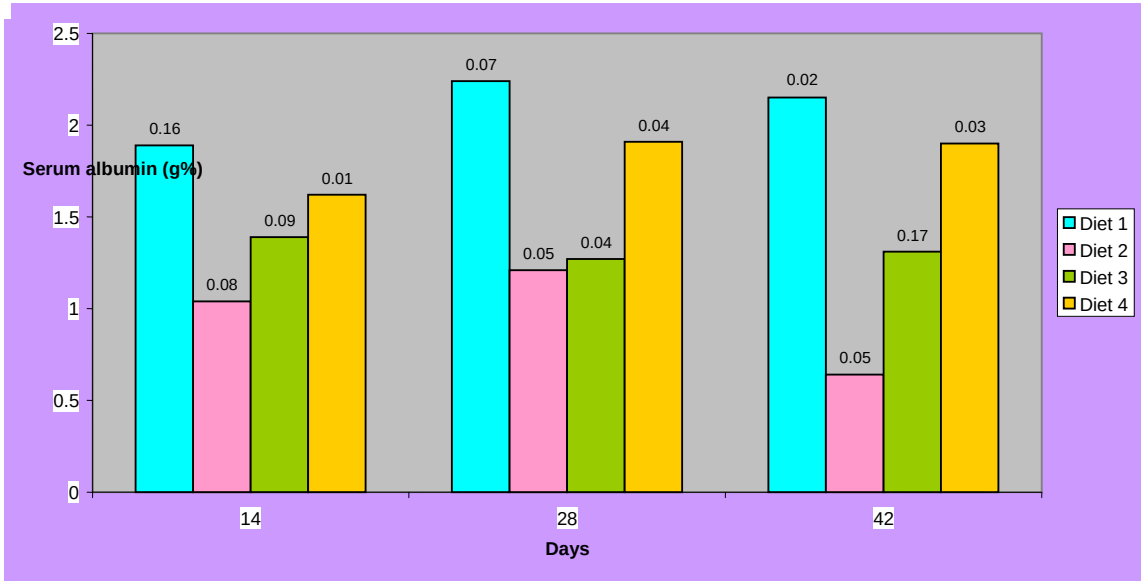


Fig:8 Mean values of Serum albumins (g%) in broilers as affected by different experimental diets and periods of collection.

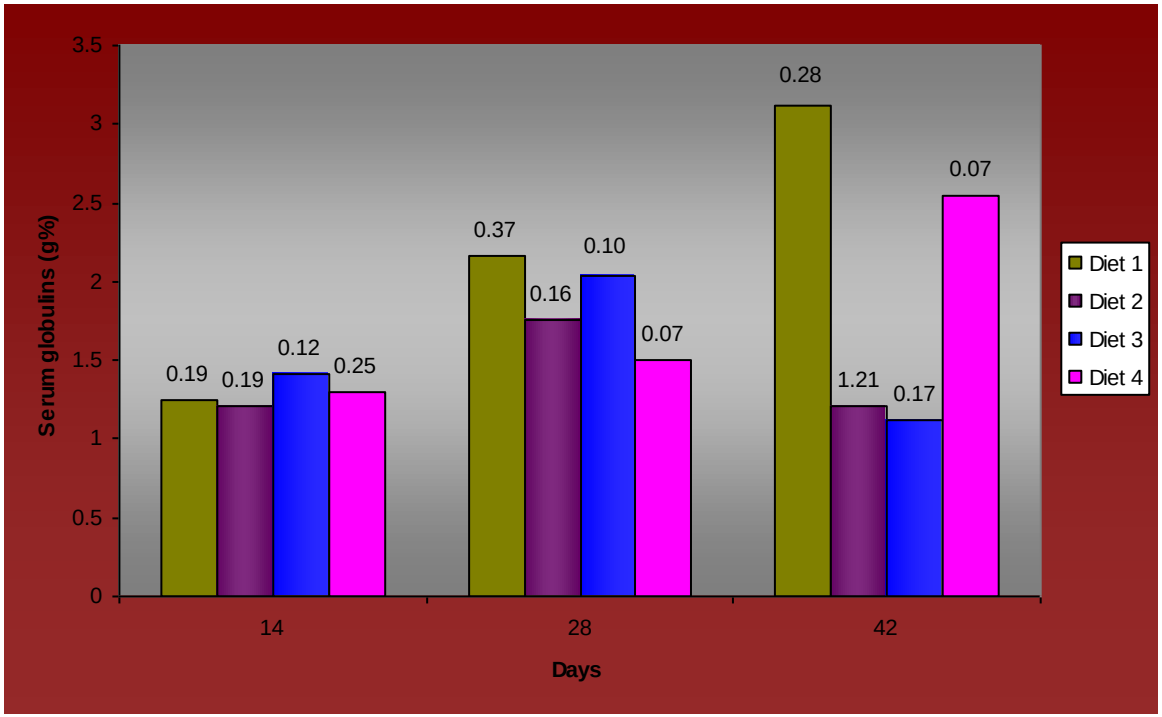


Fig:9 Mean values of Serum globulins (g%) in broilers as affected by different experimental diets and periods of collection.

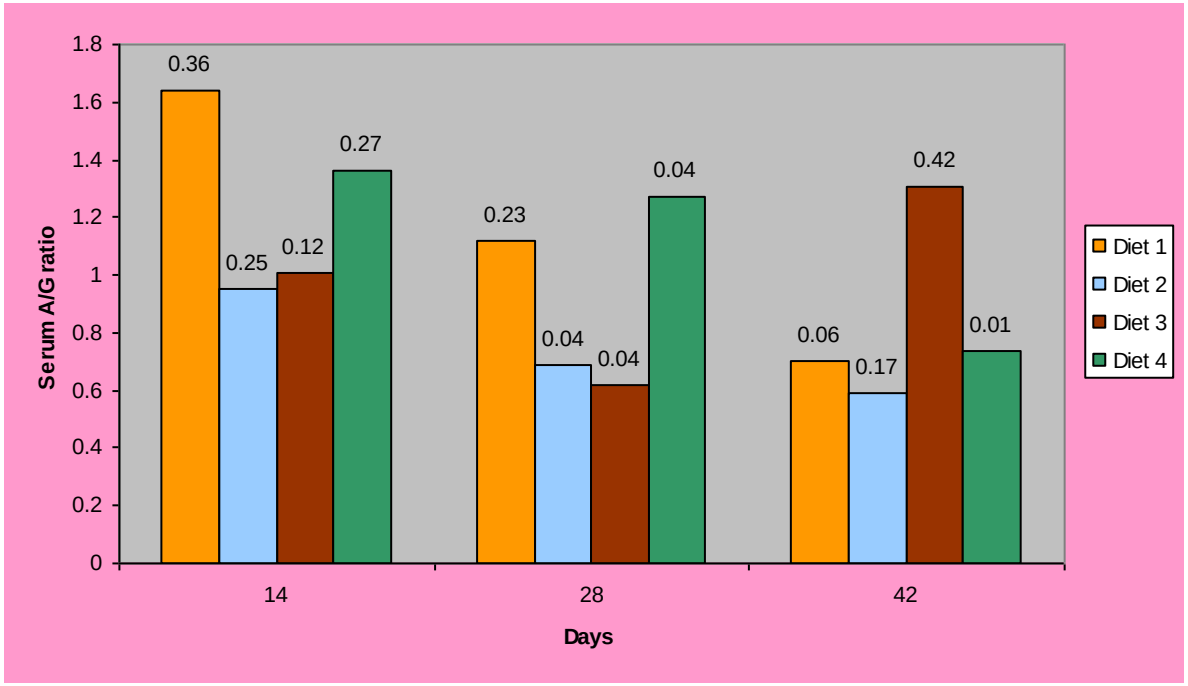


Fig:10 Mean values of Serum A/G ratio in broilers as affected by different experimental diets and periods of collection.

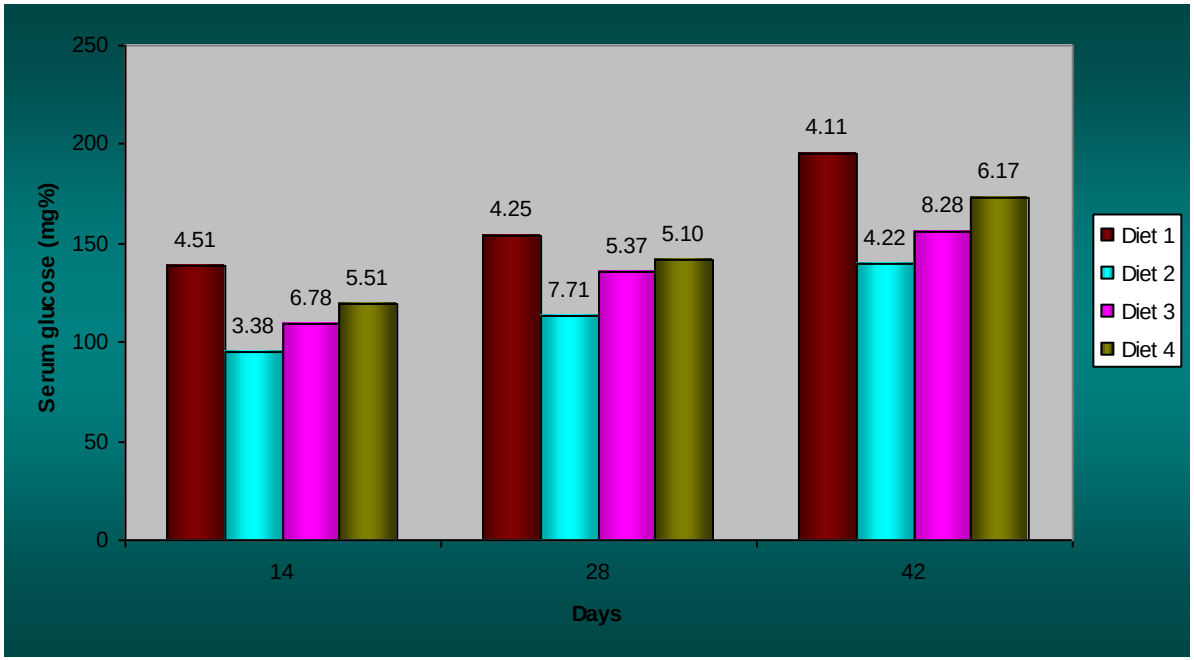


Fig:11 Mean values of Serum glucose (mg%) in broilers as affected by different experimental diets and periods of collection.

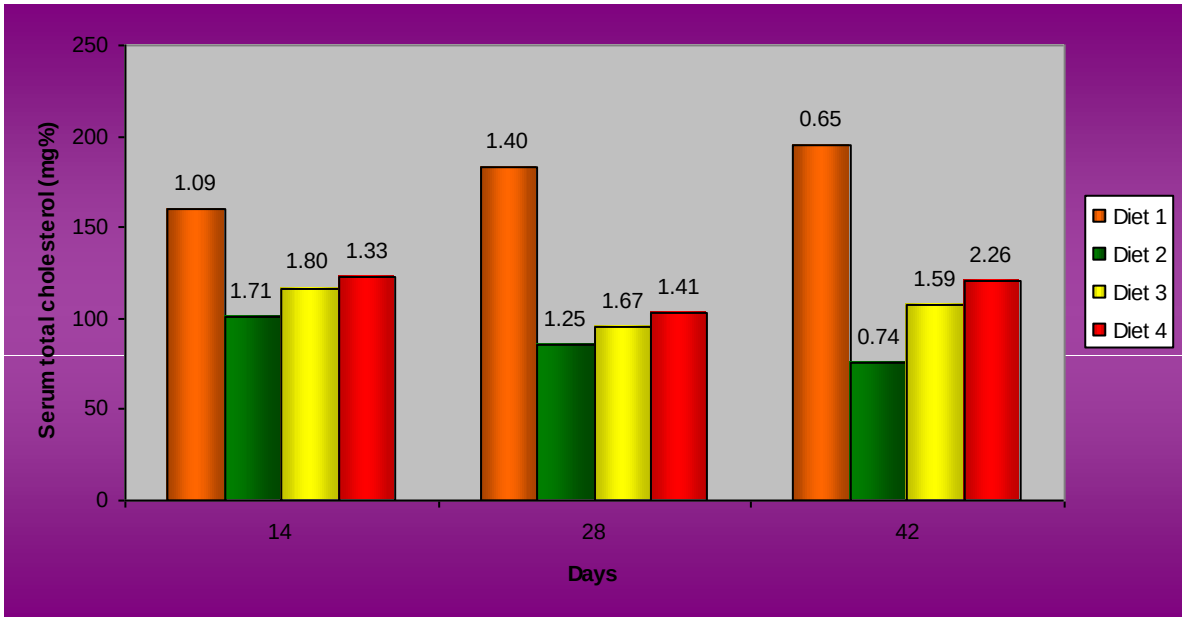


Fig:12 Mean values of Serum total cholesterol (mg%) in broilers as affected by different experimental diets and periods of collection.

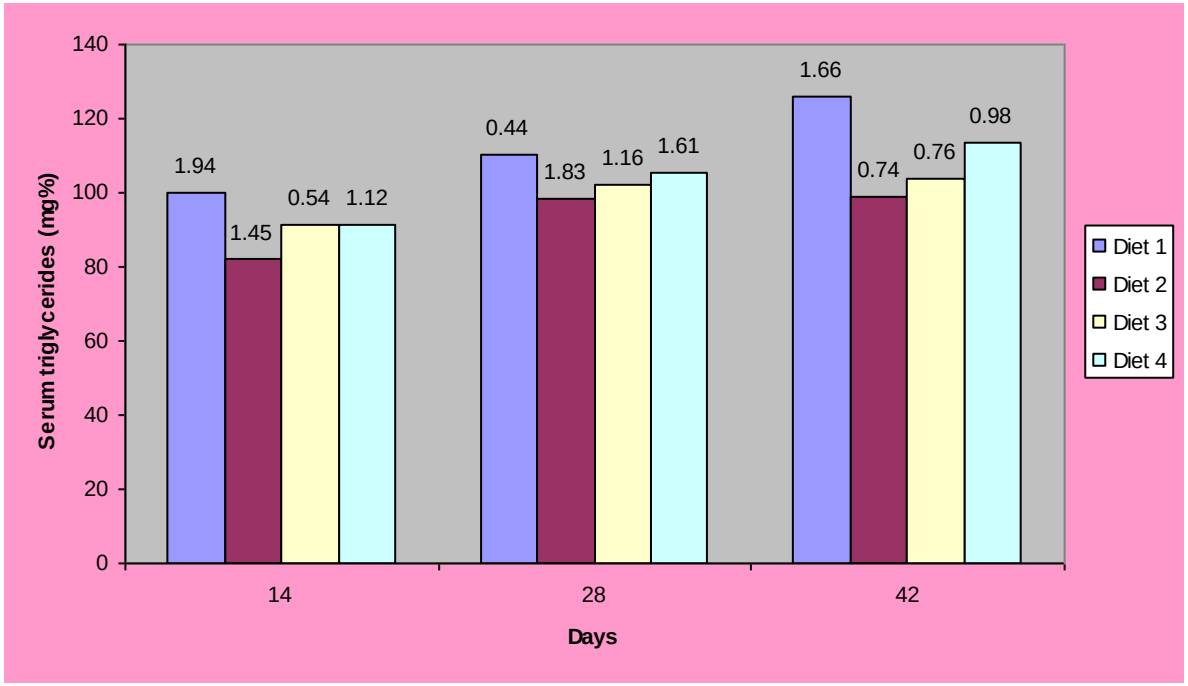


Fig:13 Mean values of Serum triglycerides (mg%) in broilers as affected by different experimental diets and periods of collection.

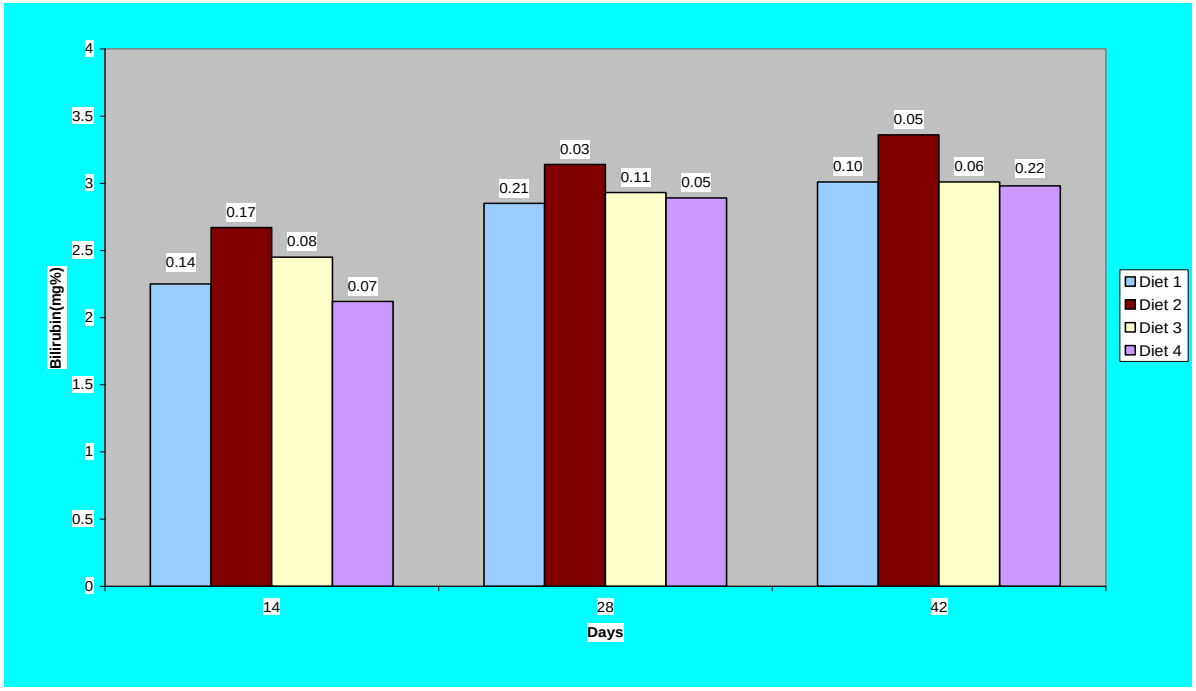


Fig:14 Mean values of Serum bilirubin (mg%) in broilers as affected by different experimental diets and periods of collection.

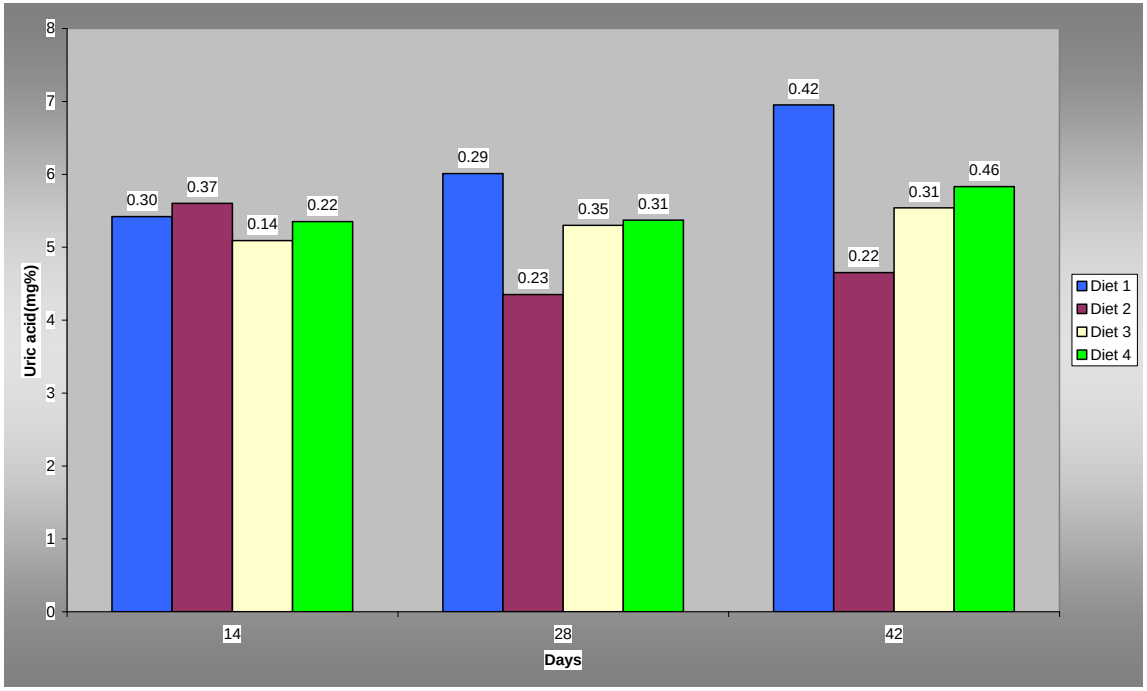


Fig:15 Mean values of Serum uric acid (mg%) in broilers as affected by different experimental diets and periods of collection.

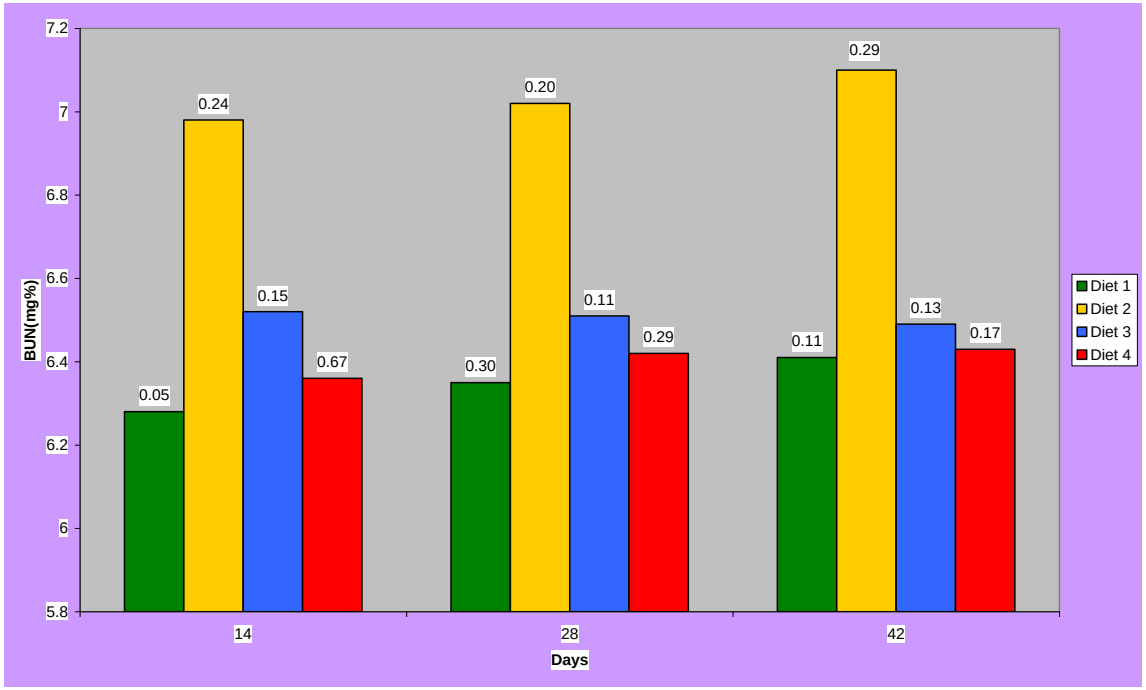


Fig:16 Mean values of Serum BUN (mg%) in broilers as affected by different experimental diets and periods of collection.

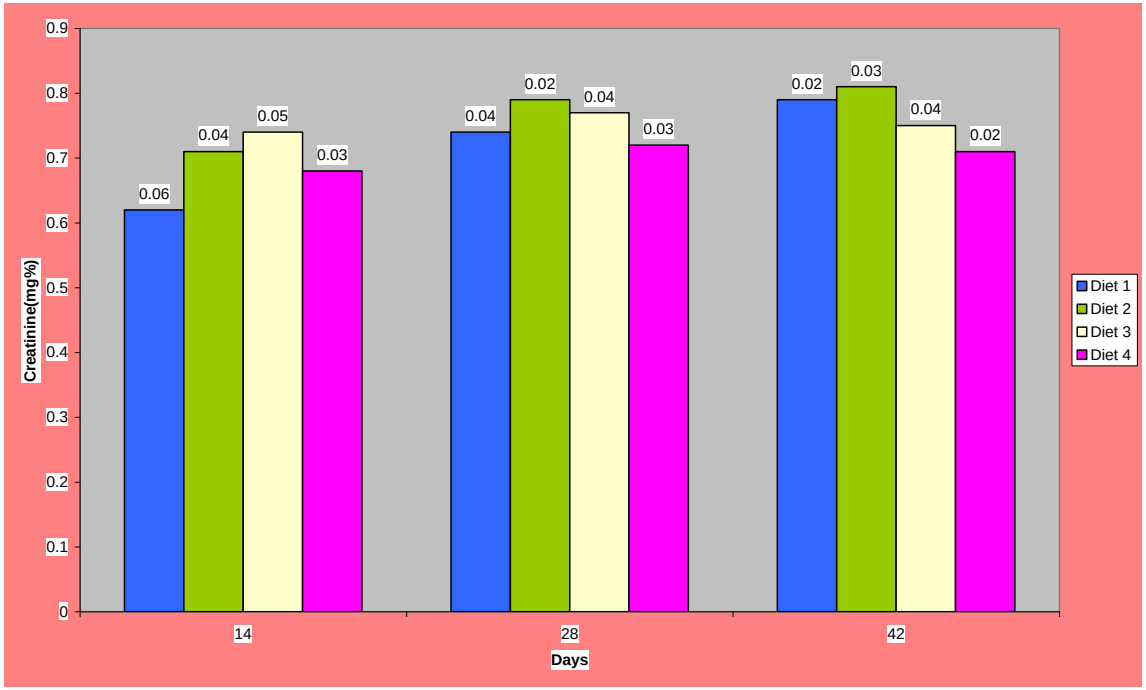


Fig:17 Mean values of Serum creatinine (mg%) in broilers as affected by different experimental diets and periods of collection.

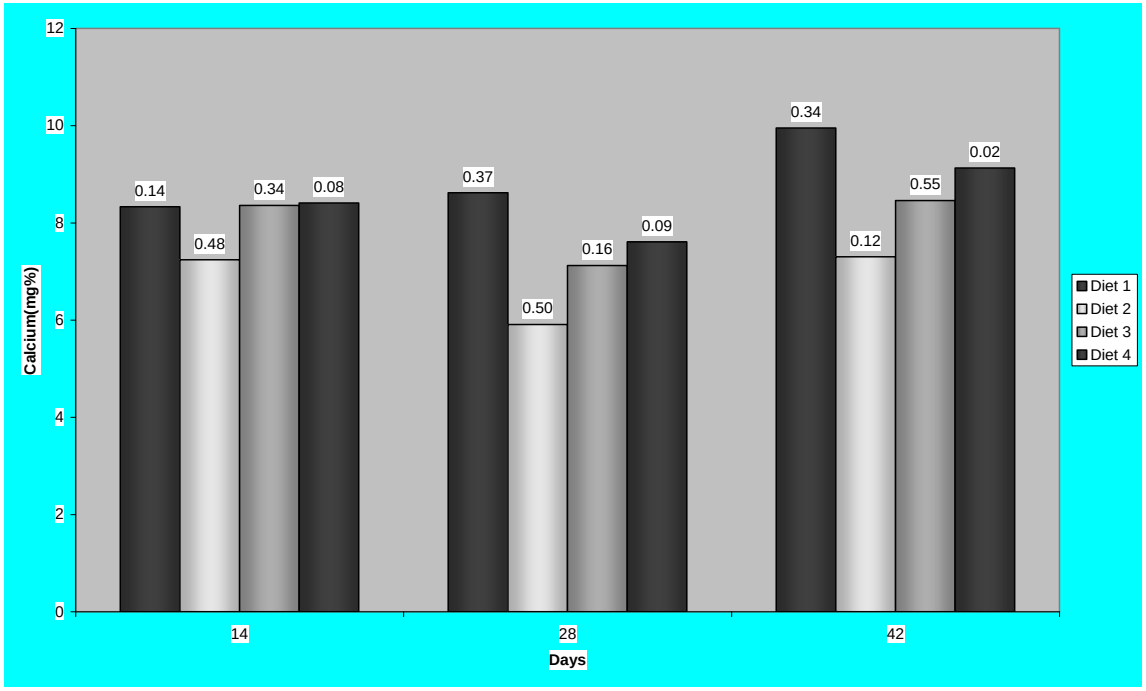


Fig:18 Mean values of Serum calcium (mg%) in broilers as affected by different experimental diets and periods of collection.

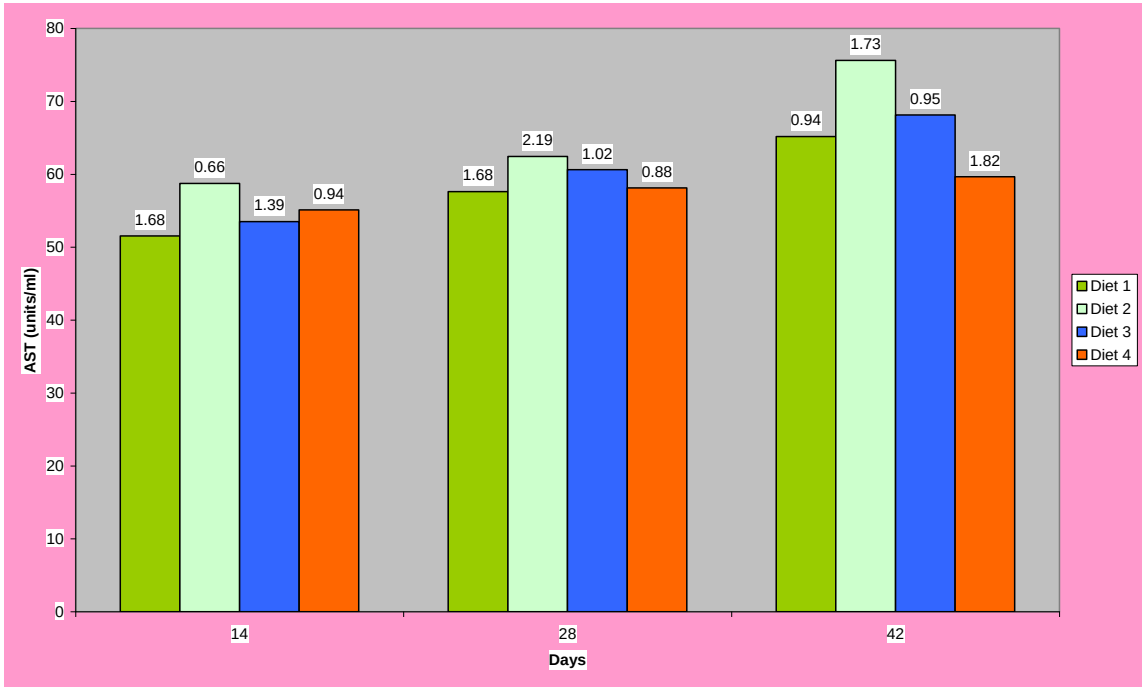


Fig:19 Mean values of Serum AST (units/ml) in broilers as affected by different experimental diets and periods of collection.

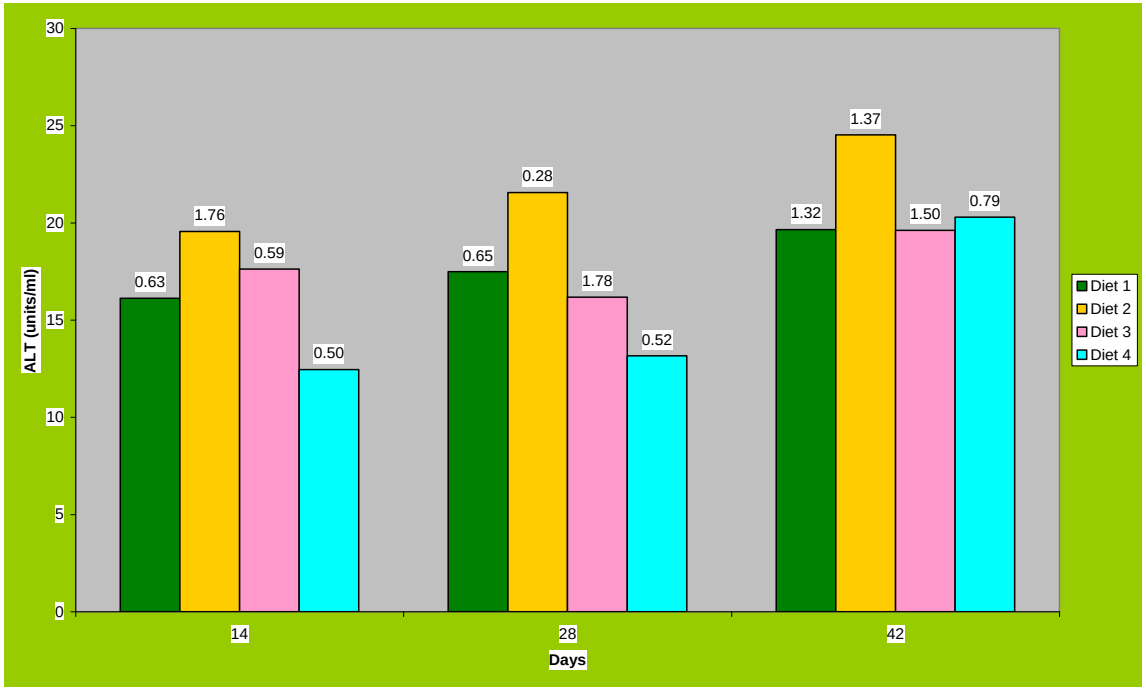


Fig:20 Mean values of Serum ALT (units/ml) in broilers as affected by different experimental diets and periods of collection.

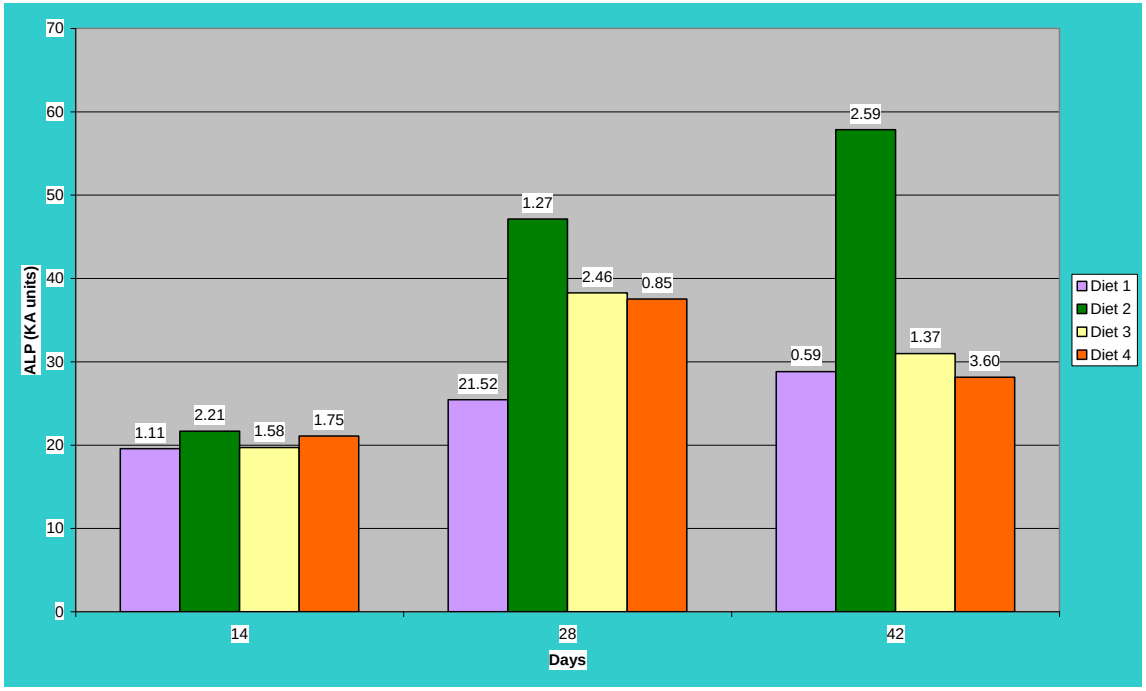


Fig:21 Mean values of Serum ALP (KA units) in broilers as affected by different experimental diets and periods of collection.

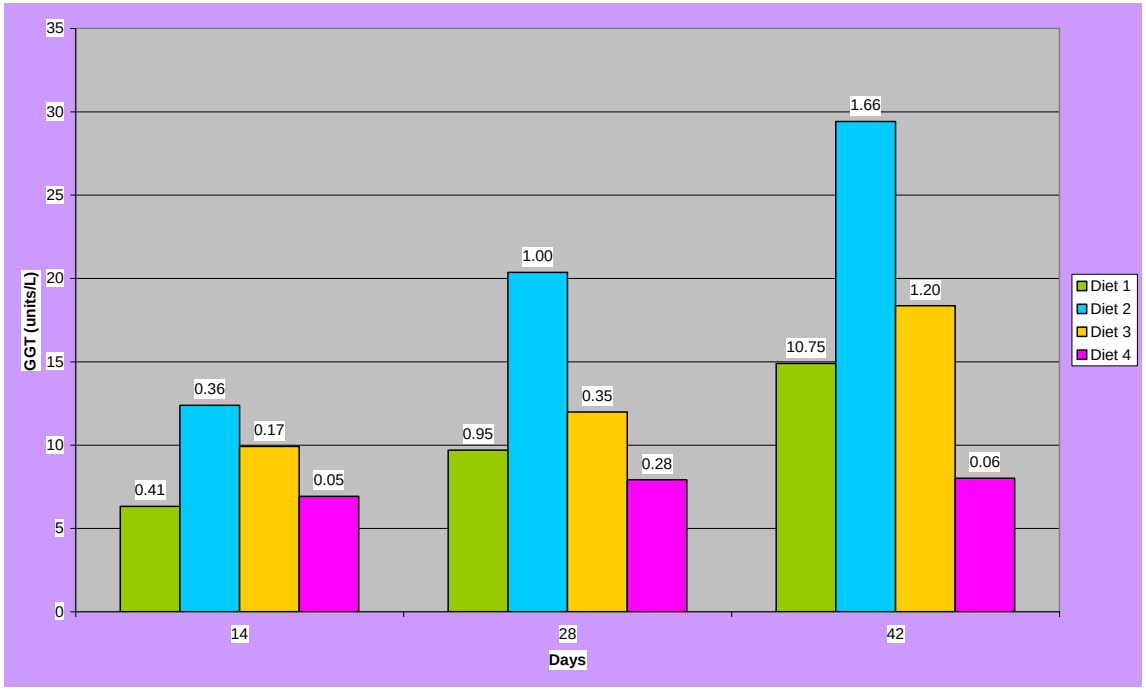


Fig:22 Mean values of Serum GGT (units/L) in broilers as affected by different experimental diets and periods of collection.

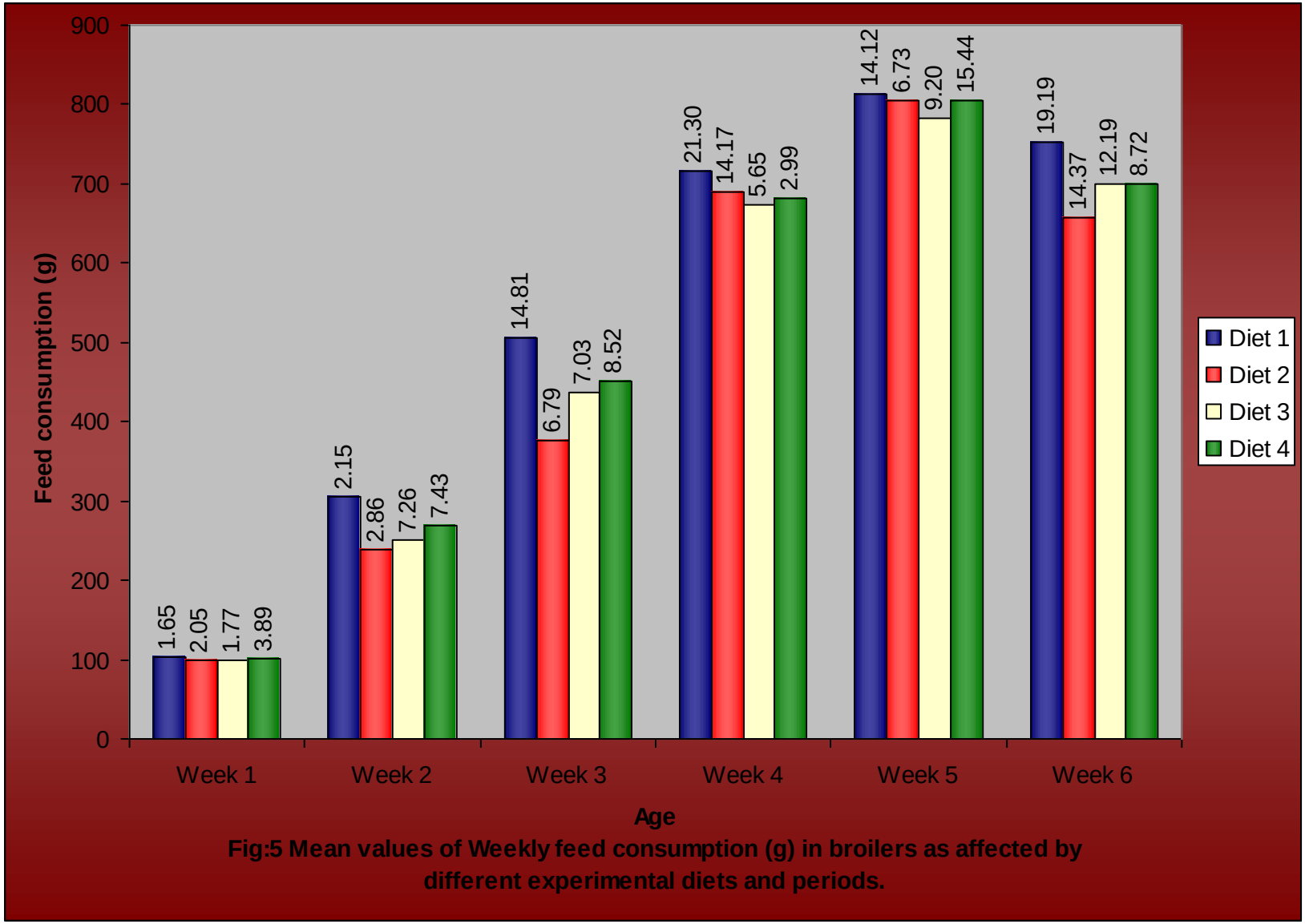


Fig:5 Mean values of Weekly feed consumption (g) in broilers as affected by different experimental diets and periods.

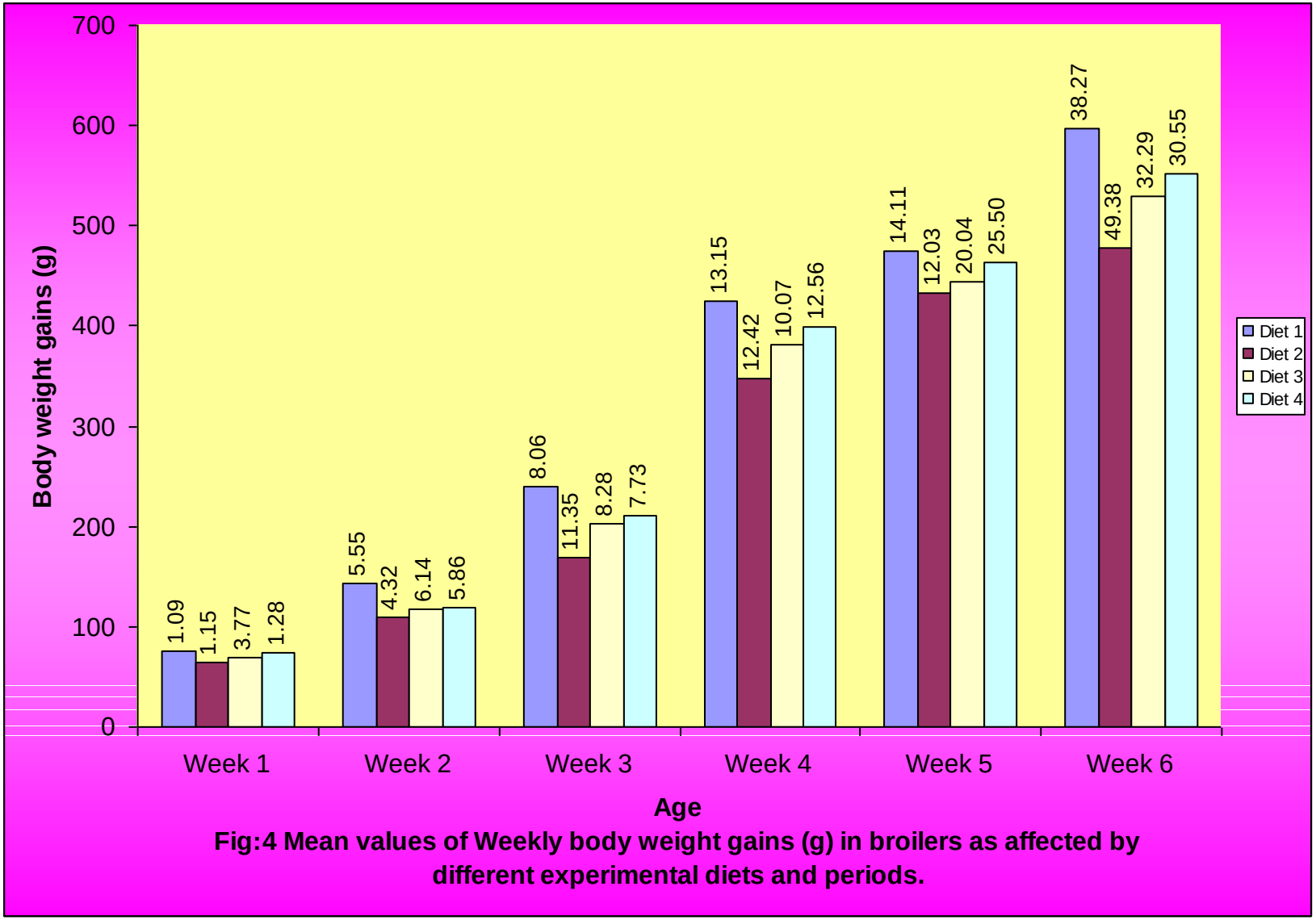


Fig:4 Mean values of Weekly body weight gains (g) in broilers as affected by different experimental diets and periods.

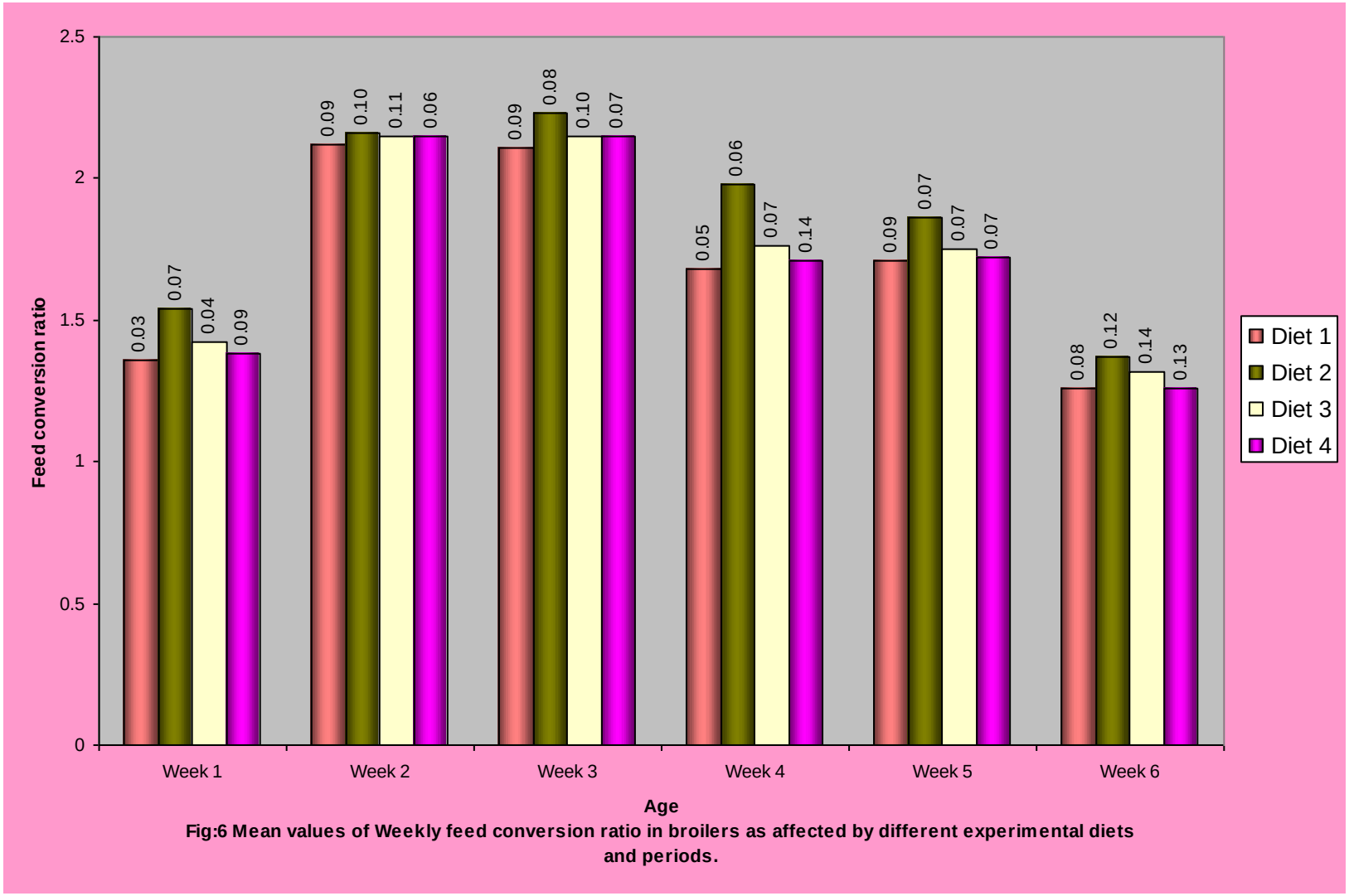


Fig:6 Mean values of Weekly feed conversion ratio in broilers as affected by different experimental diets and periods.

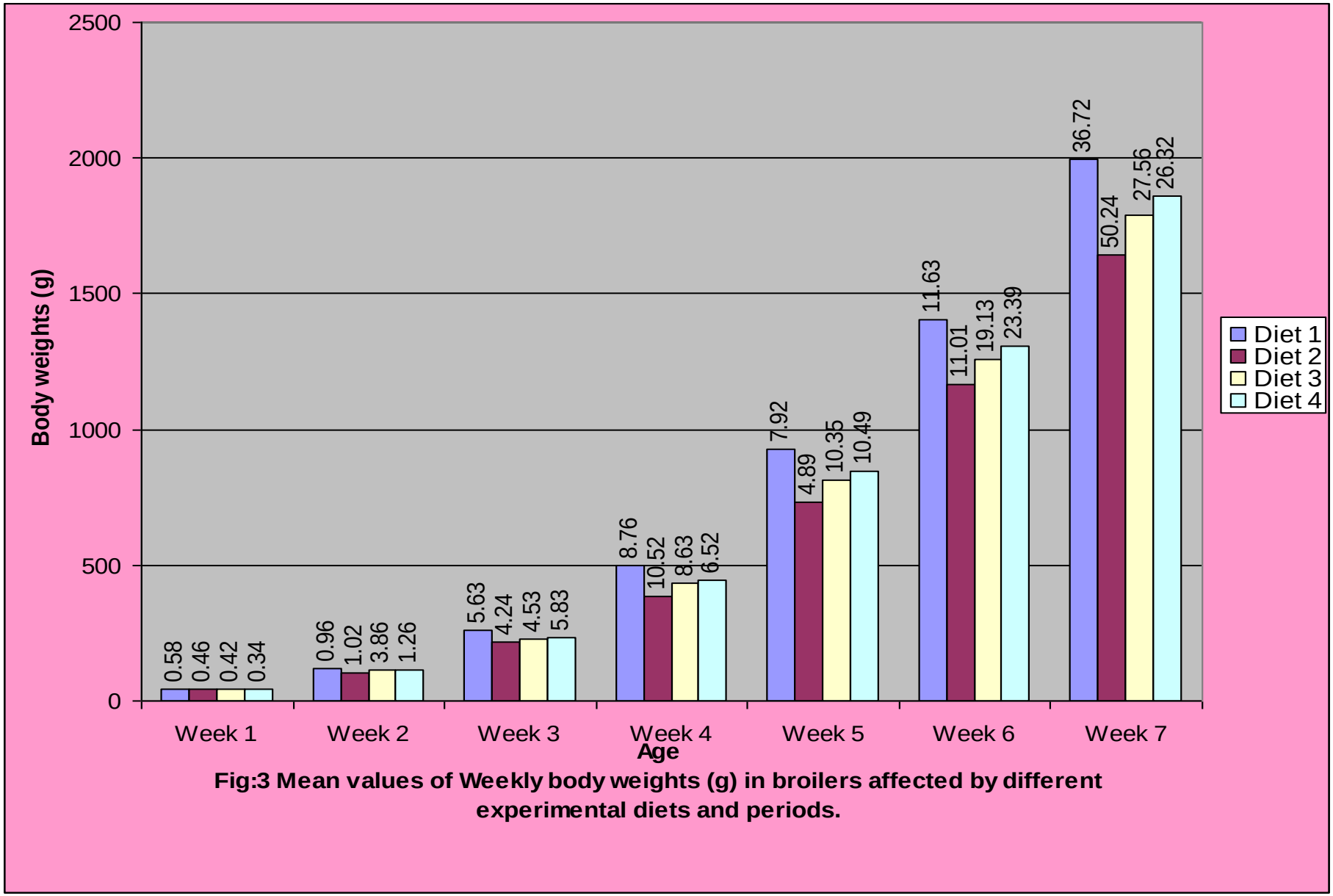


Fig:3 Mean values of Weekly body weights (g) in broilers affected by different experimental diets and periods.

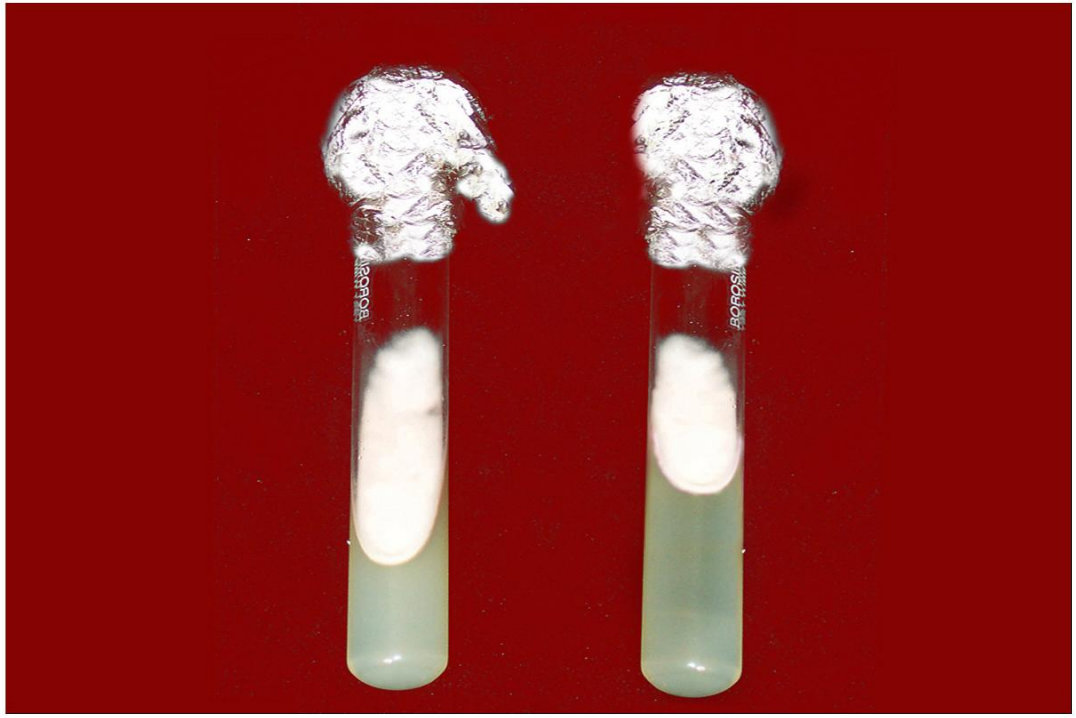


Plate 3: Cutlture slant of *Fusarium moniliforme* on potato sucrose agar.

Plate1: Culture slants of *Penicillium citrinum* on Czapak's agar

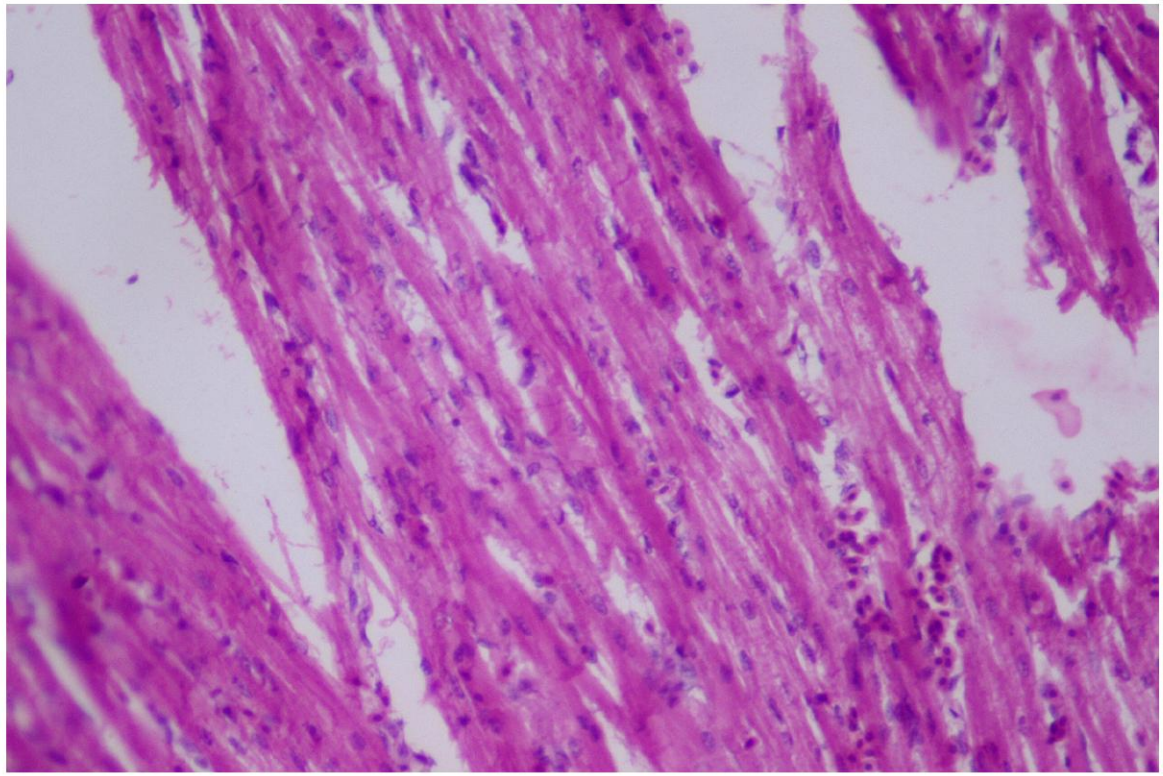
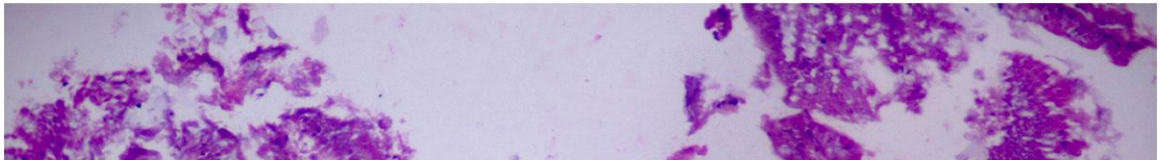


Plate21: Microphotograph of Heart (Group-2) showing mild intertubular hemorrhage and disruption of cardiac muscle fibers. HE x 200.



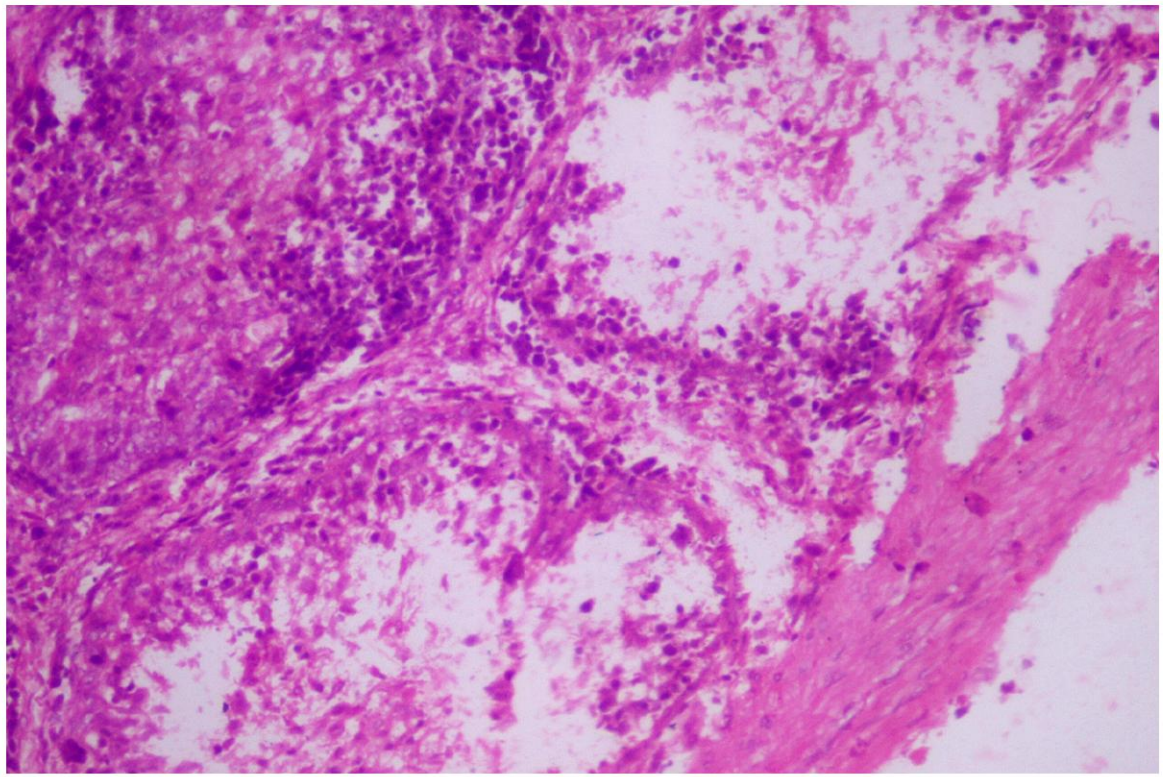
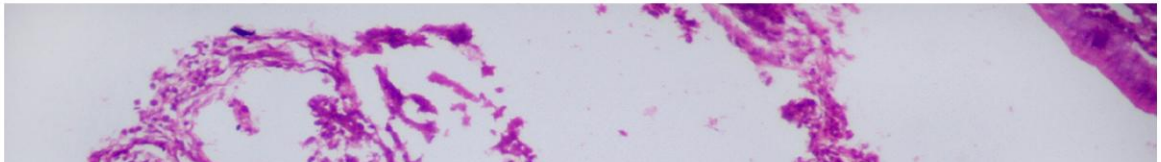


Plate19: Microphotograph of Bursa of Fabricius (Group-2) showing marked depletion of lymphoid follicles. HE x 200.



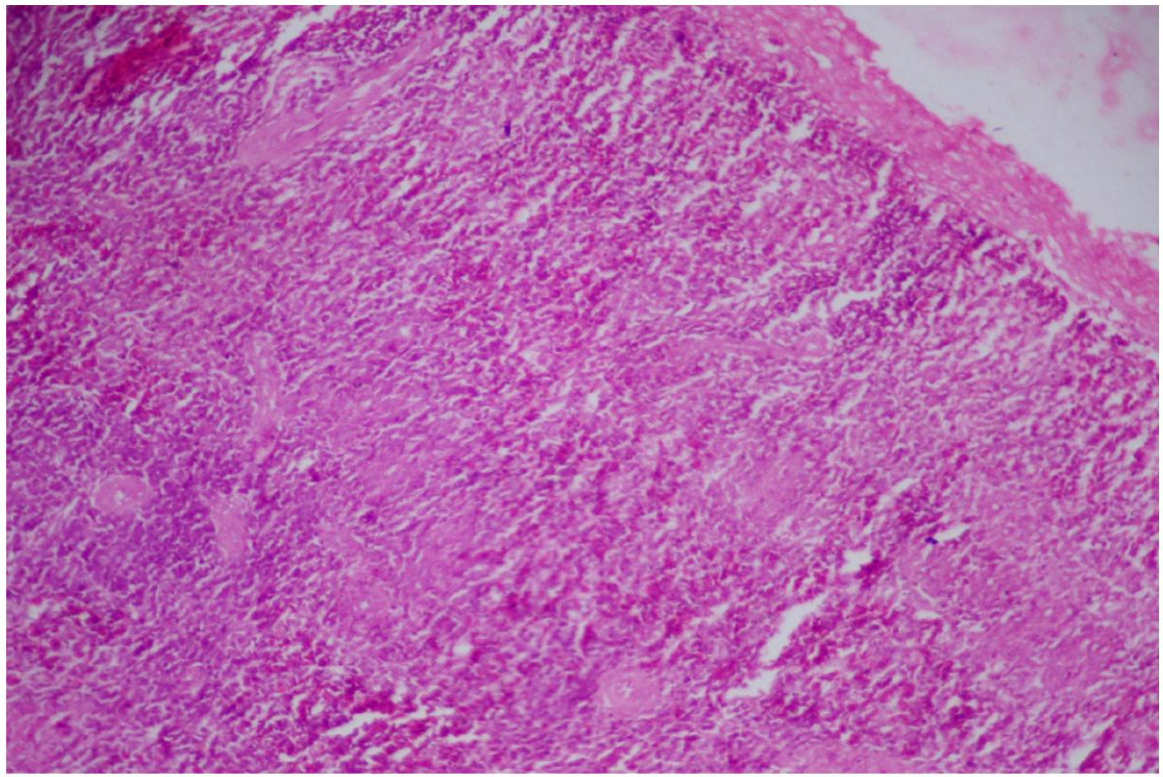
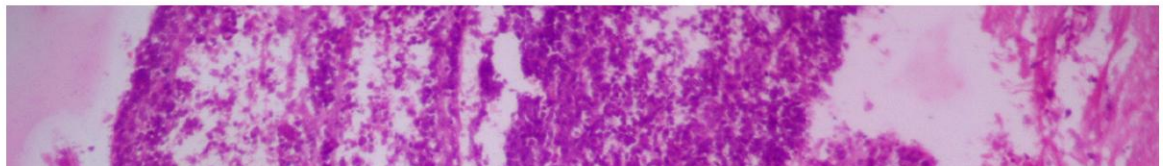


Plate17: Microphotograph of Spleen (Group-4) showing very mild sub-capsular haemorrhages. HE x 100.



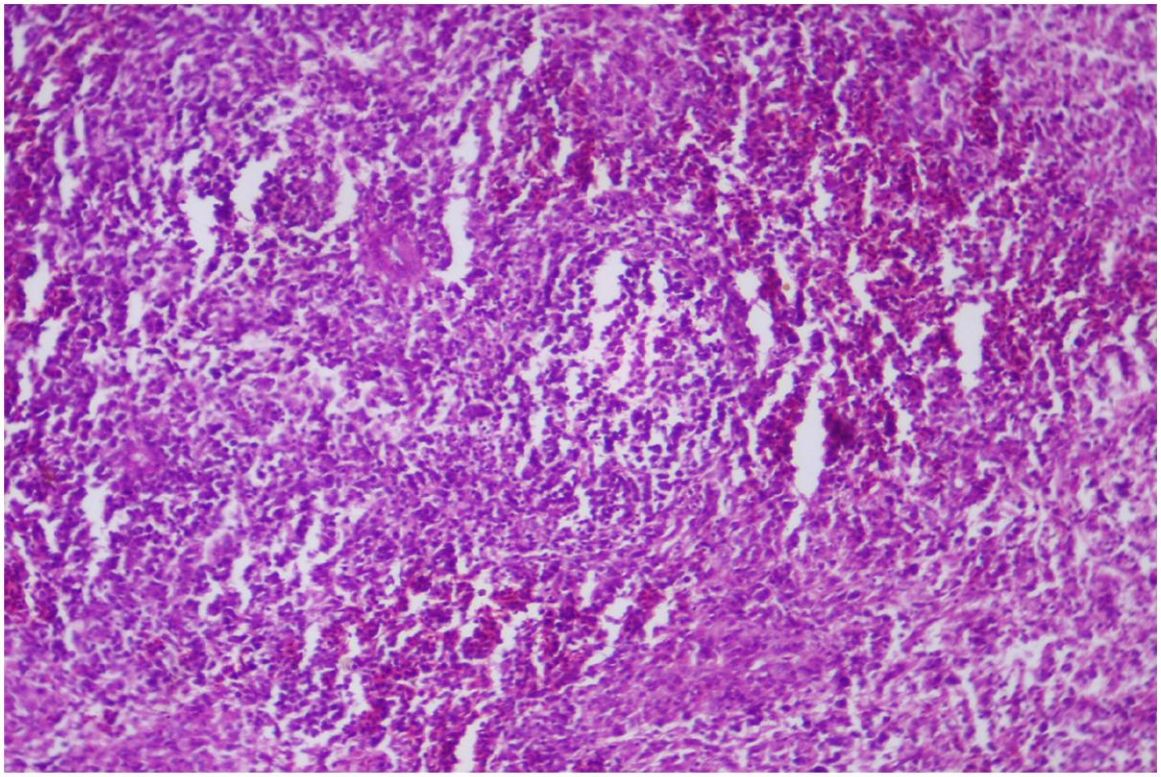
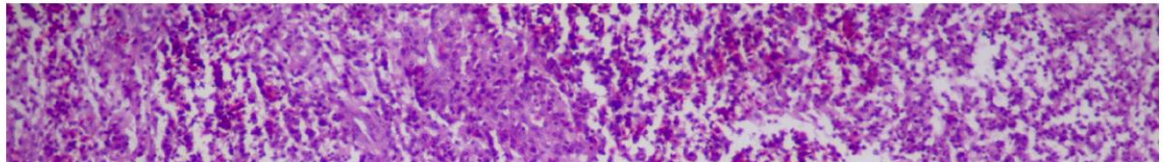


Plate15: Microphotograph of Spleen (Group-2) showing mild depletion of germinal centres. HE x 200.



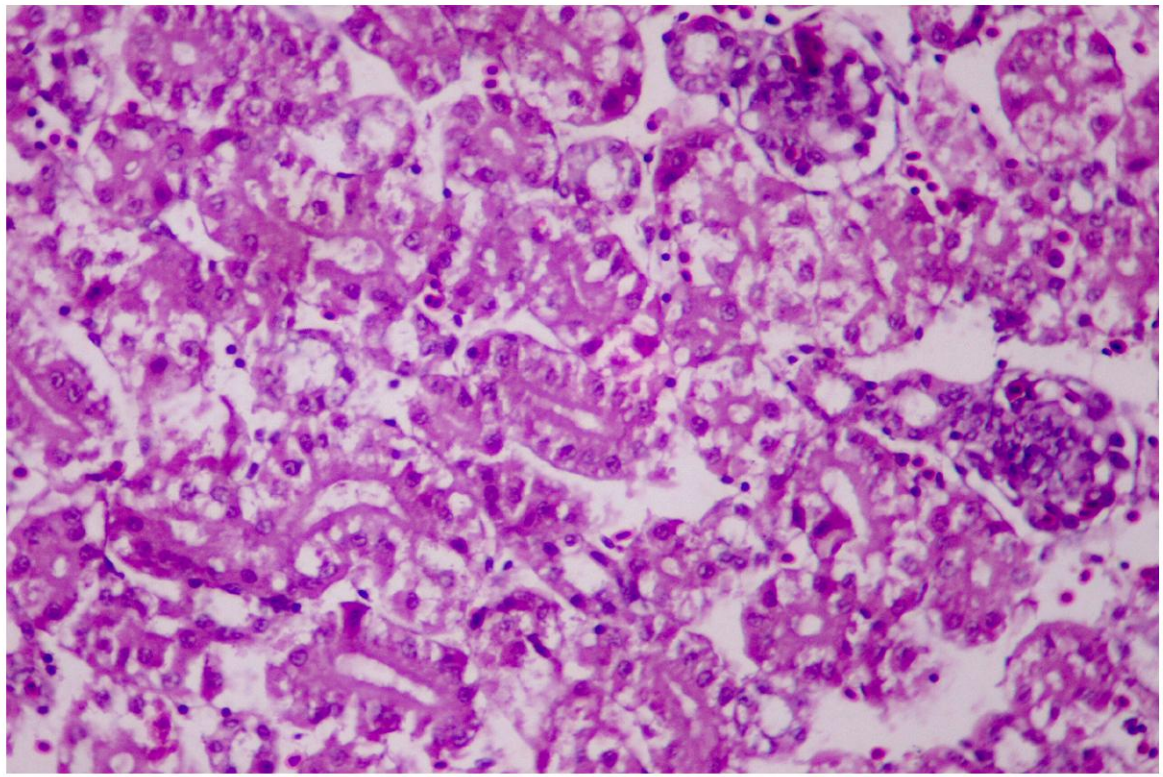
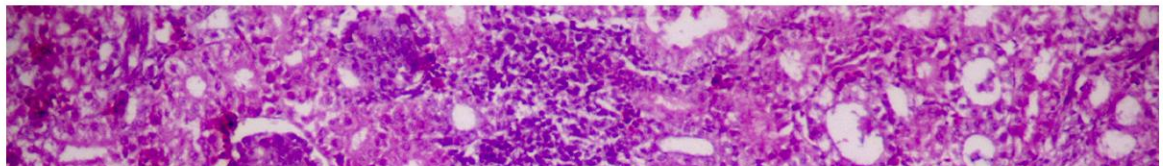


Plate13: Microphotograph of Kidney (Group-2) showing moderate degenerative changes in tubules. HE x 400.



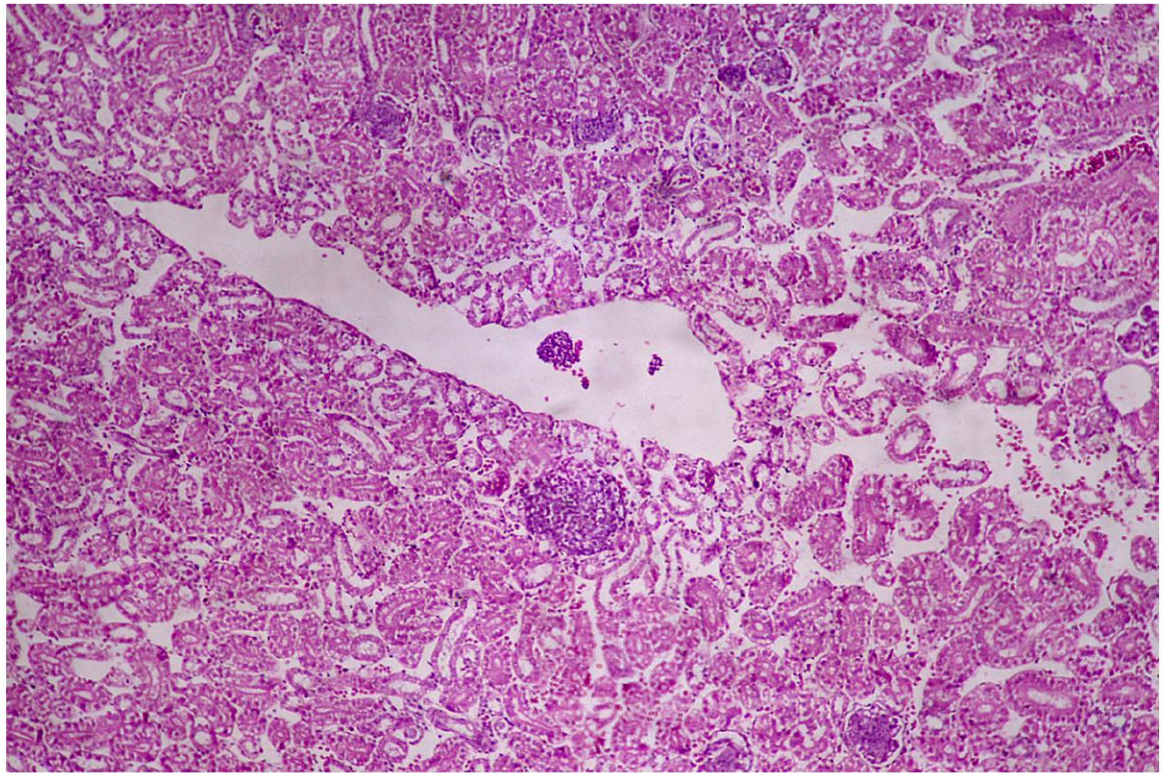
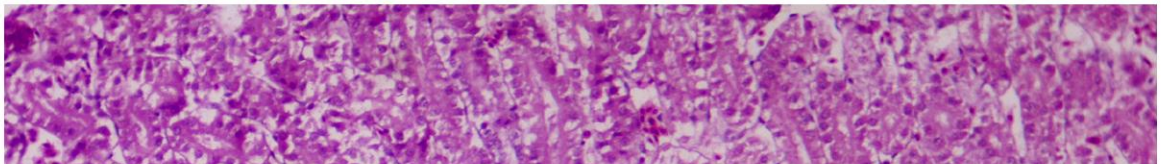


Plate11: Microphotograph of Kidney (Group-2) showing focal lymphoid aggregates and inter tubular congestion. HE x 100.



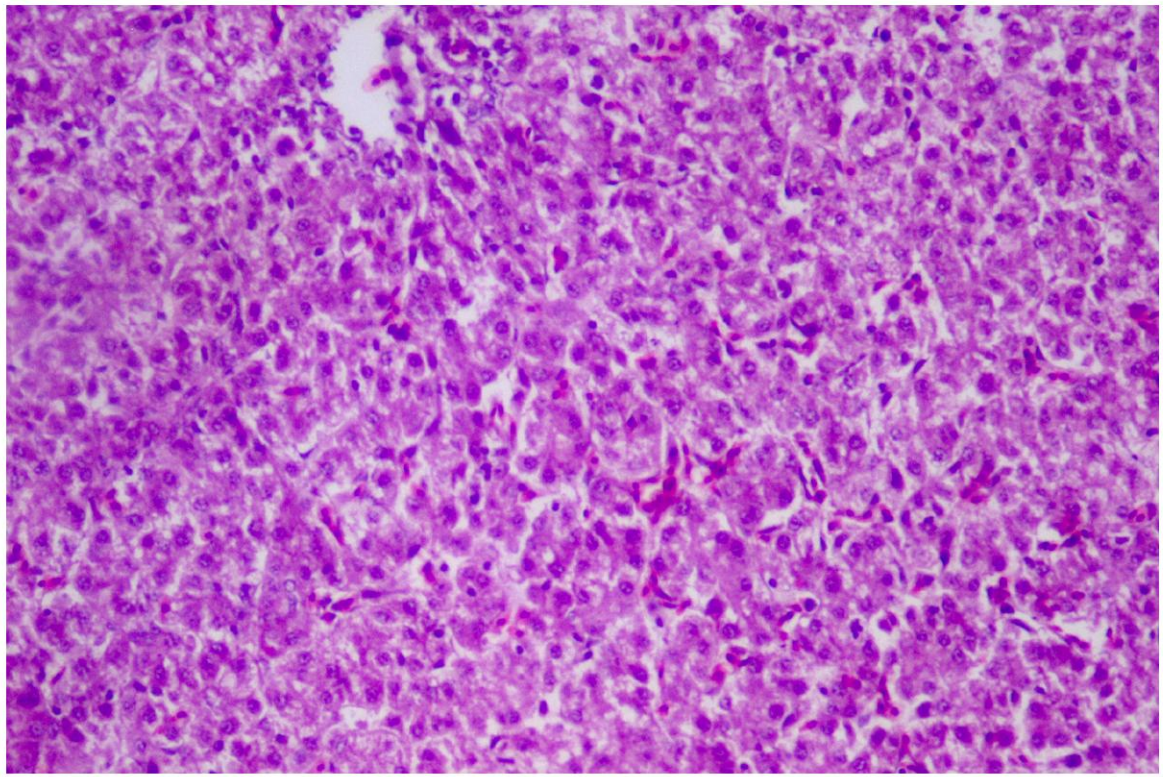
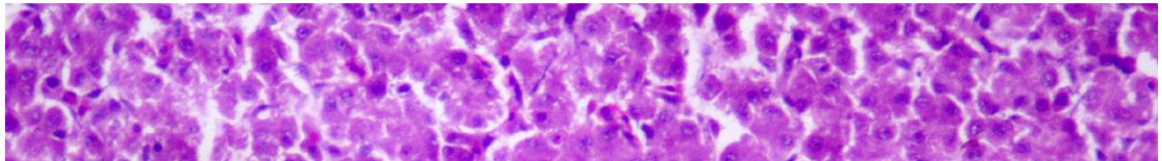


Plate9: Microphotograph of Liver (Group-3) showing very mild congestion of sinusoidal spaces. HE x 200.



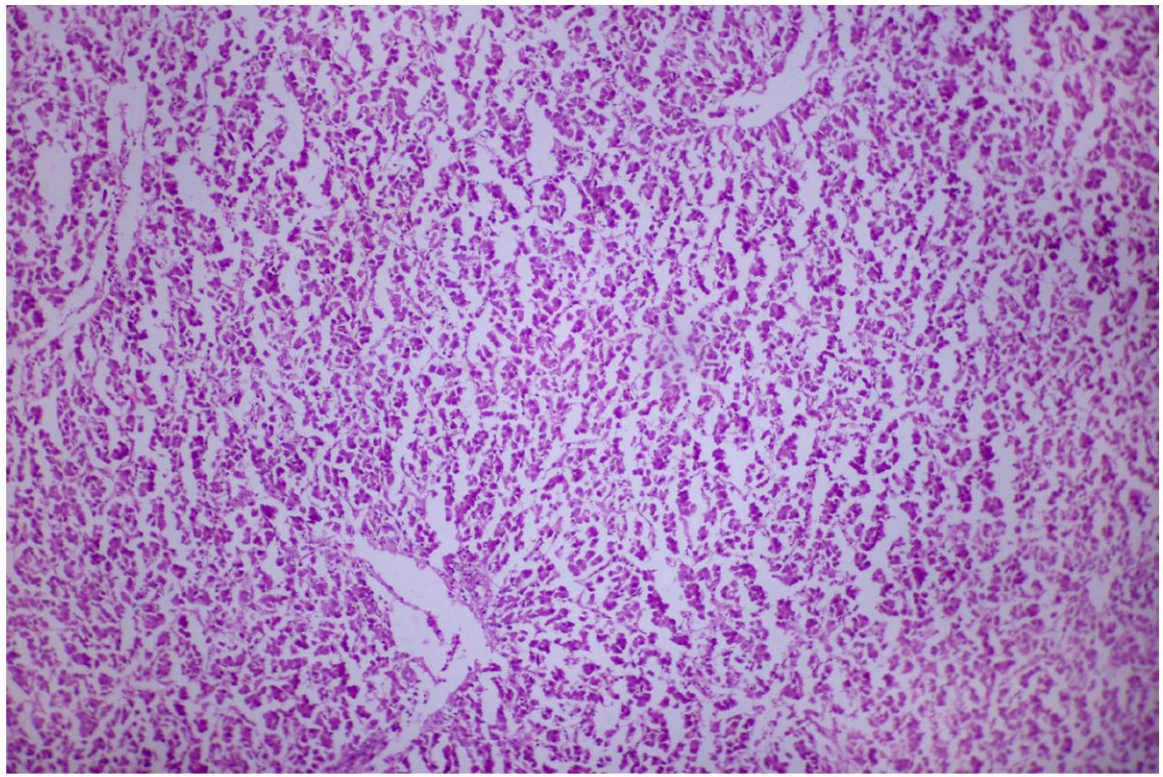
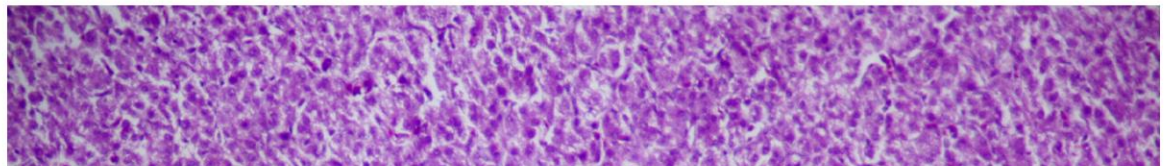


Plate7: Microphotograph of Liver (Group-2) showing dilatation of synosoidal spaces and bile duct hyperplasia. HE x 100.



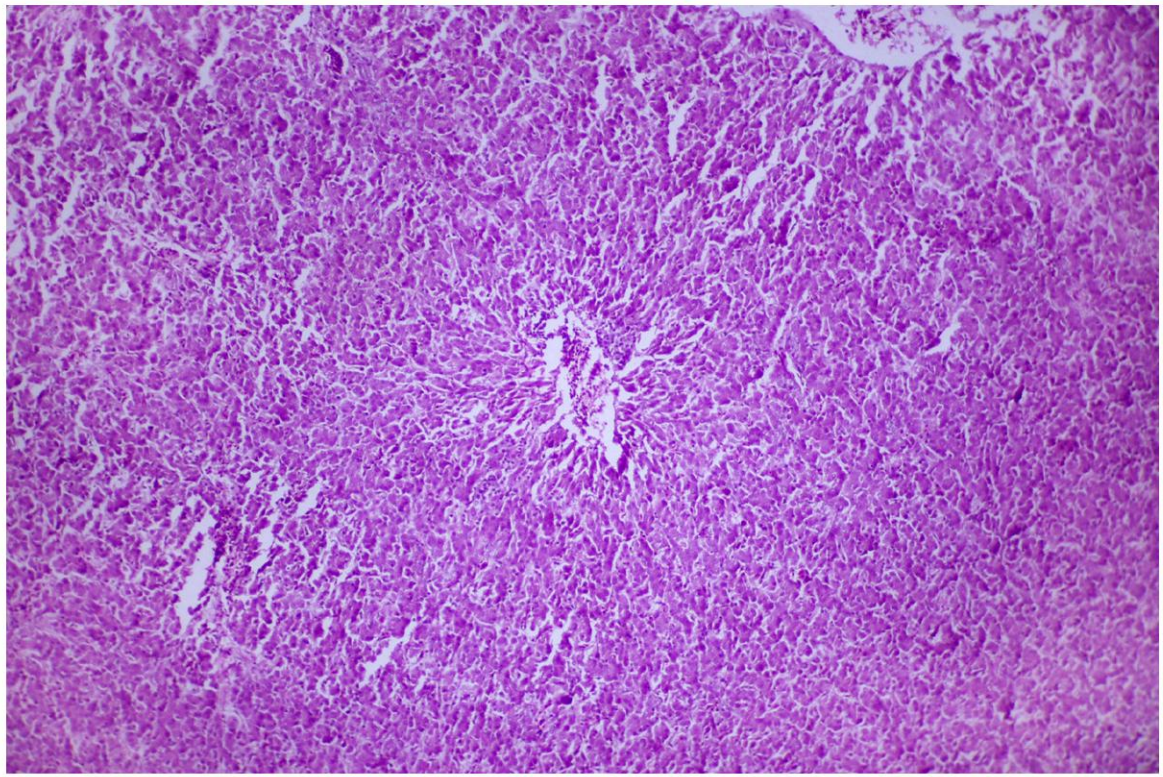


Plate5: Microphotograph of Liver (Group-2) showing mild central venous congestion and paracentral infiltration. HE x 100.