INTERACTION OF AFLATOXICOSIS WITH METHIONINE AND ZINC LEVELS IN DIET OF BROILER CHICKENS

Thesis

Submitted to the
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Veterinary Science
(Poultry Science)

May, 2013

Dedicated to

My Beloved Parents

Certificate

We the undersigned members of Advisory Committee of **Dr. Mamta Sharma,** *Roll No. 5058 a candidate for the degree of* **Master of Veterinary Science** *with the major discipline* **Poultry Science**, *agree that the thesis entitled* **"Interaction of aflatoxicosis with methionine and zinc levels in diet of broiler chickens"** *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of **Master of Veterinary Science** *Degree of this Institute.*

It is further certified that the candidate has completed all the prescribed requirements governing the award of **Master of Veterinary Science** *Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.*

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Certificate

This is to be certified that the research work embodied in this thesis entitled **"Interaction of aflatoxicosis with methionine and zinc levels in diet of broiler chickens"** *submitted by* **Dr. Mamta Sharma, Roll No. 5058,** *for the award of* **Master of Veterinary Science** *Degree in* **Poultry Science** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate herself under my supervision and guidance.*

It is further certified that **Dr. Mamta Sharma, Roll No. 5058,** *has worked for more than 21 months in the Institute and has put in more than 150 days attendance under me from the date of registration for the* **Master of Veterinary Science** *Degree in this Deemed University, as required under the relevant ordinance.*

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(Mamta Sharma)

Date: 28/05/2013

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ABBREVIATIONS

%	:	Percentage
@	:	At the rate of
AF	:	Aflatoxin
AFB_1	:	Aflatoxin B_1
ALT	:	Alanine transaminase
AST	:	Aspartate transaminase
BOD	:	Biological oxygen demand
BW/b.wt	:	Body weight
BWG	:	Body weight gain
CARI	:	Central Avian Research Institute
CMI	:	Cell mediated immunity
CP	:	Crude protein
CRD	:	Completely randomized design
Cu	:	Copper
df	:	Dilution factor
DNA	:	Deoxy-ribose nucleic acid
DOD	:	Change in optical density
EDTA	:	Ethylene diamine tetraacetic acid
et al.	:	et alii
FAO	:	Food and Agriculture Organization
FCR	:	Feed conversion ratio
g/dl	:	Gram/decilitre
GDP	:	Gross domestic production
GIT	:	Gastro-intestinal tyract
GSH	:	Reduced glutathione
H/L	:	Heterophile/ lymphocyte
HA	:	Heamaglutination
Hb	:	Haemoglobin
IB	:	Infectious bronchitis
Ig M	:	Immunoglobin M
IU	:	International unit
L	:	Litre
M	:	Milli
MAT	:	Methionine adenosyltransferase
MD	:	Marek's disease
MDA	:	Malondialdehyde
Met	:	Methionine

mg	:	Milligram
ml	:	Mililitre
Mol	:	Molar
N	:	Normal
NADPH	:	Nicotinamide adenine dinucleotide phosphate
nm	:	Nanometer
OD	:	Optical density
PBS	:	Phosphate buffer saline
PHA-P	:	Phytoheamagllutinine-P
ppb	:	Parts per billion
ppm	:	Parts per million
RBC	:	Red blood corpuscle
RNA	:	Ribose nucleic acid
ROS	:	Reactive oxygen species
rpm	:	Revolution per minute
SAMe	:	S-adenosyl methionine
SGOT	:	Serum glutamic oxaloacetic aminotransferase
SGPT	:	Serum glutamic pyruvic tansaminase
SOD	:	Superoxide dismutase
SRBCs	:	Sheep red blood corpuscles
TLC	:	Thin layer chromatography
v/v	:	Volume/volume
Vit-E	:	Vitamin E
Zn	:	Zinc
µ	:	Micro

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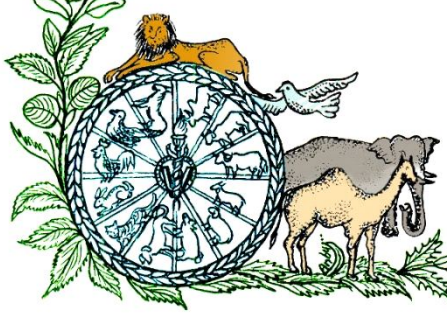


1 INTRODUCTION

Poultry farming is one of the fastest growing segments in agricultur sector in India. The annual growth rate of production of agricultural crops has been 1.5 to 2 percent per annum, while that of eggs and broilers has been 8 percent and 10 percent, respectively (FAO, 2010). The poultry industry contributes 0.7% in national GDP. Feed represents 65-80 percent of total cost of broiler production. To meet the future projection on production/requirements of meat and eggs with in the available feed resources, better utilization of intrinsic feed factors for feed-cost efficient poultry production is inevitable.

The tropical and subtropical climate with hot and humid condition prevailing in our country coupled with improper harvesting of crops, handling and processing, inadequate drying and storage facilities, and insect infestation make feedstuff susceptible to fungal contamination and production of mycotoxins. World's 25% of total grains are contaminated with mycotoxins (Fink-Gremmel, 1999). Mycotoxins not only curtail nutrient quality of the feed and nutrient utilization, but also affect health and performance of birds, and thereby increase pre-harvest losses. Supply of good quality feed is must to obtain optimum performance. Presence of mycotoxins in feed is one of the major constraints in maintaining feed quality because the mycotoxins are widely present in feedstuffs around the world and may affect production even in very low concentration.

Mycotoxins are low molecular weight secondary metabolites produced by certain strains of filamentous fungi such as *Aspergillus, Penicillium and Fusarium,* which invade crops in the field and may grow on foods during storage under favaourable conditions of temperature and humidity. These are regularly implicated in toxic syndrome in animals and humans (Smith *et al.*, 1995; Berry, 1988). Due to the diversity of their toxic effects and their

synergetic properties, mycotoxins contaminated foods and feeds are considered as risky to the consumers (Yiannikouris and Jonany, 2002; Omede, 2008).

The most widespread aflatoxins are of great concern in warm and humid climatic conditions like India (Singh *et al.*, 2010). Swamy *et al.* (2012) found that South Asian feeds are contaminated with multiple mycotoxins, along with aflatoxin. Maize, a major cereal used in poultry diet, is the most vulnerable for mould growth and production of this toxin. The occurrence of aflatoxins in agricultural commodities depends on region, season and the conditions under which particular crop is grown, harvested or stored. Crops grown under warm and moist weather in tropical or subtropical countries are especially more prone to aflatoxin contamination than those in temperate zones. Singh *et al.* (2010) reported that 90 per cent of the maize samples were positive for aflatoxin B_1 and the values ranged from non-detectable to 0.80 ppm, with an average of 0.14 ppm (140 ppb) of aflatoxin B_1.

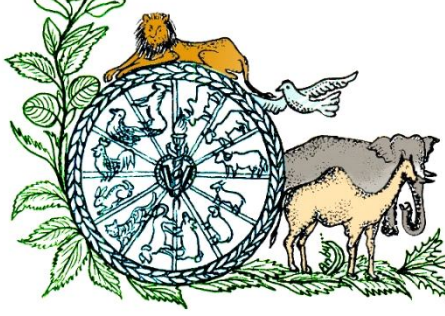
Avoidance of contaminated feed is rarely feasible and feeds that contain relatively low concentrations of AFB_1 may still have deleterious effects on sensitive species such as poultry (Doerr *et al.*, 1983; Giambrone *et al.*, 1985) especially ducklings. Even small amounts (0.25 to 0.50 ppm) of AFB_1 may cause reductions in growth parameters, hatchability and render the birds susceptible to diseases (Edds, 1979; Coulombe, 1993; Denli *et al.*, 2004; Silambarasan *et al.*, 2013).

A wide variation exists in species susceptibility to AFB_1 hepatocarcinogenesis. Fish and poultry, known to be extremely sensitive to AFB_1, responding to doses as low as 15–30 ppb (Wogan, 1992). Poultry are suggested to be the species most sensitive to the toxic effects of aflatoxin (Denli *et al.*, 2004). Aflatoxins affect energy, protein and nucleic acid metabolism (Baptista *et al.*, 2004) with described effects in a wide range of animal species such as carcinogenesis, hepatotoxicity, mutagenicity, immunosuppression and teratogeny (Busby and Wogan, 1984). Liver, the major organ involved in nutrient metabolism and detoxifying toxic materials, is the target organ for aflatoxicosis because this is the site where most aflatoxins are bio-activated to the reactive 8, 9- epoxide form, which is capable of binding both DNA and proteins. Aflatoxins contamination in feed is practically unavoidable (Coulombe *et al.*, 2005). So it is necessary to evolve the practical and suitable methods to counteract the aflatoxicosis. Extensive research has been conducted to counter mycotoxicosis by physical, chemical, nutritional or biological approaches.

A variable array of chemical factors, including nutrients e.g. dietary protein and amino acids (S-amino acid like methionine and cystine), fat, vitamins (A, D, E, K and B-complex), micro-minerals (zinc, chromium, selenium), feed additives (antibiotics, preservatives, liver tonics, herbal immunomodulaters, etc.) may interact with the aflatoxins in birds and animals. Mycotoxins can substantially decrease antioxidant assimilation from the feed and increase their requirement to prevent damaging effects of free radicals produced as a result of mycotoxin exposure. Zinc supplementation is helpful in aflatoxicosis because it acts as antioxidant by different mechanism like it is a cofactor of the main antioxidative enzyme Cu-Zn- super-oxide dismutase that inhibits the NADPH-dependent lipid peroxidation (Prasad and Kucuk, 2002), induces production of metallothionein that acts as a free radical scavenger (Oteiza *et al*., 1996) and it also interact with vitamin E and C. Addition of sulfur amino acids like methionine to diets containing aflatoxin improved performance in chickens (Veltmann *et al*. 1984). Aflatoxins are thought to be metabolized to highly reactive epoxides and phenolats that can bind and interfere with nucleic acid and proteins (Ciegler, 1975). The epoxides and phenolates are normally conjugated with glutathione and hepatic necrosis is thought to result when glutathione reserves have been drastically depleted by conjugation with toxin intermediates so that the toxin intermediates are free to bind covalently to vital cellular macromolecules. Therefore, supplementing methionine, which in turn helps to increase hepatic glutathione (GSH) concentration, may aid to protect liver against aflatoxin. Keeping in view the above, the present study has been proposed with the following objectives:

- **To evaluate the efficacy of dietary zinc or methionine in combating aflatoxicosis in broiler chickens.**
- **To evaluate the welfare aspect of birds in aflatoxicosis through dietary supplementation of methionine or zinc.**

Review of Literature



2 REVIEW OF LITERATURE

2.1 Aflatoxins

Mycotoxins comprise a group of several hundreds of chemically different toxic compounds (William, 1989; Moss, 1996; Rotter *et al.*, 1996; Sweeney and Dobson, 1998). The most common mycotoxins are aflatoxins, ochratoxin A, trichothecenes, zearalenone, and fumonisins. Out of which aflatoxins commonly contaminate a wide variety of tropical and subtropical food/feed stuffs. The discovery and isolation of aflatoxins is well known to be a result of investigations on the mysterious Turkey-X disease of 1960 which resulted in loss of several thousand turkey poults in the United Kingdom. The cause of enormous mortality in turkey poults and of similar outbreaks in other farm animals could be linked with the use of mouldy Brazilian peanut meal in the diet of affected animals (Blount, 1961). Aflatoxins are highly toxic metabolites produced mainly by *Aspegillus flavus*, *Aspegillus parasiticus*, and some other species of *Aspegillus, Penicilium and Rhizopus* (Doerr *et al.*, 1983; Hatch, 1988). In 1962, the name -aflatoxin, using first letter from *Aspergillus* and the first 3 letters from -*flavus* was proposed (Patterson, 1977). Depending upon colour of the fluorescence, AFs are divided into aflatoxin B_1 and B_2 (AFB_1, AFB_2) for blue, and G_1 and G_2 (AFG_1, AFG_2) for green (Hartley *et al.*, 1963; Dalvi, 1986). Among the various types of aflatoxins, aflatoxin B_1 (AFB_1) is most commonly encountered and it is also considered to have higher toxicity than other aflatoxins (Yunus *et. al.*, 2011). Liu (2006) also reported aflatoxin B_1 (AFB_1) as the most common of the four primary aflatoxins (B_1, B_2, G_1, and G_2) among the most potent naturally occurring carcinogens.

Aflatoxin contamination can occur in a wide variety of feedstuffs including maize, sorghum, barley, rye, wheat, peanuts, soya, rice, cottonseed and various derivative products

made from these primary feedstuffs (Busby and Wogan, 1984). Swamy *et al.* (2012) found that South Asian feeds are contaminated with multiple mycotoxins, along with aflatoxin and the major contributor of this toxin is maize. Crops grown under warm and moist weather in tropical or subtropical countries are especially more prone to aflatoxin contamination than those in temperate zones. Grains stored under high moisture or humidity at warm temperatures and inadequately dried can potentially become contaminated. Initial growth of fungi in grains can form sufficient moisture from metabolism to allow for further growth and mycotoxin formation. Aflatoxin contaminations are more likely in grains grown or handled in the tropics and sub-tropics. Stressing the host plants by insect damage, drought, poor nutrition and delayed harvest increases aflatoxin production (Brown, 1996).

2.2 Effect of aflatoxins in poultry

Aflatoxin deteriorates the quality of the feed. Therefore, it has tremendous economic impact on the poultry industry. Aflatoxins may cause serious economic losses in the poultry industry because they prevent birds from achieving optimum body weight gains (Oguz & Kurtoglu, 2000). Aflatoxin also causes economic losses to poultry industry from reductions in growth rate, hatchability, feed efficiency and immunity towards diseases (Richard *et al.*, 1986; Coulombe, 1993). As per the CAST (1989) aflatoxicosis produced severe economic losses in the poultry industry affecting ducklings, broilers, layers, turkeys and quails. Aflatoxins are the most potent mycotoxins cause wide range of clinical and sub-clinical problems in poultry. These mycotoxins are known to have strong hepatotoxic and carcinogenic effects. Aflatoxins have negative effects on animal performance and immunity (Yunus *et al.*, 2011). A wide variation exists in species susceptibility to AFB_1 hepatocarcinogenesis. Fish and poultry are known to be extremely sensitive to AFB_1, responded to doses as low as 15–30 ppb (Wogan, 1992). Susceptibility of poultry to aflatoxins varies among species, breeds and genetic lines. Toxicological studies in avian species have shown that ducklings and turkey poults are the most sensitive species to aflatoxins. Among all the mycotoxins, aflatoxins B_1 are the most potent and pathogenic to poultry. Susceptibility of chickens to toxic effects of AFB_1 varies with several factors such as breed, strain, age, nutritional status, amount of toxin intake and also the capacity of liver microsomal enzymes to detoxify AFB_1 (Edds *et al.*, 1973 and Veltmann, 1984).

Diets containing 75 to 800 ppb of AFB_1 resulted in hepatic lesions and death in chicks (Doerr *et al.*, 1983). At a concentration of 500 ppb of AF, fatty liver and increase in liver size was observed after 3 weeks of exposure (Aspalin and Carnaghan, 1961). The toxicity of aflatoxins in broiler chickens has been widely investigated by determining their teratogenic, carcinogenic, mutagenic and growth inhibitory effects (Oguz and Kurtoglu, 2000). The biochemical haematological (Oguz *et al.*, 2000), immunological (Qureshi *et al.*, 1998), pathological (Kiran *et al.*, 1998; Ortatatli and Oguz, 2001) and other toxic effects of aflatoxins have also been well described. Ghahri *et al.* (2010) reported that AF present in the naturally contaminated feed significantly depressed performance, organ morphology and most of the serum biochemical parameters.

Aflatoxicosis alters the ability of the bird to digest protein and ability to absorb amino acids. The hepatic retention of amino acids increases and ability to synthesize DNA, RNA and ribosome protein decreases. All the factors result in an increase in the protein requirement of the bird as a result of which there is delay in development of the bird. Gimeno and Martins (2000) reported that day-old chicks given a diet containing 20% crude protein (CP) and 5 ppm AFB_1, suffered a weight loss of 20% as compared to control group. However, when the CP content of the feed was 30% with the same level of AFB_1 contamination, the weight loss was only 5.4%. Thus, increase in CP content of the diet to 30% helped in ameliorating the growth suppression. Diet containing 0.2 ppm AFB_1 given to chicks for a total of 29 days resulted in an increase in the susceptibility to coccidiosis by *Eimeria tennella* and decreased the effectiveness of anticoccidial drugs (Edds *et al.*, 1976). Chickens of different ages showed differential susceptibility to different AF doses (Lanza *et al.*, 1980). At low level of contamination exposed chickens showed general weakness, failure to gain weight with concomitant decline in feed efficiency and egg production (Doerr *et al.*, 1983). Intoxicated adult laying hens have decreased egg production and reduced hatchability. In adult breeder males, testicular weight and sperm count are reduced. Insemination of hens with affected male had shown decreased fertility in some studies and no significant reduction in others (Brown, 1996). In broilers decreased water and feed intake, weight loss, dullness, stunting, ruffled feathers, poor appearance and paleness, trembling, ataxia, lameness, paralysis of the legs and wings gasping, prostration and death are frequently seen in experimental and natural outbreak of aflatoxicosis in broilers (Okoye *et al.*, 1988, Rao and Joshi, 1993 and Lesson *et al.*, 1995). Significant increase in

mortality rate was observed by Raju and Devegowda (2000) when aflatoxin B_1 (300 ppb) contaminated diet fed to broiler chickens. A 0.5 ppm concentration of AFB_1 significantly reduced the efficiency of the vaccine used against Marek's Disease (Gimeno, 2000). Metabolic alterations caused by aflatoxins in chickens resulted in elevated lipid levels (Tung *et al.*, 1972; Donaldson *et al.*, 1972), disruptions in hepatic protein synthesis (Tung *et al.*, 1975) which resulted in several blood coagulation disorders (Doerr *et al.*, 1976; Bababunmi and Bassir, 1982), immunosuppression and decreased plasma amino acid concentrations (Voight *et al.*, 1980).

2.2.1 Effect of aflatoxin on growth, feed consumption and feed efficiency of broilers

Aflatoxins affect the feed consumption and body weight gain and feed efficiency of the broiler. Modern broilers are known to gain more weight by utilizing less feed in shorter time. As AFB_1 is known as hepatotoxic, it might result in more profound negative effects in birds with more efficient nutrient conversion demanding faster hepatic metabolism. Differences in the susceptibility of broilers and layers in this regard have been already postulated to be due to differences in metabolic rate of these bird types. This is a general agreement that dietary aflatoxins reduce weight gain, feed intake, and increase feed conversion ratio in poultry birds (Yunus *et al.*, 2011; Silambarsan *et al.*, 2013). Pasha *et al.* (2007) reported that, reduction in body weight gain attributed to the presence of aflatoxin, which depressed appetite and ultimately reduced the growth rate. Dietary aflatoxin at 0.5 ppm and beyond in commercial broilers adversely affected growth in a dose dependent fashion (Reddy *et al.*, 1982, Doerr *et al.*, 1983, Johri *et al.*, 1988, Johri and Sadagopan, 1989, Verma, 1994 and Beura *et al.*, 1993). Dersjant-Li *et al.* (2003) concluded in their review that each ppm of AFB_1 would decrease the growth performance of broilers by 5%. Reduced live weight gain at 750 ppb of dietary aflatoxin in broilers was recorded by Doerr *et al.* (1983). Larsen *et al.* (1985) observed that chicks on aflatoxin contaminated feed did not gain weight as rapidly or as effectively as those on uncontaminated feed. Reddy *et al.* (1984) recorded a significant depression in weight gains and feed consumption at 0.75 ppm level in the diet. The report of the study conducted in broilers by Giambrone *et al.* (1985) indicated that a level of 0.5 ppm produced a significant decrease in weight gain and microscopic lesion indicative of aflatoxicosis at five weeks of age. Reddy *et al.* (1984) recorded a significant depression in weight gains and feed consumption at

0.75 ppm level in the diet. Churchill *et al.* (2009) reported that feeding of aflatoxin caused significant reduction in weight gain. Modirsanei *et al.* (2004) reported that the addition of 1.0 mg AFB_1, per kg in diet of broiler chicks significantly reduced body weight by 25%. Significant reduction in body weight gain was observed when the diet containing 3.5 ppm of total aflatoxin was fed to broiler chickens (Kubena *et al.*, 1990; Harvey *et al.*, 1993 and Kubena *et al.*, 1993). Scheideler (1993) reported significant decrease in the body weight gain of broilers chickens when the feed containing 2.5 ppm of aflatoxin B_1 was fed to them. Body weight gain was reduced significantly at 5 ppm of total aflatoxin (Okotie-Eboh *et al.*, 1997 and Kubena *et al.*, 1998). Azzam and Gabal (1997) fed diets containing 100 and 200 ppb aflatoxin, and observed significant reduction in weight gain. However, Raju and Devegowda (2000) reported significant decrease in the feed consumption of broiler chickens on diet containing 0.3 ppm of aflatoxin B_1. Goodarzi and Modiri (2011) found that aflatoxin more than 1ppm resulted in reducing of broiler performance and increasing feed conversion ratio. Chi and Broomhead (2011) observed that feeding 2.7 ppm aflatoxin to the broiler birds led to reduced body growth weight which is the result of significant ($P<0.05$) decrease in feed intake. Chicks receiving AF (0.5 ppm) contaminated feed had significantly ($P<0.05$) suppressed body weight, feed consumption (Pourelmi, 2013).

Patil *et al.* (2013) reported that inclusion of aflatoxin (0.5 ppm) in the basal diet markedly ($P<0.05$) reduced the feed intake at all stages of growth in broiler chickens. Modirsanei *et al.* (2004) reported that the addition of 1.0 mg AF, per kg in diet of broiler chicks significantly reduced feed intake by approximately 17%. Kubena *et al.* (1990) reported significant reduction in feed intake at 3.5 ppm of total aflatoxin level. However, Kubena *et al.* (1998) showed a significant reduction in feed consumption when total aflatoxin (5 ppm) was fed to the broiler chickens. Singh *et al.* (2011) observed that turkey poults can tolerate aflatoxin B_1 upto 50 ppb, increasing the aflatoxin content beyond 50 ppb resulted in significant reduction in body weight gain. Sapocota *et al.* (2007) observed that feeding aflatoxin B_1 @ 300 ppb resulted in decrease in the feed consumption by the birds from third week onwards. Kaoud (2012) found that the aflatoxin B_1 (1 ppm) in broiler diet significantly decreased the BW gain, feed intake, and impaired feed conversion rate. A significant ($P<0.05$) decrease in body weight, feed consumption and increase in FCR and mortality were observed due to feeding 0.05 ppm AF to broiler chickens (Indresh *et al.*, 2013).

Reduced feed intake was also reported by Ledoux *et al.* (1999) when feed was contaminated with 4 ppm of aflatoxin B_1. Santurio *et al.* (1999) observed reduced feed consumption when broilers were fed diet contaminated with 3 ppm of total aflatoxins. However, Raju and Devegowda (2000) reported 21% decrease in final body weight at 35 days of age in broilers fed on diet containing 300 ppb AFB_1. Reddy *et al.* (1982) found that growth and feed consumption at 0.5 ppm, eviscerated yields at 0.25 ppm and feed efficiency at 1.25 ppm were adversely affected. Churchill *et al.* (2009) reported lower feed consumption and inferior feed efficiency in AFB_1 contaminated diet than that of control group at eight weeks of age. Beura *et al.* (1993) reported that growth, feed consumption, retention of nutrients at 0.8 ppm and feed efficiency at 1.6 ppm of AF were adversely affected. Denli *et al.* (2009) reported that aflatoxicosis was characterized in broiler chickens by decreased feed intake, poor feed utilization and increased mortality.

Safameher (2008) reported that chickens fed 500 ppb of AF containing diet showed reduction in feed intake. Broilers diets naturally contaminated with aflatoxin decreased feed consumption and resulted in poor feed efficiency (Ghahri *et al.,* 2010). Scheideler (1993) reported that feed conversion ratio increased when broilers fed with 2.5 ppm AFB_1. Increased FCR was observed by Kubena *et al.* (1998) when broilers fed with 5 ppm total aflatoxin contaminated diet. Rosa *et al.* (2001) reported that 5 ppm of AFB_1 significantly increased the feed conversion ratio of broiler chicks. Johri *et al.* (1988) studied response of purebred broiler chicks to low level of aflatoxin and concluded that feed efficiency at 300 ppb and feed consumption at 500 ppb were reduced significantly. Aflatoxin reduced ($P < 0.05$) feed intake and body weight gain of the broiler birds when they were fed with the feed containing 1 and 2 ppm aflatoxin (Yarru, 2008). Silambarasan (2011) also concluded that addition of aflatoxin B_1 at the rate of 300 ppb in the diet impaired the performance like body weight gain, feed intake and utilization efficiency of feed, energy and protein in broiler chickens during 0-6 weeks of age. Manafi (2011) found that inclusion of 500 ppb AF in the diet significantly ($P<0.05$) reduced feed consumption, feed efficiency. Sawarkar *et al.* (2012) found that there was gradual and significant ($P<0.01$) decrease in average body weight and lower FCR was due to feeding combination of 100 ppb aflatoxin and 100 ppb ochratoxin.

2.2.2 Effect of aflatoxin on visceral organs

Liver, kidney and the immune system organs are considered to be target organs for AF and these are primarily affected in aflatoxicosis. The main effects of aflatoxins are related to liver damage and the classic symptom of aflatoxicosis is an increased liver weight (Miazzo *et al*., 2000). Renal tubular degeneration has also been reported in broilers receiving 0.5 to 1 ppm aflatoxin (Eraslan *et al.*, 2004). Aflatoxin at low doses, 150 and 300 ppb, can cause degenerative changes in several organs, including liver and kidney (Karaman *et al.*, 2010). Significant reductions in the relative weights of the bursa of Fabricius were recorded in chicks receiving 2 ppm of AF (Verma *et al.*, 2004), whereas the minimum effective dose of AF has been reported to be 1.0 or 1.25 ppm to bring about a significant reduction in the relative weight of the organ (Rao *et al.*, 1988). A severe and significant regression of bursa of Fabricius in both sexes of broilers was observed by Thaxton *et al.* (1974) at 0.75 ppm and higher levels of aflatoxin. Bailey *et al.* (1998) reported that total aflatoxin fed to broiler chickens at the rate of 5 ppm showed increase in relative weight of liver, kidney, gizzard, pancreas and spleen. A significant reduction in the relative size of bursa of fabricius was recorded at 200, 400 and 600 ppb aflatoxin levels as compared to 50 ppb (Manegar, 2010). Applegate (2009) found, intestinal crypt depth, but not villus length (thus influencing the villus:crypt ratio), increased linearly with increasing aflatoxin concentration. A significant ($P<0.05$) decrease in relative weights of bursa, thymus and increase in relative weight of liver, kidney were reported on feeding of 0.5 ppm AF to the broiler chickens (Indresh *et al*., 2013).

Karaman *et al.* (2005) reported the livers and kidneys of chicks were mostly swollen and pale yellow-red. The spleens were enlarged and congested whereas the thymus was atrophied. The bursa of Fabricius had no visible morphological changes but bursa of Fabricius and spleen had mild to moderate lymphocytic depletion in their follicles. Medullary extension and cortical atrophy in the thymus and hydropic degeneration in the tubular epithelium of kidneys were also noted in the broilers fed with feed containing 2 ppm aflatoxin. At 3.5 ppm of total aflatoxin in broilers diet relative weight of liver, kidney gizzard, spleen, pancreas, proventriculus were increased and relative weight of bursa of Fabricius was decreased (Kubena *et al.*, 1990 & 1993). Carnaghan *et al.* (1966) reported that acute toxicity of aflatoxins in chickens may be characterized by hemorrhage in many tissues and liver necrosis with icterus.

Mortality was low but marked hepatic damage was manifested by enlarged and hemorrhagic liver. The kidneys of affected birds appeared enlarged and congested (Tung *et al*., 1973) and the spleen was enlarged and mottled in appearance (Tung *et al*., 1975). Raju and Devegowda (2000) reported a significant increase in the visceral organ weight such as liver, kidney, gizzard and significant increase in the mortality rate was also observed. Relative size of liver, kidney, spleen, pancreas, proventriculus were increased in broilers fed with 5 ppm of total aflatoxin (Bailey *et al*., 1998; Kubena *et al*., 1998). Ledoux *et al*. (1999) observed that broiler fed with 4 ppm of aflatoxin B_1 contaminated diet revealed increased weight of liver, kidney and pancreas. The eviscerated carcass yield in aflatoxin treated birds was significantly ($P<0.05$) lower than that of control (Churchill *et al.*, 2009). Girish and Devegowda (2006) reported that AF caused significant increase in size of liver, kidney, spleen and gizzard (21.7%, 26.4%, 51% and 16.8%, respectively) when fed at the rate of 2 ppm. Silambarasan (2011) and Abaji (2012) reported that relative weight of liver was significantly ($P<0.05$) increased and that of bursa decreased in diet containing 300 ppb aflatoxin. Ghahri *et al.* (2010) reported that consumption of aflatoxin contaminated diet caused significant increases in the relative weights of liver and reduction in the relative weights of bursa of Fabricius. Patil *et al.* (2013) reported that aflatoxin (0.5 ppm) inclusion in the basal diet significantly ($P<0.05$) increased liver weight in broiler chickens.

2.2.3 Gross and histopathology of liver

Aflatoxin is a potent liver toxin causing hepatocarcinogenesis, hepatocellular hyperplasia, hepatic necrosis, cirrhosis, biliary hyperplasia, and acute liver damage in affected animals. Other effects include mutagenic and teratogenic effects. Large doses of aflatoxin are lethal and chronic exposure to low levels of aflatoxin can result in cancer and immunosuppression (Sharma, 1993). Primary organ affected by aflatoxin is liver and it also acts as the primary site of toxin residue. Gross and histopathological changes are useful tools for evaluating toxic effects of aflatoxin in target organs and for examining the efficacy of the detoxifying agents in broilers (Rosa *et al.*, 2001). A significant increase in the relative weight of the liver was recorded in broilers given 1 and 2 ppm of AF (Verma *et al.*, 2004). Rosa *et al.* (2001) observed significant increase in the weight of liver, kidney and spleen in broilers when fed with 0.5 ppm AFB_1. Espada *et al.* (1993) reported vacuolation of liver Cells in broiler chicks after intoxication of

aflatoxin. Karaman *et al.* (2005) reported that microscopically, the livers of chicks fed 2 ppm aflatoxin containing diet revealed moderate to severe hydropic degeneration and fatty changes in hepatocytes, bile duct proliferation and periportal fibrosis in the portal areas. Occasionally, nodular lymphoid cell accumulations were seen within the hepatic lobules. Safameher (2008) reported that the macroscopic appearance of livers from chickens fed aflatoxin contaminated feed showed a gross appearance with hypertrophy, friable and yellowish discoloration. Histopathological examination revealed accumulation of large fat droplets that displaced the nucleus in chicks given dietary aflatoxin. Denli *et al.* (2009) reported that there was a significant damage in the liver tissues of broilers received 1 ppm of aflatoxin. Liver tissues had shown vacuolar degeneration of hepatocytes, perilobular inflammation (mainly mononuclear cells), bile duct hyperplasia and hypertrophy compared with the tissue of birds fed on the uncontaminated diet. Sawarkar *et al.* (2012) found vacuolar degenerative changes and focal areas of necrosis in hepatocytes along with periportal necrosis in the liver and in the kidney cloudy swelling and severe glomeruli nephropathies along with multiple haemorrhages in the mycotoxicated (100 ppb aflatoxin and 100 ppb ochratoxin) broiler group.

Kaoud, (2012) found that liver tissue of broilers receiving AFB_1 (1 ppm) in feed had perilobular inflammation and vacuolar degeneration of hepatocytes. Ortatatli and Oguz (2001) reported that feeding 2.5 ppm of aflatoxin caused significant increase in relative weights of liver. Grossly, liver of chicks that had consumed aflatoxin were enlarged, pale yellow, friable and with rounded margin. This enlargement was more pronounced in centrilobular areas. In severe cases, some hepatocytes had pycnotic nuclei and were so swollen that several cells had ruptured. The sinusoids were shrunken or completely plugged due to swollen hepatocytes and were few or no erythrocytes in the central vein. Lethal aflatoxicosis can cause either dark red or yellow discoloration of the liver due to congestion or fat accumulation, respectively (Slowik *et al.*, 1985). Liver enlargement and discoloration in broilers had been reported by Kermanshahi *et al.* (2009). Eraslan *et al.* (2006) reported that histopathological examination demonstrated fatty degeneration of hepatocytes, mononuclear cell infiltration in periportal region, hyperplastic bile ducts and hepatocellular degeneration in the liver of aflatoxin (1 ppm) fed broilers. Silambarasan (2011) reported hypertrophy of liver and histopathological changes in liver characterized by marked destruction of hepatic cords, dilatation, and congestion of central

veins, mild adenomatous necrotic foci and mild to moderate deposition of fats in the hepatic parenchyma.

2.2.4 Effect of aflatoxin on immune system

Immune system is highly sensitive indicator of the aflatoxicosis in poultry. Aflatoxins are reported to affect the immune system because of their ability to inhibit protein synthesis (Tung *et al.*, 1975). Sharma (1993) reported immunosuppression caused by AFB1 in chickens and turkeys as well as laboratory animals by inhibiting the protein synthesis and cell proliferation. The immune system is separated into two major mechanistic groups: the innate immune system, which mediates the initial protection from infection, and the adaptive immune system, which develops more slowly but is specific and more effective in antigen elimination (Abbas and Lichtman, 2006). The aflatoxin supresses the cell mediated as well as humoral immunity of the birds. Michael *et al.* (1973) reported impairment of reticuloendothelial system of chickens during aflatoxicosis. Aflatoxin decreased the concentrations of serum protein and immunoglobulins IgM, IgG and IgA in birds which ultimately resulted in the suppression of immunity. Cell-mediated immunity, measured by a delayed hypersensitive skin test was significantly decreased in broilers receiving AF at 200 ppb or more. Patil *et al.* (2013) reported that aflatoxin inclusion 0.5 ppm in the feed caused a significant reduction in haemagglutination titer against sheep RBC and cell mediated immune responses to phytohemagglutinin (PHA-P) in broilers.

The immune responses mediated by T cells appeared to be more sensitive to AFB_1, although depending on the dose both helper T cells and suppressor T cells are affected (Hatori *et al.*, 1991). Neither humoral immunity nor the developments of the acquired immunity to Newcastle disease or fowl cholera vaccination were decreased in turkeys or in broilers by AF fed diet (Giambrone *et al.*, 1978). The presence of low level of AFB_1 in the feed decreased the vaccinal immunity and may therefore lead to the occurrence of disease even in properly vaccinated flocks (Lesson *et al.*, 1995). Thaxton *et al.* (1974) recorded reduced antibody production following injection of sheep red blood cells in chickens experiencing aflatoxicosis. Batra *et al.* (1991) found that chickens fed AFB_1 and vaccinated against Marek's disease showed a significantly higher frequency of gross and microscopical lesions of Marek's disease than chickens fed aflatoxin free diet. Consumption of aflatoxin contaminated feed resulted in

significant reduction in antibody titers against Infectious Bronchitis as compared to the control diet at 28 and 35 days of age. Aflatoxin impairs the humoral and cellular immune responses and increase susceptibility to some environmental and infectious agents (Azzam and Gabal, 1998). Ghosh and Chauhan (1991) observed that 300 ppb AFB_1 in broiler feed caused immune suppression with no apparent clinical effects, but can result in flock morbidity and/or mortality caused by secondary infections. The humoral immunity of these chicks was affected in the lower AF dose (50 ppb) in feed (Oguz *et al.*, 2003). Chickens receiving aflatoxin contaminated diets showed higher susceptibility to Marek's disease (Edds and Bortell, 1983), infectious bursal disease virus (Giambrone *et al.*, 1978), congenitally acquired salmonellosis (Wyatt and Hamilton, 1975) and duodenal and cecal coccidiosis (Edds *et al.*, 1973) than chickens receiving aflatoxin free diet. Aflatoxin affected the vaccine efficacy against Fowl cholera, MD and Infectious Bronchitis (IB) in poultry (Yegani *et al.*, 2005). Silambarasan (2011) observed that feeding of aflatoxin contamination in feed (300 ppb) from 0-6 weeks of age suppressed cell mediated and humoral immunity in broiler chickens.

2.2.5 Effect of aflatoxin on biochemical and haematological profile

Aflatoxin decreased total serum proteins, alpha, beta and gamma globulins, with IgG being more sensitive than IgM (Tung *et al.*, 1975). Decreased concentration of total serum protein was observed with feed contaminated with 3.5 ppm of total aflatoxin in broilers (Kubena *et al.*, 1990; Kubena *et al.*, 1993; Kubena *et al.*, 1998; Harvey *et al.*, 1993). Serum total protein level significantly reduced by inclusion of 5 ppm total aflatoxin in broiler diet (Okotie-Eboh *et al.*, 1997 and Bailey *et al.*, 1998). Ledoux *et al.* (1999) showed that inclusion of AFB_1 in the broiler diet at 4 ppm level decreased serum protein level. Raju and Devegowda (2000) also reported that broiler chicks fed with 0.3 ppm aflatoxin B_1 decreased the total protein level in the serum. Denli *et al.* (2009) observed an increase in ALP, AST and decrease in the concentration of uric acid and serum total protein was observed due to feeding 1 ppm of aflatoxin contaminated diet. Kececi *et al.* (1998) reported that feeding 2.5 ppm of aflatoxin in broilers caused significant decrease in the total serum protein, albumin, inorganic phosphorus, uric acid and total cholesterol. Eraslan *et al.* (2006) observed that broilers fed with 1 ppm of AF contaminated diet showed decrease in the concentrations of total protein, albumins, glucose and GGT. Moreover, he also found the increased activity of AST, ALT and LDH (lactate

dehydrogenase). Safameher (2008) reported that plasma total protein together with albumin levels were decreased in chickens fed with 0.5 ppm of aflatoxin containing diet. Serum uric acid levels and serum alkaline phosphatase levels were decreased in chickens with dietary aflatoxin. Serum LDH as well as AST activities was elevated in chickens fed aflatoxin contaminated diet. Chicks fed AF alone had increased levels of SGPT (ALT), SGOT (AST), GGT and LDH compared with control chicks (Shi *et al.,* 2009). A progressive reduction in serum protein concentration was observed in broiler chickens fed diets containing 100, 200, 400 and 600 ppb of aflatoxin (Manegar *et al.,* 2010).

Kececi *et al.* (1998) reported significant decrease in total serum protein when broilers fed with 2.5 ppm of aflatoxin in their diet. Increased ALT (alaninetransferase), AST (aspartate transferase) and GGT (Gamma glutamyl transferase) activity were also observed when chickens fed with 500 ppb of AFB_1 to broilers (Kermanshahi *et al.,* 2009). Oguz *et al.* (2002) reported that total serum protein, total cholesterol and uric acid levels were decreased with 50 ppb of AF fed diet to broiler chicks. Churchill *et al.* (2009) reported that aflatoxin caused significant ($P<0.05$) reduction in immunity against Ranikhet and infectious bursal diseases and serum biochemical values of total proteins, albumin, cholesterol and glucose. Several biochemical parameters were affected by aflatoxin exposure. Significant decrease in total serum protein level was reported by Rosa *et al.* (2001) by adding 5 ppm of aflatoxin in the diet. The level of serum protein was decreased in broilers fed with AFB_1 contaminated feed (Juan-juan *et al.,* 2010). Bailey *et al.* (1998) reported decreased level of alkaline phosphatase and uric acid concentrations in broilers when they were fed with 5 ppm of total aflatoxin. Kubena *et al.* (1993) observed significant reduction in serum AST at 3.5 ppm of total aflatoxin. Broiler chicks fed with 0.3 ppm of aflatoxin B_1 in their diet showed significant decrease in the level of serum AST (Raju and Devegowda, 2000). Santurio *et al.* (1999) showed decrease in AST when broilers fed contaminated diet with 3 ppm of aflatoxin.

Decreased values of haematocrit, haemoglobin, mean corpuscular haemoglobin, thrombocyte counts, percentage of lymphocyte and monocyte counts and increased values of white blood cell and heterophil counts were observed due to feeding of 2.5 ppm AF (Kececi *et al*., 1998). Red blood cell, haematocrit, haemoglobin, thrombocyte, and lymphocyte counts were significantly reduced, while significant increases were seen in heterophil counts by AF (2

ppm) treatment in feed (Basmacioglu *et al*., 2005). Reduction in the serum cholesterol level was observed in broilers when they were fed with aflatoxin (3.5 ppm) contaminated diet (Okotie-Eboh *et al.*, 1997; Kubena *et al.*, 1990, 1993 and 1998). Ahamad (2000) reported decreased level of serum cholesterol in broilers at 0.5 ppm AFB_1. However, at 0.3 ppm of aflatoxin B_1 significant reduction in serum cholesterol was observed (Raju and Devegowda, 2000). Decrease in serum uric acid occurred when 2.5 ppm of total aflatoxin contaminated diet was fed to broilers (Kececi *et al.*, 1998). Okotie-Eboh *et al.* (1997) reported decrease in serum glucose in broilers at 5 ppm of total aflatoxin. Significant reduction in serum glucose level was observed when broilers fed with aflatoxin B_1 (4 ppm) contaminated diet (Ledoux *et al.*, 1999). Balachandran and Ramakrishnan (1987) studied the influence of dietary aflatoxin on serum enzyme levels in broiler chicken. They measured SGPT, SGOT, serum amylase and lipase levels in Cobb broiler chickens fed 3 ppm aflatoxin. They observed an increase in serum lipase and SGOT levels, and decrease in serum amylase levels resulting in altered nutrient digestion, absorption and metabolism. Aflatoxin reduced serum total proteins, serum calcium and phosphorus but increased liver weights in a dose dependent manner (Yarru, 2008). Silambarasan (2011) reported that total serum protein was reduced significantly ($P<0.01$) in broilers. However no effect was observed on serum cholesterol and uric acid concentration when they fed with 300 ppb of aflatoxin. Sawarkar *et al.* (2012) found significant ($P<0.01$) reduction in values of haemoglobin (Hb) and reduction in serum total protein, albumin and globulin in mycotoxin (100 ppb aflatoxin and 100 ppb ochratoxin) fed broilers. Patil *et al*. (2013) reported that the inclusion of aflatoxin (0.5 ppm) in diet induced a significant ($P<0.05$) hypoproteinemic state in broiler chickens.

2.3 Amelioration of aflatoxicosis through dietary approaches

The toxicity of mycotoxins may be strongly influenced by nutritional intervention. Changes in the composition of poultry diets alleviate some of the adverse effects attributed to the consumption of aflatoxin. Dietary fortification with certain vitamins (Hamilton *et al.*, 1972), protein (Smith *et al.*, 1971), fat (Hamilton *et al.*, 1972), fatty acids (Lanza *et al.*, 1981) and trace mineral have shown to lessen the effect of aflatoxin on the performance of poultry. Dietary approaches hold great potential for protecting birds against the effects of mycotoxins.

2.3.1 Effect of dietary zinc on aflatoxicosis

Zinc is present in all cells and participates in a wide variety of metabolic processes by virtue of its diverse catalytic roles in over 200 enzymes. Zn enzymes are involved in the synthesis and/or degradation of carbohydrates, lipids, proteins, and nucleic acids and encompass all known classes of enzymes (Falchuk and Vallee, 1985; Kaim and Schwederski, 1994). Zinc must be supplemented to most diets of poultry to meet the nutritional requirements for this element, because of the poor availability of zinc in plant feed ingredients caused by the binding of Zn by phytate (Oberleas *et al.*, 1962; O'Dell *et al.*, 1964; Ellis *et al.*, 1982; Fordyce *et al.*, 1987).

It is the second most abundant trace element in mammals and is a component of many enzymes such as superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, nuclear (A) polymerase, leucine aminopeptidase etc. and taking part in antioxidant defence as an integral part of superoxide dismutase; hormone secretion; keratin generation and epithelial tissue integrity; nucleic acid synthesis; protein synthesis; sexual development and spermatogenesis and immune function. Mycotoxins can substantially decrease antioxidant assimilation from the feed and increase their requirement to prevent damaging effects of free radicals produced as a result of mycotoxin exposure. According to Surai (2002) the mycotoxin toxicity can be decreased as a result of increased antioxidant supplementation.

There are three different mechanisms by which Zinc acts as an antioxidant:

- The first and important mechanism by which Zinc acts as an antioxidant is that it plays a key role in suppression of free radicals because it is a cofactor of the main antioxidative enzyme Cu-Zn- SOD and it also inhibits the NADPH-dependent lipid peroxidation (Prasad and Kucuk, 2002). Superoxide dismutase is present in intermitochondrial space of hepatocyte, brain and erythrocyte. It prevents lipid peroxidation via inhibiting glutathione depletion as well (Prasad, 1997). Due to the ability to replace Fe and Cu from binding sites, Zn can compete with these transition metals to bind to the cell membrane and decrease the production of free radicals and thus exert a direct antioxidant action (Oteiza *et al.*, 1996; Powell, 2000; Prasad and Kucuk, 2002).

- Zinc also induces production of metallothionein, a cystine-rich protein that acts as a free radical scavenger (Oteiza *et al.*, 1996). Zinc is absorbed in the small intestine and an intestinal pool of Zn may be formed by binding the metal to the intestinal metallothionein or Zn may be transported by albumin in plasma to the liver (Prasad, 1993). More than one isoform of metallothionein is found in different tissues in animal species. But only, a single isoform of metallothionein in the chicken has been found in liver, pancreas, kidney, and intestinal mucosa (Mc Cormick, 1984; Sandoval *et al.* 1998; Cao *et al.* 2000). Metallothionin, which is an effective scavenger for hydroxyl radical; it has been suggested that Zn-metallothionein complexes in the islet cells provide protection against immune-mediated free radical attack (Salgueiro *et al.*, 2000; Prasad and Kucuk, 2002).
- Another mode of action proposed for Zn as an antioxidant is its interaction with vitamin E. During Zn deficiency, probably due to defective formation of chylomicrons in the enterocyte, absorption of lipid-soluble vitamins such as E and A is impaired.

Liver is the site of high zinc metabolic activity and the organ most affected by aflatoxicosis. Wyatt *et al.* (1985) studied the effect of supplementation of zinc (40 ppm diet) in calves fed 5 ppm aflatoxin on some plasma enzymes. The concentration of plasma glutamic-oxaloacetatic acid transaminase and alkaline phosphatase were increased substantially and lactic dehydrogenase was reduced in aflatoxin fed calves means supplementation of zinc partially counteract the effect on aflatoxin on these enzyme. Hegazy and Adachi (2000) found that Zn-fortified diet (60 ppm Zn supplemented) resulted in significant improvement in relative body growth and feed efficiency in chicks exposed to combination of *Salmonella* and aflatoxin inoculation. This result may due to an increase plasma concentration of insulin-like growth factor-1, which depends mainly on an adequate level of Zn in the blood. This level may be altered by *Salmonella* and aflatoxin treatments. In this way Zn also help in body growth performance which is reduced in aflatoxicosis.

2.3.2 Effect of dietary methionine on aflatoxicosis

Aflatoxicosis, one of the important mycotoxicosis of poultry, causing economic losses due to reduced production or mortality of the birds. One of the alternatives to prevent and control the aflatoxicosis is supplementation of methionine. Methionine is a lipotropic agent so

that, helps to prevent the accumulation of fat in the liver and thus ensure normal liver function, which is essential for the elimination of toxins from the body. When we can add methionine; as a methyl donor, for amelioration of aflatoxicosis then it act by two ways:

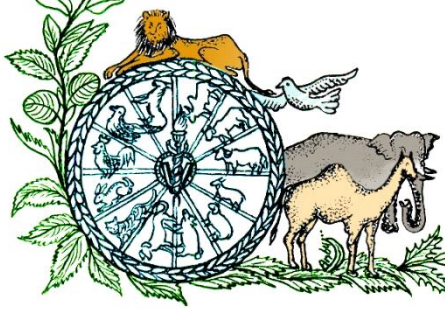
First, methionine is an essential amino acid that contains sulphur, a substance that is required for the production of the body's most abundant natural antioxidant, glutathione (GSH).The enzymatic biotransformation changes undergone by a toxin (mycotoxin) in the body usually result in its detoxification consisting of a loss of toxicological activity (Galtier *et al*., 2008). Mycotoxins are well known for undergoing liver biotransformation in humans and animal species. Aflatoxins are thought to be metabolized to highly reactive epoxides and phenolats (the furan system of aflatoxin is involved here) that can bind and interfere with nucleic acid and proteins (Ciegler, 1975). Thus not only the parent molecule capable of binding of cellular structures, but is also its metabolites epoxides and phenolates. The epoxides and phenolates are normally conjugated with glutathione that serves to protect vital macromolecules from these toxin intermediates. Hepatic necrosis is thought to result when glutathione reserves have been drastically depleted by conjugation with toxin intermediates so that the toxin intermediates are free to bind covalently to vital cellular macromolecules. Therefore supplementing methionine, which in turn helps to increase hepatic glutathione concentration, may aid to protect liver against aflatoxin.

Secondly, S-adenosyl Methionine (SAMe), a metabolite of methionine, is an important molecule that is required for many vital functions and survival of cells in the body. It is the principal biological methyl donor required for methylation of DNA, RNA, biogenic amines, phospholipids, histones, and other proteins. In the liver, SAM is a precursor for glutathione. Thus, SAM deficiency can impair many vital functions of the liver, which render it susceptible to injury by toxic agents such as aflatoxins. Biosynthesis of SAMe occurs in all mammalian cells as the first step in methionine catabolism in a reaction catalyzed by methionine adenosyltransferase (MAT). Decreased hepatic SAMe biosynthesis is a consequence of all forms of chronic liver injury (Mato and Lu, 2007). Carcinogens cause DNA hypomethylation by decreasing the availability of SAMe. Since methionine increases the liver,content of SAMe, provision of methionine may prevent carcinogen-induced DNA hypomethylation (Tao *et al*., 2000).

Sulfur amino acids, i.e., cysteine and methionine (which can be converted to cysteine via the cystathionine pathway in the liver), are the essential components for glutathione synthesis. Any attempt to increase intracellular cysteine by supplementing with methionine, which can be actively converted to cysteine via the cystathionine pathway, would be effective in elevating the glutathione level in hepatocytes (Wang *et al.*, 1997), this may help to protect the liver cells from toxic effects of mycotoxins including aflatoxins. It has been postulated that higher levels of methionine supplementation would counteract the methionine depletion due to the fact that glutathione is composed of methionine and cystine (Devegowda *et al.*, 1998). Naveenkumar *et al.* (2005) found that 0.5% supplementation of methionine was beneficial in preventing the aflatoxin (1 ppm)-induced toxicity.

Sapocota *et al*. (2007) found that the supplementation of methionine, 0.8 per cent showed the best result on performance of broilers (growth, feed intake and feed efficiency) and reduced the pathological alterations of the vital organs due to feeding 300 ppb aflatoxin. Veltman *et al*. (1983) observed that increasing dietary total sulphur amino acids to level in excess of NRC protected chicks form the growth depressing effects of aflatoxin, possibly through an increased rate of detoxification by glutathione, a sulphur amino acid metabolite. Permana *et al*. (2011) evaluate the effect of supplementation of DL-methionine on aflatoxin contaminated diet on broiler performance and showed the result that the level of aflatoxin in the diet varied from 19.24 - 34.50 ppb in starter diet and 66.67 - 78.50 ppb in finisher diet. During finishing period, the supplementation of 0.25% DL-methionine increased significantly ($P<0.01$) feed intake from 2,578 g/chick in control group to 2,746 g/chick, however the diet supplemented with 0.35% DL-methionine was not significantly different from the control diet. The supplementation of 0.25% DL-methionine during starter period increased significantly ($P<0.01$) body weight gain (1,498 g to 1,726 g). Adding methionine at 400 or 600 mg/kg abolished the effect of aflatoxin in body weight gain (Al-Jubory, 2000).

Materials and Methods



3 MATERIALS AND METHODS

Present study is the evaluation of zinc and methionine supplementation in the aflatoxin contaminated feed of the broiler chickens. Different levels of zinc and methionine were incorporated in aflatoxin contaminated diet and their effect on growth rate, feed consumption, feed conversion ratio, immune response, carcass traits, haematology, blood biochemical parameters and histopathology has been studied. The experimental procedures and analytical techniques employed during the course of study have been described below. The work of this study was undertaken in the Division of Avian Nutrition and Feed Technology, Central Avian Research Institute, Izatnagar, U.P-243122.

3.1 Production of aflatoxin

3.1.1 Fungal culture

Aflatoxin was produced using the fungal strain *Aspergillus flavus* NRRL 6513 that was obtained from U.S Department of Agriculture, Illinois, U.S.A and maintained at mycotoxin laboratory, CARI, Izatnagar. To get the fresh spores the culture was regularly subcultured on Potato Dextrose Agar (PDA) medium slants and stored at 5ºC.

3.1.2 Sub-culturing and production of fresh spores

Potato-agar medium was prepared according to the procedure described by Shotwell *et al*. (1966). Three flasks were taken. Flask contained distilled water, 100 ml; dextrose, 20 g; $CaCO_3$, 0.2 g; and $MgSO_4$. $7H_2O$, 0.2 g. Flask 2 contained 400 ml of distilled water and 15 g of agar. Flask 3 contained 200 g of potatoes (Peeled and sliced) and 500 ml of distilled water. Contents of flask 3 were brought momentarily to 121^{0}C in an autoclave and filtered through cheese cloth. The solution was brought up to the original volume.

Simultaneously, the agar in flask 2 was melted and the solution in flask 1 was heated to boiling. Contents of all the three flasks were mixed; the whole medium was autoclaved at 15 lbs pressure for 10- 15 minutes. The medium was poured to test tubes which after proper plugging were kept overnight in slanting position. Inoculation was done under a horizontal laminar flow, which was switched on 2 hr before. Fresh spores were held and streaked with the help of a sterilized platinum loop before the flame. The test tubes were incubated at a temperature of 28ºC±1ºC inside the BOD incubator for 8 days. The fungal growth was noticed by whitish appearance during the initial 3 days and subsequently turned to greenish and finally the dark green colouration confirmed the formation of fresh spores. The spores were scraped loose with a loop. The slants were shaken to give a uniform suspension of spores. The spore's suspension was used to inoculate the substrate.

3.1.3 Aflatoxin production on maize

Aflatoxin was produced on maize substrate. Fermentations were carried out in batches as per the method described by Shotwell *et al.* (1966). Hundred grams of cracked maize free from all possible extraneous materials was taken in Erlenmeyer flasks plugged with cotton and autoclaved at 15 lbs pressure for 15 minutes and cooled. To this half cooked maize, 6-10 ml of sterilized distilled water was added (according to the ambient temperature) so as to maintain the desired amount of moisture and humidity. Each flask after shaking was inoculated with fresh spores obtained from sub-culturing of the tubes. The flasks containing inoculated maize were incubated at 28±1ºC for 8 days in a BOD incubator. Regular shaking was done to avoid sticking of maize to the walls of flasks and to promote quick and easy fungal growth. After 3 days, the mould growth was confirmed by the appearance of whitish growth on maize, gradually changing to greenish colour and finally to dark green conidia showing extensive moulds growth. The flasks containing mouldy maize were removed from the incubator and were autoclaved at 15 lbs pressure for 15 minutes to destroy the fungus and live spores. The mouldy maize was dried in hot air oven at 80ºC for 24 hours. The contaminated maize after drying was ground to fine powder of maize. The mixture was stored in large polythene bags. The representative samples in triplicate were drawn for estimating the aflatoxin content.

3.2 Aflatoxin analysis

The extraction and estimation of aflatoxin was done as per the procedure of Pons *et al.* (1966). Aqueous acetone was used for extraction of the toxin. Aflatoxin contents were finally quantified using a spectrophotometer.

3.2.1 Preparation of crude extract of the sample

Known quantity of representative sample of the material (mouldy maize) was taken in a 500 ml Erlenmeyer flask and extracted with 70 per cent aqueous acetone for 1 hr with the help of wrist action horizontal shaker. The contents were filtered and the filtrate was collected in a 500 ml beaker. The volume of the filtrate was reduced to 140 ml on a water bath. Twenty percent lead acetate (20 ml) and distilled water (60 ml) were added after cooling. The contents were filtered through Whatman No. 1 filter paper and the filtrate was centrifuged at 10,000 rpm for 10 minutes. The obtained supernatant was extracted with 50 ml of chloroform in a separating funnel and was kept overnight after shaking for complete separation. The chloroform layer (bottom layer) was collected and passed successively through anhydrous sodium chloride and sodium sulphate. The collected liquid should evaporate to dryness. This was the crude extract of aflatoxin. TLC plates were prepared using silica gel-G and it was placed into stopper flask and 100 ml of distilled water was added to it. The contents of flask were shaken vigorously and poured into an applicator. Five glass plates (20×20cm) were immediately coated with a 0.25 m thick of slurry. The coated plates were placed in dust free atmosphere until get settled and they were heated in an oven at 100°C for one hour. After cooling, the plates were stored in a pate chamber. Before use, the plates were activated on hot air oven at 80°C for one hour. The crude extracts were dissolved in 5 ml chloroform. The solutions prepared in the chloroform for TLC were applied to the chromatoplates using micropipettes. The extract solutions were spotted in a line 2 cm from the bottom of the chromatoplates leaving at least 2 cm margin from either side. The solutions containing known quantity of AFB_1 were spotted along with the sample extracts. While applying the extracts the solvent was not allowed to spread more than 5 mm in diameter. The solutions were spotted as quickly as possible in subdued light. The plates were developed by standing them in a chromatography tank containing toluene-ethyl acetate-formic acid (60:30:10) solvent mixture to a depth of not more than 1 cm and the tank was saturated with the solvent vapour before use. The solvent front was allowed to reach 10 to 12 cm. it took about 30 minutes at 27°C. After development, the plates were dried in

horizontal position and viewed under long wave ultraviolet lamp in a dark cabinet. Fluorescent intensities of the spots of sample extract with that of the standard gave the concentration for further calculation. If the spots were not matching, the sample extract was diluted further with chloroform until sample AF fluorescence matches with standard fluorescence. Concentration of AFB_1 in µg/g (ppm) was calculated from following expression.

$$\text{Aflatoxin µg/g (ppm)} = \frac{S \times Y \times V}{X \times W}$$

Where,

S = Standard AFB_1 which matched the test sample (µl)

Y = Concentrations of standard AFB_1 (µg/ml)

V = Solvent used dilution of sample extract (ml)

X = Sample extract spotted giving florescent intensity equivalent to AFB_1 standard (µl)

W = Weight of original sample contained in the final extract (gm)

3.2.2 Aflatoxin standards

The AFB_1 standard was procured from M/s Sigma Aldrich Chemicals Ltd., USA. A solvent containing acetonitrile and benzene (2:98) was used for preparation of stock and working solutions of standards.

3.2.3 Quantitative estimation of aflatoxin

The area covering the spots were marked by a sharp needle under the UV light and the silica gel covering each spot was scraped off with a blade and collected in tubes. The toxin was extracted with 2 ml cold methanol for 3 minutes and filtered through sintered glass. The filtrate was washed three times with methanol and the combined filtrate was made to 5 ml volume. Optical density at 363 nm was measured on an UV spectrophotometer. The concentration of each aflatoxin in µg was calculated from the following expression:

$$\text{Amount of aflatoxin present (µg)} = \frac{\text{Optical density x factor x eluant volume x original Volume of chloroform extract}}{\text{Volume of chloroform extract spotted}}$$

The factor used for AFB_1 in the above formula was 14.18 and were calculated from extinction coefficient of AF (Nabney and Nesbitt, 1965).

3.3 Experimental design

Experimental design was completely randomized design (CRD). The study was completed in two experiments each consisted of six dietary treatments. Each dietary treatment had 5 replicates and each replicate had 8 chicks. The experiment was conducted in white broiler chickens (Strain-SDL) from day- old to 6 weeks of age. The basal diet was mixed with the required quantity of mouldy maize to get the desired concentration of 250 ppb AFB_1.

Table 3.1: Experimental groups and treatment

Group	Dietary Treatment
Experiment 1 (Zinc)	
T_1	Basal diet
T_2	Basal diet + 250 ppb Aflatoxin B_1
T_3	Basal diet + 20 ppm zinc
T_4	Basal diet + 40 ppm zinc
T_5	T_2 + 20 ppm zinc
T_6	T_2 + 40 ppm zinc
Experiment 2 (Methionine)	
T_1	Basal diet
T_2	Basal diet + 250 ppb Aflatoxin B_1
T_3	Basal diet + 0.05% methionine
T_4	Basal diet + 0.1% methionine
T_5	T_2 + 0.05% methionine
T_6	T_2 + 0.1% methionine

3.3.1 Biological feeding trial

Day-old broiler chicks were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into various groups. The details of various experimental groups are given in Table 3.1 All birds were reared under standard managemental conditions from 0-6 weeks of age. All birds were fed with broiler

starter ration for 1-21 days and broiler finisher ration from 22 to 42 days. The compositions of broiler starter and broiler finisher ration are presented in Table 3.2.

Table 3.2: Ingredients and chemical composition of basal feed

Ingredient	Starter (%)	Finisher (%)
Maize	55.505	61.715
Soybean	41	35
Limestone	1	1.1
Di-calcium phosphate	1.75	1.5
Common salt	0.3	0.3
DL-methionine	0.11	0.02
TM premix	0.1	0.1
Vitamin premix	0.15	0.15
B complex	0.015	0.015
Choline chloride	0.05	0.05
Chemical composition of basal feed		
Crude protein (%)	21.50	19.50
ME (kcal/kg)	2859.82	2919.78
Calcium (%)	1.04	0.99
Available phosphorus (%)	0.45	0.40
Lysine (%)	1.29	1.14
Methionine (%)	0.52	0.43

Weekly individual body weight, feed consumption of each group and daily mortality were recorded. Week-wise liveability percentages of chicks kept on different dietary treatment were calculated. At the end of experiment, 10 birds per dietary treatment were sacrificed at random in order to record organ weights and liver and intestine samples were collected for histopathological examination. Weights of liver, spleen, bursa of Fabricius and thymus and carcass traits as per cent of body weight were calculated.

3.4 Immunological studies

3.4.1 Humoral immune response to sheep red blood cells

3.4.1.1 Total haemagglutinin (HA) antibody titre

The microtitre haemagglutination procedure as described by Siegel and Gross (1980) with slight modifications was followed to measure total HA antibody titres in chickens.

3.4.1.2 Reagents

a. Alsever's solution

Dextrose	2.05 g
Trisodium citrate dehydrate	0.80 g
Sodium chloride	0.42 g
Citric acid	0.055 g
Distilled water	100 ml

*pH 6.5

**Stored in a refrigerator at 4°C till further use

b. PBS (Phosphate buffer saline)

Sodium chloride (NaCl)	8.0 g
Potassium chloride (KCl)	0.20 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.20 g
Disodium hydrogen phosphate ($Na_2PO_4.2H_2O$)	1.44 g
Distilled water	1 L

pH 7.20

3.4.1.3 Procedure

Preparation of sheep red blood cells suspension

Blood from jugular vein of healthy sheep was collected in Alsever's solution. The Red blood cells were washed thrice in PBS (Phosphate buffer saline, pH 7.2). Finally 1% suspension of SRBCs in PBS (v/v) was prepared.

Immunization and harvesting of immune serum

On 30th day of experiment trial, 1 ml of 1% (v/v) of SRBCs suspension was injected intravenously to all birds. On day 5 post-immunization about 2 ml of blood was collected from 10 birds per dietary treatment. The blood was kept in incubator at 37^{0}C for 1 hour to clot. The clot was allowed to retract after detaching from sides of its container and left at 4^{0}C. Centrifugation of blood was carried out at 2000 rpm for 5-10 minutes to facilitate rapid collection of the serum. Required quantity of immune serum was harvested and stored at -20^{0}C for subsequent testing.

Haemagglutination test (HA test)

Haemagglutination (HA) test was used to determine antibody titre. The microtitre plates (U bottom) were cleaned, rinsed with PBS and dried. The HA test was done in duplicate for each sample. The following steps were followed.

i) 50 µl of PBS was distributed in each well of the microtitre plate.
ii) 50 µl of serum was added in the first well.
iii) Two fold serial dilutions were made upto row 12.
iv) 50 µl of 1% SRBC in PBS was added in each well.
v) The plates were covered with a cover.
vi) Plates were shaken on automatic shaking machine to enhance proper mixing.
vii) The microtitre plates were then kept at 30^0C for 1 hour for incubation.
viii) The plates were read under bright light.
ix) The titre was expressed as $\log_2$ of the highest dilution in which there was complete haemagglutination.

3.4.2 *In vivo* cell mediated immune response (CMI)

The cell mediated immune response to PHA-P antigen was evaluated by the method described by Corrier and DeLoach (1990). On 24th day of experiment trial, 10 birds per group were randomly selected and 0.5 ml (1 mg/ml of PBS) of phytohaemagglutinin-P (PHA-P) was injected intradermally in the right foot web. Left foot web of the same bird received 0.5 ml of sterile PBS and thus served as control. The skin thicknesses of right and left foot webs were measured using micrometer just before injection and 24 hours after injection. Foot web index was calculated using the following formula:

$$\text{Foot web index} = (D - C) - (B - A)$$

Where,

D = skin thickness of right foot web 24 hours after injection of PHA-P.
C = skin thickness of right foot web just before injection of PHA-P.
B = skin thickness of left foot web 24 hours after injection of sterile PBS.
A = skin thickness of left foot web just before injection of sterile PBS.

3.5 Biochemical and heamatological parameters

After 6 weeks, the blood samples from each treatment group were collected. The serum was separated and stored at -20^{0}C and analyzed for various biochemical parameters.

3.5.1 Total protein

Total serum protein was estimated by Biuret method using commercial kit manufactured by Span Diagnostics Ltd, SACHIN, Surat.

3.5.2 Total cholesterol

Total cholesterol was estimated by Wybenga and Pileggi method using commercial kit manufactured by Span Diagnostics Ltd, SACHIN, Surat.

3.5.3 Uric acid

Serum uric acid was estimated by uricase/POD method using commercial kit manufactured by Span Diagnostics Ltd, SACHIN, Surat.

3.5.4 SGPT

Serum GPT values estimated by 2,4-DNPH (Reitman and Frankel Method) method using commercial kit manufactured by Span Diagnostics Ltd, SACHIN, Surat.

3.5.5 SGOT

Serum GOT values estimated by 2,4-DNPH(Reitman and Frankel Method) method using commercial kit manufactured by Span Diagnostics Ltd, SACHIN, Surat.

3.5.6 Haemoglobin (Hb)

The haemoglobin concentration in blood was estimated by Sahli's method. Haemoglobin is converted into acid haematin by addition of 0.1 N HCl. The resultant solution is then matched against a reference solution (Sahli's Haemoglobinometer). Reading on the graduated tube noted and this is expressed as haemoglobin level in g/dl

3.5.7 Heterophile/Lymphocyte ratio

The percentage distribution of different leukocytes was determined from a blood smear stained with Giemsa stain. The blood smear was prepared in a clean and dry glass slide using

anticoagulant added blood. The blood smears were fixed with methanol. Then, blood smears were covered uniformly with enough Giemsa stain for 30-45 minutes. Afterwards, the stained blood smears were washed with running tap water and air dried. The smears were examined under microscope using oil immersion objective lens after adding one or two drops of cedar wood oil on the stained blood smear. The percentage of different leucocytes was calculated after counting of 100 leucocytes and the heterophil/lymphocyte (H/L) ratio was calculated by dividing the number of heterophils by that of lymphocyte.

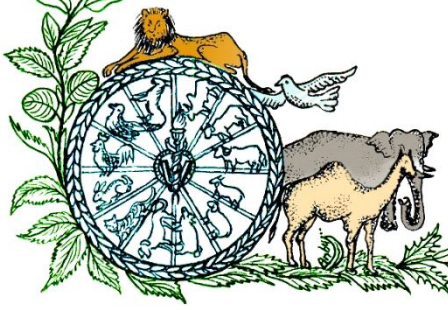
3.6 Histopathology of liver and intestine

At the end of experiment, liver and intestine samples were collected and fixed in 10% formal saline. The formal saline fixed samples were cut into pieces of 2-3 mm thickness and washed thoroughly in tap water overnight before dehydrating the tissues in ascending grades of alcohol (50%, 60%, 70%, 80%, 90% absolute alcohol I and II). The dehydrated tissues were cleared in benzene and embedded in paraffin blocks. Serial sections of 5-micron thickness were cut and stained with hematoxyline and eosin (Culling, 1968) and examined for various histopathological changes. Morphometric study was done by microscop-OLYMTUS, BH41 using softwere Crog Rex C5.

3.7 Statistical analysis

The data collected were subjected to statistical analysis following the methods suggested by Snedecor and Cochran (1989) and Dunccan's range test was used for calculating differeneces amongst different means. The statistical package SPSS 16.0 was used for analysis of data.

Results and Discussion



4 RESULTS AND DISCUSSION

The present investigation was undertaken to evaluate the efficacy of dietary zinc and methionine in combating aflatoxicosis in broiler chickens. Two experiments, involving supplementation of zinc and methionine were conducted separately. The effect of supplementation of zinc and methionine (alone) on broiler performance, carcass traits, blood biochemicals, organ weight, immune response and histopathology under experimental aflatoxicosis was studied from 0-6 weeks of age in broiler chickens. The results of the study are presented in this chapter.

4.1 Broiler performance

4.1.1 Body weight gain (BWG)

Experiment 1 (Zinc)

The weekly and phase-wise body weight gain (BWG) as influenced by various dietary treatments is presented in Table 4.1 and 4.2. The graphical representation of BWG in different growth phases is given in Figure 4.1. At first week of age, the body weight gain did not differ significantly among various dietary treatments. Significant differences in BWG among dietary treatments were recorded from second week of age. At second week of age, the average BWG in control group (T_1) was 156.36 g as against 132.82 in aflatoxin alone fed group (T_2) which was significantly ($P<0.05$) lower. The BWG in groups T_3, T_4, and T_6 was statistically similar to that of control, however the BWG in group T_5 was statistically similar to that of aflatoxin fed group, indicating that the supplementation of zinc at 20 ppm level may not be sufficient to ameliorate the adverse effects of 250 ppb aflatoxin B_1. At third week of age, the BWG in control group was 208.39 g which significantly ($P<0.05$) reduced to 177.57 g in T_2. The average BWG in T_3 to T_6 was comparable to that of control (T_1). At forth week of age,

the average BWG of control group was 264.32 g which significantly (P<0.05) reduced to 214.34 g in aflatoxin fed group (T_2). The BWG in groups T_3 and T_4 was similar to that of control, whereas the BWG in groups T_5 and T_6 was significantly (P<0.05) lower than that of control but significantly (P<0.05) higher than that of aflatoxin fed group. Thus, addition of zinc at both levels significantly improved the BWG. During fifth week of age, the BWG of control group (T_1) was 297.98 g which significantly (P<0.05) reduced to 234.10 g in aflatoxin fed group (T_2). The BWG in groups T_3, T_4 and T_6 was statistically similar to that of control, however, the BWG of group T_5 was significantly (P<0.05) higher than T_2 but lower than that of control. During sixth week of age, the average BWG in control group was 338.75 g as against 293.32 g in aflatoxin fed group (T_2). The BWG in other treatments (T_3 to T_6) was statistically similar to that of control diet. Thus, supplementation of zinc at both levels in the aflatoxin contaminated diet significantly (P<0.05) improved BWG which was statistically similar to that of control diet (T_1).

During starter phase of growth (0-3 weeks) the weight gain of birds in control group was 440.83 g which significantly (P<0.05) reduced to 381.65 g due to aflatoxin feeding in T_2. The BWG in other treatments (T_3 to T_6) was statistically similar to that of control. During 4-6 weeks of age, the average BWG of control group was 901.06 g which significantly (P<0.05) reduced to 741.77 g due to aflatoxin feeding in T_2. The BWG in groups T_3, T_4, and T_6 was statistically similar to that of control, however the BWG of group T_5 was higher (P<0.05) than T_2 but lower (P<0.05) than that of control diet. During overall growth phase (0-6 weeks) the weight gain of broilers in control group was 1341.90 g as against 1123.43 g in aflatoxin alone fed group which was significantly (P<0.05) lower. The overall BWG in groups T_3, T_4 and T_6 was statistically similar to that of control, however the weight gain in group T_5 was higher (P<0.05) than T_2 but could not match with that of control diet.

The present study indicated that inclusion of 250 ppb of aflatoxin in the diet of broilers resulted in significant reduction in BWG. Significant reduction in BWG of broilers at 300 ppb level of dietary aflatoxin was also reported by previous researchers (Silambarsan *et al.*, 2013; Sapocota *et al.*, 2007; Abaji, 2012; Raju and Dewegowda, 2000). Earlier studies also indicated that dietary aflatoxin at 0.5 ppm or more in commercial broiler diet adversely affected growth in a dose-dependent fashion (Johri *et al.*, 1988; Beura *et al.*, 1993; Verma, 1994; Rosa *et al.*, 2001; Oguz and Parlat, 2004).

Table 4.1: Weekly body weight gain (g/b) of broilers fed various dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	7 6.07 ±1.24	156.36 ±1.24^{c}	208.39 ±8.16^{b}	264.32±3.93^{d}	297.98 ±5.16^{c}	338.75±6.10^{b}
T_2	C+ AF 250 ppm	71.25±0.54	132.82±5.73^{a}	177.57±13.97^{a}	214.34±3.92^{a}	234.10±5.66^{a}	293.32±10.41^{a}
T_3	C+20 ppm zinc	76.32 ±1.17	151.02±2.74^{bc}	209.15 ±5.61^{b}	265.36 ±1.37^{d}	302.98±6.47^{c}	346.11±16.75^{b}
T_4	C+40 ppm zinc	77.29±5.19	148.37±6.95^{bc}	203.30±11.46^{ab}	267.24±5.53^{d}	302.89±10.47^{c}	350.25±14.37^{b}
T_5	T2+20 ppm zinc	73.35 ±2.35	137.53 ±5.50^{ab}	203.86±7.63^{ab}	229.25±4.89^{b}	266.52±6.55^{b}	325.78±7.50^{b}
T_6	T2+40 ppm zinc	76.86±1.32	151.15±4.74^{bc}	206.35±2.71^{b}	251.19±5.46^{c}	288.45±13.96^{bc}	330.93±6.54^{b}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

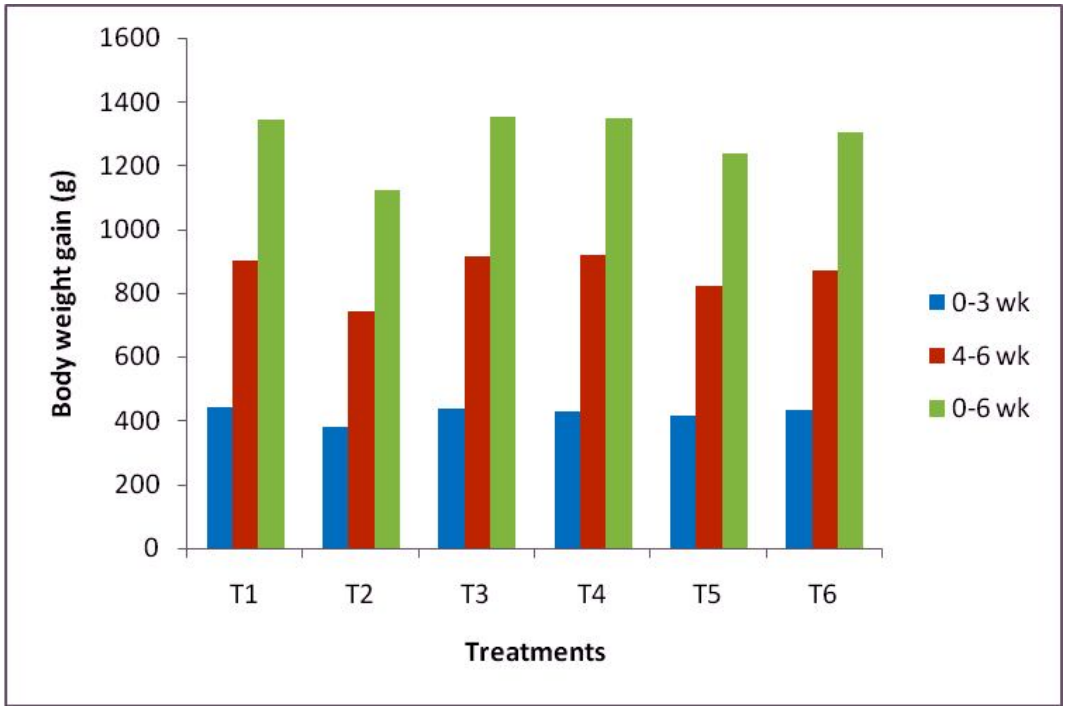


Fig. 4.1 : Body weight gain in different growth phases

The present study revealed that supplementation of zinc at 40 ppm levels in the aflatoxin contaminated diet caused significant (P<0.05) increase in the overall weight gain which was statistically similar to that of control. Similar finding was reported by Hegazy and Adachi (2000) where supplementation of 60 ppm zinc resulted in significant improvement in weight gain of chicks exposed to aflatoxin.

Table 4.2: Body weight gain (g/b) in different growth phases of broilers fed dietary treatments

Treatment identification		0-3 wk	4-6 wk	0-6 wk
T1	Control (C)	440.83±7.80^{b}	901.06±7.96^{c}	1341.90±8.50^{c}
T2	C+ AF 250 ppm	381.65±17.88^{a}	741.77±15.90^{a}	1123.43±29.00^{a}
T3	C+20 ppm zinc	436.49±49.00^{b}	914.45±16.15^{c}	1350.95±20.46^{c}
T4	C+40 ppm zinc	428.96±20.69^{b}	920.39±24.03^{c}	1349.36±43.68^{c}
T5	T2+20 ppm zinc	414.76±14.85^{ab}	821.56±15.32^{b}	1236.32±26.16^{b}
T6	T2+40 ppm zinc	434.37±7.90^{b}	870.58±25.52^{bc}	1304.95±28.11^{bc}

Values bearing different superscripts in a column differ significantly (P<0.05).

Experiment 2 (Methionine)

The weekly and phase-wise body weight gain as influenced by various dietary treatments is presented in Table 4.3 and 4.4. BWG of different growth phases is represented in Figure 4.2. During first week of age, the BWG in various dietary treatments did not differ significantly (P<0.05) from that of control. During second and fourth weeks of age, the BWG of groups T_2 and T_5 was significantly (P<0.05) lower than that of control but the BWG in other treatment groups was statistically similar to that of control. During third and fifth week of age, the BWG of group T_2 was significantly lower than that of control, however the BWG of other treatment groups was statistically (P<0.05) similar to that of control diet, indicating that the methionine supplementation to the aflatoxin contaminated diet significantly ameliorated the adverse effects of aflatoxicosis. At sixth week of age, the BWG in groups T_2 and T_5 was significantly (P<0.05) lower than that of control, however, the BWG in groups T_3, T_4 and T_6 was statistically similar to that of control.

During starter phase of growth (0-3 weeks), the BWG of broilers in groups T_2 and T_5 was significantly ($P<0.05$) lower than that of control, however, the BWG of groups T_3, T_4 and T_6 was statistically similar to that of control. During 4-6 and 0-6 weeks of age, the average BWG of the broilers in various dietary treatments showed almost similar trend. Significantly ($P<0.05$) reduced BWG was reported in T_2 and T_5 in both the growth phases (i.e. 0-3 and 4-6 weeks). The average BWG in T_3, T_4 and T_6 was statistically similar to that of control, indicating that addition of methionine to the basal diet did not produce any positive effect on weight gain of broilers. This validates the requirement of methionine as suggested by NRC (1994). Furthermore, supplementation of methionine (0.1%) to the aflatoxin contaminated diet ameliorated the adverse effect of aflatoxicosis on BWG.

The present study revealed that supplementation of methionine at 0.05% level failed to ameliorate the adverse effect of aflatoxicosis caused by 250 ppb aflatoxin B_1, but 0.1% methionine supplementation to the aflatoxin contaminated diet significantly ($P<0.05$) increased the overall BWG and the gain was statistically similar to that of control. Naveenkumar *et al.* (2007) and Sapocota *et al.* (2007) also reported significant improvement in BWG of broiler chickens due to methionine supplementation in diet during aflatoxicosis. Veltman *et al.* (1983) also reported that increasing dietary total sulphur amino acids to level in excess of NRC protected chicks from the growth depressing effects of aflatoxin, possibly through an increased rate of detoxification by glutathione.

Table 4.4: Body weight gain (g/b) in different growth phases of broilers fed various dietary treatments

Treatment	Identification	0-3 wk	4-6 wk	0-6 wk
T1	Control (C)	440.83 ± 7.80^{c}	901.06 ± 7.96^{b}	1341.90 ± 8.50^{b}
T2	C+ AF 250 ppm	381.65 ± 17.88^{a}	741.77 ± 15.90^{a}	1123.43 ± 29.00^{a}
T3	C+0.05% methionine	437.88 ± 11.67^{c}	881.42 ± 23.15^{b}	1319.31 ± 32.38^{b}
T4	C+0.1% methionine	446.72 ± 14.37^{c}	882.37 ± 20.31^{b}	1329.09 ± 30.85^{b}
T5	T2+0.05% methionine	400.85 ± 6.36^{ab}	800.31 ± 30.62^{a}	1201.16 ± 25.96^{a}
T6	T2+0.1% methionine	433.81 ± 8.04^{bc}	875.63 ± 28.27^{b}	1309.45 ± 31.92^{b}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

Table 4.3: Weekly body weight gain (g/b) of broilers fed various dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	76.07 ±1.24	156.36 ±1.24^{b}	208.39 ±8.16^{b}	264.32±3.93^{b}	297.98 ±5.16^{b}	338.75±6.10^{c}
T_2	C+ AF 250 ppm	71.25±0.54	132.82±5.73^{a}	177.57±13.97^{a}	214.34±3.92^{a}	234.10±5.66a	293.32±10.41^{a}
T_3	C+0.05% methionine	75.37±1.63	154.99±4.01^{b}	207.52±6.19^{b}	257.81±5.96^{b}	289.97±6.85^{b}	333.63±12.05^{c}
T_4	C+ 0.1% methionine	77.18±1.68	159.28±6.02^{b}	210.26±9.50^{b}	263.23±7.46^{b}	292.47±7.08^{b}	326.66±7.64^{bc}
T_5	T2+ 0.05% methionine	70.21±2.26	132.06±2.87^{a}	198.58±1.85^{ab}	217.62±6.27^{a}	279.27±30.55^{b}	303.41±6.54^{ab}
T_6	T2+0.1% methionine	75.91±2.23	152.18±3.82^{b}	205.70±2.49^{b}	262.53±22.02^{b}	286.67±14.11^{b}	326.42±3.70^{bc}

Values bearing different superscripts in a column differ significantly (P<0.05).

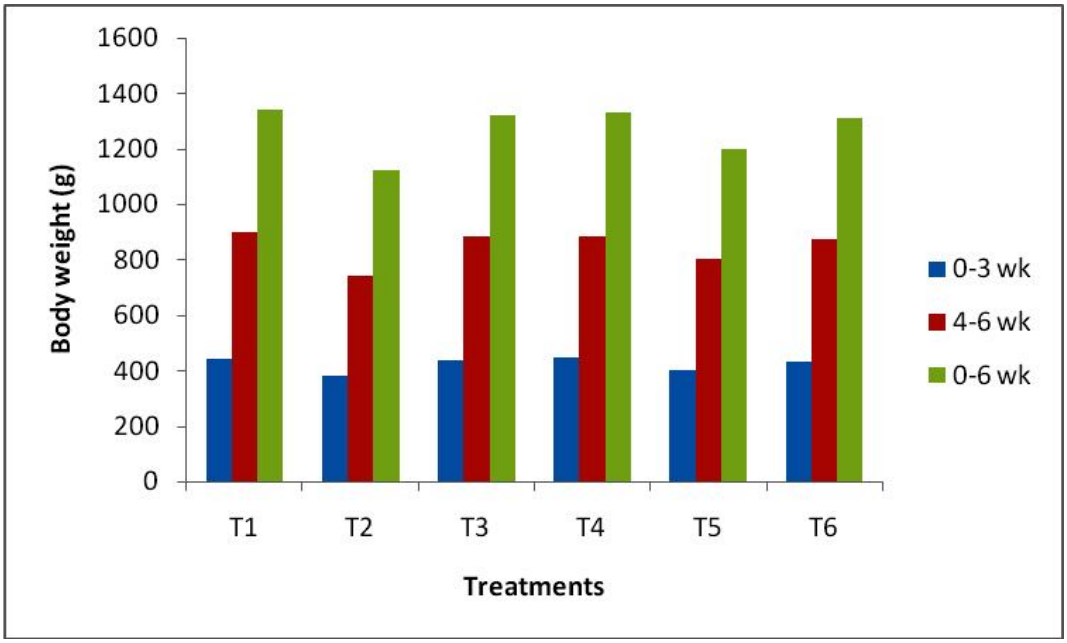


Fig. 4.2 : Body weight gain in different growth phase

Table 4.5: Weekly feed intake (g/b) of broilers fed different dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	113.82±2.41	251.37±14.28	374.05±11.80	522.60±12.38	610.54±3.32^{b}	751.97±14.31^{ab}
T_2	C+ AF 250 ppm	112.82±2.36	243.70±10.90	364.74±9.03	498.65±9.96	545.35±12.07^{a}	706.63±11.22^{a}
T_3	C+20 ppm zinc	116.72±0.96	257.40±5.55	380.95±5.00	524.98±9.53	608.45±2.68^{b}	761.92±6.65^{b}
T_4	C+40 ppm zinc	116.32±4.64	243.89±7.22	363.63±5.29	522.82±11.62	615.16±6.40^{b}	755.37±14.37^{ab}
T_5	T2+20 ppm zinc	113.72±2.90	250.34±6.07	375.64±5.69	495.61±10.87	580.35±26.80^{ab}	718.04±17.98^{ab}
T_6	T2+40 ppm zinc	115.72±1.48	264.25±7.73	372.39±4.89	502.75±20.61	603.37±17.80^{b}	720.18±23.44^{ab}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

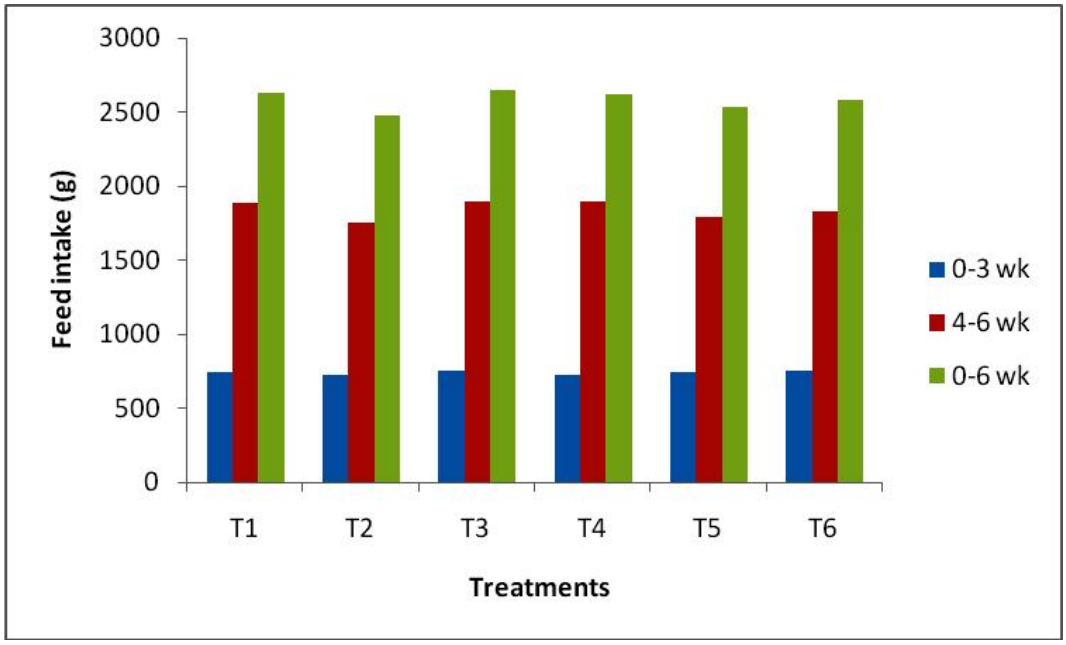


Fig. 4.3 : Feed intake in different growth phases

4.1.2 Feed intake (FI)

Experiment 1 (Zinc)

The data pertaining to weekly and phase-wise feed consumption are presented in Table 4.5 and 4.6. The graphical presentation of feed consumption during different growth phases is given in Figure 4.3. During first, second, third and fourth week of age, the FI in all the treatment groups was significantly ($P<0.05$) similar. During fifth week of age, the FI in control group was 610.54 g which significantly ($P<0.05$) reduced to 545.35 g due to aflatoxin feeding in group T_2. The FI in other treatments was statistically similar to that of control. During sixth week of age, the FI of T_3 group was significantly ($P<0.05$) higher than that of aflatoxin fed group (T_2), however, the FI of all other treatment was comparable to that of control diet.

During starter phase (0-3 weeks) of the trial, the FI in various treatment groups did not differ significantly. During 4-6 weeks of age, the FI in control group was 1885.11 g which significantly ($P<0.05$) reduced to 1750.64 g in aflatoxin alone fed group (T_2). The FI of other treatment groups was statistically comparable to that of control. During overall growth period (0-6 weeks), the FI in control group was 2624.36 g as against 2471.91 g in aflatoxin fed group (T_2) which was significantly ($P<0.05$) lower. The FI in all other treatment groups was statistically comparable to the control.

The present study revealed that aflatoxin contamination in diet resulted in significantly reduction in feed consumption. Similar observation was reported by Beura *et al.* (1993), who also reported reduced feed consumption in pure bred and commercial broiler chicken at 0.3 and 0.8 ppm, respectively. Significantly reduced feed consumption at 0.3 ppm aflatoxin was also reported by Silambarasan *et al.* (2013); Abaji (2012) and Raju and Devegowda (2000). Several researchers (Kubena *et al.*, 1990; Kubena *et al.*, 1998; Ledoux *et al.*, 1999; Verma *et al.*, 1994; Santurio *et al.*, 1999) also reported decreased feed consumption due to aflatoxin contamination ranging from 1 to 5 ppm. In presented study, supplementation of zinc to basal diet did not produce any positive effect on feed consumption of broilers, however, supplementation of zinc to the aflatoxin contaminated diet resulted in improved feed consumption in broiler chickens. Hegazy and Adachi (2000) also reported improved feed consumption due to zinc supplementation during aflatoxicosis in chickens.

Table 4.6: Feed intake (g/b) in different growth phases of broilers fed different dietary treatments

Treatment	Identification	0-3 wk	4-6 wk	0-6 wk
T1	Control (C)	739.25±15.97	1885.11±25.55^{b}	2624.36±35.50^{b}
T2	C+ AF 250 ppm	721.26±18.26	1750.64±22.64^{a}	2471.91±29.74^{a}
T3	C+20 ppm zinc	755.08±6.63	1895.35 ±16.18^{b}	2650.43±20.025^{b}
T4	C+40 ppm zinc	723.84±9.07	1893.36±25.86^{b}	2617.20 ±30.73^{b}
T5	T2+20 ppm zinc	739.71±11.57	1794.02 ±54.23^{ab}	25.33.73±64.37^{ab}
T6	T2+40 ppm zinc	752.36±11.82	1826.31±57.18^{ab}	2578.67±49.30^{ab}

Values bearing different superscripts in a column differ significantly (P<0.05).

Experiment 2 (Methionine)

The data pertaining to weekly and phase-wise feed consumption as influenced by various dietary treatments are presented in Table 4.7 and 4.8. The graphical representation of feed consumption during different growth phases is given in Figure 4.4.

During first, second and third weeks of age, the feed intake in all the treatment groups was statistically (P<0.05) similar. During fourth week of age, the feed consumption of group T_5 was significantly (P<0.05) lower than that of control, however the FI in T_5 did not differ significantly from that of aflatoxin fed group (T_2). The FI in all other treatments was statistically comparable to that of control. During fifth week of age, the FI in control group was 610.54 g which significantly (P<0.05) reduced to 545.35 g due to feeding of aflatoxin in T_2. The FI in groups T_3 to T_6 was statistically similar to that of control . During sixth week of age, the FI in control group (T_1) was 751.95 g as against 706.63 g in aflatoxin alone fed group (T_2). The FI in other treatment groups was statistically comparable to that of control.

During starter phase of growth (0-3 weeks), there was no significant difference in feed consumption of various dietary treatments. During 4-6 weeks of age, the FI in control group was 1885.11 g which significantly (P<0.05) reduced to 1750.69 g in aflatoxin fed group (T_2). The FI in T_3 to T_6 groups was statistically (P<0.05) similar to that of control.

Table 4.7: Weekly feed intake (g/b) of broilers fed different dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	113.82±2.41	251.37±14.28	374.05±11.80	522.60±12.38	610.54±3.32^{b}	751.97±14.31^{b}
T_2	C+ AF 250 ppm	112.82±2.36	243.70±10.90	364.74±9.03	498.65±9.96	545.35±12.07^{a}	706.63±11.22^{a}
T_3	C+ 0.05% methionine	113.38±2.89	250.63±1.73	371.45±8.20	523.28±9.24	608.91±4.74^{b}	743.04±17.67^{ab}
T_4	C+ 0.1% methionine	115.42±2.34	258.54±5.52	376.12±6.71	521.95±8.54	629.62±13.93^{b}	737.60±5.21^{ab}
T_5	T2+ 0.05% methionine	115.65±3.06	245.48±4.06	375.47±5.20	469.07±9.38	618.18±4.00^{b}	733.49±18.68^{ab}
T_6	T2+ 0.1% methionine	112.12±3.14	247.03±6.93	371.59±8.84	514.11±7.75	604.45±5.60^{b}	750.44±5.49^{b}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

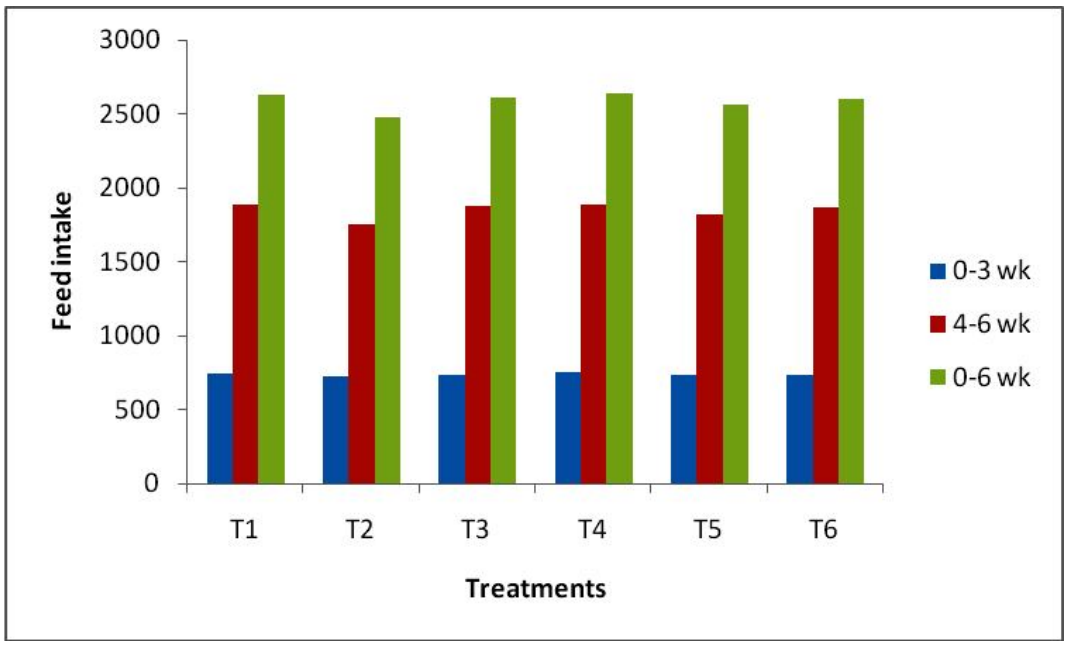


Fig. 4.4 : Feed intake in different growth phases

During overall growth phase (0-6 weeks), the FI of control group broilers was 2624.36 g which significantly (P<0.05) reduced to 2471.91 g due to aflatoxin feeding in group T_2. The FI in other treatment groups was statistically similar to that of control. The results showed that addition of methionine to the basal diet did not produce any positive effect on feed consumption of broilers. Supplementation of methionine to the aflatoxin contaminated diet significantly ameliorated the adverse effects of aflatoxin on feed consumption of broiler chickens.

In present study, methionine supplementation (0.1%) to aflatoxin contaminated diet resulted in significant improvement in feed consumption of broilers which was statistically equal to that of control. Sapocota *et al*. (2007) also reported significant improvement in feed consumption of broilers due to methionine supplementation in aflatoxin contaminated diet.

Table 4.8: Feed intake (g/b) in different growth phases of broilers fed different dietary treatment

Treatment	Identification	0-3wk	4-6 wk	0-6 wk
T1	Control (C)	739.25±15.97	1885.11±25.55^{b}	2624.36±35.50^{b}
T2	C+ AF 250ppm	721.26±18.26	1750.64±22.64^{a}	2471.91±29.74^{a}
T3	C+ 0.05% methionine	735.47±10.91	1875.23±28.33^{b}	2610.71±39.15^{b}
T4	C+ 0.1% methionine	750.08±12.32	1889.18±20.36^{b}	2639.26±31.56^{b}
T5	T2+ 0.05% methionine	736.61±11.76	1820.75±18.28^{b}	2557.36±20.02^{ab}
T6	T2+ 0.1% methionine	730.74±17.30	1869.01±27.20^{b}	2599.75±32.17^{b}

Values bearing different superscripts in a column differ significantly (P<0.05).

4.1.3 Feed conversion ratio (FCR)

Experiment 1 (Zinc)

The data pertaining to feed conversion ratio in different weeks and growth phases is given Table 4.9 and 4.10. The graphical representation of FCR in various growth phases is given in Figure 4.5.

During first week of age, there was no significant difference among various dietary treatments in FCR which varied between 1.49 and 1.58. During second week of trial, the

FCR in control group (T_1) was 1.61 which significantly ($P<0.05$) increased to 1.83 due to aflatoxin feeding in group (T_2). The FCR in groups T_3, T_4 and T_6 was statistically comparable to that of control, however, the FCR of group T_5 was statistically similar to that of aflatoxin fed group (T_2) and significantly ($P<0.05$) higher than that of control, indicating that supplementation of 20 ppm diet zinc to the aflatoxin contaminated diet may not be sufficient to ameliorate the harmful effect of aflatoxicosis on FCR in broiler chickens. During third and sixth week of growth period, the FCR in various treatment groups did not vary significantly from that of control. During fourth week of age, the FCR in control group (T_1) was 1.96 which significantly ($P<0.05$) increased to 2.32 in aflatoxin alone fed group (T_2). The FCR of groups T_5 and T_6 was significantly lower than that of aflatoxin alone fed group (T_2) but significantly ($P<0.05$) higher than that of control, indicating that supplementation of zinc to the aflatoxin contaminated diet partially ameliorated the ill effects of aflatoxin on FCR. At fifth week of age, the FCR of control birds was 2.05 which significantly ($P<0.05$) increased to 2.33 due to administration of aflatoxin. The FCR in groups T_3 to T_6 did not vary significantly ($P<0.05$) from that of control.

With regard to feed conversion ratio in different growth phases, the FCR during 0-3 weeks in control group was 1.67 which significantly ($P<0.05$) increased to 1.90 due to aflatoxin feeding in group T_2. The FCR in other treatment groups (T_3 to T_6) was statistically ($P<0.05$) similar to that of control, indicating that supplementation of zinc at both levels ameliorated the adverse effects of aflatoxicosis in broiler chickens. The FCR during 4-6 weeks in control group was 2.09 which significantly ($P<0.05$) increased to 2.36 due to administration of aflatoxin in T_2. The FCR in other treatment groups (T_3 to T_6) was statistically ($P<0.05$) comparable to that of control, indicating that supplementation of zinc to aflatoxin contaminated feed ameliorated the adverse effects of aflatoxin on FCR in broiler chickens. With regard to overall FCR (0-6 weeks), the FCR in control group was 1.95 which significantly ($P<0.05$) increased to 2.20 due to aflatoxin feeding in group T_2. The overall FCR in groups T_3 and T_4 was statistically similar to that of control, indicating that addition of zinc to the basal diet did not have any positive effect on FCR. The overall FCR of groups T_5 and T_6 was also statistically ($P<0.05$) similar to that of control, indicating that addition of zinc (20 and 40 ppm) to the aflatoxin contaminated diet significantly ($P<0.05$) ameliorated the adverse effects of aflatoxin on overall FCR in broiler chickens.

Table 4.9: Weekly feed conversion ratio of broilers fed different dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	1.49±0.30	1.61±0.10^{a}	1.80±0.07	1.96±0.02^{a}	2.05±0.03^{a}	2.22±0.02
T_2	C+ AF 250 ppm	1.58±0.03	1.83±0.01^{b}	2.12±0.23	2.32±0.05^{c}	2.33±0.08^{b}	2.41±0.04
T_3	C+20 ppm zinc	1.53±0.02	1.70±0.03^{b}	1.82±0.05	1.97±0.04^{a}	2.01±0.04^{a}	2.22±0.11
T_4	C+40 ppm zinc	1.52±0.08	1.65±0.03^{a}	1.81±0.09	1.95±0.02^{a}	2.03±0.05^{a}	2.17±0.11
T_5	T2+20 ppm zinc	1.55±0.04	1.82± 0.04^{b}	1.85±0.06	2.16±0.04^{b}	2.18±0.09^{ab}	2.20±0.08
T_6	T2+40 ppm zinc	1.50±0.01	1.73±0.05^{ab}	1.80±0.01	2.09±0.04^{b}	2.11±0.11^{ab}	2.18±0.09

Values bearing different superscripts in a column differ significantly ($P<0.05$)

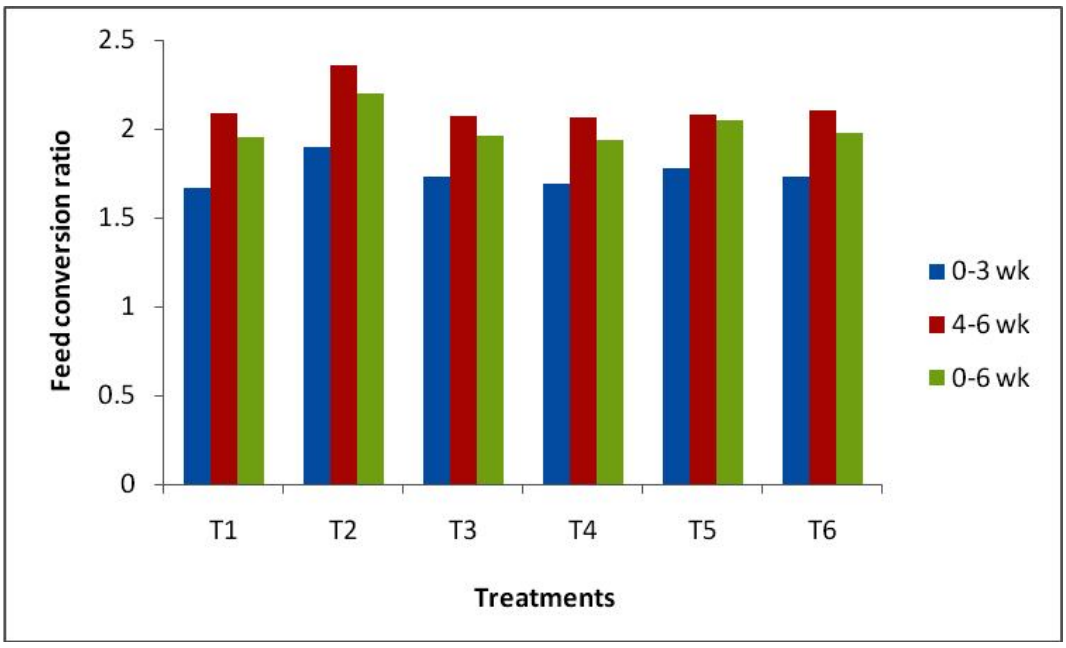


Fig. 4.5 : Feed conversion ratio in different growth phases

Impaired feed efficiency is a common feature in poultry during aflatoxicosis. In the present study, aflatoxin contamination in feed resulted in poor feed efficiency in broilers during 0-6 weeks of age. Silambarasan *et al.* (2013); Abaji (2012) and Raju and Devegowda (2000) also reported significantly poor feed efficiency in broiler chickens with 0.3 ppm level of dietary aflatoxin. Scheideler (1993) and Rosa *et al.* (2001) also reported impaired feed efficiency due to 2.5 ppm and 5.0 ppm aflatoxin feeding, respectively. Similarly, other researchers have also reported a dose dependent significant reduction in feed efficiency due to presence of aflatoxin in diet (Verma, 1994; Reddy *et al.,* 1982; Rosa *et al.,* 2001).

In present study, addition of zinc (20 and 40 ppm) resulted in significant improvement in feed conversion ratio during aflatoxicosis. This finding is in agreement with Hegazy and Adachi (2000) where zinc fortified diet (60 ppm zinc supplementated) resulted in significant improvement in feed efficiency of chicks exposed to aflatoxicosis.

Table 4.10: Feed conversion ratio in different growth phases of broilers fed different dietary treatments

Treatment	Identification	0-3wk	4-6 wk	0-6 wk
T1	Control (C)	1.67±0.01^{a}	2.09±0.02^{a}	1.95±0.01^{a}
T2	C+ AF 250 ppm	1.90±0.08^{b}	2.36±0.04^{b}	2.20±0.05^{b}
T3	C+20 ppm zinc	1.73±0.03^{a}	2.07±0.05^{a}	1.96±0.04^{a}
T4	C+40 ppm zinc	1.69±0.06^{a}	2.06±0.04^{a}	1.94±0.05^{a}
T5	T2+20 ppm zinc	1.78±0.04^{ab}	2.08±0.07^{ab}	2.05±0.04^{a}
T6	T2+40 ppm zinc	1.73±0.02^{a}	2.10±0.09^{a}	1.98±0.06^{a}

Values bearing different superscripts in a column differ significantly (P<0.05).

Experiment 2 (Methionine)

The data pertaining to feed conversion ratio in different weeks and growth phases as influenced by various dietary treatments is presented in Table 4.11 and 4.12. The graphical representation of FCR in various growth phases is given in Figure 4.6. During first week of age, the FCR in various treatment groups varied between 1.47 to 1.65. The FCR in various treatment groups was statistically (P<0.05) similar to that of control barring treatment T_5 wherein

higher FCR compared to control was observed. During second week of growth period, the FCR in control group was 1.61 which significantly ($P<0.05$) increased to 1.83 in aflatoxin alone fed group (T_2). The FCR in treatment groups T_3, T_4 and T_6 was statistically similar to that of control. The FCR of T_5 was significantly ($P<0.05$) higher than that of control group (T_1), indicating that supplementation of 0.05% methionine to aflatoxin contaminated diet may not be sufficient to curb the ill effects of aflatoxin on feed efficiency. During third and fifth weeks of trial period, there was no significant ($P<0.05$) difference among the various dietary treatments in feed conversion ratio. During fourth week of age, the FCR in control group was 1.96 which significantly ($P<0.05$) increased to 2.32 in aflatoxin alone fed group (T_2). The FCR in other treatment groups (T_3 to T_6) was statistically similar to that of control. During sixth week of growth period, the FCR of control birds was 2.22 as against 2.41 in aflatoxin fed group which was significantly ($P<0.05$) lower. The FCR of groups T_3, T_4 and T_6 was statistically comparable with that of control. The FCR of group T_5 was significantly ($P<0.05$) higher than that of control and statistically similar to that of aflatoxin fed group, indicating that lower level of methionine (0.05%) supplementation to the aflatoxin contaminated feed failed to remove the adverse effect of aflatoxin on FCR in broiler chickens.

With regard to FCR in different growth phases, the FCR during 0-3 weeks, in groups T_2 and T_5 was statistically similar and higher than that of control. The FCR of groups T_3, T_4 and T_6 was statistically ($P<0.05$) similar to that of control. During 4-6 weeks of growth period, the FCR of groups T_3 to T_6 was statistically ($P<0.05$) similar to that of control and the FCR of aflatoxin fed group (T_2) was significantly ($P<0.05$) higher compared to that of control. During overall growth period (0-6 weeks), the FCR of group T_5 was statistically similar to that of aflatoxin alone fed group (T_2), indicating that supplementation of 0.05% methionine to the aflatoxin contaminated diet did not curb the ill effects of aflatoxin on overall FCR in broiler chickens. The overall FCR of groups T_3 and T_4 was statistically similar to that of control, suggesting that addition of methionine to the basal diet did not produce any positive effect on FCR of broilers. The FCR of group T_6 was statistically ($P<0.05$) similar to that of control, indicating that supplementation of 0.1% methionine to the aflatoxin contaminated diet curbed the ill effect of aflatoxin on feed efficiency of broilers during 0-6 weeks of age.

Table 4.11: Weekly feed conversion ratio of broilers fed different dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	1.49±0.30	1.61±0.10^{a}	1.80±0.07	1.96±0.02^{a}	2.05±0.03	2.22±0.02^{a}
T_2	C+ AF 250 ppm	1.58±0.03	1.83±0.01^{b}	2.12±0.23	2.32±0.05^{b}	2.33±0.08	2.41±0.04^{c}
T_3	C+0.05% methionine	1.50±0.02	1.62±0.03^{a}	1.79±0.04	2.03±0.04^{a}	2.10±0.03	2.23±0.04^{a}
T_4	C+0.1% methionine	1.49±0.00	1.62±0.03^{a}	1.80±0.06	1.98±0.02^{a}	2.15±0.03	2.26±0.06^{ab}
T_5	T2+0.05% methionine	1.65±0.05	1.86±0.02^{b}	1.89±0.00	2.15±0.02^{ab}	2.32±0.25	2.42±0.07^{c}
T_6	T2+0.1% methionine	1.47±0.01	1.62±0.06^{a}	1.80±0.02	2.00±0.14^{a}	2.12±0.08	2.29±0.01^{ab}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

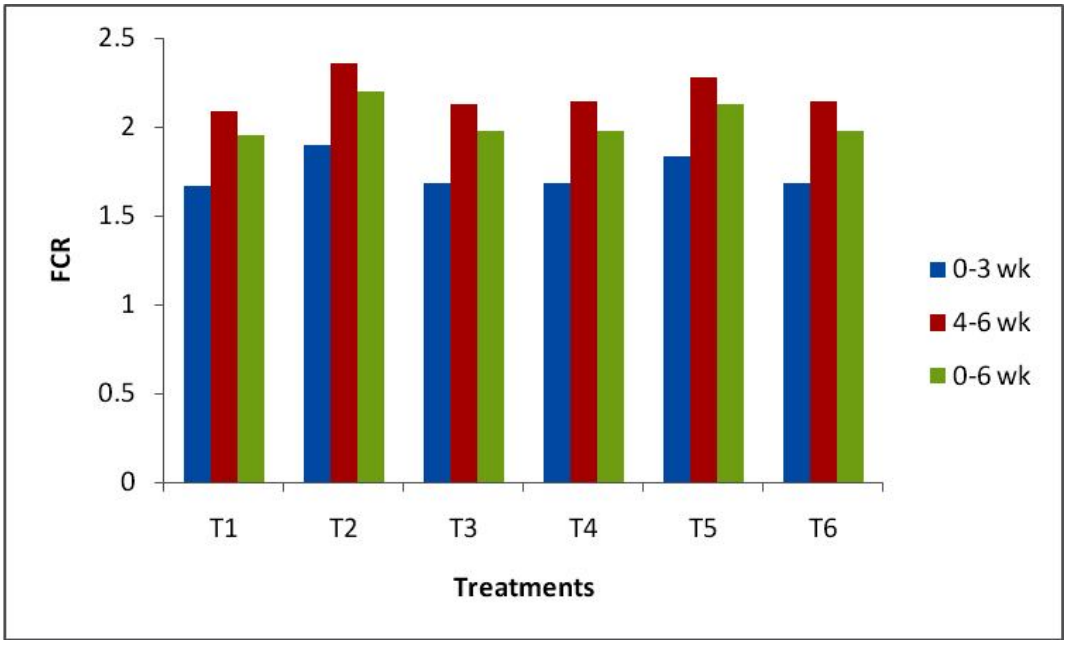


Fig. 4.6 : Feed conversion ratio in different growth phases

In the present study, supplementation of methionine at 0.1% level to aflatoxin contaminated diet resulted in significant improvement in feed efficiency that was equal to that of control. These reports are in agreement with literature (Sapocota *et al*., 2007; Naveenkumar *et al*., 2007), wherein methionine supplementation to the aflatoxin contaminated feed resulted in improved feed efficiency in broiler chickens.

Table 4.12: Feed conversion ratio in different growth phases of broilers fed different dietary treatments

Treatment	Identification	0-3 wk	4-6 wk	0-6 wk
T1	Control (C)	1.67±0.01^{a}	2.09±0.02^{a}	1.95±0.01^{a}
T2	C+ AF 250 ppm	1.90±0.08^{b}	2.36±0.04^{c}	2.20±0.05^{b}
T3	C+0.05% methionine	1.68±0.03^{a}	2.13±0.02^{ab}	1.98±0.02^{a}
T4	C+0.1% methionine	1.68±0.03^{a}	2.14±0.02^{ab}	1.98±0.02^{a}
T5	T2+0.05% methionine	1.83±0.01^{b}	2.28±0.09^{ab}	2.13±0.05^{b}
T6	T2+0.1% methionine	1.68±0.02^{a}	2.14±0.05^{ab}	1.98±0.03^{a}

Values bearing different superscripts in a column differ significantly (P<0.05).

4.1.4 Liveability percentage

Experiment 1(Zinc)

The results on week-wise liveability percentage in broilers kept on different dietary treatments are presented in Table 4.13.

During first week of age, no mortality was recorded as the dead chicks were replaced with chicks of equal weights. During second and third weeks of age, the liveability percentage varied from 97.50 to 92.50 and 95.00 to 90.00, respectively. The week-wise liveability percentage did not vary significantly (P<0.05) among various treatment groups. During fourth and fifth weeks of age, the liveability percentage varies from 95.00 to 87.50. During sixth week of age, the liveability percentage in control group was 92.50 which numerically reduced to 85.00 in toxin fed group (T_2). The liveability percentage in groups T_3, T_4 and T_6 was higher compared to toxin alone fed group (T_2). The liveability percentage in group T_5 was numerically

equal to that of T_2 and lower than that of T_1, whereas the liveability percentage of group T_6 was closer to that of control group.

In the present study, 250 ppb aflatoxin level in the diet resulted in higher mortality compared to control. Inclusion of aflatoxin at 250 ppb did not produce heavy mortality, which might be due to the low level of aflatoxin inclusion in the diet. Silambarasan (2011) and Abaji (2012) also reported an increase in mortality due to 300 ppb level of aflatoxin in the diet of broilers. Occurrence of mortality due to aflatoxin contamination in the diet has also been reported by Denli *et al.* (2009); Reddy *et al.* (1982) and Gopi (2006).

In the present study, supplementation of zinc (40 ppm) improved the liveability percentage in broiler chickens.

Experiment 2 (Methionine)

The results on week-wise liveability percentage in broilers kept on different dietary treatments are presented in Table 4.14.

During first week of age, no mortality was recorded as the dead chicks were replaced with chicks of equal weights. During second and third weeks of age, the liveability percentage varied from 97.50 to 92.50 and 95.00 to 90.00, respectively. The week-wise liveability percentage did not vary significantly ($P<0.05$) among various treatment groups. During fourth and fifth weeks of age, the liveability percentage varied from 95.00 to 87.50. During sixth week of age, the liveability percentage in control group was 92.50 which numerically reduced to 85.00 in toxin fed group (T_2). The liveability percentage in groups T_3, T_4 and T_6 was higher compared to toxin alone fed group (T_2). The liveability percentage in group T_5 was numerically equal to that of T_2 and lower than that of T_1, whereas the liveability percentage of group T_6 was closer to that of control group.

In the present study, supplementation of methionine (0.1%) improved the livability percentage in broiler chickens.

Table 4.13: Livability percentage as influenced by various dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	100.00	97.50±2.50	95.00±3.06	95.00±3.06	95.00±3.06	92.50±3.06
T_2	C+AF 250 ppm	100.00	92.50±3.06	90.00±2.00	87.50±0.00	87.50±0.00	82.00±2.50
T_3	C+20 ppm zinc	100.00	97.50±2.50	95.00±3.06	95.00±3.06	90.00±2.50	87.50±0.00
T_4	C+40 ppm zinc	100.00	97.50±2.50	95.00±3.06	95.00±3.06	92.50±3.06	90.00±2.50
T_5	T2+20 ppm zinc	100.00	95.00±3.06	95.00±3.06	92.50±3.06	90.00±2.50	82.00±2.50
T_6	T2+40 ppm zinc	100.00	97.50±2.50	97.50±2.50	95.00±3.06	92.50±3.06	90.00±2.50

Table 4.14: Liveability percentage as influenced by various dietary treatments

Treatment	Identification	I wk	II wk	III wk	IV wk	V wk	VI wk
T_1	Control (C)	100.00	97.50±2.50	95.00±3.06	95.00±3.06	95.00±3.06	92.50±3.06
T_2	C+ AF 250 ppm	100.00	92.50±3.06	90.00±2.00	87.50±0.00	87.50±0.00	82.00±2.50
T_3	C+0.05% methionine	100.00	97.50±2.50	95.00±3.06	95.00±3.06	92.00±2.50	90.00±2.50
T_4	C+0.1% methionine	100.00	97.50±2.50	95.00±3.06	95.00±3.06	95.50±3.06	90.00±3.06
T_5	T2+0.05% methionine	100.00	95.00±3.06	92.00±3.06	90.00±2.50	90.00±2.50	82.00±2.50
T_6	T2+0.1% methionine	100.00	97.50±2.50	95.50±3.06	95.00±3.06	95.00±3.06	90.00±2.50

4.2 Carcass traits

4.2.1 Slaughter Traits

Experiment 1 (Zinc)

The data pertaining to shrinkage loss, dressed yield and eviscerated yield were statistically analysed and presented in Table 4.15. The shrinkage loss, dressed yield and eviscerated yield varied from 3.91 to 4.18, 75.94 to 76.78 and 66.77 to 68.14%, respectively. There was no significant difference among various dietary treatments with respect to shrinkage loss, dressing yield and eviscerated yield.

The present study indicated no significant ($P<0.05$) change in the shrinkage loss, dressing yield and eviscerated yield due to contamination of 250 ppb of aflatoxin B_1 in the feed. Similar results were reported by Silambarasan (2011) and Abaji (2012). They also reported no significant change in slaughter traits (Shrinkage loss, dressing percentage and eviscerated yield) due to addition of 300 ppb aflatoxin B_1 in the diet of broiler chickens. Contrary to this, Pasha *et al.* (2007) reported decreased dressing percentage due to feeding of aflatoxin contaminated diet. A reduction in eviscerated yield due to feeding of aflatoxin contaminated diet was reported by Churchill *et al.* (2009). Doerr *et al.* (1983) also reported reduced eviscerated yield at aflatoxin levels of 0.065 to 2.7 ppm. In the present study, supplementation of zinc had no effect on the slaughter traits of broiler chickens.

Table 4.15: Slaughter traits (% of live weight) as influenced by various dietary treatments

Treatment	Identification	Shrinkage loss	Dressing yield	Eviscerated yield
T1	Control (C)	4.10±0.20	76.55±0.75	67.69±2.08
T2	C+ AF 250 ppm	3.93±0.24	75.94±1.13	66.77±0.43
T3	C+20 ppm zinc	4.04±0.13	76.62±0.59	68.14±0.37
T4	C+40 ppm zinc	4.18±0.38	76.78±0.96	67.53±0.56
T5	T2+20 ppm zinc	3.91±0.99	76.28±0.88	67.62±0.40
T6	T2+40 ppm zinc	3.99±0.19	76.46±0.34	67.75±0.60

Experiment 2 (Methionine)

The data pertaining to slaughter traits was statistically analysed and presented in Table 4.16. The shrinkage loss, dressing yield and eviscerated yield varied from 3.93 to 4.32, 75.94 to 76.96, and 66.74 to 67.97%, respectively. There was no significant difference among various dietary treatments with respect to shrinkage loss, dressing yield and eviscerated yield. In the present study supplementation of methionine had no effect on the slaughter traits of broiler chickens. The information on effect of methionine supplementation on slaughter traits during aflatoxicosis is lacking in literature.

Table 4.16: Slaughter traits (% of live weight) as influenced by various dietary treatments

Treatment	Identification	Shrinkage loss	Dressing yield	Eviscerated yield
T1	Control (C)	4.10±0.20	76.55±0.75	67.69±2.08
T2	C+ AF 250 ppm	3.93±0.24	75.94±1.13	66.77±0.43
T3	C+0.05% methionine	4.07±0.29	76.80±0.90	67.86±0.50
T4	C+0.1% methionine	4.32±0.11	76.96±0.79	67.97±0.50
T5	T2+0.05% methionine	3.98±0.14	75.64±0.68	66.74±0.54
T6	T2+0.1% methionine	4.11±0.11	76.15±0.76	66.85±0.32

4.2.2 Cut–up parts

Experiment 1 (Zinc)

The relative values of cut-up parts (thigh, drumstick, breast, back, neck and wing) expressed as percentage of pre-slaughter live weight taken at the end of 6th week of the trail period in various dietary treatments are presented in Table 4.17. The present study revealed that the relative weight of cut-up parts did not vary significantly ($P<0.05$) among various dietary treatments. Inclusion of aflatoxin B_1 (250 ppb) in the diet of birds did not produce any significant ($P<0.05$) effect in the cut-up parts of birds. The relative yield of cut-up parts measured in different dietary treatments did not vary significantly ($P<0.05$) from that of control.

The present study revealed that the relative yield of cut-up parts, measured as percentage of pre-slaughter live weight did not vary significantly ($P<0.05$) due to various dietary treatments.

Table 4.17: Cut-up parts yield (% of live weight) of broilers fed different dietary treatments

Treatment	Identification	Thigh	Drumstick	Breast	Back	Neck	Wing
T_1	Control (C)	10.06±0.18	9.71±0.50	18.40±0.86	19.86±1.16	3.92±0.40	8.61±0.19
T_2	C+ AF 250 ppm	9.74±0.16	9.49±0.46	16.55±1.03	17.25±0.48	3.24±0.48	8.48±0.25
T_3	C+20 ppm zinc	10.13±0.17	9.25±0.14	16.91±0.60	18.00±0.77	3.69±0.42	9.18±0.25
T_4	C+40 ppm zinc	10.01±0.29	10.10±0.32	17.62±1.11	18.09±0.86	4.35±0.50	8.82±0.24
T_5	T2+20 ppm zinc	9.92±0.39	9.24±0.40	16.98±0.63	17.26±1.00	3.80±0.40	8.35±0.21
T_6	T2+40 ppm zinc	9.84±0.13	9.81±0.49	16.55±0.88	18.89±0.23	3.89±0.58	9.20±0.26

Table 4.18: Cut-up parts yield (% of live weight) of broilers fed different dietary treatments

Treatment	Identification	Thigh	Drumstick	Breast	Back	Neck	Wing
T_1	Control (C)	10.06±0.18	9.71±0.50	18.40±0.86	19.86±1.16	3.92±0.40	8.61±0.19
T_2	C+ AF 250 ppm	9.74±0.16	9.49±0.46	16.55±1.03	17.25±0.48	3.24±0.48	8.48±0.25
T_3	C+0.05% methionine	10.19±0.23	10.71±0.37	15.81±0.57	17.83±0.86	4.23±0.32	8.75±0.24
T_4	C+0.1% methionine	10.10±0.32	11.53±0.93	15.54±1.96	19.36±0.70	3.39±0.27	9.17±0.56
T_5	T2+0.05% methionine	9.44±0.15	10.30±0.42	14.16±1.34	18.29±1.24	4.04±0.38	9.53±0.37
T_6	T2+0.1% methionine	9.41±0.18	9.44±0.86	16.20±0.81	19.16±1.88	3.72±0.38	9.16±0.25

Incorporation of 250 ppb aflatoxin B_1 in the diet of broilers did not produce any significant change on cut-up parts of broilers. Similar results were also reported earlier by Silambarasan (2011) and Abaji (2012). They also observed no significant effect in relative yield of cut-up parts (thigh, drumstick, breast, back, neck and wing) due to addition of 300 ppb aflatoxin in the diet of broiler chickens. They also reported that non-significant effect on cut-up parts may be due to low levels of aflatoxin in the diet. Shamsudeen (2007) reported that cut-up parts yield decreased as the diet aflatoxin level increased (0.5 to 1.0 ppm) in the diet of broiler chickens. Huff and Doerr (1981) also reported that the cut-up parts yields were decreased with decreased in body weight and breast yield was mainly decreased due to dietary contamination of aflatoxin. In the present study, supplementation of zinc at any level to the aflatoxin (250 ppb) contaminated diet did not produce any significant alteration in cut-up parts of broiler chickens.

Experiment 2 (Methionine)

The relative weights of cut-up parts (thigh, drumstick, breast, back, neck and wing) expressed as percentage of pre-slaughter live weight taken at the end of 6th week of the trail period in various dietary treatments are presented in Table 4.18. No significant ($P<0.05$) differences were reported in the relative weight of cut-up parts among various dietary treatments. The relative yield of cut-up parts reported in various dietary treatments did not vary significantly ($P<0.05$) from that of control. The results of the present study revealed that supplementation of methionine at either level to the 250 ppb aflatoxin contaminated diet did not reveal any significant change in cut-up parts of broiler chickens. The information on effect of methionine supplementation on cut-up parts during aflatoxicosis is lacking in literature.

4.2.3 Organ weights

The data pertaining to relative organ weights (liver, spleen, bursa and thymus) expressed as percentage of live weight were statistically analyzed and are presented in Table 4.19 (experiment 1) and 4.20 (experiment 2). The Figure 4.7 (experiment 1) and 4.8 (experiment 2) gives the graphical representation of various organ weights.

Experiment 1 (Zinc)

4.2.3.1 Liver

The relative weight of liver (Percent of live body weight) was 2.37 in the control group (T_1), which significantly ($P<0.05$) increased to 3.03 in aflatoxin fed group (T_2). The relative weight of liver in group T_5 was significantly ($P<0.05$) lower than that of T_2 but significantly higher than that of control, indicating that supplementation of 20 ppm to the aflatoxin contaminated diet partially ameliorated the adverse effects of aflatoxin on relative liver weight. The relative weight of liver of group T_6 was statistically similar to that of control, indicating that addition of 40 ppm zinc to the aflatoxin contaminated feed ameliorated the adverse effects of aflatoxin on relative weight of liver.

In the present study, contamination of aflatoxin at 250 ppb level in the diet of broiler chickens resulted in significant ($P<0.05$) increased in the relative weight of liver. Similar observations were also reported by Silambarasan (2011); Abaji (2012); Smith and Hamilton (1970) and Verma (1994). Raju and Devegowda (2000) also reported a significant increase in the relative weight of liver due to 300 ppb of aflatoxin contamination in the diet of broilers. Several researchers have reported a significant increase in the relative weight of liver (Kubena *et al.*, 1993; Kubena *et al.*, 1998; Miazzo *et al.*, 2000; Rosa *et al.*, 2001).

In the present study, supplementation of zinc at 40 ppm level ameliorated the adverse effects of aflatoxin on relative weight of liver.

4.2.3.2 Spleen

The relative weight of spleen in control group was 0.24% which significantly ($P<0.05$) increased to 0.35% in the aflatoxin alone fed group (T_2). The relative weight of spleen in other groups was statistically similar to that of control. In the present study, a significant increase in the relative spleen weight was recorded at 250 ppb level of dietary aflatoxin. Abaji (2012) also reported a significant increase in the relative weight of spleen at 300 ppb level of dietary aflatoxin. Significant increase in relative spleen weight due to dietary aflatoxin content ranging from 3.5 to 5 ppm has also been reported by earlier researchers (Kubena *et al.*, 1990; Kubena *et al.*, 1993; Bailey *et al.*, 1998; Kubena *et al.*, 1998 and Rosa *et al.*, 2001).

In the present study, supplementation of zinc to the aflatoxin contaminated diet ameliorated the ill effects of aflatoxin on relative weight of spleen.

4.2.3.3 Bursa of Fabricius

The relative weight of bursa of Fabricius in control group was 0.19% which significantly ($P<0.05$) reduced to 0.10% in the aflatoxin alone fed group (T_2). The relative weight of bursa in other treatment groups was statistically similar to that of control. Aflatoxin being a potent immunosuppressant causes reduction in size of bursa of Fabricius. In the present study, a significant ($P<0.05$) decrease in the weight of bursa was reported at 250 ppb level of dietary aflatoxin. These results corroborate well with earlier reports of Silambarasan (2011) and Abaji (2012). In their study, a significant decrease in the relative bursa weight was observed at 300 ppb level of aflatoxin in the diet. Significant reduction in the relative weight of bursa of Fabricius was reported in chicks receiving 2 ppm of aflatoxin (Verma *et al.*, 2004). A severe and significant regression of bursa of Fabricius in broilers was observed by Thaxton *et al.* (1974) at 0.75 ppm and higher level of aflatoxin. Similar results have also been reported by Chattopadhyay *et al.* (1985). Gopi (2006) and Beura (1988) also observed a significant reduction in bursal weight due to dietary aflatoxin.

In the present study, supplementation of zinc ameliorated the ill effect of aflatoxin on relative weight of bursa of Fabricius.

4.2.3.4 Thymus

The relative weight of thymus varied between 0.28 to 0.34 % among various dietary treatments. There was no statistical difference in relative weight of thymus among various dietary treatments. Ortatatli *et al*. (2005) also reported no significant difference in relative weight of thymus when diet containing 100 ppb AFB_1 was fed to broiler chickens.

In the present study, supplementation of zinc had no effect on the relative weight of thymus in broiler chickens.

Table 4.19: Relative weights (% of live weight) of organs fed different dietary treatments

Treatment	Identification	Liver	Spleen	Bursa	Thymus
T1	Control (C)	2.37±0.04^{a}	0.24±0.01^{a}	0.19±0.02^{b}	0.31±0.02
T2	C+ AF 250ppm	3.03±0.09^{c}	0.35±0.01^{b}	0.10±0.01^{a}	0.34±0.02
T3	C+20ppm zinc	2.40±0.08^{ab}	0.26±0.02^{a}	0.17±0.01^{b}	0.28±0.03
T4	C+40ppm zinc	2.37±0.07^{a}	0.25±0.02^{a}	0.19±0.04^{b}	0.29±0.02
T5	T2+20ppm zinc	2.49±0.06^{b}	0.27±0.02^{a}	0.15±0.01^{ab}	0.32±0.03
T6	T2+40ppm zinc	2.50±0.05^{ab}	0.27±0.02^{a}	0.17±0.02^{b}	0.31±0.03

Values bearing different superscripts in a column differ significantly (P<0.05).

Experiment 2 (Methionine)

4.2.3.5 Liver

The relative weight of liver (Percent of live body weight) in the control group was significantly (P<0.05) lower than that of aflatoxin fed group (T_2). The relative weight of liver in T_5 was significantly (P<0.05) lower than that of T_2 but significantly higher than that of control, suggesting that supplementation of methionine (0.05%) to the aflatoxin contaminated feed partially removed the adverse effects of aflatoxin on relative liver weight. The relative weight of liver in group T_6 was statistically similar to that of control, indicating that incorporation of 0.1% methionine to the aflatoxin contaminated diet ameliorated the adverse effects of aflatoxin on relative weight of liver.

In the present study, supplementation of methionine at 0.1% level ameliorated the adverse effects of aflatoxin on relative weight of liver. These results are in agreement with Sapocota *et al.* (2007).

4.2.3.6 Spleen

The relative weight of spleen in control group was significantly (P<0.05) lower than that of aflatoxin fed group (T_2). The relative weight of spleen in group T_5 was statistically similar to that of T_2, indicating that supplementation of methionine at 0.05% level could not ameliorate the ill effect of aflatoxin on relative spleen weight. The relative weight of spleen in group T_6 was significantly (P<0.05) lower than that of T_2 and statistically similar to that of

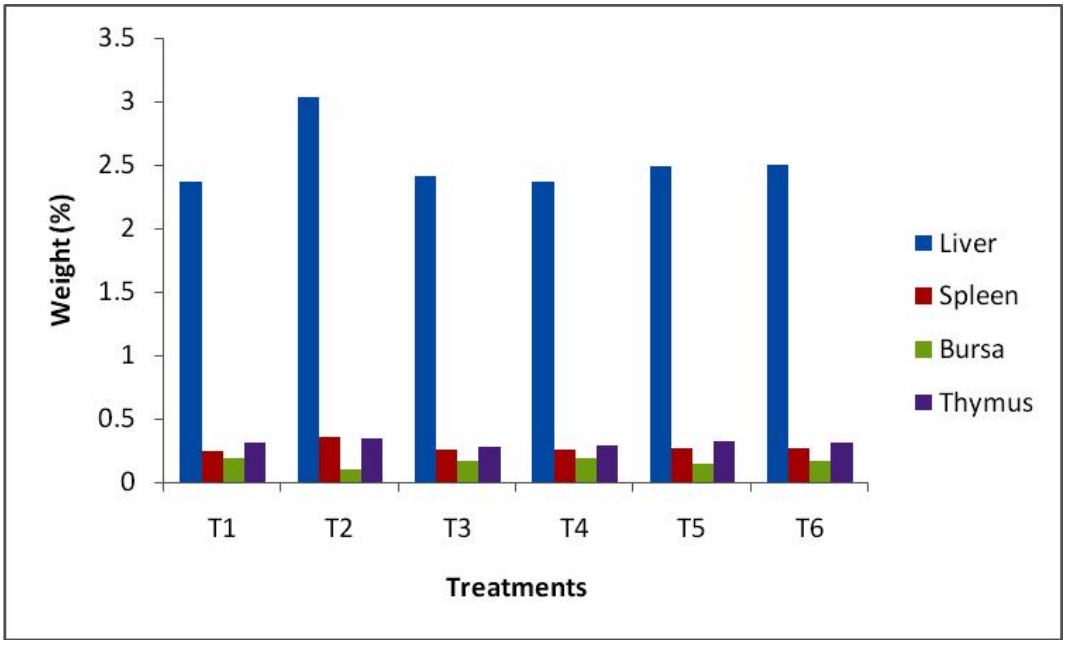


Fig. 4.7 : Relative weight (% of body weight) of liver, spleen, bursa and thymus

control, suggesting that incorporation of methionine (0.1%) to the aflatoxin contaminated feed ameliorated the adverse effect of aflatoxin on relative weight of spleen.

In the present study, supplementation of methionine (0.1%) to the aflatoxin contaminated diet reversed the effect of aflatoxin on relative weight of spleen.

4.2.3.7 Bursa of Fabricius

The relative weight of bursa of Fabricius in control group (T_1) was significantly ($P<0.05$) higher than that of aflatoxin alone fed group (T_2). The relative weight of bursa of Fabricius in other treatment groups was statistically similar to that of control.

In the present study, supplementation of methionine to the aflatoxin contaminated diet ameliorated the ill effect of aflatoxin on relative weight of bursa of Fabricius.

4.2.3.8 Thymus

The relative weight of thymus varied between 0.29 to 0.34% among various dietary treatments. There was no significant difference in relative weight of thymus among various dietary treatments.

In the present study, supplementation of methionine had no effects on the relative weight of thymus in broiler chickens.

Table 4.20: Relative weights (% of live weight) of organs fed different dietary treatments

Treatment	Identification	Liver	Spleen	Bursa	Thymus
T1	Control (C)	2.37±0.04^{a}	0.24±0.01^{a}	0.19±0.02^{b}	0.31±0.02
T2	C+ AF 250ppm	3.03±0.09^{b}	0.35±0.01^{c}	0.10±0.01^{a}	0.34±0.02
T3	C+0.05% methionine	2.36±0.10^{a}	0.26±0.02^{ab}	0.19±0.01^{b}	0.31±0.02
T4	C+0.1% methionine	2.33±0.06^{a}	0.25±0.02^{a}	0.19±0.01^{b}	0.31±0.03
T5	T2+0.05% methionine	2.67±0.10^{b}	0.31±0.02^{bc}	0.15±0.01^{b}	0.29±0.03
T6	T2+0.1% methionine	2.44±0.07^{ab}	0.28±0.00^{ab}	0.16±0.00^{b}	0.32±0.04

Values bearing different superscripts in a column differ significantly ($P<0.05$).

4.3 Effect on biochemical and haematological parameters

The data pertaining to serum protein, cholesterol, uric acid, SGPT and SGOT were statistically analyzed and the mean values are presented in Table 4.21 (experiment 1) and 4.22 (experiment 2).

Experiment 1 (Zinc)

4.3.1 Total serum protein

The total serum protein value in control group was 5.31 g/dl which significantly ($P<0.05$) reduced to 4.73 g/dl in aflatoxin fed group (T_2). The serum protein in group T_3 and T_4 was statistically similar to that of control. The serum protein in T_5 was statistically similar to that of AF fed group indicating that supplementation of zinc at 20 ppm level did not improve serum protein content. The serum protein in group T_6 was significantly ($P<0.05$) higher than that of T_2 and statistically similar to that of control, indicating that supplementation of zinc at 40 ppm diet level improved the serum protein content equal to that of control.

In the present study, inclusion of aflatoxin at 250 ppb level in feed caused significant ($P<0.05$) reduction in serum protein level. Significant decrease in serum protein content due to feeding of aflatoxin contaminated diet has also been reported by earlier researchers (Abaji, 2012; Bakshi, 1991; Jassar and Singh, 1993; Shukla and Pachuri, 1995; Vasan *et al*., 1998; Gopi, 2006 and Silambarasan, (2011). The decrease in total serum protein by aflatoxin feeding has been reported due to reduced content of albumin and β globulin (Pier, 1992). Reduced value of serum albumin and globulin has also been reported by Huff *et al*. (1992). Other researchers reported that decrease in serum protein by aflatoxin feeding was attributed to failure in digestion and absorption of protein in gastro-intestinal tract (Voight *et al*., 1980) and inhibition of protein synthesis due to aflatoxin contamination in diet (Sarasin and Moule, 1973). Groopman *et al*. (1996) also reported that the decline in serum protein may be due to decline in protein synthesis by forming adduct with DNA, RNA and protein and inhibit RNA synthesis and DNA-dependent RNA polymerase activity as well as causing degranulation of endoplasmic reticulum.

In the present study, addition of zinc (40 ppm) to the aflatoxin contaminated diet, significantly ($P<0.05$) increased the serum protein content equal to that of control.

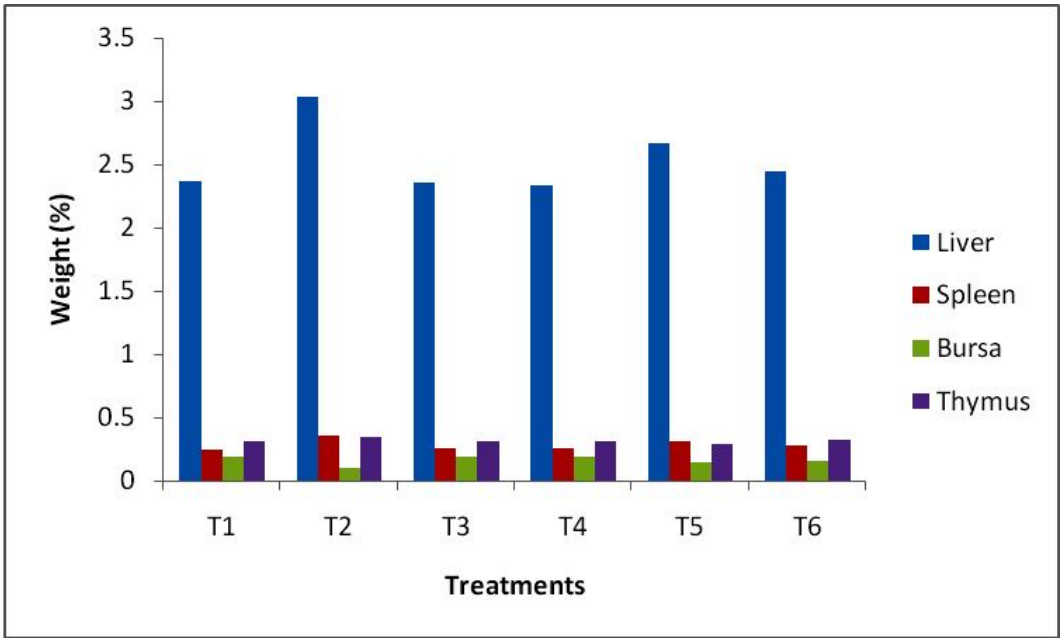


Fig. 4.8 : Relative weight (% of body weight) of liver, spleen, bursa and thymus

Table 4.21: Certain blood biochemical and haematological constituents of broilers fed different treatments

Treatment	Identification	Protein (g/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	SGPT (IU/L)	SGOT (IU/L)	Hb (g/dl)	H/L ratio
T_1	Control (C)	5.31±0.21^{b}	189.42±9.48^{b}	6.87±0.49^{b}	31.00±1.87^{a}	134.46±3.81^{a}	7.93±0.24^{c}	0.57±0.24^{a}
T_2	C+ AF 250 ppm	4.73±0.24^{a}	131.96±5.00^{a}	4.07±0.94^{a}	47.00±4.63^{b}	163.69±4.80^{c}	5.46±0.06^{a}	1.08±0.06^{c}
T_3	C+20 ppm zinc	5.81±0.13^{b}	187.87±15.81^{b}	6.01±0.60^{b}	30.00±4.18^{a}	138.55±4.39^{a}	7.10±0.26^{bc}	0.65±0.06^{ab}
T_4	C+40 ppm zinc	6.04±0.27^{b}	179.82±20.43^{b}	6.27±0.40^{b}	31.00±5.33^{a}	137.97±10.35^{a}	7.26±0.65^{bc}	0.67±0.00^{ab}
T_5	T2+20 ppm zinc	4.89±0.30^{a}	148.78±19.48^{ab}	5.23±0.88^{ab}	41.00±4.30^{ab}	159.01±6.95^{bc}	5.93±0.58^{ab}	0.84±0.02b
T_6	T2+40 ppm zinc	5.38±0.24^{b}	155.43±22.92^{ab}	6.21±0.35^{b}	40.00±6.51^{ab}	141.47±4.39^{ab}	7.06±0.54^{bc}	0.75±0.06^{ab}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

4.3.2 Total serum cholesterol

The serum cholesterol content of control group (T_1) was 189.42 mg/dl which significantly ($P<0.05$) reduced to 131.96 mg/dl in aflatoxin alone fed group (T_2). The serum cholesterol concentration in groups T_3 to T_6 was statistically similar to that of control. The cholesterol concentration of groups T_5 and T_6 did not differ significantly ($P<0.05$) from that of T_2 and control, indicating that the supplementation of zinc at both levels to AF contaminated diet partially improved the serum cholesterol content.

The present study revealed that there was significantly ($P<0.05$) reduction in serum cholesterol content due to feeding of 250 ppb dietary aflatoxin. Raju and Devegowda (2000); Yunus *et al.* (2011) and Abaji (2012) also reported significant reduction in serum cholesterol content due to 0.3 ppm aflatoxin in the diet of broiler chickens. Silambarasan (2011) howerever, reported no significant change in serum cholesterol level due to 0.3 ppm dietary AF. A significant reduction in serum cholesterol has also been observed by several researchers (Vasan *et al.*, 1998; Reddy *et al.*, 1984; Shukla and Pachuri, 1995; and Fazal *et al.*, 1980). A dose-dependent decrease in serum cholesterol at and beyond 0.25 ppm level of dietary aflatoxin was reported by Reddy *et al.* (1984). In contrary, Chattopadhyay *et al.* (1985) could not find any change in serum cholesterol at dietary aflatoxin ranging from 0.5 to 2.5 ppm.

In the present study, supplementation of zinc at both levels to the aflatoxin contaminated feed partially improved the serum cholesterol content.

4.3.3 Serum uric acid

The serum uric acid content in control group was 6.87 mg/dl which significantly ($P<0.05$) reduced to 4.07 mg/dl in AF alone fed group (T_2). The uric acid concentration in group T_5 did not differ significantly ($P<0.05$) from that of T_2 and control, indicating that inclusion of 20 ppm diet zinc to the aflatoxin contaminated feed partially improved the serum uric acid content. The uric acid content of group T_6 was statistically similar to that of control and significantly ($P<0.05$) higher than that of aflatoxin fed group, indicating of 40 ppm zinc to the aflatoxin contaminated feed improved the serum uric acid content equal to that of control.

In the present study, 250 ppb aflatoxin contamination resulted in significant ($P<0.05$) reduction in serum uric acid content. Safameher (2008) also reported that significant reduction

in the content of serum uric acid with 0.5 ppm of aflatoxin containing diet. Oguz *et al.* (2000) reported that serum uric acid was decreased when 50 ppb AF containing diet was fed to broiler chickens. Denli *et al*. (2009) also observed that 1 ppm AF containing diet resulted in decrease in serum uric acid concentration. A significant decrease in the uric acid concentration was also reported by several other researchers (Bailey *et al*., 1998; Kececi *et al*., 1998).

In the present study, supplementation of 40 ppm diet zinc to the 250 ppb aflatoxin contaminated feed improved the serum uric acid concentration.

4.3.4 Serum glutamic oxaloacetic transferase (SGOT)

The SGOT activities in control group was 134.46 IU/L which significantly ($P<0.05$) increased to 163.69 IU/L in the aflatoxin alone fed group (T_2). The SGOT activities of T_5 were statistically similar to that of T_2, indicating that the supplementation of 20 ppm diet zinc to the AF contaminated feed failed to remove the effects of aflatoxin in broiler chickens. The SGOT activities in group T_6 were statistically similar to that of control, indicating that supplementation of 40 ppm diet zinc to the aflatoxin contaminated diet reversed the effect of aflatoxicosis on SGOT activities in broiler chickens. In the present study, 250 ppb aflatoxin resulted in increased activities of SGOT. These results are in agreement with earlier reports (Abaji, 2012). Safameher (2008) also observed elevated SGOT activities in chickens with 0.5 ppm of aflatoxin contaminated diet. Denli *et al.* (2009) and Eraslan *et al.* (2006) also reported an increase in the activities of SGOT with 1 ppm of aflatoxin contaminated diet. Increased activities of SGOT due to aflatoxin in diet were also reported by Shi *et al.* (2009); Raju and Devegowda (2000) and Balachandran and Ramakrishnan (2004).

In the present study, supplementation of zinc (40 ppm) significantly reduced the ill effects of aflatoxicosis, indicating that zinc supplementation in the diet containing 250 ppb aflatoxin may ameliorate the adverse effects of aflatoxin on SGOT activities.

4.3.5 Serum glutamic pyruvic transeferase (SGPT)

The SGPT values in control group was 31.00 IU/L which significantly ($P<0.05$) increased to 47.00 IU/L in the aflatoxin alone fed group (T_2). The SGPT values in all other groups were statistically similar to that of control.

In the present study, increased activity of SGPT due to 250 ppb dietary aflatoxin was earlier reported (Abaji, 2012). Denli *et al.* (2009) and Eraslan *et al.* (2006) also reported an increase in the activity of SGPT with 1 ppm of aflatoxin contaminated diet. Increased level of SGPT due to aflatoxin was also reported by several researchers (Shi *et al.*, 2009; Kermanshahi *et al.*, 2009; Balachandran and Ramakrishnan, 1987).

In the present study, addition of zinc partially ameliorated the ill effects of aflatoxin on SGPT activity.

4.3.6 Haemoglobin and H/L ratio

The haemoglobin value in control group was 7.93 g/dl which significantly ($P<0.05$) reduced to 5.46 g/dl in the aflatoxin alone fed group (T_2). The Hb value in T_3, T_4 and T_6 was statistically similar to that of control. The Hb value in T5 did not differ significantly ($P<0.05$) from that of T_2 and was significantly ($P<0.05$) lower than that of control, indicating that supplementation of 20 ppm diet zinc may not be sufficient to reverse the haemoglobin concentration in blood. In the present study, aflatoxin contamination resulted in reduced haemoglobin level in broiler chickens. Similar result was also found by Kececi *et al.* (2010), who also reported reduced Hb level in broiler chickens at 2.5 ppm AF. Basmacioglu *et al.* (2005) also reported significantly ($P<0.05$) reduced Hb level at 2 ppm aflatoxin contamination. In the present study, supplementation of zinc to basal diet did not produce any positive effect on haematology of broilers, however, supplementation of 40 ppm diet zinc to aflatoxin contaminated diet resulted in significant improvement of Hb level equal to that of control.

H/L ratio in control group was 0.57 which significantly ($P<0.05$) increased to 1.08 in AF fed group (T_2). The H/L ratio in T_3, T_4 and T_6 was statistically similar to that of control. The H/L ratio in T_5 was significantly ($P<0.05$) lower than that of T2 but significantly ($P<0.05$) higher than that of control, indicating that supplementation of 20 ppm diet zinc may not be sufficient to reverse the H/L ratio. In the present study, aflatoxin contamination resulted in increased H/L ratio in broiler chickens. These findings agreed with the other reports that explain the suppressive effects of aflatoxin on haematopoiesis and immune responses (Huff *et al.*, 1986; Oguz *et al.*, 2003). The increase in heterophil counts suggested that the toxin was elicited the inflammatory response of broiler chickens (Kececi *et al.*, 1998).

The present study revealed that aflatoxin contamination in feed resulted in significantly increased H/L ratio in broilers. Basmacioglu *et al*. (2005) reported elevated H/L ratio in broilers due to feeding 2 ppm AF.

In presented study, supplementation of zinc at any level to basal diet did not produce any positive effect on haematology of broilers, however, supplementation of zinc (40 ppm) to the aflatoxin contaminated diet resulted in decreased H/L ratio in broiler chickens equal to that of control.

Experiment 2 (Methionine)

4.3.7 Total serum protein

The total serum protein content of aflatoxin fed group (T_2) was lower ($P<0.05$) than that of control, however the serum protein content of groups T_3 to T_6 was statistically similar to that of control.

The present study indicated that supplementation of methionine at both levels to the aflatoxin contaminated diet significantly improved the serum protein content.

4.3.8 Total serum cholesterol

The serum cholesterol content in control group was significantly ($P<0.05$) higher than that of aflatoxin alone fed group (T_2). The serum cholesterol contents in groups T_3 to T_6 was statistically similar to that of control. The serum cholesterol content in groups T_5 and T_6 did not differ significantly ($P<0.05$) from that of T_2 and control, indicating that the inclusion of methionine at both levels to the aflatoxin contaminated feed partially improved the serum cholesterol content.

In present study, inclusion of both levels of methionine to the 250 ppb aflatoxin contaminated diet partially improved the serum cholesterol content.

4.3.9 Serum uric acid

The serum uric acid content of aflatoxin fed group (T_2) was significantly ($P<0.05$) lower than that of control. The uric acid content of groups T_5 and T_6 did not differ significantly ($P<0.05$) from that of T_2 and control, indicating that the addition of both levels of methionine to the aflatoxin contaminated feed resulted in partial improvement in serum uric acid content.

Table 4.22: Certain blood biochemical and haematological constituents of broilers fed different treatments

Treatment	Identification	Protein (g/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	SGPT (IU/L)	SGOT (IU/L)	Hb (g/dl)	H/L ratio
T_1	Control (C)	5.31±0.21^{b}	189.42±9.48^{b}	6.87±0.49^{b}	31.00±1.87^{a}	134.46±3.81^{a}	7.93±0.24^{c}	0.57±0.24^{a}
T_2	C+ AF 250 ppm	4.73±0.24^{a}	131.96±5.00^{a}	4.07±0.94^{a}	47.00±4.63^{b}	163.69±4.80^{c}	5.46±0.06^{a}	1.08±0.06^{c}
T_3	C+0.05% methionine	5.66±0.27^{b}	184.67±4.46^{b}	6.27±0.36^{b}	30.00±3.53^{a}	133.29±3.28^{a}	7.53±0.56^{bc}	0.63±0.05^{a}
T_4	C+0.1% methionine	5.48±0.13^{b}	185.60±5.43^{b}	6.21±0.27^{b}	30.00±3.53^{a}	137.97±4.07^{a}	7.80±0.52^{c}	0.69±0.04^{a}
T_5	T2+0.05% methionine	4.96±0.31^{ab}	149.44±5.53^{ab}	5.34±0.36^{ab}	45.00±5.70^{b}	152.58±1.09^{b}	6.56±0.26^{ab}	0.91±0.03^{bc}
T_6	T2+0.1% methionine	5.03±0.26^{b}	142±12.68^{ab}	5.40±0.48^{ab}	33.00±2.54^{a}	139±3.96^{a}	6.93±0.29^{bc}	0.76±0.04^{ab}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

In the present study, supplementation of methionine to the aflatoxin contaminated diet partially improved the serum uric acid content.

4.3.10 Serum glutamic oxaloacetic amino transferase (SGOT)

The SGOT activities in aflatoxin fed group were statistically higher than that of control. The SGOT activities in group T_5 were significantly ($P<0.05$) lower than that of T_2 but significantly higher than that of control, indicating that the supplementation of 0.05% methionine to the aflatoxin contaminated feed partially improved the SGOT values. The SGOT value in group T_6 was statistically similar to that of control, indicating that the supplementation of 0.1% methionine ameliorated the adverse effects of aflatoxin on SGOT activities.

The present study revealed that 0.1% methionine supplementation in the diet containing 250 ppb aflatoxin may ameliorate the adverse effects of aflatoxin on SGOT activities.

4.3.11 Serum glutamic pyruvic transeferase (SGPT)

The SGPT value in control group was significantly ($P<0.05$) lower than that of AF alone fed group (T_2). The SGPT values in group T_5 was statistically similar to that of aflatoxin fed group and significantly higher than that of control, indicating that addition of 0.05% methionine failed to remove the ill effects of aflatoxicosis. The SGPT value of group T_6 was significantly ($P<0.05$) lower than that of T_2 and statistically similar to that of control, suggesting that supplementation of 0.1% methionine to the aflatoxin contaminated diet reversed the SGPT value.

In the present study, supplementation of 0.1% methionine ameliorated the adverse effects of aflatoxin on SGPT activity.

4.3.12 Haemoglobin and H/L ratio

Aflatoxin contamination, in the present study, resulted in reduced haemoglobin level in broiler chickens. The Hb value in T_3, T_4 and T_6 was statistically similar to that of control. The Hb value in T_5 did not differ significantly ($P<0.05$) from that of T_2 and was significantly ($P<0.05$) lower than that of control, indicating that supplementation of 0.05% methionine may not be sufficient to reverse the haemoglobin concentration in blood. In the present study, supplementation of methionine at any levels to the basal diet did not produce any positive

effect on haematology of broilers, however, supplementation of 100 ppm methionine to aflatoxin contaminated diet resulted in significant improvement of Hb level equal to that of control.

The present study revealed that aflatoxin contamination in feed resulted in significantly increased H/L ratio in broilers. The H/L ratio in T_3, T_4 and T_6 was statistically similar to that of control. The H/L ratio in T_5 was significantly ($P<0.05$) lower than that of T_2 but significantly ($P<0.05$) higher than that of control, indicating that supplementation of 0.05% methionine may not be sufficient to reverse the H/L ratio. In the presented study, supplementation of methionine at any level to basal diet did not produce any positive effect on haematology of broilers, however, supplementation of 0.1% methionine to the aflatoxin contaminated diet resulted in decreased H/L ratio in broiler chickens equal to that of control.

4.4 Immune response

Aflatoxicosis suppresses both humoral and cell mediated immunity. Immunosuppression caused by aflatoxicosis has been demonstrated in poultry as well as laboratory animals (Sharma, 1993).

Experiment 1 (Zinc)

4.4.1 Effect on cell mediated immunity (CMI)

The data pertaining to CMI response to PHA-P measured as foot web index and humoral immune response measured as haemagglutination titre (HA) against SRBCs of broiler chickens fed different dietary treatments was statistically analyzed and presented in Table 4.23 while its graphical representation is given in Figure 4.9 and 4.10 respectively. The CMI value in control group (T_1) was 0.42 mm which significantly ($P<0.05$) decreased to 0.29 mm in the aflatoxin fed group (T_2). This revealed that inclusion of dietary aflatoxin at 250 ppb level in feed significantly ($P<0.05$) decreased the CMI response to PHA-P compared to control. The CMI value in T_3 and T_4 groups was statistically similar to that of control. The CMI values of group T5 did not vary significantly ($P<0.05$) from T_1 and T_2, indicating that supplementation of zinc at 20 ppm diet level partially ameliorated the adverse effect of aflatoxin on CMI response. The CMI value in group T_6 was significantly ($P<0.05$) higher than that of T_2 and statistically similar to that of control, indicating that supplementation of zinc at 40 ppm diet level ameliorated the ill effects of aflatoxin on CMI response.

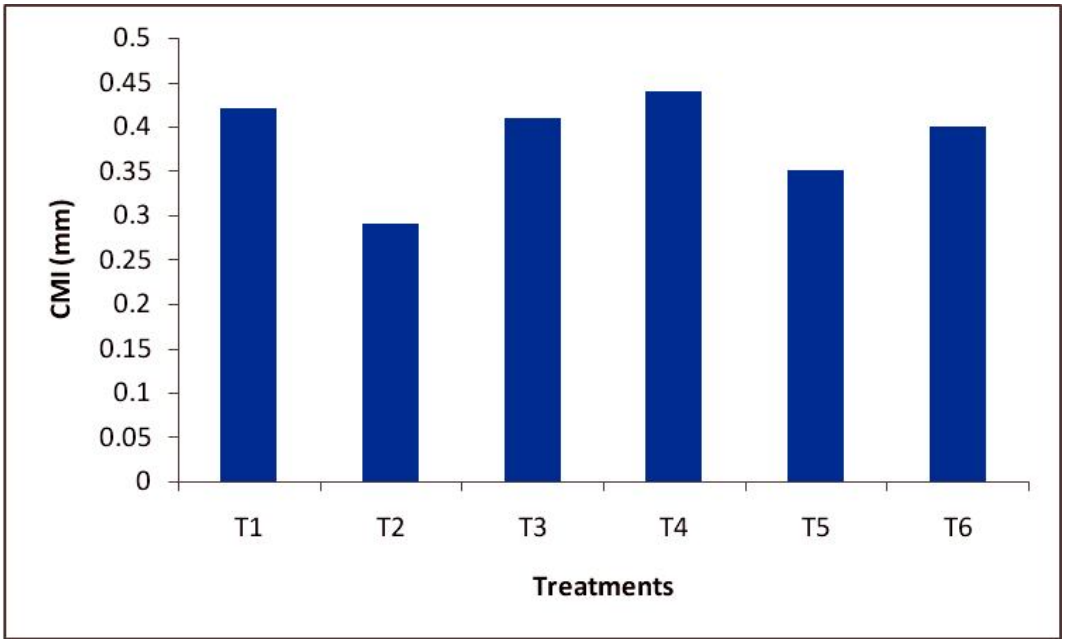


Fig. 4.9 : Cell mediated immune response to PHA-P

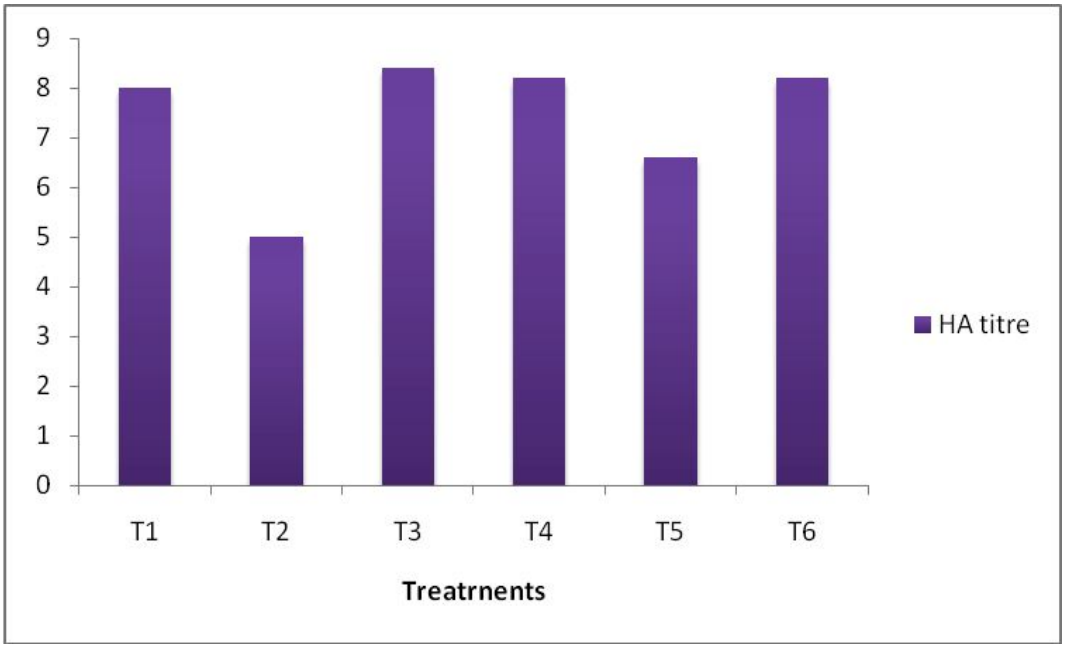


Fig. 4.10 : HA titre against Sheep RBCs

The present study showed that aflatoxin contamination at 250 ppb level in diet caused significant ($P<0.05$) reduction in CMI response to PHA-P. Silambarasan (2011) and Abaji (2012) also reported a significant decrease in the CMI response at 300 ppb level of dietary aflatoxin in broiler chickens. Poor CMI responses in chickens due to aflatoxin feeding were also earlier reported by Kadian *et al.* (1988); Deo *et al.* (1998) and Bakshi (1991). Suppression of CMI response may be due to impaired lymphoblastogenesis (Chang *et al.*, 1976) and impairment of lymphokine production (McLouglin *et al.*, 1984).

In the present study, supplementation of zinc (40 ppm diet) ameliorated the ill effect of aflatoxin in broiler chickens.

4.4.2 Humoral immune response

The HA titre value in aflatoxin alone fed group (T_2) was significantly ($P<0.05$) lower than that of control (T_1). The HA titre value of other groups did not vary significantly ($P<0.05$) from that of control.

Aflatoxicosis suppresses both humoral and cell mediated immunity and immune system is highly sensitive indicator of aflatoxicosis in poultry (Giambrone *et al.*, 1985). In the present study, dietary AF at 250 ppb level significantly ($P<0.05$) reduced the HA titre against sheep RBCs. This report is in agreement with Verma (1994) where significant ($P<0.05$) decrease in HA titre against SRBCs with inclusion of 0.5 and 1 ppm level of AF in feed was reported in broiler chickens. During experimental aflatoxicosis, reduced humoral immune response has also been reported by many workers (Virdi *et al.*, 1989 and Bakshi, 1991).

In present study, the HA titre value of group T_5 did not vary ($P<0.05$) from T_1 and T_2, indicating that supplementation of zinc (20 ppm) to the AF contaminated diet partially ameliorated the ill effect of AF on humoral immunity. The HA titre value in group T_6 was significantly ($P<0.05$) higher than that of T_2 but statistically similar to that of control, indicating that inclusion of zinc (40 ppm diet) to the AF contaminated diet ameliorated the ill effect of aflatoxicosis on humoral immune response.

In the present study, supplementation of 40 ppm zinc to the 250 ppb aflatoxin contaminated feed ameliorated the ill effect of aflatoxicosis on humoral immune response of broiler chickens.

Table 4.23: Cellular and humoral immunity of broilers fed different treatments

Treatment	Identification	CMI (mm)	HA Titre
T1	Control (C)	0.42 ± 0.40^{b}	8.00 ± 0.54^{b}
T2	C+ AF 250 ppm	0.29 ± 0.04^{a}	5.00 ± 0.63^{a}
T3	C+20 ppm zinc	0.41 ± 0.06^{b}	8.40 ± 0.50^{b}
T4	C+40 ppm zinc	0.44 ± 0.04^{b}	8.20 ± 0.58^{b}
T5	T2+20 ppm zinc	0.35 ± 0.05^{ab}	6.60 ± 0.67^{ab}
T6	T2+40 ppm zinc	0.40 ± 0.02^{b}	8.20 ± 0.86^{b}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

Experiment 2 (Methionine)

4.4.3 Effect on cell mediated immunity (CMI)

The data pertaining to CMI response to PHA-P measured as foot web index and humoral immune response measured as haemagglutination titre (HA) against SRBCs of broilers fed different dietary treatments was statistically analyzed and presented in Table 4.24 while its graphical representation is given in Figure 4.11 and 4.12 respectively. The CMI value in control group (T_1) was significantly ($P<0.05$) higher than that of aflatoxin alone fed group (T_2). The present investigation revealed that addition of aflatoxin (250 ppb) in the diet of broiler chicken significantly ($P<0.05$) decreased the CMI response to PHA-P compare to control.

The CMI value of T_5 did not vary significantly ($P<0.05$) from T_1 and T_2, suggesting that supplementation of methionine at 0.05% level to the AF contaminated diet partially ameliorated the adverse effect of aflatoxin on CMI response. The CMI value in group T_6 was significantly ($P<0.05$) higher than that of T_2 and statistically similar to that of control, suggesting that addition of methionine at 0.1% level ameliorated the ill effect of AF on CMI response.

In present study, supplementation of 0.1% methionine to the 250 ppb AF contaminated diet ameliorated the ill effect of aflatoxin on CMI response.

4.4.4 Humoral immune response

The HA titre value of control group (T_1) was significantly ($P<0.05$) higher than that of AF fed group. The HA titre value in other groups did not vary significantly ($P<0.05$) from that of control.

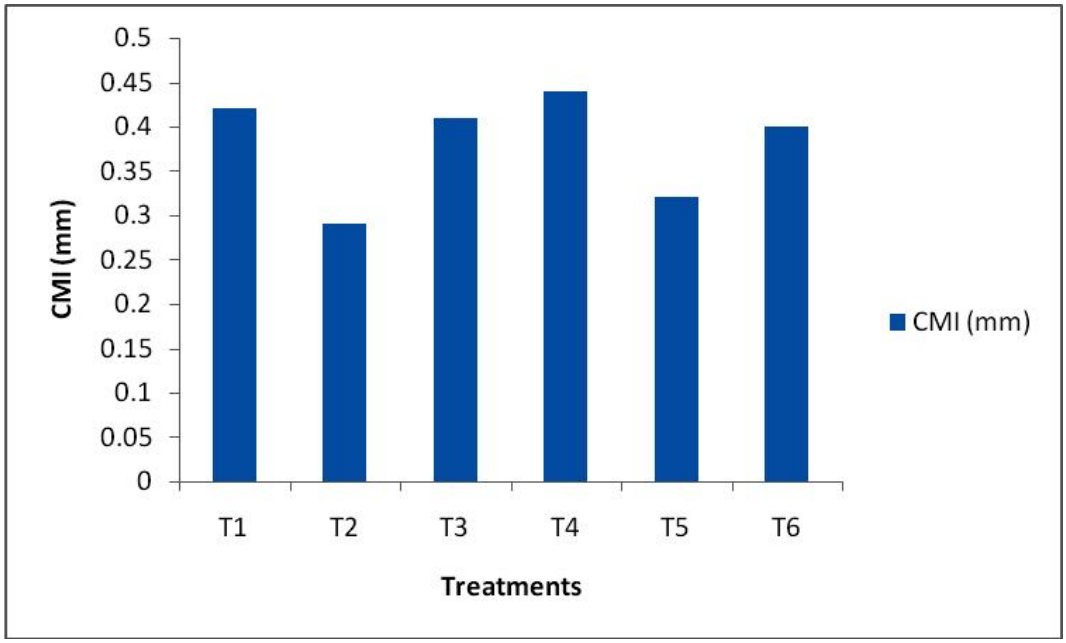


Fig. 4.11 : Cell mediated immune response to PHA-P

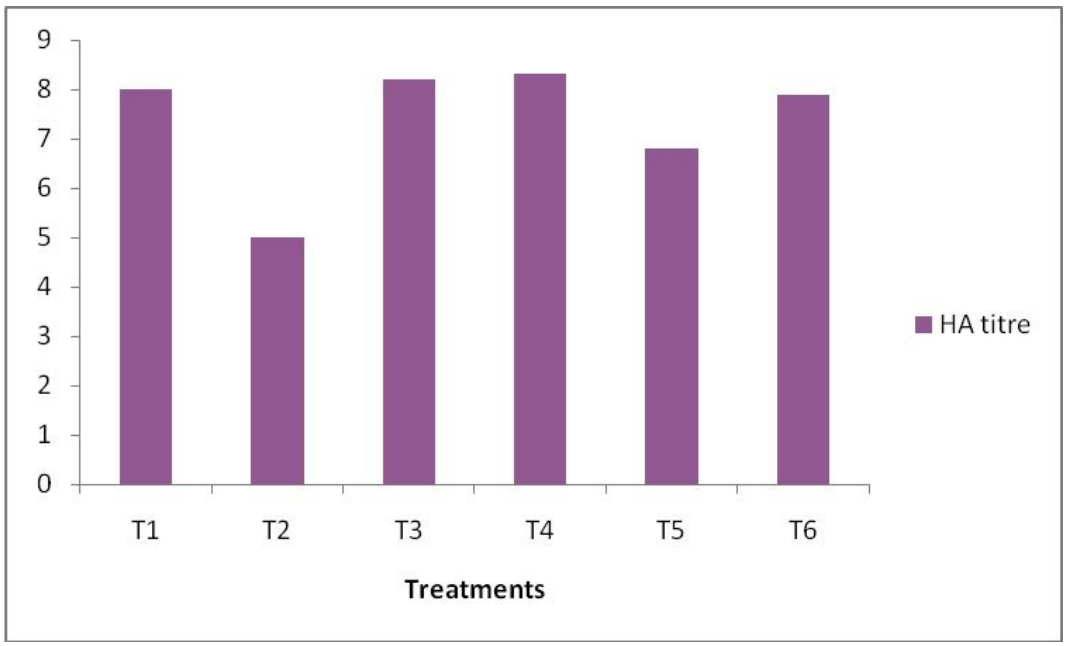


Fig. 4.12 : HA titre against Sheep RBCs

In the present study, the HA titre value of group T_5 did not vary significantly ($P<0.05$) from T_1 and T_2, indicating that supplementation of methionine (0.05%) to the aflatoxin contaminated diet partially ameliorated the ill effect of aflatoxicosis on humoral immune response. The HA titre value in group T_6 was significantly ($P<0.05$) higher than that of T_2 but statistically similar to that of control (T_1), suggesting that inclusion of 0.1% methionine to the AF contaminated diet ameliorated the ill effect of aflatoxicosis on humoral immune response.

In the present study, supplementation of methionine (0.1%) to the 250 ppb AF contaminated feed ameliorated the ill effect of aflatoxicosis on humoral immune response of broiler chickens.

Table 4.24: Cellular and humoral immunity of broilers fed different treatments

Treatment	Identification	CMI (mm)	HA Titre
T_1	Control (C)	0.42 ± 0.04^{b}	8.00 ± 0.54^{b}
T_2	C+ AF 250 ppm	0.29 ± 0.04^{a}	5.00 ± 0.63^{a}
T_3	C+0.05% methionine	0.41 ± 0.05^{b}	8.2 ± 0.58^{b}
T_4	C+0.1% methionine	0.44 ± 0.11^{b}	8.30 ± 0.50^{b}
T_5	T2+0.05% methionine	0.32 ± 0.06^{ab}	6.80 ± 1.15^{ab}
T_6	T2+0.1% methionine	0.40 ± 0.07^{b}	7.9 ± 0.81^{b}

Values bearing different superscripts in a column differ significantly ($P<0.05$).

4.5.1 Histopathology of liver and intestine

Experiment 1 (Zinc)

Liver is the primary organ for the metabolism of aflatoxin therefore the alterations were observed in the liver parenchyma as liver is main target organ in aflatoxicosis. The results of histopathology of liver and intestine were illustrated in Figure 4.13 to 4.26.

The liver sample of control group was normal. The liver samples collected from the birds fed AF grossly showed swelling, enlargement and paleness with focal dark area on liver. Figure 4.15 illustrates the micrograph of liver tissue of control group (T_1). The architectural and cellular organization of liver parenchyma was normal in group T_1, therefore this group was

adopted as a reference standard for comparison of tissues from other groups. The Fig.4.16 illustrates the results regarding the histopathology of the T_2 group. Tissues showed moderate to severe degenerative changes in hepatic cells with greater disorganization in tissue marking the hepatotoxicity. This section showed the proliferation of bile ducts along with periportal infiltration of heterophils and MNCs (mononuclear cells). The cellular organization was again normal in T_3 and T_4 groups. Micrograph of liver of group T_5 showed mild changes like sinusoidal dilatation with mild changes in hepatocytes. Therefore, addition of zinc at 20 ppm diet level partially diminished the toxic effect of aflatoxin on liver tissues. Supplementation of 40 ppm diet zinc to the AF contaminated diet (T_6) revealed only mild congestion with normal appearing hepatocytes.

In the present study, diet containing 250 ppb of aflatoxin resulted in alterations in the liver tissue like degeneration, focal areas of necrosis, bile duct hyperplasia, and MNCs infiltration around portal vein in broiler chickens. Similar histological changes were observed by Tessari *et al*. (2006); Karaman *et al*. (2010); Abaji (2012); Silambarasan (2011) in broiler chicken fed 300 ppb aflatoxin. The present study revealed that supplementation of zinc at 40 ppm diet level to the aflatoxin contaminated diet ameliorated the ill effects of aflatoxin on liver.

Cellular organization of intestine was normal in control group (T_1). Degenerative changes, sloughing and focal area of severe necrosis along with infiltration of inflammatory cells were seen in aflatoxin fed group (T_2). T_3 and T_4 groups showed normal histopathology as control. In T_5 group degeneration and severe necrosis in villus were seen and in T_6, focal area of necrosis with normal histology as control was seen. It indicated that 40 ppm diet zinc level was effective in amelioration of aflatoxicosis on intestinal histopathology in broiler chickens.

In the present study, toxic effects of aflatoxin were also seen in intestine. Histopathologically it showed sever necrosis, degenerative changes and infiltration of inflammatory cells. Similar histological changes were also observed by Kumar and Balachandran (2009) wherein they reported catarrhal enteritis with lymphocytic or mononuclear cell infiltrations in the intestine of broilers fed on AF (1 ppm) contaminated feed.

The parameters pertaining to morphometry of distal jejunum of GIT as influenced by various dietary treatments are given in Table 4.25. The histopathology was normal in control group (T_1); therefore this group was adopted as a reference standard for comparison of

Fig.4.13 : Liver of control group

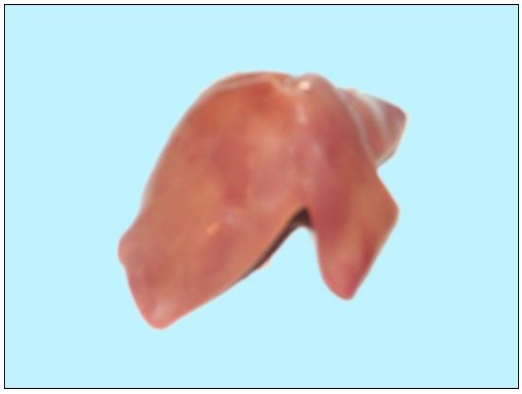


Fig.4.14 : Liver of AF fed group Paleness, haemorrhage and rounded margin

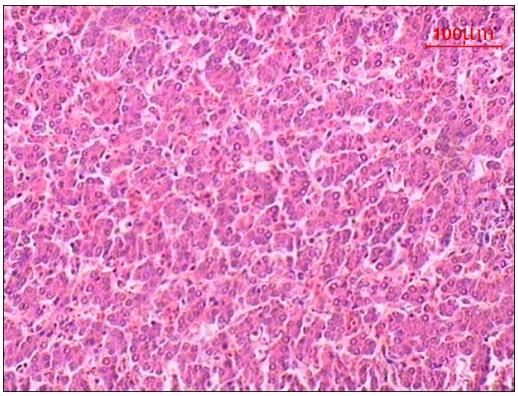


Fig.4.15 : Basal diet Normal liver parenchyma

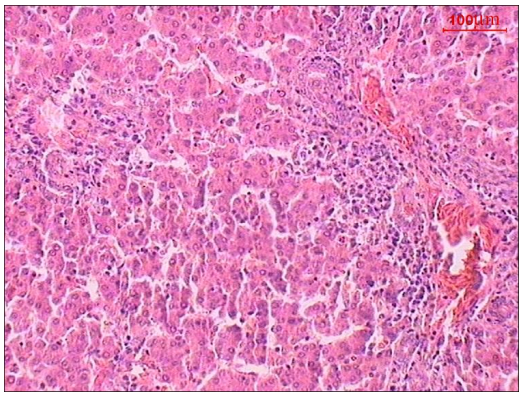


Fig.4.16 : Basal diet and AF Proliferation of bile duct along with periportal infiltration

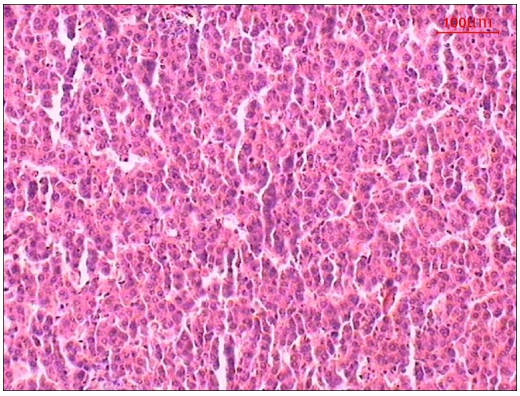


Fig.4.17 : Basal diet +20 ppm zinc Normal liver parenchyma

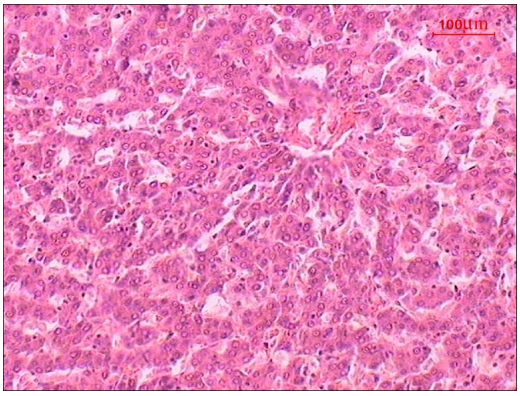


Fig.4.18 : Basal diet +40 ppm zinc Normal liver parenchyma

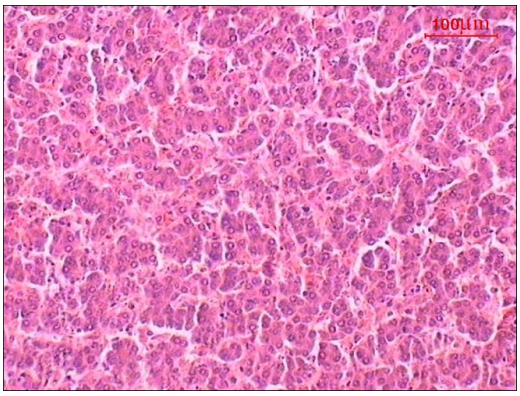


Fig.4.19 : Basal diet +AF+20 ppm zinc Mild sinusoidal dilatation with mild changes in hepatocytes

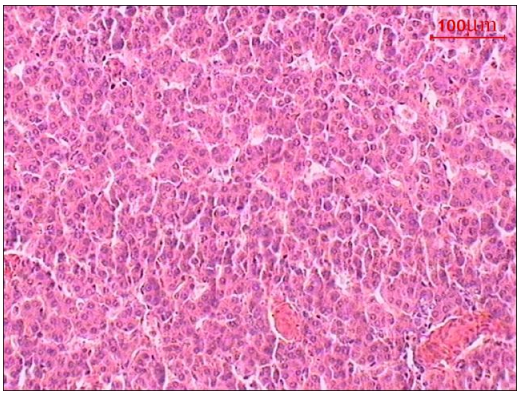


Fig.4.20 : Basal diet+AF+40 ppm zinc Mild congestion with normal appearing hepatocytes

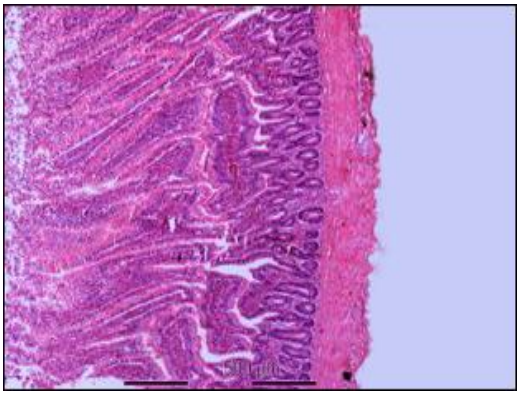


Fig.4.21 : Basal diet
Normal intestinal tissue

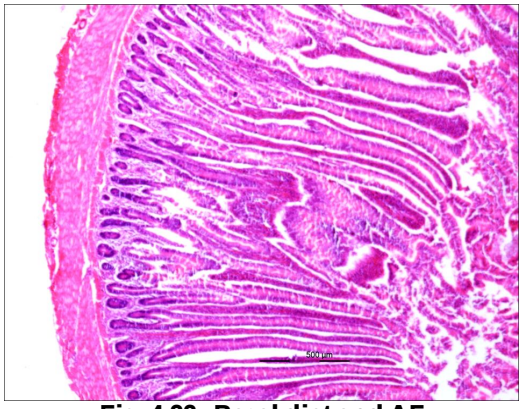


Fig. 4.22 : Basal diet and AF
Degeneration and sloughing of villus of intestinal tissue

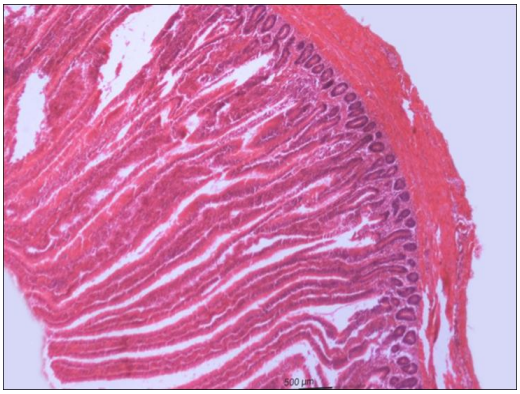


Fig. 4.23 : Basal diet +20 ppm zinc
Normal intestinal tissue

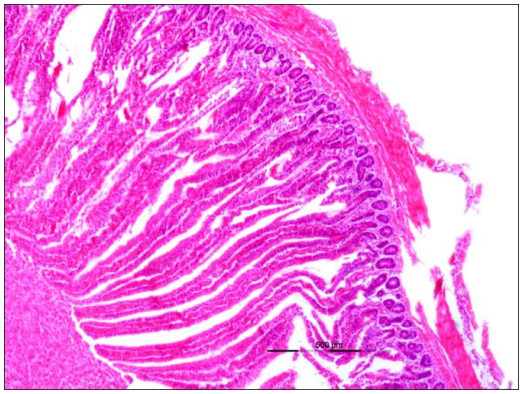


Fig.4.24 : Basal diet +40 ppm zinc
Normal intestinal tissue

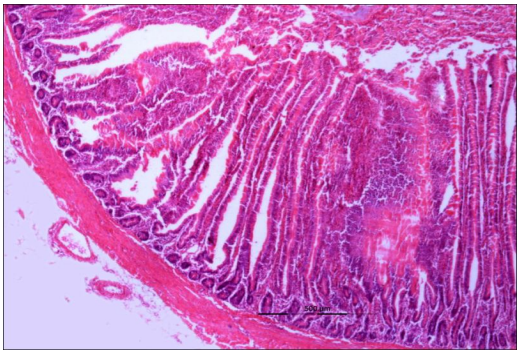


Fig.4.25 : Basal diet+AF+20 ppm zinc
Degeneration and severe necrosis in villus

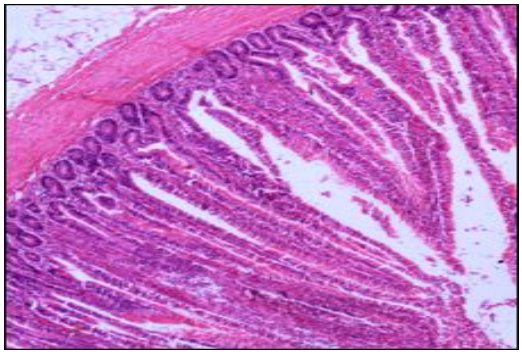


Fig.4.26 : Basal diet +AF+40 ppm zinc
Focal area of necrosis with normal villus

morphometry with other groups. There was no significant change in villus length of various treatment groups. In AF fed group (T_2), the intestinal crypt depth was significantly ($P<0.05$) higher (129.66 µm) than the control (109.66 µm), the crypt depth in all other treatments was statistically similar to that of control. Addition of zinc to the basal diet did not produce any effect on the crypt depth. However, supplementation of zinc (20 ppm diet and 40 ppm diet) to the aflatoxin contaminated feed restored the crypt depth equal to that of control.

The villus length/ crypt depth ratio in control group was 7.42 which significantly ($P<0.05$) reduced to 6.34 in aflatoxin fed group (T_2). Similar result was found by Applegate *et al*. (2009), intestinal crypt depth, but not villus length (thus influencing the villus: crypt ratio), increased linearly with increasing AF concentration in the diet (0, 0.6, 1.2, and 2.5 mg/kg). The villus length/ crypt depth ratio in other treatment groups was statistically similar to that of control. Supplementation of zinc (20 ppm diet and 40 ppm diet) to the aflatoxin contaminated feed restored the villus length/crypt depth ratio equal to that of control. The present study, supplementation of zinc at both 20 ppm and 40 ppm levels to the aflatoxin contaminated diet significantly ($P<0.05$) improved the villus length/crypt depth ratio equal to that of control.

Table 4.25: Morphometric study of distal jejunum of broilers fed different treatments

Treatment	Villus length (µm)	Crypt Depth (µm)	Villus length/ Crypt Depth
T_1	813.33±2.40	109.66±2.90[a]	7.42±0.19[a]
T_2	822.33±5.83	129.66±1.20[a]	6.34±0.09[b]
T_3	815.33±2.18	113.76±1.86[a]	7.16±0.10[a]
T_4	815.30±2.81	112.86±5.38[a]	7.26±0.36[a]
T_5	817.03±4.07	117.53±1.52[a]	6.95±0.12[a]
T_6	817.16±4.18	115.86±2.45[a]	7.06±0.16[a]

Experiment 2 (Methionine)

The results of histopathology of liver and intestine were illustrated in Fig. 4.27 to 4.38. Figure 4.27 illustrates the micrograph of liver tissue from T_1 group which was fed with the

basal diet. The architectural and cellular organization was normal in the liver parenchyma; therefore this group was adopted as a reference standard for comparison of tissues from other groups. The Figure 4.28 illustrates the results regarding the histopathology of the T_2 group. Tissues showed moderate to severe degenerative changes in hepatic cells with greater disorganization in tissue marking the hepatotoxicity. The cellular organization in groups T_3 and T_4 was normal as control (T_1). Sinusoidal dilatation with mild focal infiltration of inflammatory cells was seen in T_5 group, suggesting that supplementation of 0.05% methionine to the toxin contaminated diet partially ameliorated the adverse effects of aflatoxin on liver. In group T_6, liver parenchyma was normal as that of control (T_1) indicating that supplementation of 0.1% methionine to the toxin contaminated diet ameliorated the adverse effects of aflatoxin on liver.

Cellular organization was normal in control group (T_1). Degenerative changes, sloughing and focal area showed sever necrosis along with infiltration of inflammatory cells were seen in aflatoxin fed group (T_2). T_3 and T_4 groups showed normal histopathology as control. The micrograph of group T_5 shows dilatation of villi with desquamation of lining epithelial cells. The micrograph of group T_6 shows mild degenerative change with normal villus as control (T_1). The present study indicated that supplementation of 0.1% methionine to the aflatoxin contaminated diet ameliorated the ill effects of aflatoxin on intestine histopathology in broiler chickens.

The parameters pertaining to morphometry of distal jejunum of GIT as influenced by various dietary treatments are given in Table 4.26. The histopathology was normal in control group (T_1); therefore this group was adopted as a reference standard for comparison of morphometry with other groups. There was no significant change in villus length of various treatment groups. In AF fed group (T_2), the intestinal crypt depth was significantly ($P<0.05$) higher than that of control. The crypt depth in all other treatments was statistically similar to that of control. Addition of methinine to the basal diet did not produce any effect on the crypt depth. However, supplementation of methionine (0.05% and 0.1%) to the aflatoxin contaminated feed restored the crypt depth equal to that of control.

The villus length/ crypt depth ratio in control group was significantly ($P<0.05$) higher than that of aflatoxin fed group (T_2). The villus length/ crypt depth ratio in other treatment

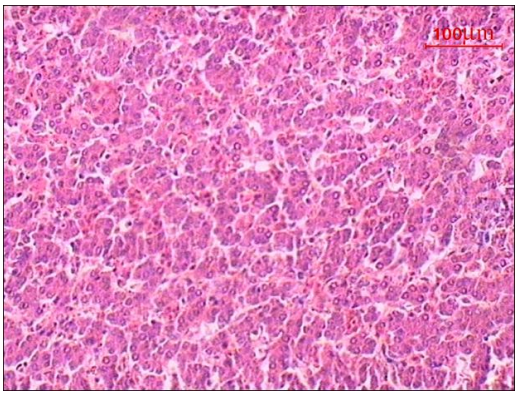


Fig.4.27 : Basal diet
Normal liver parenchyma

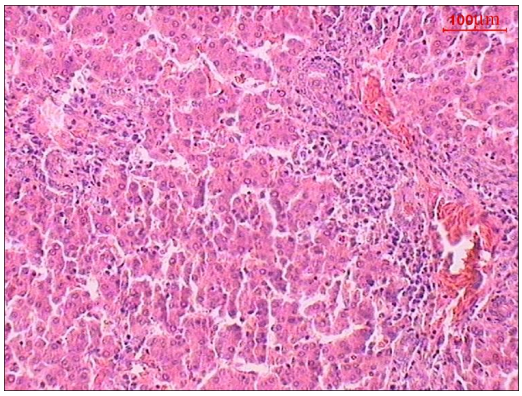


Fig.4.28 : Basal diet and AF
Proliferation of bile duct along with periportal infiltration

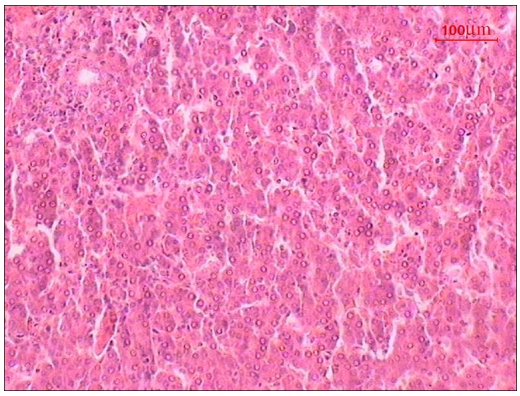


Fig. 4.29 : Basal diet+500 ppm methionine
Normal liver parenchyma

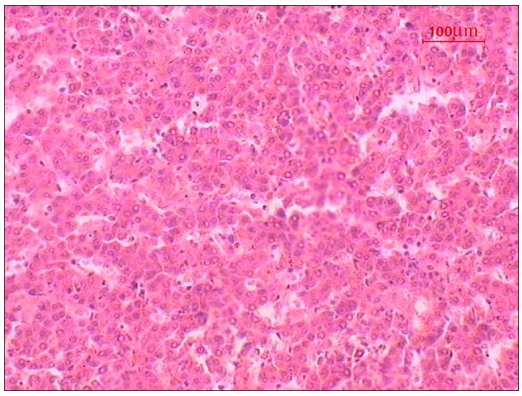


Fig. 4.30 : Basal diet+1000 ppm methionine
Normal liver parenchyma

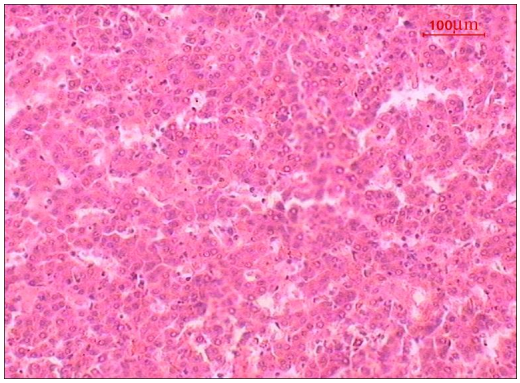


Fig.4.31 : Basal diet+AF+500 ppm methionine
Mild sinusoidal dilatation with normal liver parenchyma

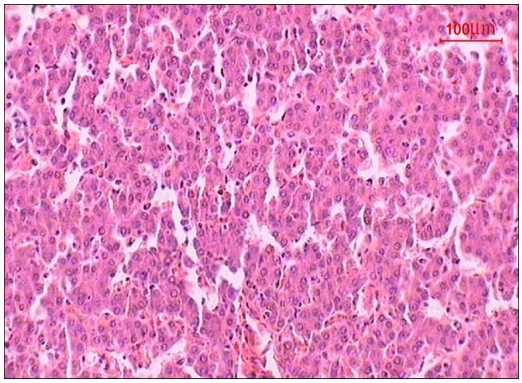


Fig.4.32 : Basal diet+AF+1000 ppm methionine
Mild sinusoidal dilatation with normal liver parenchyma

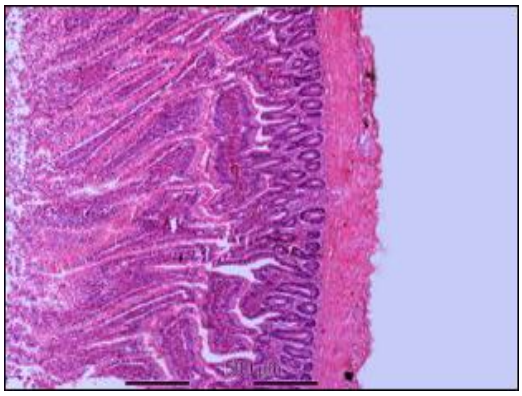


Fig.4.33 : Basal diet
Normal intestinal tissue

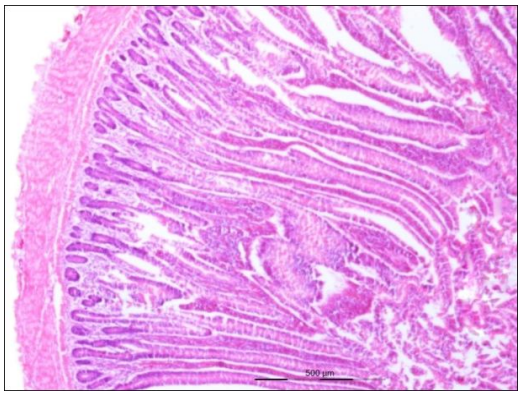


Fig.4.34 : Basal + diet and AF
Degeneration and slughing of villus of intestinal tissue

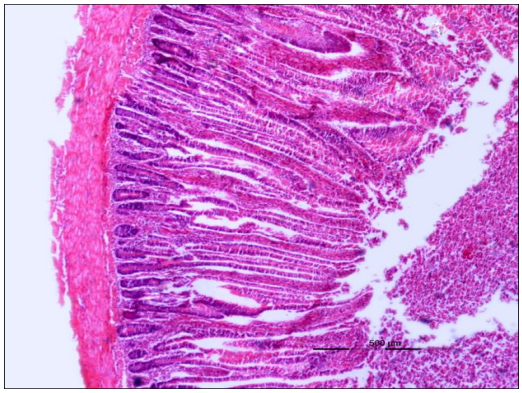


Fig.4.35 : Basal diet+500 ppm methionine
Normal intestinal tissue

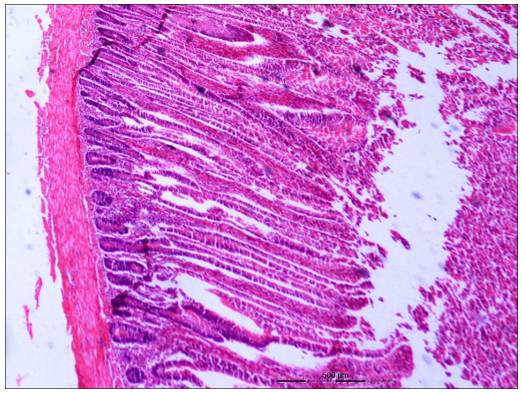


Fig.4.36 : Basal diet+1000 ppm methionine
Normal intestinal tissue

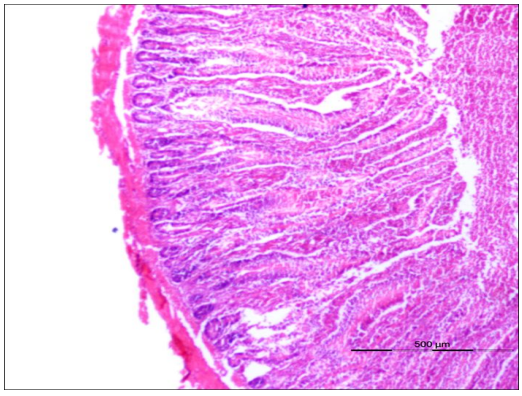


Fig. 4.37 : Basal diet +AF+500 ppm methionine
Dilatation of villus with desquamation of lining epithelial cells

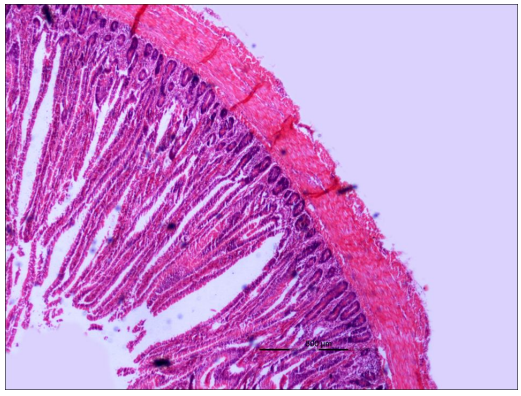


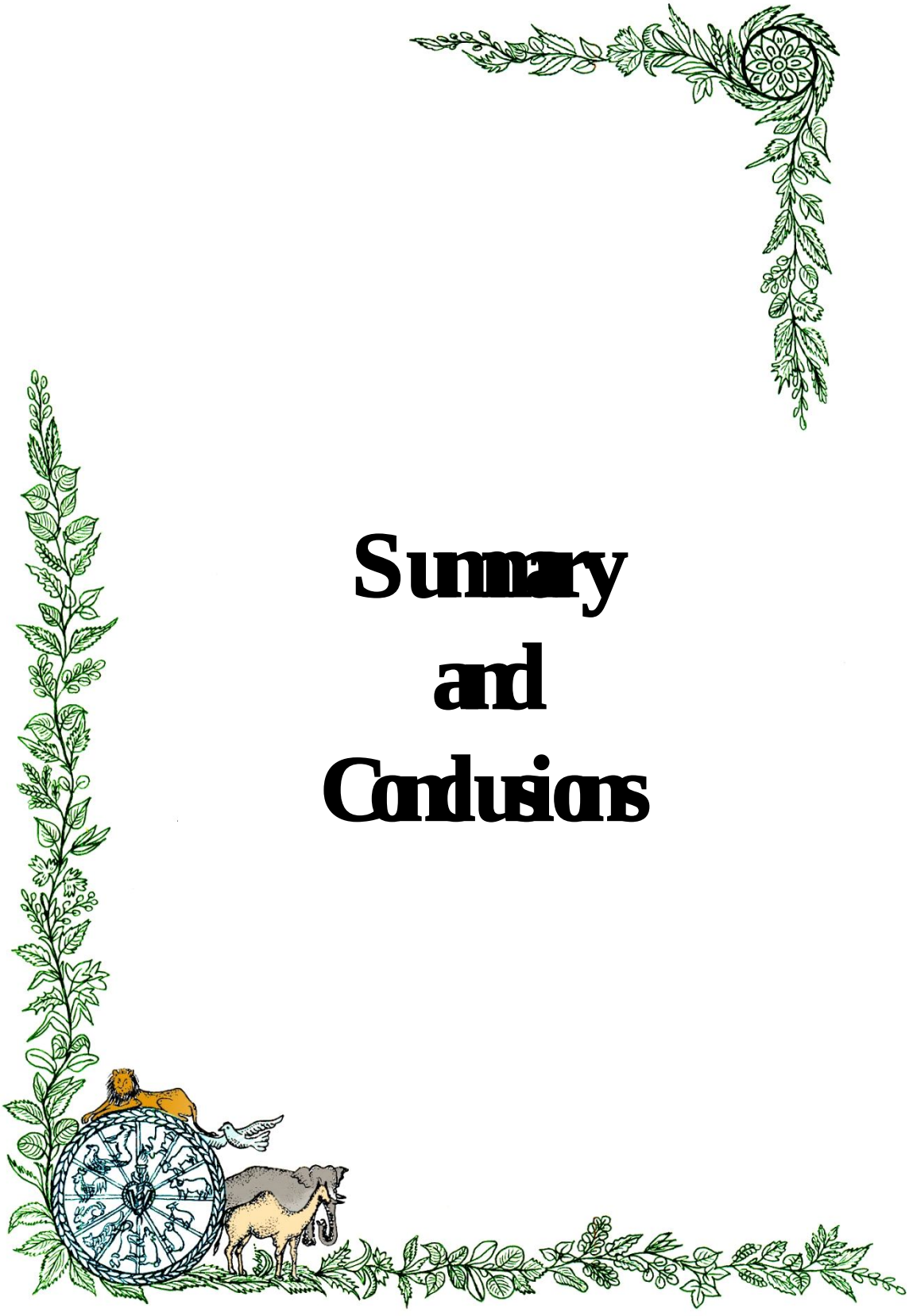
Fig.4.38 : Basal diet+AF+1000 ppm methionine
Mild degenerative change with normal villus

groups was statistically similar to that of control. Supplementation of methionine (0.05% and 0.1%) to the aflatoxin contaminated feed restored the villus length/crypt depth ratio equal to that of control. The present study, supplementation of methionine at 0.05% and 0.1% levels to the aflatoxin contaminated diet significantly (P<0.05) improved the villus length/crypt depth ratio equal to that of control.

Table 4.26: Morphometric study of distal jejunum of broilers fed different treatments

Treatment	Villus length (µm)	Crypt Depth (µm)	Villus length/ Crypt Depth
T_1	813.33±2.40	109.66±2.60^{a}	7.42±0.19^{a}
T_2	822.33±5.83	129.66±1.20^{c}	6.34±0.09^{b}
T_3	816.66±1.45	112.50±2.46^{ab}	7.26±0.17^{a}
T_4	813.45±2.01	111.70±2.26^{ab}	7.28±0.14^{a}
T_5	820.45±2.30	118.03±1.96^{b}	6.95±0.12^{a}
T_6	818.86±3.36	116.38±2.82^{ab}	7.02±0.16^{a}

Values bearing different superscripts in a column differ significantly (P<0.05).



Summary and Conclusions

5 SUMMARY AND CONCLUSIONS

Aflatoxins are the most potent mycotoxins causing wide range of clinical and sub-clinical infestations in poultry. Zinc participates in antioxidant defence mechanism as cofactor of super-oxide dismutase. Therefore, its supplementation may be helpful in ameliorating aflatoxicosis. Similarly, methionine helps in increasing hepatic glutathione concentration and thus may aid to protect liver against aflatoxicosis. Accordingly, this study was planned to investigate the effect of supplementation of zinc or methionine in ameliorating aflatoxicosis in broiler chickens.

Aspergillus flavus NRRL 6513 strain was grown in Mycotoxin Laboratory of CARI, Izatnagar and fresh spores were produced by sub-culturing on potato dextrose agar and the aflatoxin was produced on maize substrate. The mouldy maize was mixed with the feed to obtain the desired concentration of 250 ppb AFB_1. In this study, two experiments were conducted. Each experiment consisted of 6 dietary treatments, formulated with or without aflatoxin (250 ppb), and each dietary treatment consisted of five replicated groups of 8 birds each and reared up to 6 weeks of age. In experiment 1, T_1 was control diet and T_2 was control diet mixed with 250 ppb of aflatoxin. Other dietary treatments were T_3-T_1+20 ppm diet zinc, T_4-T_1+40 ppm diet zinc, T_5-T_2+20 ppm diet zinc, T_6-T_2+40 ppm diet zinc. In experiment 2, T_1 was control without aflatoxin and T_2 was control diet mixed with 250 ppb of aflatoxin. Other dietary treatments were T_3-T_1+0.05% methionine, T_4-T_1+0.1% methionine, T_5-T_2+0.05% methionine, and T6-T2+0.1% methionine. During trial period weekly body weight, feed intake and liveability were recorded. At the end of the experiment, 8 birds were sacrificed randomly from each treatment to study the carcass traits and organ weights as well as for collection of samples of liver and intestine tissue for histpathological examination. Total serum

protein, cholesterol, uric acid, SGOT and SGPT and heamoglobin and H/L ratio were also evaluated. Immunological studies (cell mediated immunity and humoral immunity) was done.

Experiment 1 (Zinc)

During 0-3 weeks, 4-6 weeks and overall growth phases the weight gain of broilers in toxin fed group (T_2) was significantly ($P<0.05$) lower than the control group (T_1). Zinc supplementation to the basal diet did not produce any change in weight gain of birds. Zinc supplementation at both the levels of 20 and 40 ppm diet had an ameliorative effect on body weight gain against aflatoxicosis but the effect of 40 ppm diet zinc level (T_6) was more pronounced than 20 ppm diet level (T_5). During starter phase (0-3 weeks) of the trial, the feed intake among various treatment groups did not differ significantly. During 4-6 and 0-6 weeks of age, the feed intake in control group (T_1) was significantly ($P<0.05$) higher than that of aflatoxin fed group (T_2). The feed intake of other treatment groups was statistically comparable to that of control. Supplementation of zinc to the aflatoxin contaminated diet reversed the harmful effect of aflatoxicosis on feed intake. With regard to FCR, addition of aflatoxin in the diet of broilers deteriorated the feed efficiency during all the growth phases. Zinc supplementation at both levels (20 and 40 ppm diet) to the aflatoxin contaminated feed ameliorated the adverse effect of aflatoxin on FCR in broiler chickens. The week-wise liveability percentage did not vary significantly among various treatments. However, numerically lowest liveability percentage was observed in toxin fed group (T2).

In the present study, there was no significant change in the slaughter traits (shrinkage loss, dressing yield and eviscerated yield) as well as cut-up parts yield (thigh, drumstick, breast, back, neck and wing) due to 250 ppb of aflatoxin in the diet. Aflatoxin in feed significantly ($P<0.05$) increased the relative weight of liver and spleen and decreased the relative weight of bursa but there was no effect on relative weight of thymus. The results showed that supplementation of zinc at 40 ppm diet levels reversed the adverse effects of aflatoxin on relative weight of liver and spleen. Supplementation of 40 ppm diet zinc ameliorated the ill effect of aflatoxicosis on relative weight of bursa. Addition of 250 ppb of aflatoxin in the diet, significantly ($P<0.05$) reduced the serum protein, cholesterol and uric acid content and increased the serum SGPT and SGOT activities. Supplementation of zinc (40 ppm diet) to the aflatoxin contaminated diet significantly ($P<0.05$) reversed the effects of aflatoxicosis on these biochemical

parameters. The present study revealed that aflatoxin contamination in diet resulted in significantly reduced Hb level and elevated H/L ratio in broilers. Supplementation of zinc (40 ppm diet) to the aflatoxin contaminated diet restored these parameters equal to that of control.

Dietary addition of aflatoxin at 250 ppb level significantly ($P<0.05$) decreased the HA titre value against sheep red blood cells (SRBCs) and cell mediated immune response to PHA-P compared to control group. Supplementation of zinc (40 ppm diet) ameliorated the adverse effect of aflatoxin on CMI response as well as humoral immunity. The liver samples in AF fed group grossly showed enlargement and paleness with focal dark area and histopathologically, moderate to severe degenerative change in hepatic cells, proliferation of bile ducts along with periportal infiltration of heterophils and MNCs in liver. The micrograph of intestine samples of aflatoxin fed group showed degenerative changes, sloughing and focal area of severe necrosis along with infiltration of inflammatory cells in intestine. The supplementation of 40 ppm diet zinc ameliorated the damage caused by 250 ppb of aflatoxin in liver and intestine of broiler chickens. The results of morphometric study indicated that, significant ($P<0.05$) decrease in villus length/ crypt depth ratio was due to aflatoxin feeding in T_2 group compared to that of control (T_1). Supplementation of zinc at 20 and 40 ppm diet levels to the aflatoxin contaminated diet significantly ($P<0.05$) improved the villus length/crypt depth ratio equal to that of control.

Experiment 2 (Methionine)

The weight gain of broilers during 0-3, 4-6 and 0-6 weeks (overall growth phase) of age was significantly ($P<0.05$) lower in aflatoxin fed group (T_2) than that in control group (T_1). Methionine supplementation to the basal diet did not produce any change in weight gain of birds. However, methionine supplementation at 0.1% level ameliorated the adverse effect of aflatoxin on body weight gain of broilers. During 0-3 week of age, the FI among various treatment groups did not differ significantly. During 4-6 and 0-6 weeks of age, the feed consumption in control group (T_1) was significantly higher than that of aflatoxin fed group (T_2). The feed consumption in all the treatments groups was statistically similar to that of control group. Supplementation of methionine at 0.1% level to the aflatoxin contaminated feed ameliorated the ill effect of aflatoxin on feed consumption during overall growth phase in broiler chickens. During overall growth period (0-6 weeks), the FCR of group T_5 was statistically

similar to that of aflatoxin alone fed group (T_2), indicating that supplementation of 0.05% methionine to the aflatoxin contaminated diet did not curb the ill effects of aflatoxin on overall FCR in broiler chickens. The overall FCR of groups T_3 and T_4 was statistically similar to that of control, suggesting that addition of methionine to the basal diet did not produce any positive effect on FCR of broilers. The FCR of group T_6 was statistically ($P<0.05$) similar to that of control, indicating that supplementation of 0.1% methionine to the aflatoxin contaminated diet curbed the ill effect of aflatoxin on feed efficiency of broilers during 0-6 weeks of age. The week-wise liveability percentage did not vary significantly among various treatments. However, numerically lowest liveability percentage was observed in toxin fed group (T2).

There was no significant difference among various dietary treatments with respect to shrinkage loss, dressing yield and eviscerated yield and in cut-up parts yield (thigh, drumstick, breast, back, neck and wing). Aflatoxin in feed significantly ($P<0.05$) increased the relative weight of liver and spleen and decreased the relative weight of bursa but there was no effect on relative weight of thymus. Supplementation of methionine at 0.1% level to the aflatoxin contaminated feed improved ($P<0.05$) the relative weights of liver, spleen and bursa. Addition of 250 ppb of aflatoxin in the diet, significantly ($P<0.05$) reduced the serum protein, cholesterol and uric acid content and increased the serum SGPT and SGOT activities. Supplementation of methionine (0.1%) to the aflatoxin contaminated diet significantly ($P<0.05$) reversed the effects of aflatoxicosis on these biochemical parameters. The results revealed that aflatoxin contamination in diet resulted in significantly reduced Hb level and elevation in H/L ratio in broilers. Supplementation of methionine (0.1%) to the aflatoxin contaminated diet restored these parameters equal to that of control.

Dietary addition of aflatoxin at 250 ppb level significantly ($P<0.05$) decreased the HA titre value against sheep red blood cells (SRBCs) and cell mediated immunity response to PHA-P compared to control group. Supplementation of methionine (0.1%) ameliorated the adverse effect of aflatoxin on CMI response as well as humoral immunity registering their values equal to that of control. The liver samples in aflatoxin fed group grossly showed enlargement and paleness with focal dark area and histopathologically, moderate to severe degenerative change in hepatic cells, proliferation of bile ducts along with periportal infiltration of heterophils and mononuclear cells in liver. The micrograph of intestine samples of aflatoxin

fed group showed degenerative changes, sloughing and focal area of severe necrosis along with infiltration of inflammatory cells in intestine. The supplementation of 0.1% methionine ameliorated the ill effects caused by 250 ppb of aflatoxin in liver and intestine of broiler chickens. The result of morphometric study indicated that, significant ($P<0.05$) decrease in villus length/ crypt depth ratio was due to aflatoxin feeding in T_2 group compared to that of control (T_1). Supplementation of methionine at 500 and 0.1% levels to the aflatoxin contaminated diet significantly ($P<0.05$) improved the villus length/crypt depth ratio equal to that of control.

Conclusions

- Addition of zinc above the NRC reccomondation of 40 ppm during both 0-3 weeks and 4-6 weeks of age did not prove beneficial thus the present study validates its requirement as 40 ppm per kg diet of broilers.
- Similarly, the dietary levels of 0.5% and 0.38% methionine for 0-3 weeks and 4-6 weeks of age, respectively were optimum for growth performance, feed conversion efficiency, carcass traits and immuno-compitance of birds.
- Induced aflatoxicosis with 250 ppb aflatoxin resulted in depression of growth, feed intake, feed conversion efficiency, enlargement of liver and spleen, regression of bursa, decreased total protein and uric acid, cholesterol, haemoglobin, increased level of SGOT and SGPT and H/L ratio and immuno-suppression.
- Supplementation of additional zinc at 40 ppm diet (total 80mg/kg diet) to the aflatoxin contaminated diet ameliorated the ill effects of 250 ppb aflatoxin on body weight gain, feed intake and feed conversion ratio, relative weight of liver, spleen and bursa, blood protein and uric acid, haemoglobin, SGPT, SGOT, H/L ratio and immune response of broiler chickens during 0-6 weeks of age.
- Addition of methionine 0.1% above the NRC recommendation in starting (total 0.6% Met) and finishing diet (total 0.48% Met) in the AF contaminated diet improved the production performance, relative weight of liver, spleen and bursa, blood biochemical and haematological parameters and immune responce during 0-6 weeks age of broiler chickens experimentally induced with aflatoxicosis (250 ppb).

- Aflatoxins in feed increasd the requirement of both dietary zinc and methionine.
- Addition of 40 ppm zinc in diet or methionine at 0.1% above the NRC reccomondation reduced the severity of the hepatic and intestinal histopathological changes associated with aflatoxicosis and improved the intestinal health of broiler chickens.
- A dietary level of 80 ppm of zinc in AF contaminated diet improved the welfare aspects like growth, feed intake, H/L ratio, immunity and gut integrity and health of stressed birds.
- Similarly, Supplementation of 0.1% methionine above the requirement of birds during aflatoxicosis resulted in welfare of birds.

Recommendation

Supplementation of 40 ppm diet zinc or 0.1% methionine above the NRC reccomondation is effective in counteracting the aflatoxicosis in broiler chickens and thus supplementation of 40 ppm diet zinc or 0.1% methionine above the NRC recommendation along with other traditional measures could be an effective means for managing aflatoxicosis in broiler chickens.

Future study

- The interactive effect of methionine and zinc with other aflatoxicosis amelioration agents should be investigated.
- Aflatoxicosis amelioration effect of methionine and zinc at various levels of aflatoxin in different poultry species should be investigated.

Mini Abstract

6 MINI ABSTRACT

The present study was conducted to evaluate the efficacy of zinc and methionine in ameliorating induced aflatoxicosis (250 ppb aflatoxin B_1) in broiler chickens from 0-6 weeks of age. First experiment included six dietary treatments (T_1-control; T_2-T_1+250 ppb AFB_1; T_3-T_1+20 mg Zn /kg; T_4-T_1+40 mg Zn/kg; T_5-T_2+20 mg Zn/kg and T_6-T_2+40 mg Zn/kg diet), each offered to 5 replicated groups of 8 birds. Addition of AFB_1 at 250 ppb level significantly ($P<0.05$) decreased the body weight gain, feed intake, and impaired feed conversion ratio. Supplementation of zinc at 40 ppm diet level effectively reduced the adverse effect of aflatoxicosis on such production parameters of broilers. AF increased the relative weights of liver and spleen while decreased in weight of bursa of Fabricius. These effects of AF were ameliorated by supplementation of 40 mg Zn/kg diet. Addition of aflatoxin in the diet decreased the serum protein, cholesterol, uric acid and haemoglobin levels and increased the serum SGPT and SGOT activities and H/L ratio. Supplementation of 40 ppm diet zinc to the AF contaminated diet ameliorated the ill effects on blood biochemical and hematological parameters. Supplementation of 40 ppm diet zinc significantly improved the CMI response as well as humoral immunity, which was reduced because of AF contamination in feed. Histopathological study showed degenerative change in hepatic cells, proliferation of bile ducts along with periportal infiltration of MNCs in liver and degenerative changes, sloughing and focal area of necrosis along with infiltration of inflammatory cells in intestine. Supplementation of zinc reduced the severity of the hepatic and intestinal histopathological changes associated with aflatoxicosis and improved the villus length/crypt depth ratio. In experiment 2, six treatments (T_1-control; T_2-T_1+250 ppb AFB_1; T_3-T_1+0.05% Met; T_4-T_1+0.1% Met; T_5-T_2+0.05% Met; T_6-T_2+0.1% Met) were formulated and each diet was fed to 5 replicated groups of 8 birds upto 6 weeks of age. Supplementation of 0.1% methionine in AF contaminated diet improved ($P<0.05$) the body weight gain (BWG). Met supplementation at 0.1% level ameliorated the ill effects of aflatoxin on BWG, feed consumption and feed conversion efficiency. Supplementation of 0.1% Met to the AF contaminated diet ameliorated the ill effects of aflatoxin on relative organ weight, blood biochemical and haematological parameters. Supplementation of 0.1% Met to the AF contaminated diet significantly improved the CMI response as well as humoral immunity. Supplementation of methionine also reduced the incidence and severity of the hepatic and intestinal histopathology changes associated with aflatoxicosis and improved the villus length/ crypt depth ratio. It is concluded that supplementation of 80 mg zinc/kg diet or 0.6% Met and .48% Met in starting and finishing diets ameliorated the adverse effects of aflatoxin in broiler chickens.

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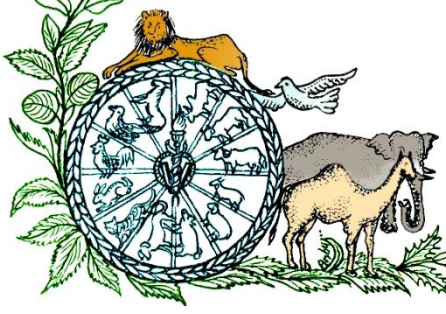

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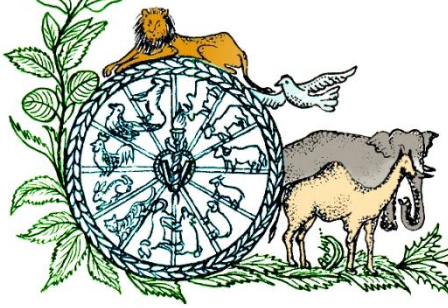

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