CHAPTER II

REVIEW OF LITERATURE
CHAPTER 2

REVIEW OF LITERATURE

Presence of mycotoxins in poultry feed can result in severe economic losses to the poultry producer and occurrence of mycotoxin residues in animal product can produce hazard to public health. There are many reports of the toxic effects of aflatoxin and ochratoxin in poultry through feeds, however, literature on the synergistic effect of these two is rare. The literature most pertinent to the subject is reviewed in this chapter.

2.1 Incidence

Aflatoxin

Greater incidence of contamination of poultry feed stuffs by aflatoxin is experienced by warm and humid regions of the world (Krough, 1987 and Devegowda et al., 1998b). Jelinek et al. (1989) reported aflatoxin levels to be higher than 5-20 ppb in grains and 20-50 ppb in most of the feed samples monitored.

Jindal et al. (1993a) analyzed 240 feed samples from various poultry farms of Haryana and found none to be free of aflatoxin. They found aflatoxins ranged between 7 and 11,600 ppb in these samples.

Natrajan et al. (1999) analyzed 18,374 feed stuffs samples for presences of aflatoxin B1 out of which 691 samples of maize were contaminated with low
level of aflatoxin (1 – 50 ppb) and 341 maize samples were contaminated with high level of aflatoxin (51-500 ppb and above). Whereas in groundnut capa, 2947 samples were contaminated with low level of aflatoxin B1 (1 – 100 ppb) and high level (above 100 ppb) was observed in 2676 samples.

Moregsonkar (2002) surveyed poultry feed and feed ingredients of broiler ration in Marathwada region for presence of mycotoxin. Out of which 21 samples analyzed were found positive for aflatoxin (12 ppb – 120 ppb).

Ochratoxicosis

The natural contamination with ochratoxin was first reported by Shotwell et al. (1969) in corn sample.

Spontaneous ochratoxin induced nephropathy has been recorded in natural field outbreaks on poultry farms in many countries. The Aspergillus ochraceus, the main ochratoxin forming fungus has been isolated from stored grains and from wide variety of feed and food stuff (Dwivedi and Bums, 1986).

Ochratoxins are produced by the toxigenic fungi at varied environmental conditions that include temperature ranging from 4 to 37°C. The incidence is more common in temperate regions of the world (Krough, 1987 and Deveciowa et al. 1998c).

Wood, (1992) reported in surveys conducted by USDA and FDA in Canada that less than 2% samples of corn, wheat, barley, sorghum and oats
were contaminated by ochratoxin A (OA). The incidence was highest in barley.

Chandrasekaran, (1996) screened poultry feed stuffs in southern India and found high concentration of ochratoxin in (78%) in sunflower oil cakes and in layer mash samples.

**Co-contamination**

Huff and Doerr, (1981) reported common occurrence of both aflatoxin and ochratoxin in animal feed stuffs.

Chandrasekaran, (1996) reported 8% co-occurrence of ochratoxin and T2 toxin in sunflower oil cakes all over India. Similar co-occurrence of aflatoxin, fumonisin and zearalenone in Australian maize is reported by Ravindran et al. (1996).

Sundaram et al. (1999) analyzed 1968 SFOC samples and found 113 samples highly contaminated with both ochratoxin and aflatoxin B1. Again out of 1405 feed samples, they found 63 samples contained both ochratoxin A and aflatoxin B1 upto 20 ppb level and in 48 samples co-occurrence was at higher level (21 to 200 ppb).

### 2.2 Production of Mycotoxins

Mycotoxins are produced by fungi usually as secondary metabolites in grains or forages. Most susceptible feed grains are corn, wheat, rice and oil
Devegowda *et al.* (1998b) reported that warm and humid climatic condition like those existing in Asian countries favour the production of aflatoxins.

**Ochratoxicosis**

Hugh *et al.* (1971) studied, the effect of temperature and length of incubation on ochratoxin A (OA) production by using various substrates. The optimal temperature for toxin production was found to be around 28°C. A very low level of OA (3 μg) was obtained in corn, rice and wheat bran after incubation at 4°C for 28 days. Whereas, 1 – 2 mg OA was obtained after incubation at 15°C for 2 weeks.

Krogh, (1987) suggested that, the ochratoxins were produced by toxigenic fungi at a variety of environmental conditions that included a temperature range of 4 – 37°C and grain moisture content of 18.5 – 40%.

Devegowda *et al.* (1998b) reported that, winter season, which was accompanied with high moisture in tropical countries, favoured the production of ochratoxin, zearalenone, vomitoxin and T2 toxin.

Ominski *et al.* (1994) observed that peanut and soybean were much better substrates than rape seed, wheat or corn for production of ochratoxin by *A. alutaceus*, whereas ochratoxin production by *P. verrucosum* was better on wheat than on corn or the oil seeds. Author in same study pointed out that there was positive correlation between protein concentration of barley and
production of ochratoxin by both *A. alternatus* and *P. verrucosum* at 12° and 25°C. Further they stated that amount of inoculum (spore load) also affected the production of ochratoxin by *A. ochraceus* considerably.

2.3 Mycotoxicosis in broilers

Aflatoxicosis

Aflatoxicosis may be acute, sub acute or chronic type. Increased mortality, decreased organ weights and liver damage are important effects of aflatoxicosis.

Lancaster *et al.* (1961) discovered aflatoxicosis in the year 1960. Gopal *et al.* (1968) were the first to report an outbreak of aflatoxicosis in India in 1968.

Smith and Hamilton (1970) reported that the young birds were sensitive when measured in terms of growth rate.

Verma (1982) reported reduced egg production and hatchability, damaged haemopoietic and haemostatic system due to aflatoxicosis.

Devegowda *et al.* (1997) suggested that the aflatoxicosis was mainly responsible for reduction in feed intake and feed efficiency resulting in depressed growth and less finished weight which caused heavy economic losses to broiler industry. The economic losses are estimated to run in millions of rupees.
Ochratoxicosis

The ochratoxins were first described by Van Der et al. (1965) as a group of low molecular weight toxic metabolites of Aspergillus ochraceus. They designated ochratoxin as A, B and C types of which ochratoxin A was found to be more prevalent and extremely toxic.

Doerr and et al. (1980) reported reduced growth rate and impaired coagulation of blood in as specific effect of ochratoxicosis.

Chang and Hamilton, (1980) observed decreased skeletal integrity, anemia, leucocytopenia and impaired phagocytosis.

Combined toxicity

Mycotoxins offer a potential threat to broilers in view of their performance. If the feed is contaminated with multiple mycotoxin then it adversely reflect the feed consumption, growth rate and feed conversion ratio.

Huff et al. (1992) observed that the toxicity resulting from combination of aflatoxin and ochratoxin was more severe than when either of these mycotoxins were present alone.

Devegowda et al. (1998a) reported that the mycotoxins in combination appeared to exert greater negative impact on the health and productivity of livestock and poultry in comparison to their individual effects.
2.4 Body weight gain, feed intake and feed conversion ratios

Aflatoxicosis

The economically significant effects of aflatoxicosis in broilers are decreased growth and poor feed conversion ratio which results in less finished weight.

Smith and Hamilton (1970) studied effect of different levels of aflatoxin (0.625 to 10 ppm) on body weight for 3 weeks and found that threshold dose above 1.25 ppm affected body weight. They further noted that extent of depression of the growth rate depended on the dose level.

Lanza et al. (1980) noticed that dietary aflatoxin levels at 2.5 and 5.0 µg of aflatoxin per gram of diet depressed body weight.

Reddy et al. (1982) found that dietary aflatoxin ranging from 0.5 ppm to 5 ppm significantly depressed the weight gain and feed consumption in broilers. They observed that the feed efficacy at 1.25 ppm and livability at 1.0 ppm level were adversely affected.

Reddy et al. (1984) observed that weight gain and feed consumption were depressed significantly at and beyond 0.75 ppm aflatoxin in feed for 28 days.

Hoff et al. (1986) noticed that there was significant decrease in body weight by feeding 5.0 ppm aflatoxin at 6 days of age in broilers and at 2.5 ppm aflatoxin level significant decrease was seen at 17 days of age.
Kubena et al. (1990) observed reduced body weight gain by 21 to 38% in broilers fed 5 mg aflatoxin per kg feed at 0 to 3 weeks of age.

Jindal et al. (1993b) noticed significant decrease in body weight in broilers fed 0.5 ppm aflatoxin from five week onwards. They also noticed decrease in feed intake and increase in FCR in aflatoxin fed group.

Mishra et al. (1996) reported dose related reduced feed intake and weight gain in broilers, fed with feed containing 0.25 ppm to 2.0 ppm aflatoxin.

Poor feed efficiency and significant decrease in body weight in aflatoxin fed groups (0.1 to 0.5 ppm were noticed at the 8 weeks of age by Sundaresan and Mani (1996).

Johi (1999) reported that the aflatoxin levels of 0.8 ppm and 1.6 ppm caused significant depression of growth and poor efficiency of feed conversion in broilers.

Mani et al. (2000) demonstrated that intermittent feeding of aflatoxin B1 at 0.20 ppm for a period of 8 weeks suppressed the body weight gain, feed consumption and poor feed efficiency in broilers but not up to significant level.

Chede and Pashak (2001) observed that dietary aflatoxin (0.25 and 0.50 ppm) decreased body weight and increased feed conversion ratio in broilers.
Moregaonkar (2002) reported decrease in feed intake, weight gain and increase in feed conversion ratio in broiler birds fed with aflatoxin B₁ @ 1 ppm in feed for 42 days.

Dersjant et al. (2003) reviewed and evaluated quantitative impact of dietary aflatoxin concentration on performance of broilers and concluded that each mg/kg increase of aflatoxin in the diet, would depress growth rate by 5% in broilers.

Verma et al. (2004) studied effect of different dietary levels of aflatoxin (0.5, 1.0 and 2.0 mg/kg) and found significant growth depression, reduced feed consumption and poor feed conversion efficiency in broilers fed a diet containing the concentrations of 1 mg/kg and 2 mg/kg of aflatoxin.

Kumar et al. (2005) found that aflatoxin when fed @ 0.5 ppm for 0 to 35 days of age in broilers significantly reduced the body weights and efficiency of feed utilization.

**Ochratoxicosis**

Huff et al. (1974) reported significant reduction in growth in broilers when fed with graded dietary levels of ochratoxin A (0.5 to 5.0 μg/g) upto three weeks of age at and above 2 μg/g dose rate.

Prior et al. (1980) observed reduction in body weight gain and feed consumption in broilers fed with ochratoxin A (0.5 to 2 ppm) for eight weeks.
They further observed dose dependent depression in the parameters and it was more marked in males than in females.

Significant reduction in body weight and numerical reduction in FCR in broilers fed 2 ppm ochratoxin for 0 to 3 weeks of age was found by Huff and Doerr (1981).

Hamikon et al. (1982) found decrease in feed consumption, poor growth rate and poor feed conversion efficiency, in ochratoxin intoxication in broiler.

Kubena et al. (1985) observed that feeding of OA at 2 ppm decreased body weights and efficiency of feed utilization in broiler chicks.

Niemiec and Scholtyssek (1989) observed reduction in weight gain, feed consumption and higher FCR values in broilers fed ochratoxin at the rate of 1.5 ppm for six weeks.

Mohiuddin et al. (1993) fed different levels of ochratoxin (0.75 to 3.0 μg/kg body weight) in the diet from 4 to 8 weeks of age and found that there was significant weight loss in broilers at 3.0 μg/ dose of ochratoxin.

A dose related growth depression was reported by Verma et al. (1995) and Johri et al. (1996) in broilers fed with feed containing 1 to 4 ppm OA for seven weeks.

Thyagarajan et al. (1996) observed growth depression and poor feed efficiency in broilers fed 1 μg/g of ochratoxin A at 8 weeks of age, whereas,
1.5 μg/g of ochratoxin A depressed the body weight from second week onwards.

Rama Devi et al. (1999) fed ochratoxin @ 1.0 ppm and 2.0 ppm levels for six weeks and observed significant reduction in weight gain, feed consumption and higher values of feed conversion ratio at both the dose levels.

Moregaonkar (2002) reported reduction in feed intake, weight gain and increase in feed conversion ratio in broiler birds fed with ochratoxin A @ 1 ppm in feed for 42 days.

Sawale (2002) recorded significant decrease in body weight gain, feed consumption and feed conversion efficiency in birds fed with OA @ 1 ppm of the feed.

Verma et al. (2004) studied effects of different dietary concentrations of ochratoxin (1.0, 2.0 and 4.0 mg/kg) and found significant growth depression, reduced feed consumption and poor feed conversion efficiency at 2 mg/kg and 4 mg/kg dose levels.

Kumar et al. (2005) observed significant reduction in body weights and efficiency of feed utilization in birds fed ochratoxin @ 0.5 ppm level for 35 days of age in broiler chicken. They observed 32.62 per cent reduction in body weight as compared to control group.
Combined toxicity

Huff and Doerr (1981) found synergistic effect of aflatoxin and ochratoxin A and recorded significant reduction in growth rate in broilers.

Huff et al. (1992) observed that the toxicity resulting from the combination of aflatoxin and ochratoxin A was more severe than either of these mycotoxins.

Devegowda et al. (1998a) reported that mycotoxins in combination appear to exert greater negative impact on the health and productivity of livestock and poultry in comparison to their individual effects.

Moregaonkar (2002) studied the toxicity of feeding aflatoxia B1, ochratoxin A and their combination in broilers and found additive interaction between them indicated their synergistic effect on growth performance.

Verma et al. (2004) studied effect of combination of aflatoxin and ochratoxin on the performance of broiler and found that combination of 2 mg/kg aflatoxin and 4 mg/kg ochratoxin in the diet exerted maximum adverse effect on growth, feed intake and feed efficiency, indicating a synergistic effect on performance.

Kumar et al. (2005) reported that the body weights and efficiency of feed utilization were reduced significantly in birds fed 0.5 ppm aflatoxin and 1 ppm ochratoxin in combination for 35 days to broiler chicken. There was 41.46% reduction in body weight in birds as compared to control group.
2.5 Relative organ weights

**Aflatoxicosis**

Reddy *et al.* (1982) added different levels of aflatoxin (0.50 – 4.00 ppm) in broiler feed and observed significant increase in weights of liver, kidney, spleen and pancreas, while bursa of fabricius regressed at 1.25 ppm aflatoxin level.

Huff *et al.* (1986) reported enlargement in liver, kidney, gizzard, proventriculus and spleen in broilers fed with diet containing 2.5 to 5.0 ppm of aflatoxin where no significant effect on liver and pancreas weight was observed by Barmase *et al.* (1990) in birds fed 0.25 and 0.5 ppm levels of aflatoxin.

Kubena *et al.* (1990 and 1993) fed feed containing 2.5 to 5.0 ppm aflatoxins to broiler and found that relative weights of visceral organs of broilers viz, liver, kidney, proventriculus, pancreas and gizzard were increased. Similar increase in weights of vital organs were also reported by Bakshi *et al.* (1995).

Moregaonkar (2002) reported significant decrease in weights of liver, kidney, spleen and bursa of fabricius in birds fed aflatoxin @ 1 ppm in diet for 42 days of age.
Ochratoxicosis

Huff et al. (1974) reported increase in the relative weights of kidneys in chicks fed with ochratoxin A @ 1 ppm and liver at 0.8 ppm level.

Huff and Doerr (1981) observed increased relative weight of liver, kidney and proventriculus in broilers fed ochratoxin ranging from 2 – 4 ppm.

Campbell et al. (1983) reported that, relative weights of bursa of fabricius significantly decreased in birds fed with ochratoxin A @ 2 μg/g of feed.

Enlargement of liver and kidney in broilers received ochratoxin at @ 3.0 ppm was observed by Manning and Wyatt, (1984). Dwivedi and Burns, (1984a) observed enlargement of kidney, liver and proventriculus in broilers fed ochratoxin @ 2 – 4 ppm from 0 – 20 days of age.

Shreemannarayana et al. (1989) fed ochratoxin to broilers @ 1 ppm in feed and found significant decrease in relative weights of liver, pancreas and gizzard.

Gibson et al. (1990) reported increase in relative weights of liver, proventriculus, gizzard and heart in birds fed ochratoxin @ 4.0 ppm in feed.

Sakhivelan and George (2002) observed increase in liver, spleen weights and reduction in bursa of fabricius in broilers when ochratoxin A was incorporated in the diet @ 2 ppm.
Combined toxicity

Huff and Doerr (1981) found no interactive effects between aflatoxin and ochratoxin on weights of spleen, pancreas and proventriculus. Authors in same study found that the kidney and gizzard were sensitive to the coincident exposure to these mycotoxins and were significantly enlarged, whereas, there was no synergistic effect on size of liver, spleen, pancreas and proventriculus.

Cambell et al. (1983) fed aflatoxin @ 2.5 ppm and ochratoxin A @ 2.0 ppm in diet for 3 weeks and found significant decrease in weight of bursa of fabricius.

Significant increase in relative weight of liver, kidney, proventriculus and heart in birds fed aflatoxin @ 3.5 μg/gm and ochratoxin @ 2.0 μg/gm for 3 week was found by Huff et al. (1992).

Moregaonkar, (2002) found significant decrease in weights of liver, spleen, kidney and bursa in birds fed aflatoxin @ 1 ppm and ochratoxin @ 1 ppm in the diet for 42 days.

2.6 Serum Biochemistry

Aflatoxicosis

Lanza et al. (1980) reported significant decrease in plasma protein and cholesterol levels in birds fed graded levels of aflatoxin 1.25 to 5.0 ppm in three population of chicken. The plasma cholesterol and total protein were
found more sensitive to aflatoxin treatment at the later ages than body weight and other blood values.

Reddy et al. (1984) recorded significant decrease in serum total protein, cholesterol and uric acid at and beyond 0.75 ppm of aflatoxin in birds fed toxin containing diet for 28 days.

Huff et al. (1986) fed birds with a diet containing aflatoxin B1 at doses of 2.5 and 5.0 ppm level and found decrease in serum total protein and albumin by 3rd and 6th day of age and decrease in serum cholesterol by 21st day of age.

Highly significant reduction in serum protein and cholesterol levels in birds fed with aflatoxin B1, at doses of 1.0 and 2.0 ppm upto 8 weeks of age was observed by Mani et al. (1993).

Jassar and Singh (1993) observed significant decrease in serum total protein at all intervals in cobb broiler chicks fed aflatoxin B1 @ 6.00 ppm in diet, whereas gradual decrease in serum protein upto 21 days at 3.00 ppm aflatoxin dose and increase in concentration of cholesterol and alkaline phosphatase at both the doses was reported.

Johri (1998; reported that a dietary aflatoxin level of 0.6 ppm caused a significant decrease in serum total protein and serum cholesterol and increase in serum uric acid level in birds from 8th day to 47th day of age.

Mishra (1999) fed damaged feed containing aflatoxin level ranging from 0.0049 to 0.0220 ppm to broiler birds for five weeks and found no
significant change in total serum protein, albumin and globulin. However, he reported higher serum bilirubin values at 1st and 5th week of experiment.

Serum cholesterol and serum total protein were decreased when aflatoxin B₁ (@ 0.3 mg/kg of feed) was fed to broiler chicks for a period of 1 to 35 days (Raju and Devegowda, 2000).

Mani et al. (2000) observed gradual decrease in serum protein and cholesterol levels in broilers fed aflatoxin B₁ ranging from 100 to 500 ppb in feed.

Moregaonkar (2002) observed significant decrease in serum total protein, albumin, globulin and cholesterol levels and increase in serum uric acid levels at different intervals in broilers fed with aflatoxin B₁ @ 1.00 ppm/kg of feed for 42 days.

Shivachandra et al. (2003) recorded lowered levels of total serum protein, albumin and globulin and significant increase in the levels of SGPT, SGOT and alkaline phosphatase in broiler chicken fed aflatoxin @ 1 ppm levels in the feed from 7th day to 7th week of age.

Kumar et al. (2005) fed aflatoxin @ 0.5 ppm in feed to broiler chicken from 0 to 35 days of age and observed that the serum total protein and albumin values were significantly reduced and values of the serum alkaline phosphatase were significantly elevated.
Ochratoxicosis

Huff and Hamilton (1974) found increase in serum levels of uric acid in birds fed with ochratoxin due to disturbed renal functions. The impairment of renal function due to nephrotoxic effect of ochratoxin was confirmed by phenol red clearance test.

Huff et al. (1975) fed broilers with diet containing 4 ppm and 8 ppm of ochratoxin from day 1 to 3 weeks of age and found increased plasma uric acid levels.

Decreased levels of serum total protein, albumin, globulin, cholesterol, phosphorus and increased uric acid concentration were observed in broiler birds fed ochratoxin A @ 3 µg per gm of feed by Manning and Wyatt (1984).

Huff et al. (1988) reported significant reduction in serum levels of total protein, albumin and globulin. They also found significant increase in serum uric acid and creatinine levels indicative of nephrotoxicity in broiler birds fed with ochratoxin @ 1.0, 2.0 and 4.0µg/gm of feed.

Sreemannarayana et al. (1989) fed broilers with ochratoxin A @ 1.5 and 10 mg/kg of diet up to four weeks of age and observed decreased total serum protein, albumin and cholesterol, whereas increase in serum uric acid, creatinine and alkaline phosphatase.
Bagouy and Khalek (1997) reported decrease in total serum protein and cholesterol and significant increase in bilirubin in naturally Ochratoxicated birds in their study.

Stoev et al. (1999) observed increased serum levels of uric acid and glucose accompanied with decreased serum levels of cholesterol and total protein in broiler chicks fed a diet containing 130, 300 or 800 ppb ochratoxin A.

Raju and Devegowda (2000) reported decrease in cholesterol content and increase in serum gamma glutamyl transferase (G.G.T.) in broilers exposed to ochratoxin 2 mg/kg, for 35 days period.

Ramadevi et al. (2000a) studied effect of ochratoxin A on some serum biochemical levels and observed decreased total protein, albumin and cholesterol levels in serum and increased alkaline phosphatase and uric acid levels in broilers fed diet containing ochratoxin A at 1 ppm and 2 ppm levels.

Moregaonkar (2002) observed decrease in serum total protein, albumin, globulin and cholesterol levels and increase in serum uric acid levels in broiler birds fed with ochratoxin A @ 1.00 ppm/kg of feed for 42 days at different intervals of study.

Sakthivelan and George (2002) reported significant decrease in serum total proteins from 7th day onwards in broiler chicks fed ochratoxin A (2 mg/kg feed) and 21st day onwards in broilers chicks fed ochratoxin A (1 mg/kg feed).
Further, they observed low values for serum cholesterol and higher values for serum uric acid levels in birds fed with ochratoxin @ 2 mg/kg feed.

Kumar et al. (2005) observed significant reduction in serum total proteins and albumin values and elevation of serum creatinine, GGT and ALP values in broiler chicken fed ochratoxin @ 1 ppm.

**Combined toxicity**

On review of literature on effects of aflatoxin B₁ and ochratoxin A fed together on biochemical parameters of broilers is scanty.

Huff et al. (1984) fed aflatoxin @ 2.5 ppm and ochratoxin A @ 2.0 ppm in combination for 3 weeks and found significant reduction in plasma carotenoids.

Huff et al. (1992) reported significant decrease in levels of total serum protein, albumin and cholesterol in broiler fed aflatoxin @ 3.5 μg/gm and ochratoxin @ 2.0 μg/gm in combination in diet.

Raju and Devegowda (2000) studied combined effects of aflatoxin B₁ (0.3 mg/kg), ochratoxin A (2 mg/kg) and T-2 toxin (3 mg/kg) on serum biochemistry and found significant interaction between any two toxins for their additive effects on serum GGT activity at 21 days. Conversely, antagonistic interactions were observed among any two of the toxins for their effects on variables such as serum protein and serum cholesterol content.
Moregoankar (2002) fed aflatoxin B₁ @ 1 ppm and ochratoxin A @ 1 ppm in combination in diet to broiler birds from 0 to 42 days of age and found that additive interaction between aflatoxin B₁ and ochratoxin A had synergistic effect on biochemical parameters resulting in significant decrease in serum total protein, albumin and cholesterol.

Kumar et al. (2005) in their studies with combined feeding of aflatoxin 0.5 ppm and ochratoxin 1 ppm level in diet for 0 to 35 days of age reported marked reduction in serum total protein and albumin and significant higher levels of GGT and ALP suggestive of synergistic effect of two toxins on these biochemical parameters.

2.7 Haematological studies

Aflatoxicosis

Doerr et al. (1974) found that the whole blood clotting time was significantly increased in birds fed aflatoxin (0.625 to 10.0 ppm). Further they stated that aflatoxin altered hemostasis more severely than ochratoxin and T₂ toxin.

Lanza et al. (1980) fed different levels of aflatoxin @ 1.25, 2.50 and 5.0 ppm in BR₁ and BR₂ broiler strain and found significant reduction in Hb, PCV, and RBC count and levels in a dose related fashion.
Reddy et al. (1984) studied effect of dietary aflatoxin on commercial broiler chicks by feeding 0.25 to 1.00 ppm level aflatoxin and observed that both Hb and PCV were decreased as the aflatoxin dose was increased. Effect was seen in birds fed aflatoxin for 21 days and above.

Huff et al. (1986) found significant decrease in Hb and PCV levels at 5.0 ppm level of aflatoxin at 12 days and by 2.5 ppm aflatoxin at 21 days of age. Similar findings were also recorded by Mohiuddin et al. (1986).

Shukla and Pachauri (1987) recorded significantly lowered values of Hb, PCV and total RBC count. The fall in haemoglobin was high in pullets fed with 5 μg and 10 μg aflatoxin/gm of feed. Further, they observed no effect on TLC, DLC, ESR, MCV, MCH and MCHC values.

Significant reduction in mean values of Hb, PCV and TEC values in chicks fed aflatoxin B1 @ 0.5 ppm for 4 to 5 days was reported by Singh et al. (1992).

Mani et al. (1992) and Mani et al. (1993) reported significant reduction in Hb and PCV values in aflatoxin fed birds (0.75 and 1.5 ppm).

Moregaonker (2002) observed significant decrease in Hb, PCV, TEC, TLC values and lymphocyte and monocyte counts and increase in heterophil count in broiler fed with 1.0 ppm of OA for 42 days.

Chede et al. (2000) and Mani et al. (2000) reported reduction in Hb, PCV and TEC values in birds fed aflatoxin in diet.
Ochratoxicosis

Significant increase in recalcification and prothrombin but no change in blood clotting time in broilers fed Ochratoxin was reported by Doer et al. (1974).

Huff et al. (1979) found significant decrease in Hb and PCV values in chicks fed 8 ppm dietary OA for 3 weeks. Total erythrocyte count was unaltered.

Chang et al. (1979) fed diet containing 0.5 to 8 ppm OA to broiler birds from day old to three weeks of age and found significant reduction in leukocyte count at all doses and the leucocytopenia was characterized by increase in relative concentration of heterophils and decrease in that of lymphocytes.

Doerr et al. (1981) reported severe coagulopathy with impaired blood clotting, in broilers fed diet containing 0.5 to 8 ppm OA from hatching to three weeks of age.

Mohiuddin et al. (1993) fed broiler birds with different dietary levels of OA, 0.75, 1.58 and 3.0 ppm for four weeks and reported significant decrease in Hb, PCV, TEC, TLC and heterophilic counts.

Rama Devi et al. (2000) observed significant reduction in Hb, PCV and TEC values in broilers fed with ochratoxin @ 1 and 2 ppm for six weeks.
Significant decrease in Hb and TEC values in broilers fed with diet containing 130, 365 and 790 ppb of OA was recorded by Stoev et al. (2000).

Moregoankar (2002) fed broilers with feed containing 1 ppm ochratoxin A from 0 to 42 days of age and found decrease in Hb, PCV, TEC, and TLC values and lymphocyte counts and increase in heterophil counts.

**Combined toxicity**

Combined toxicity of aflatoxin and ochratoxin in broilers was studied by Doerr and Huff (1980) and they observed significant decrease in PCV and Hb and 53 per cent increase in prothrombin time by dose of aflatoxin @ 2.5 and 2.0 ppm ochratoxin for three weeks.

Campbell et al. (1983) fed broiler birds with aflatoxin 2.5 ppm and ochratoxin 2.0 ppm in combination in feed for 3 weeks of age and observed an anemia with lymphocytopenia and heterophilia.

Elevated prothrombin time in birds fed aflatoxin @ 2.5 ppm and ochratoxin @ 2.0 ppm for 3 weeks was reported by Huff et al. (1983).

Moregoankar (2002) reported significant decrease in Hb, PCV, TEC, TLC and increase in blood clotting time in broiler birds fed aflatoxin @ 1 ppm and ochratoxin @ 1 ppm in combination for 42 days of age.
2.8 Pathological studies

2.8.1 Clinical signs and symptoms

Aflatoxicosis

Symptoms such as retarded growth, ruffled feathers, closed eyes and weakness were reported by Tapia et al. (1980) in broiler fed with diet containing 6 ppm of aflatoxin B₁ for four weeks. However, they did not notice any symptoms at 3 ppm level of aflatoxin.

Chattopadhyaya et al. (1985) recorded retarded growth, jaundice, coagulopathy in the form of bleeding and dehydration of combs and shanks were the prominent symptoms in aflatoxicosis. Loss of appetite, rapid emaciation and death within three weeks were the prominent findings seen in broilers fed 6.25 ppm of aflatoxin, whereas in birds fed 3.25 ppm of aflatoxin inco-ordination of movements, weakness of legs and paralysis after 6 weeks were the symptoms noticed by Moorothy et al. (1985).

Ghosh et al. (1989) fed broilers with feed containing 1.0 ppm of aflatoxin for the period of 6 weeks and observed weakness, stunted growth, ruffled feathers, anorexia, dullness and pendulous abdomen.

Moregoackar (2002) fed dietary aflatoxin @ 1 ppm to broilers and observed dull, depressed, anorexic birds with ruffled feathers and somnolence were the prominent symptoms from second week onwards.
**Ochratoxicosis**

Listlessness, huddling, diarrhoea and nervous symptoms like tonic convulsions, tremors and loss of rightening reflex were the symptoms observed by Huff *et al.* (1974) in acute ochratoxicosis in broiler birds.

Dwivedi and Burns (1984a) reported dullness, huddling, weakness, decreased appetite and diarrhoea in broiler birds given 2 to 4 ppm of ochratoxin in feed.

Manning and Wyatt (1984) observed listlessness, emaciation, dehydration and diarrhoea in birds fed with feed containing 3 ppm of ochratoxin.

Niemiec and Scholtyssek (1989) noticed disorders of balance and diarrhoea in birds fed ochratoxin at the level of 1.5 ppm for 6 weeks of period.

Moregaonkar (2002) fed ochratoxin \( \lambda \) @ 1.0 mg/kg of feed for 42 days to broiler birds and observed symptoms like huddling, ruffled feathers, dullness, trembling of wings, emaciation, reduced appetite, giddiness and diarrhoea.

**Combined toxicity**

Huff and Doer (1981) studied synergism of toxicity between aflatoxin and ochratoxin \( \lambda \) in broiler chickens and they inferred from their data that synergistic effects existed between these mycotoxins. Further, they stated that
symptom pattern could be altered, confounding preliminary diagnosis during multiple mycotoxicosis.

Moregaonkar (2002) observed huddling in corner, reluctance to move, somnolence, severe diarrhoea, trembling of the wings and muscles, ruffled feathers, dullness, depression and emaciation in broiler chickens fed with 1 ppm each of aflatoxin and ochratoxin in combination from first week onwards.

2.8.2 Mortality

Aflatoxicosis

Gopal et al. (1968) was first to report outbreak of aflatoxicosis in India; death of 2219 chicks was recorded. Smith and Hamilton (1970) reported 25 per cent mortality in broilers fed 10 ppm aflatoxin for three weeks. Reddy et al. (1982) fed dietary aflatoxin from 1-6 ppm of broiler chicks from 0-3 weeks of age and found high mortality in chicks from day old onwards at 1-0 ppm level of aflatoxin.

Ghosh et al. (1989) recorded 20 per cent mortality in chicks fed 1.0 ppm of dietary aflatoxin whereas no mortality in chicks fed 0.3 ppm of aflatoxin. Kubena et al. (1990) observed 10 per cent mortality in broiler chicks fed 3.5 ppm aflatoxin for three weeks. Feeding of 1.0 ppm of aflatoxin for 32 days in diet caused 4 per cent mortality in birds. (Parbharaa and George, 1999)
Ochratoxicosis

Manning and Wyatt (1984) compared the toxicity of A. ochraceus contaminated wheat and three chemical forms of ochratoxin and observed higher rate of mortality (10-17 per cent) in feeding 3 ppm level ochratoxin for four week. Niemiec and Scholtyssek (1989) observed increased mortality in broilers, receiving a diet contains 1.5 ppm ochratoxin for six weeks. Huff et al (1992) observed 12.5 per cent mortality in game birds fed 4 ppm of ochratoxin during third week. In broilers fed 4 ppm ochratoxin, 20 per cent mortality was reported by Verma et al. (1995)

Combined toxicity

Huff and Doerr (1981) observed numerical increase in mortality in birds in combined toxicity by aflatoxin and ochratoxin in the diet.

Gupta et al. (1999) reported 25-30 per cent mortality in rabbits when fed aflatoxin B (40-90 ppb) and ochratoxin (8-100 ppb) for 4-5 month. Moregaonkar (2002) fed aflatoxin (1ppm) and ochratoxin (1 ppm) in diet of broiler birds for 42 days and reported that there was 24 per cent mortality.

2.8.3 Macroscopic and microscopic lesions

Aflatoxicosis

Balachandran and Ramakrishan (1987) studied pathological effects of aflatoxicosis in cobb broiler chickens by feeding dietary aflatoxin @ 1 ppm and 3 ppm from 0 to 28 days and grossly observed that liver was enlarged,
mottled, soft, friable and yellow coloured. Histopathologically, liver showed hepatomegaly and retrograde changes, bile duct hyperplasia and focal necrosis. In kidney, tubular degeneration, hemorrhages and lymphocytic infiltration were noticed.

Ghosh et al. (1989) fed aflatoxin @ 0.3 ppm and 1 ppm in diet for 6 weeks and observed gross lesions such as mottled liver, pale kidney, petechiae on heart, splenomegaly and atrophy of thymus and bursa of Fabricius. Histopathologically, they recorded bile duct hyperplasia, focal necrosis, disruption of architecture, fatty changes and foci of regeneration with ductular hyperplasia in liver. In kidney, tubular degenerative changes with fibroblastic proliferation and cellular infiltration in the interstitium were noticed. Hemorrhages in myocardial muscles, lymphoid depletion and reticular hyperplasia of spleen and catarhal enteritis were the additional observations noticed.

Bakshi et al. (1995) induced aflatoxicosis in broiler chickens by feeding different levels of aflatoxin in diet (0.75, 1.5 and 3.0 ppm) for six weeks and noticed hepatomegaly with petechial hemorrhages, splenomegaly and regression of bursa and thymus. Histopathologically, degenerative and necrotic changes were observed in liver at 1.5 ppm and 3.0 ppm levels of aflatoxin. Degenerative changes and depletion of lymphoid cells were observed in spleen, bursa and thymus. Reticuloendothelial cell hyperplasia in
spleen and necrotic changes in lymphoid cells of bursa were observed. These changes were dose dependent.

Enlargement and congestion of kidney, pancreas and spleen were the changes noticed at the dose level of 2.5 and 5 ppm aflatoxin for 3 weeks of age (Baigh et al., 1998).

Mlazzo et al. (2000) incorporated aflatoxin B1 @ 2.5 mg/kg of diets for 21 to 41 days of age and observed liver hypertrophy, friable and yellowish discolouration. Histopathologically, liver showed multifocal and varied cytoplasmic vacuolization with perilobular location and confirmed hepatic fatty degeneration a characteristic of aflatoxicosis.

Moregaonkar (2002) induced aflatoxicosis in broiler birds and observed that liver was enlarged, fragile and yellowish grossly. Histopathologically, centrlobular congestion, degeneration, fatty changes, biliary hyperplasia and coagulative necrosis were seen. Kidney was congested and slightly enlarged, pale with petechial hemorrhages. Histopathologically, kidney showed granular and vacuolar degenerative changes, necrobioitc changes, hemorrhages and increased glomerular cellularity. Bursa was atrophied and microscopically regenerative changes, depopulation of lymphocytes, interfollcular edema and disruption of bursal mucosa were noticed. In heart, congestion, petechial hemorrhages and focal areas of necrosis were gross changes noted.
Histopathologically, congestion hemorrhages, disruption of myocardial muscle and loss of fibrilar striations were observed.

Peroze and Rivera (2003) reported severe lymphoid depletion in more than 50% of follicles with vacuolization and increased connective tissue in bursa of Fabricious in broilers exposed to aflatoxin B₁.

Grossly, mottled, enlarged spleen and bursa and microscopic lesions such as congestion, petechial haemorrhages, degenerative changes in liver, lymphoid cell depletion in spleen and bursa were the observations made by several workers in aflatoxicosis such as Moorhy et al. (1985), Somvansi and Mohanty (1991), Mani et al. (1992) and Sandhu et al. (1995).

Ochratoxicosis

Kidney is the principle organ affected in most of the studies on ochratoxicosis in broilers. Peckham et al. (1971) grossly observed visceral gout with white flake like deposits in the kidney, ureters, heart, pericardium, liver and spleen in acute ochratoxicity in broiler by feeding ochratoxin A from one to seven days of age. Histopathologically, acute nephrosis with proteinacious casts, urates, scattered heterophils and localized necrosis of renal tubules were seen. In liver, from mild diffuse vacuolation of hepatocytes to necrotic foci, suppressed hemopoiesis in bone marrow and depletion of lymphoid elements from spleen and bursa were prominent findings.
Swelling of tubular epithelial cells, tubular dilatation and proteinaceous casts in the lumen were the microscopic changes in kidney in broilers fed dietary ochratoxin A (Huff et al. 1975).

Dwivedi and Burns (1984a) recorded that feeding of 2 and 4 ppm ochratoxin A in broilers for three weeks caused significant enlargement of kidney and liver. Histopathologically, these doses produced congestion, degeneration, vacuolation of hepatocytes and hemorrhages in the liver parenchyma. In birds fed 4 ppm ochratoxin, necrotic changes were seen in renal tubules, while thickening of glomerular basement membrane at both the dose levels. Severe lymphoid depletion from bursal follicles, which appeared small rounded often surrounded by connective tissue, was noticed. Marked depletion of lymphoid cells in thymus, spleen and plasma cell population in Harderian glands was observed.

Glomerular hyalinosis, increased cellularity and thickening of basement membrane of glomeruli, occasional nodular hyaline droplets in the tubular lumen were the renal lesions in ochratoxicosis in broiler birds, described by Jubb et al. (1991).

Stoev et al. (2000) fed ochratoxin at lower levels of 305 and 790 ppb in diet for 10 weeks and noticed pale, enlarged kidneys, enlarged liver, distended gall bladder and slight catarhal enteritis grossly. Histopathologically, degenerative changes in epithelial cells of proximal convoluted tubules with
slight proliferation of connective tissue and mononuclear cell infiltration in renal interstitium, vacuolar degeneration and fatty changes and mononuclear cell infiltration in liver and degenerative changes and depletion of lymphoid tissue in thymus, bursa and spleen. In brain, slight degenerative changes and oedema were observed, whereas perivascular oedema was seen in heart.

Moregaonkar (2002) fed broiler chicken dietary ochratoxin @ 1 ppm from 0 to 42 day of age and grossly observed pale, enlarged and bulged out from the lumbar fossa with diffuse petechial hemorrhages in kidneys, whereas microscopically, there were diffuse necrobiotic and necrotic changes in tubular epithelial cells, marked fibrous connective tissue proliferation in intertubular spaces and increased glomerular cellularity. Grossly, liver was pale and enlarged with rounded borders. Histopathologically, diffuse fatty and necrobiotic changes, hepatocellular coagulative necrosis with marked fibrous connective tissue proliferation in liver were seen. Bile duct hyperplasia, marked disruption of hepatic artery and lymphocytic aggregates in the periportal areas were the additional changes noticed in liver. Bursa was almost normal initially, later on slightly congested, enlarged and edematous. Microscopically, marked depopulation and proliferation of fibrous connective tissue in interfollicular tissue and necrotic changes in lymphoid cells.

Similar response in terms of gross and microscopic changes were observed in ochratoxicosis by many workers such as Huff et al. (1979);

**Combined toxicity**

Huff and Doerr (1980) studied synergism of ochratoxin A and aflatoxin B1 in broiler chicken by feeding 2 ppm ochratoxin A and 2.5 ppm aflatoxin in the diet. They observed that the tan – yellow colour characteristic of liver during aflatoxicosis was absent in coinfectedated birds and further they stated that this finding was of practical significance since a macroscopic diagnostic marker of aflatoxicosis was lost during aflatoxin-ochratoxin synergism.

Huff and Doerr (1981) reported that individual toxins significantly altered the size of liver, spleen, pancreas and proventriculus, however synergistic effect on the size of these organs was not observed. The kidney and gizzard were sensitive to the coincident exposure to these mycotoxins. They further opined that the kidney was the most sensitive organ to the combined toxicity and nephropathy was the characteristic of this interaction.

Moregaonkar (2002) observed pale and enlarged liver and pale atrophied kidneys with petechial and ecchymotic hemorrhages. Heart, brain, spleen, lungs and intestine were congested in broiler birds fed aflatoxin @ 1 ppm and ochratoxin @ 1 ppm in combination from 0 to 42 days of age.
2.9 Counteraction of mycotoxins

Mycotoxins contaminated feed greatly reduces broiler performance resulting in serious economic losses to broiler producer. Mycotoxins can enter into human food chain directly through foods of plant origin or indirectly through foods of animal origin and thereby increasing the risks of public health. Extensive research has been conducted to develop the methodologies for counteraction of mycotoxins with varying success. Different methods for counteraction of mycotoxins are chiefly based upon degrading, destroying, inactivating or removing the mycotoxins. Some of the important measures used to control mycotoxins in feed such as dietary manipulation, addition of mold inhibitors, non-nutritive adsorbents or sorbents and antioxidants to the poultry feed.

2.9.1 Use of toxin binders

Mycotoxin binding agents seem to be very promising approach to the detoxification of mycotoxin. Many chemicals have been tested for their ability to reduce mycotoxin concentration in a variety of grains and grain products and very few of them proved to be successful and being used commercially. Mineral clays such as bentonite, zeolite and aluminosilicate, activated charcoal and yeast cell wall products are source of the important adsorbents used to bind mycotoxins in feed. These substances upon their activation in vivo,
bind/adsorb the mycotoxins and isolate them from digestive process thereby preventing them from entering into circulation (Devegowda et al., 1998b).

**Aflatoxicosis**

Dalvi and Ademoyero (1984) observed moderate increased weight gain and feed intake by addition of activated charcoal along with aflatoxin in poultry diet.

Kubena et al. (1990) studied the efficacy of hydrated sodium calcium aluminosilicate (HSCAS 0.5 % of diet) to reduce toxicity of aflatoxin (3.5 mg/kg of diet) in broilers and concluded that HSCAS diminished many of the adverse effects of dietary aflatoxins. They observed improvement in body weight gain, lower mortality, partial improvement in serum total protein, cholesterol and creatinine kinase protective effect against relative weights of proventriculus and bursa and partial protective effect against relative weights of kidney and gizzard, whereas no improvement in relative weights of spleen, feed intake, serum levels of albumin, cholesterol, triglycerides, uric acid and creatinine in turkey poults.

Huff et al. (1992) observed 65% improvement in body weights, improvement in relative weights of liver, kidney, proventriculus and heart. They also observed improvement in serum levels of albumin, total protein and cholesterol in broiler birds fed AFB (3.5 ppm) along with HSCAS (0.5%) in diet for 3 weeks.
Rama Rao and Anjaneyula (1993) observed moderate improvement in body weight gain, feed consumption, feed conversion ratio, Hb, PCV and TSP values in birds fed AFB₁ (1.0 ppm) along with 0.5% and 0.1% charcoal in the diet from 5 to 45 days. Gross and microscopic lesions in liver, kidney, heart, spleen and intestine were comparatively minimum and mild indicating efficacy of charcoal in reducing toxic effects of aflatoxin by acting as an adsorbent.

Activated charcoal resulted in beneficial effect on broilers exposed to dietary aflatoxins reported by Rao and Joshi (1993).

Devegowda et al. (1994) added *saccharomyces cerevisiae* yeast culture 10.1 per cent and 0.2 per cent along with aflatoxin (500 or 1000 ppb) and observed improved body weight, feed conversion efficacy, serum levels of total protein and albumin. Ameliorating in effect of aflatoxin by addition of *saccharomyces cerevisiae* in diet was also found by churchill et al. (2001).

Jindal et al. (1994) found moderate improvement in body weights, feed consumption and feed conversion ratios when 200 ppm activated charcoal was added in diet containing 0.5 ppm aflatoxin. It also protected the birds from AFB₁ induced liver damage.

Mahesh and Devegowda (1996) in an *in vitro* study compared the aflatoxin binding ability of the modified MOS and hydrated sodium calcium aluminosilicate (HSCAS) using aflatoxin contaminated poultry feed. At highest level of inclusion, both products were found effective in binding
aflatoxin up to 80 per cent. Whereas, at lower levels, they found modified MOS was more effective as compared to HSCAS.

Park et al. (1996) reported that aflatoxin stressed chickens that were supplemented with dietary mannanoigosaccharides (MOS) and showed a significant reduction in liver cholesterol and fat levels. Increase in these parameters was typically observed during aflatoxicosis hence reduction was indicator of the detoxifying effect of the MOS.

Edrington et al. (1997) observed partial improvement in body weight gain, improvement in relative weights of liver, and hematocrit values in birds fed AFB1 (4 mg/kg) along with super activated charcoal (0.5%) in the diet upto 21 days of age.

Devegowda et al. (1998b) stated that HSCAS clays had the disadvantages of high inclusion rates.

Raju and Devegowda (2000) studied the influence of esterified glucomannan on different parameters in broiler exposed to aflatoxin 0.3 mg / kg and found that esterified glucomannan increased body weight (2.26 per cent), feed intake (1.6 per cent), serum protein (14.7 per cent) and cholesterol (21.9 per cent) and decreased liver weight (32.5 per cent).

Moregaonkar (2002) observed partial improvement by adding Toxi Bind Dry in aflatoxicated birds in growth performance, value of haemoglobin, PCV, TEC, serum protein and cholesterol.
Huff (2004) stated that there was no practical safe method for the detoxication of mycotoxins contaminated commodities. However, the clays that were specially manufactured such as hydrated sodium calcium aluminosilicate could be added to reduce the toxicity of aflatoxin.

Kumar et al. (2005) studied the effect of vitamin E and selenium in aflatoxin fed birds and found significant gain in body weight, decline in levels of serum GGT and ALP values and increase in the levels of serum protein and albumin.

**Ochratoxicosis**

Rotter et al. (1989) found failure of activated charcoal in preventing toxic effects of ochratoxin when added in ochratoxicated feed.

Huff et al. (1992) studied the efficacy of hydrated sodium calcium aluminosilicate (HSCAS) to reduce ochratoxicity (2.0ppm) and found little effect. There was no improvement in body weight, relative weights of liver, kidney, proventriculus and heart and serum levels of total protein, albumin and cholesterol in ochratoxicated birds.

Rama Devi et al. (1999) studied efficacy of bentonite (0.5 per cent) in ameliorating ochratoxicosis at 1 and 2 ppm level in broilers. Results indicated that bentonite could not ameliorate the effects of OA on the basis of haematological and biochemical changes. There was no improvement in body weight gain, feed intake, feed conversion ratio, values of Rb, PCV, TEC,
serum levels of total protein, albumin, cholesterol and uric acid and hepatic and renal lesions produced by OA in the birds.

Improved body weight and feed intake, lower weights of liver, adrenals and serum gamma glutamyl transferase levels and improved serum protein and blood haemoglobin values indicated the beneficial effects of esterified glucomannan on Ochratoxicated chickens (Raju and Devegowade 2000)

Kumac et al. (2005) studied the effect of Vitamin E and selenium on ochratoxic (1ppm) fed birds and found significant gain in body weight, increase in serum proteins, albumin and decline in serum GGT and ALP values indicating beneficial effects of Vitamin E and Se in Ochratoxicated birds.

Combined toxicity

Huff et al. (1992) studied the efficacy of hydrated sodium calcium aluminosilicate (HSCAS) when fed @ 0.5 per cent to reduce combined toxicity of aflatoxin 3.5 ppm and ochratoxin A (2.0 ppm) and found little effect on toxicity resulting from combination of aflatoxin and ochratoxin. They found that addition of HSCAS had slight ameliorating effect on body weight, whereas no improvement in serum levels of total protein, albumin and cholesterol and relative weights of liver, kidney, proventriculus and heart.