Journal of Animal Feed Science and Technology 3 (2015) 21-26

Aflatoxicosis in livestock and poultry

P. Kavitha*, N. Mounika, J.V. Ramana***, B. Sreedevi******

*Assistant Professor **MVSc. Scholar ***Professor and Head, Dept. of Animal Nutrition ****Professor, Department of Veterinary Microbiology, College of Veterinary Science, Tirupati, AP, India.

Keywords:
Aflatoxins
Aflatoxicosis
Livestock and Poultry
Prevention
Treatment.

Abstract

Aflatoxin is the most studied mycotoxin, due to both its toxicity to animals and people and its high carcinogenic potential. Out of aflatoxins group, AFB1 is the most toxic. The main biological effects on farm animals, including malabsorption of various nutrients, coagulopathy, decreased tissue integrity, poor growth, poor efficiency of feed conversion, enhanced susceptibility to infection, vaccine failures, drug failures, reproductive problems in males and females and Increased sensitivity to temperature extremes. Toxic residues of aflatoxin in animal products present a hazard to public health. A variety of physical, chemical and biological approaches to counteract the aflatoxin problem have been reported in the literature on mycotoxins; but large-scale, practical and cost-effective methods for detoxifying aflatoxin-containing feedstuffs are currently not available.

Introduction

Aflatoxicosis is a disease condition caused by the consumption of aflatoxins. The name ''aflatoxin" derives from the first letter of the word Aspergillus and the first three letters of flavus. Aflatoxins are the most dangerous secondary mould metabolites produced by *fungi Aspergillus flavus* and other related species of *Aspergillus* fungi. Aflatoxins show fluoresce strongly in ultra violet light. The major members of aflotoxins are designated as B1, B2, G1 and G2. B1 and B2 fluoresces blue, while G1 and G2 fluoresces green. B1 is most hepato toxic. All four have been detected as contaminants of crops before harvest, between harvesting and drying, during storage, and after processing and manufacturing (Council for Agricultural Science and Technology, 1989).

Types of aflatoxins

Around 17 aflatoxins have been isolated (WHO, 1979), only 4 of them are well known and studied extensively from toxicological point of view. Being intensely fluorescent in ultraviolet light, the four are designated by letters B1, B2, G1 and G2 representing their blue and green fluorescence in UV light. Two other familiar aflatoxins are M1 and M2, because of their presence in milk of animals, which were exposed to B1 and B2. Of all the above-named aflatoxins, aflatoxin B I (AFB1) is most acutely toxic to various species. Toxigenic *A. flavus* isolates generally produce only aflatoxins B1 and B2, whereas *A. parasiticus* isolates generally produce aflatoxins B1, B2, G1 and G2. Aflatoxin M1 is a metabolite of aflatoxin B1 in humans and animals, which is equally potent as that of AFB1. Aflatoxin M2 is a metabolite of aflatoxin B1 in milk of cattle fed on contaminated foods. Although aflatoxins B1, B2 and G1 are common in the same food sample, AFB1 predominates (60-80% of the total aflatoxin content).

Etiology

Aflatoxins are secondary metabolites produced by the common moulds of Aspergillusflavus, A. parasiticus and A.nominus. The development of aflatoxins depends on the infestation and growth of

Corresponding Author: J.V. Ramana, Professor and Head, Department of Animal Nutrition, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupathi - 517 502, Andhra Pradesh, India.
E-mail: jvenkataramana@rediffmail.com

the *Aspergillus* mould in grain. High carbohydrate containing grains and feedstuffs, such as peanut meal, corn, sorghum, and cottonseed are favoured by *Aspergillus* spp. Groundnuts and groundnut meal are the two agricultural commodities that seem to have the highest risk of aflatoxin contamination. Aflatoxin production is also stimulated by high zinc concentration in feed (Pattison et al., 2008).

Crops grown under warm and moist weather in tropical or subtropical countries are especially more prone to aflatoxin contamination than those in temperate zones. Water stress, high-temperature stress and insect damage of the host plant are major determining factors in mould infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities and weed competition have been associated with increased mould growth and toxin production. The moisture content of the substrate and temperature are the main factors regulating the fungal growth and toxin formation. A moisture content of 18% for starchy cereal grains and 9-10% for oil-rich nuts and seeds has been established for maximum production of the toxin (WHO, 1979). Below-normal soil moisture (drought stress) has also been found to increase the number of *Aspergillus* spores in the air. Therefore, when drought stress occurs during pollination, the increased inoculum load (spores in the air) greatly increases the chances of infection.

Host susceptibility

Poultry is more susceptible to aflatoxins (Austwick, 1983). Susceptibility of poultry to aflatoxins varies among species, breeds and genetic lines. Comparative toxicological studies in avian species have shown that ducklings and turkey poultry are the most sensitive species to aflatoxins. The susceptibility ranges from ducklings > turkey poults > goslings > pheasant chicks > chickens (Muller *et al.*, 1970). Young poultry are more sensitive to aflatoxin than adults. Ducks being 10 times more sensitive than chickens. Dairy and beef cattle are more susceptible to aflatoxicosis than sheep. Young animals of all species are more susceptible to the effects of aflatoxins than mature animals. Pregnant and growing animals are less susceptible than young animals but more susceptible than mature animals. The lethal levels are different in different species (Table 1).

Table 1: Comparative LD50 or lethal values for aflatoxin B1 (WHO, 1979)

Species	Oral LD50/Lethal dose (mg/Kg)
Chick embryo	0.025
Duckling	0.3
Turkey poultry	0.5
Chicken, New Hampshire	2.0
Chicken, Rhode Island	6.3
Sheep	5.0
Pig	0.6
Cattle	0.5-1.5

Pathogenesis

The principal target organ for aflatoxins is the liver. After the absorption, highest concentration of the toxin is found in the liver (Mintzlaff *et al.*, 1974). In liver, aflatoxin B1 is metabolized by microsomal enzymes to different metabolites through hydroxylation, hydration, demethylation and epoxidation. In liver enzymatic degrada-tion of toxins takes place via the mixed function oxidase system (MFO), where toxins are converted into a more polar structure. In aflatoxicosis, however, the MFO system in the liver seems to oxidize the afla-toxin to another metabolite that reacts with the chromatin of the nucleolus protoblast, thus impairing the template activity of the chromatin to produce M-RNA. Aflatoxin binds to both RNA and DNA and blocks transcrip-tion. Aflatoxin B1 inhibit tRNA binding activity of some amino acids in protein synthesis especially the essential amino acids such as lysine, leucine, arginine and glycine. The tRNA binding, have different inhibitory effect, which interfere with the translation level of protein biosynthesis and affect cell metabolism.

In day-old chicks, AFB1 reduces the activity of liver UDP glucose-glycogen transglucosylase resulting in depletion of hepatic glycogen stores (Shankaran *et al.*, 1970). On the other hand, there is lipid accumulation in the liver of chickens and ducklings exposed to aflatoxin (Carnaghan *et al.*, 1966; Shank and Wogan, 1966). With regard to its toxic effects on liver microsomal enzymes, AFB1 is known to decrease microsomal glucose-6-phosphatase activity (Shankaran *et al.*, 1970) whereas stimulation of microsomal enzyme activity by inducers seems to be unaffected by AFB1 (Kato *et al.*, 1970). Another effect of aflatoxin is that it causes anticoagulation of blood. This is probably because AFB1 inhibits synthesis of factors II and VII involved in prothrombin synthesis

and clotting mechanism (Bababunmi and Bassir, 1969).

Immuno-suppression is observed in animals fed aflatoxin.Aflatoxins appear to de-crease the lymphocyte response to mitogens, inhibit macrophage migration, and decrease the effectiveness of humoral mediators such as complement(Hoerr, FJ and D'Andrea,1983).

Carcinogenicity of aflatoxin has not been thoroughly studied, although trout and rat hepatomas and occasional swine undifferentiated neoplasms have been linked to aflatoxicosis. (Heathcote, JG, and Hibben,1978; Hoerr,FJ and D'Andrea,1983).

Aflatoxin B1 is excreted in urine and feces, and also in milk of lactating animals either unchanged or as various metabolites (Nabney *et al.,* 1967; Allcroft *et al.,* 1968). Only one milk metabolite, AFM1, appears to be the major metabolite of AFB1 that has shown appreciable oral toxicity (Holzapfel *et al.,* 1966).

Symptoms

Effects of aflatoxin consumption are similar in all animals; the animal's susceptibility to aflatoxin, however, varies by species, age, and individual variation (Pier, 1987). In acute clinical aflatoxicosis general signs include edema of the lower extremities, abdominal pain, and vomiting. Blood pigments may appear in the urine and mucous membranes are icteric. Feed refusal, reduced growth rate, decreased milk production and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning.

In cattle most commonly reported signs with acute toxicosis include anorexia, depression, dramatic drop in milk production, weight loss, lethargy, ascitis, icterus, tenesmus, abdominal pain, bloody diarrhea, abortion, hepatoencephalopathy, photosensitization and bleeding (Eaton and Groopman, 1994; Reagor, 1996). Other signs associated with acute aflatoxicosis include blindness, walking in circles, ear twitching, frothy at the mouth, keratoconjunctivitis and rectal prolapse (Radostits *et al.,* 2000).

In addition, chronic aflatoxicosis may impair reproductive efficiency including abnormal estrous cycle and abortions, induce immunosuppression and increase susceptibility to disease (Cassel *et al.,* 1988). The immunotoxic effect of AFB1 was expressed via the cell-mediated immune system (Raisbeck *et al.,* 1991).

In sheep and goats anorexia, depression and icterus were observed exposed to aflatoxin. The goats also developed a nasal discharge and dark brown urine was noted in the sheep (Abdelsalam *et al.,* 1989).

The clinical syndrome in pigs include rough coat, depression, anorexia, decreased feed conversion, decreased rate of gain, weight loss, muscular weakness and shivering, tremors, bloody rectal discharge and icterus (Hoerr and D' Andrea, 1983; Radostits *et al.,* 2000). Aflatoxins also suppress the immune system and thus make pigs more susceptible to bacterial viral or parasitic diseases (Diekman *et al.,* 1992).

In poultry, aflatoxin impairs all important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, male and female reproductive performance. Some influences are direct effects of intoxication, while others are indirect, such as from reduced feed intake (Calnek *et al.,* 1997). The direct and indirect effects of aflatoxicosis include increased mortality from heat stress (broiler breeders, Dafalla *et al.,* 1987), decreased egg production in leghorns, (Bryden *et al.,* 1980), anemia, hemorrhages and liver condemnations (Lamont, 1979), paralysis and lameness (Okoye *et al.,* 1988), impaired performance in broilers, increased mortality rate in ducks, (Bryden *et al.,* 1980), impaired ambulation and paralysis in quail, (Wilson *et al.,* 1975), impaired immunization in turkeys, (Hegazy *et al.,* 1991), and increased susceptibility to infectious diseases (Bryden *et al.,* 1980 and Calnek *et al.,* 1997).

Clinical laboratory findings

Clinical laboratory findings vary with the animal species, level of aflatoxin in the ration, and the duration of feeding. There are no consistent diagnostic changes in hematocrit, hemoglobin, and differential cell counts in animals fed aflatoxin. Leukocytosis may occur in animals with secondary bacterial infections. Serum bilirubin levels may be elevated and typically serum protein levels are decreased.

Lesions

Lesions observed at necropsy related to either acute or chronic liver disease are dependent upon the level of aflatoxin and the duration of feeding. A majority of acute liver damage observed has been the result of experimentally high doses, while chronic liver damage is a more common field observation. The liver is usually pale tan, yellow or orange. Hepatic fibrosis and edema of the gallbladder may also be observed.

Diagnosis

The diagnosis of aflatoxicosis is often difficult because of the variation in clinical signs, gross pathological conditions and the presence of infectious diseases due to the suppression of the immune system. On the farm, more than one mould

or toxin may be present in the contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult. A quick screening test for aflatoxin level in shelled corn or ground feed is the Woods' light test. A black light is held over the sample and flourescing of a metabolite in the production of aflatoxin might be observed.

Diagnosis is based on history and clinical signs, lab tests such as thin layer chromatography (TLC), mycological examination, culture samples in lab, lesions in post mortem examination, PCR, detection of aflatoxins by high pressure liquid chromatography (HPLC) and ELISA. Chromatographic methods such as TLC and HPLC are considered the gold standard and are thus the most widely used techniques in aflatoxins analysis.

Treatment

Aflatoxicosis is typically a herd rather than an individual animal problem. If aflatoxin is suspected, analyze the ration immediately. Eliminate the source at once, if aflatoxins are present. Increase levels of protein and vitamins A, D, E, and K in the ration as the toxin binds vitamins and affects protein synthesis. Practice good management to alleviate stress, reducing the risk of secondary infections. Provide immediate attention and treatment for secondary infections. Environmental stress should also be minimized.

Hydrated sodium calcium aluminosilicate (HSCAS), a sorbent compound obtained from natural zeolite, has demonstrated an ability to adsorb mycotoxins with a high affinity. Addition of this compound to feedstuffs contaminated with aflatoxins has shown a protective effect against the development of aflatoxicosis in farm animals.

Prevention

Aflatoxin levels which are considered safe in animal feedstuffs are 20 ppb or lower. A concentration of aflatoxin in feed at 100-300 ppb caused chronic intoxication signs in swine, whereas acute lethal intoxication of swine was observed at feed levels of 1,000 ppb or greater (Hoerr, FJ, and D'Andrea, 1983). Cattle and sheep are relatively more refractory to the effects of alfatoxin, pos-sibly due to rumenal microbial degeneration, whereas poultry are more sensitive to afla-toxin than swine Aflatoxicosis can only be prevented by feeding rations free of aflatoxin. Preventing aflatoxin contamination requires an on-going and thorough sampling and testing program.

Control strategies for aflatoxicosis prevention

Moisture/temperature

Monitoring and control of moisture is critical in the prevention of fungal growth and mycotoxin production. Moisture level of grains should be kept at below 13%. Aflatoxins and other mycotoxins produced by *Aspergillus* spp. are not likely to be produced at temperatures below 5 to 8^0C (Rajendra Damu et al ; 2014).

Cleaning

Periodic cleaning of all feed handling equipments with 5 to 10% bleach solution will help control mould growth as well as actually destroy, to some extent the aflatoxins present.

Pre-harvest control measures

Preharvest control measures include prevention of insect infestation, crop residues and crop rotation, irrigation and soil condition and effective drying and storage regimens.

Harvest measures

Timing of harvesting greatly influences mycotoxin production, harvesting should take place as soon as the crop is fully grown and the crop cycle is completed.

Post-harvest measures

Post-harvest strategies involved various physical, chemical and biological methods to inactivate, destroy, or remove the mycotoxin (Galvano *et al*., 2001).

Physical Methods

Antimycotic agents

Antimycotic agents like sorbic acid and sorbate; propionic acid and propionate, benzoic acid, benzoates and parabens; and acetic acid and its derivatives are the chemicals that prevent mould growth and interfere with mycotoxin production. 1% propionic acid, incorporation of 0.2% potassium sorbate, 0.7% methyl paraben and 0.2% sodium propionate completely inhibited fungal growth (Tong and Draughon, 1985).

Irradiation

Gamma or electronic irradiation is highly effective

for destroying the fungal spores. Simple exposure of contaminated grains to sunlight (UV) substantially reduces mycotoxin levels.

Processing of food

Most of the mycotoxins are generally stable at room temperature. Processing of food has been found to decrease the prior concentration. Wet milling, malting, brewing, cooking and dry and oil roasting are methods to eliminate the mycotoxins, effectively.

Chemical Methods

Ammoniation

Treatment with aqueous and gaseous ammonia or ammonium hydroxide, with or without heat and pressure to destroy the mycotoxin in contaminated food and feed is currently the best and effective method. Ammoniation not only detoxified several mycotoxins (85-100% reduction), but also inhibited mould growth (Madson *et al*., 1983).

Sodium hydroxide

Warming of grain to 1050C in the presence of 0.5% sodium hydroxide detoxified various mycotoxins in the feed.

Mycotoxin-binding agents

Numerous agents like, activated carbons (charcoal), bentonites, clay, hydrated sodium calcium alumino silicate, and zeolite, have currently been used to counteract the mycotoxicosis These sorbents are nutritionally inert and reduce the bioavailability of various mycotoxins by absorption on their surface in intestinal tract. Charcoal at 2% level had shown beneficial effects, during *in vivo* studies., HSCAS (0.5%) was effective at reducing the toxicity of aflatoxin (Harvey *et al*., 1993; Abo-Norag *et al*., 1995)

Biological Methods

Mannan oligosaccharide (MOS) extracted from the cell wall of *Saccharomyces cerevisiae* has shown broad-spectrum efficacy against most of the mycotoxins (Raju and Reddy, 2000).

Feed additives

Vitamins

Vitamin A, E and C possesses the antioxidant properties against the mycotoxin-induced damage.

Lipids

The higher levels of dietary fat reduced mortality and in some instances, improved the body weights. Lipids exerted their effects in part by interfering with absorption of the aflatoxin.

References

1. Abdelsalam, E. B., Eltayeb, A.F., Noreidin, A.A. and , A. M. Abdulmagid. Aflatoxicosis in fattening sheep. *Vet. Rec.* 1989; 124: 487-488.
2. Abo-Norag, M., Edrington, T.S., Kubena, L.F., Harvey, R.B. and T.D. Phillips. Influence of a hydrated sodium calcium aluminosilicate and virginiamycin on aflatoxicosis in broiler chicks. *Poultry Science*. 1995; 7: 626-632.
3. Allcroft, R., Roberts, B. A. and M. K Lloyd. Excretion of aflatoxin in a lactating cow.*Food Cosmet. Toxicol,* 1968; 6: 619- 625.
4. Austwick, P.K.C. Fungal Nephrotoxins. *Veterinary Research Communication.* 1983; 7: 145-154.
5. Bababunmi, E. A. and O. Bassir. The effect of aflatoxin on blood clotting in the rat. *Brit. J. Pharmacol.* 1969; 37: 497-500.
6. Bryden, W.L., Lioyd, A.B. and R.B. Cumming. Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis. *Aust. Vet. J,* 1980; 56: 176-180.
7. Calnek, B.C., Barnes, H.J., McDougald, L.R. and Y.M. Saif. *Diseases of poultry.* 10th ed. 1997; 951-979. Mosby-Wolfe, Iowa state Univ. press, Ames, Iowa, USA.
8. Carnaghan, R. B. A., Lewis, G., Patterson, D. S. P. and R. Allcroft. (1966). Biochemical and pathological aspects of groundnut poisoning in chickens. *Pathol. Vet.* 1966; 3: 601-615.
9. Cassel, E.K., Barao, S.M. and D.K. Carma. (1988). Aflatoxicosis and ruminants. *Texas Vet. Med. Diagnostic lab, Tesas college.*
10. Council for Agricultural Science and Technology. In: K.A. Nisi (Editor), Mycotoxins: Economical and Health Risks. Council for Agricultural Science and Technology, Ames. 1989; 1-91.
11. Dafalla, R., Hassan, Y.M. and S.E.I. Adam. Fatty and hemorrhagic liver and kidney syndrome in breeding hens caused by AFB1 and heat stress in the Sudan. *Vet. Hum. Toxicol.* 1987; 29: 222-226.
12. Diekman, A., Coffey, M.T., Purkhiser, E.D., Reeves, D.E. and L.G Young. (1992). Mycotoxins and swine performance. CES, PTH-129, Purdue Univ., West Lafayette, Indian.
13. Eaton, D.L. and J.D. Groopman. (1994). The toxicology of aflatoxins. Human health,veterinary

and agricultural significance. pp. 6-8 Academic press, San Diego, Ca.

14. Edds, G.T., Nair, N.P. and C.F. Simpson. Effect of AFB1 on resistance in poultry against cecal coccidiosis and Marek's disease. *Am. J. Vet.* Res, 1976; 34: 819-826.
15. Galvano, F., Piva A., Ritienia and G. Galvano. Dietary strategies to counteract the effects of mycotoxins : A review. *Journal of Food Protection.* 2001; 64: 120-131.
16. Harvey, R.B., Kubena, L.F., Ellisalde, M.H. and T.D. Phillips. Efficacy of zeolitic ore compounds on the toxicity of aflatoxin to growing broiler chickens. *Avian Diseases*. 1993; 37: 6773.
17. Heathcote and J.R. Hibben.(1978) *Aflatoxins Chemual and Biological Aspects*. Amsterdam, Elsevier Scientific Publishing Co.
18. Hegazy, S.M., Azzam, A. and M.A. Gabal. Interaction of naturally occurring aflatoxins in poultry feed and immunization against fowl cholera. *Poult. Sci. 1991;* 70: 2425--2428.
19. Hoerr, FJ, and D'Andrea, G.H. Bidogkal Effects of Aflatoxin in Swine. *Aflatoxin and A. Flazms in Corn.* Auburn University Ag. Ex. Sta, Res. Bui. 1983; 279: 51 - 55.
20. Holzapfel, C. W., Steyn, P. S. and I. F. H. Purchase. Isolation and structure of aflatoxins M I and M 2. *Tetrahedron Lett.* 1966; 25 : 2799-2803.
21. Kato, R., Takaoka, A., Onoda, K. and Y. Omori. (1970). Different effect of aflatoxin on the induction of tryptophan oxygenase and of microsomal hydroxylase system. *J. Biochem,* (Tokyo). 68 : 589-592.
22. Lamont, M.H. Cases of suspected mycotoxicosis as reported by veterinary investigation centers. *Proc. Mycotoxins Anim. Dis.* 1979; 3 : 38-39.
23. Madsen, A., Hald, B. and H.P. Mortensen. Feeding experiment with ochratoxin A contaminated barley for bacon pigs-3. Detoxification by ammonia heating +NaOH or autoclaving. *Acta Agriculturae Scandinavica.* 1983; 33: 171-175.
24. Mintzlaff, H. J., Lotzsch, R., Tauchmann, F., Meyer, W. and L. Leistner. Aflatoxin residues in the liver of broiler chicken given aflatoxin-containing feed. *Fleischwirtschaft.* 1974; 54 : 774-778.
25. Muller, R.D., Carlson, C.W., Semeniuk, G. and G.S. Harshfield. (1970). The response of chicks, duckling, goslings, pheasants and poults to graded levels of aflatoxins. *Poult. Sci.* 1970; 49: 1346-1350.
26. Nabney, J., Burbage, M. B., Allcroft, R. and G. Lewis. Metabolism of aflatoxin in sheep. Excretion pattern in the lactating ewe. *Food Cosmet. Toxicol,* 1967; 5 :11-17.
27. Okoye, J.O.A., Asuzu, I.U. and J.C. Gugnani. Paralysis and lameness associated with aflatoxicosis in broilers. *Avian Pathol.*1988; 17: 731-734.
28. Pattison M., Mc Mullin P., Bradbury J. and D. Alexander. (2008).*Poultry Diseases*(Sixth Edition), Chapter 38, pages 435-442.
29. Pier, A. C. Aflatoxicosis and immunosuppression in mam-malian animals. In: M. S. Zuber, E. B. Lillehoj, and B. L. Renfro (Ed.) Aflatoxin in Maize. 1987; 58-65. CIMMYT, Mex-ico.
30. Radostits, O.M., Gay, C.C., Blood, D.C. and K.W. Hinchcliff. *Veterinary medicine,* 2000; 1684-1688, W.B. Saunders Co. Ltd., London.
31. Raisbeck, M.F., Rottinghaus, G.E. and J.D. Kendall. Effects of naturally occurring mycotoxins on ruminants. 1991; 647-677.
32. Rajendra Damu Patil, Rinku Sharma and Rajesh Kumar Asrani. Mycotoxicosis and its control in poultry: A review. *Journal of Poultry Science and Technology.* 2014; 2 : 1-10.
33. Raju, M.V.L.N. and M.R. Reddy. Prevention and control of mycotoxins in poultry diets. *Poultry Punch,* Oct 2000; 36-63.
34. Reagor, J.C. (1996). *Implications of mycotoxins in horses.* WEVR, 96, Cybersteed, Ine. Reddy, D.N.; Rao, P.V. Reddy, V.R. and Yadgiri, B. (1984). Effect of selected levels of dietary aflatoxin on the performance of broiler chickens. *Indian, J. Anim.* Sci. 1996; 54: 68-73.
35. Shank, R. C. and G.N. Wogan. Acute effects of aflatoxin B1 on liver composition and metabolism in the rat and duckling. *Toxicol. Appl. Pharmacol.* 1966; 9: 468-476.
36. Shankaran, R., Raj, H. G. and T.A. Venkatasubramanian. (1970). Effect of aflatoxin on carbohydrate metabolism in chick liver. *Enzymlogia.* 39: 371-378.
37. Tong, C.H. and F.A. Draughon. Inhibition by antimicrobial food additives of ochratoxin A production by *Aspergillus sulphureus* and *Penicillium viridicatum. Applied and EnvironmentalMicrobiology.* 1985; 49: 1407-1411.
38. WHO, World Health Organization. Environmental Health Criteria, Safety evaluation of certain food additives. 1979; 1-127.
39. Wilson, H.R., Douglas, C.R., Harms, R.H. and G.T. Edds. Reduction of aflatoxin effects on quail. *Poult. Sci.* 1975; 54: 923-925.