International Journal of Research and Development in Applied Science and Engineering (IJRDASE)
ISSN: 2454-6844

Effect of Mycotoxins on Aquaculture

Shambhu Prasad,
Directorate of Distance Education
L.N. Mithila University Darbhanga, 846008,
prasad4dde@gmail.com

Abstract-- Adequate health management is the key of intensive fish farming. Recent technological applications that make artificial feeding inevitable during aquaculture have increased the risk of mycotoxin exposure to fish. Almost all fish feed ingredients are potent substrates facilitating mold invasion and subsequent toxic metabolite production. But, in haste to raise production "aquaculture feed quarantine" has largely been ignored. The reason may arguably be attributed to the fact that any epidemic outbreak, as witnessed during the last century in many parts of the world due to mycotoxin contaminated feed supply, has not so far been registered here. It cannot be argued that our fish feeds are free from mycotoxins. Mass mortality of fishes in our country is a recurrent phenomenon, but we do not inspect the death of fishes from the mycotoxicosis point of view. Toxic molds are ubiquitous in occurrence with variable magnitudes of incidence depending upon climatic factors. Mycotoxin is a generic term used to define toxic mold metabolites in food/feeds, which can cause illness or death on ingestion, skin contact or inhalation. The disease caused in general is mycotoxicosis. With the establishment of aflatoxicosis , a distinct disease syndrome caused due to aflatoxins. secondary toxic metabolites of Aspergillus flavus group of fungi and their recognition as storage fungi, a thrilling awareness rippled among the researchers and people concerned with livestock production Almost simultaneously many other mycotoxins, viz, ochratoxins, citrinin, sterigmatocystin, zeralenone, trichothecenes. paulin. penicillic acid, ergot alkaloids. PR toxin, kojic acid, viriditoxin and rubratoxin were also added to the list.

1. Introduction:

The Aquaculture in India is an essential subsector of agriculture that provides food, employment, and other economic resources for the state (Prasad 2002). Fish production can be threatened when feeds are contaminated by fungi and their toxic metabolites. Several mycotoxins, including aflatoxins (AFs), cyclopiazonic acid (CPA), fumonisins (FUMs), nivalenol (N IV) and zearalenone (ZEN) have been reported to contaminate fish feed and their ingredients (Prasad 2002,Das 2002,Verma 1990, Labuda et al. 2005; De Boevre et al. 2012; Ezekiel et al. 2012a; Njobeh et al. 2012; Rodrigues and Naehrer 2012;). The occurrence of mycotoxins in feed ingredients depends on several factors that include climatic conditions, diversity of fungi contaminating the crops, harvesting methods of the individual crops, storage practices, and seasonal variations, while the types and levels of mycotoxins in the feed largely depend on the mycotoxins in the individual feed ingredients, the mix/ proportion of feed ingredients, feed processing techniques, and storage practices (Warth et al. 2012;). Mycotoxins pose a huge threat to the safety and security of livestock first and then to human beings that consume them due to their different toxic effects and their probable synergistic properties (Shephard 2008; Hossain et al. 2011; Njobeh et al. 2012). When animals ingest feed contaminated with high mycotoxin concentrations, mycotoxicoses, often marked by reduced animal productivity (reduced body weight gain, reduced litter sizes, deformed offspring, reduced egg production) and immune suppression (Shareef 2010; Hossain et al. 2011), could result to severe economic losses. Fish feed ingredients are derived from a variety of raw materials that originate from plants and animals. It is usually a mixture of cereals that serves as energy source, animal protein sources (fish meal, meat, and bone meal), and plant protein sources (soybean meal). Maize, the predominant grain used in Fish feeds, can be contaminated by mycotoxins from Aspergillus, Fusarium and Penicillium during processing and storage (Zinedine and Manes 2009;).
Globally, mycotoxins in finished Fish feed have been reported (Labuda et al. 2005; De Boevre et al. 2012; Ezekiel et al. 2012a; Njobeh et al. 2012; Rodrigues and Naehrer 2012), but there is sparse information on the source tracking of mycotoxin contamination of the feed by individual ingredients. Hence, this study aimed at investigating mycotoxins in Fish feed and the ingredients used in locally formulating the feed in Bihar with a view to associate contamination of major ingredients to overall contamination in finished feed. This survey provides snapshot data for the consideration of other cereal-based ingredients and protein sources that are less prone to AFs and FUMs contamination in feed formulation.

2. Mycotoxins in Human Food Chain:

Studies till date have amply proved the mycotoxins to be toxic to all microorganisms, animal models and sometimes to plants as well tested so far but are not infectious. Before the discovery of aflatoxins, the role of mycotoxins played in disease of livestock and other animals was uncertain. But subsequent demonstrations of carcinogenic potentials of aflatoxins and many other toxic mould metabolites, the next question asked was "Are mycotoxins involved in human health?"

The present level of social awareness towards livelihood practices are such that moldy food are generally rejected but visual appearance of mould on food stuffs does not necessarily indicate mycotoxin contamination. Similarly, the reverse is also true. Mycotoxins may be present in foods that do not present such visual warnings.
The involvement of mycotoxins as etiologic agents of human disease is difficult to determine because direct

evidence of such involvement in terms of controlled human experiments does not exist. The fact that different animal species are susceptible to both acute and chronic effects of a mycotoxin offers indirect evidence that man may be adversely affected by the mycotoxin (Hayes, 1981).

Possible appearance of mycotoxins in human food chain has been widely reviewed (Goldblatt, 1969). Entry of these mycotoxins in human food chain via commodities of agriculture/plant origin is quite indisputable. During 1970s several experiments were conducted to divulge the possibilities of mycotoxin exposure to man through dietary intakes of animal origin (Keyl and Masni, 1966; Frank, 1966) showing least possibilities of mycotoxin uptake through animal tissues. It was further explained that while animal tissue may not contain any aflatoxin, there is a problem with milk of lactating mammals fed on aflatoxin contaminated diet (de-longh et al., 1965). Cows (Alcroft and Caraghan, 1963), rats (de longh et. al., 1964) and sheep (Nabney et al., 1967) when fed aflatoxin contaminated rations, excrete in their milk toxic metabolites, aflatoxin M (refer Introduction: Aflatoxins) with almost comparable toxic potentials to aflatoxin B. Buttler and Clifford (1965) suggested that aflatoxin B, is metabolized, in part, in the liver of rats to aflatoxin M or "milk toxin". Metabolite conversion of aflatoxin B into aflatoxin M, and some other metabolic forms inside the animal system have amply been proved in various animals (Holzapfel et al., 1966) but the bioaccumulation of these metabolites in the tissue system was not thoroughly clarified till 1970s.

During early 1980s methods of tissue residues analaysis of mycotoxins and their metabolite (Stolof, 1982) was worked out because epidemeological associations are best made when exposure to the agent under study can be established for each member of the affected cohort and the reverse for the controls. Incidentally, metabolism of almost all mycotoxins investigated so far are very rapid. Residues of parent compound and metabolites can be detected for study only either with exaggerated acute dose level or chronic prolonged exposure, and that too only for a short period rather in a matter of days after administration is ceased. There is no evident storage of either the parent compound or a metabolite in any tissue, such as seen in case of chlorinated pesticides in adipose tissue (Stolof, 1982). However, subsequent works at least in case of ochratoxin A, revealed that the toxin after being bound with serum macromolecules constitute a "mobile reserve that can be released to the tissue over long period of time (Stojkovic et al., 1984) (refer Introduction: Ochratoxins).

Trace of various mycotoxins and their edible metabolites in edible tissues, milk, and eggs of farm animals fed rations containing small concentration of mycotoxins, particularly aflatoxins, ochratoxins, zeralenone (Hayes, 1981; Stolof, 1982) have been shown. Patterson et al. (1978) have reviewed the potential hazard to man to this direct exposure, as compared with direct dietary intake of mycotoxins, and have presented data relating to the measurement of aflatoxin, ochratoxin A and zeralenone in milk and tissues of cattle fed naturally occurring contaminated rations. Valenta (1998) has described that intake of OA by contaminated feed may lead to residues in the blood, the kidney and the liver of pigs and poultry and to a lesser extent in muscle tissue, adipose and eggs. Thus, product of animal origin can contribute to the Ochratoxin A intake of humans.

Recently tissue retention of diet-related mycotoxins in channel catfish has been investigated (Wu, 1999). Subchronic and toxic concentration of aflatoxin B: (1, 5 and 50 ppm). fumonisin B1 (10 and 100 ppm) and Ochratoxin A (5 and 50 ppm) were incorporated into practical diets fed to young channel catfish (*Ictalurus punctatus*). Mycotoxin residue in tissues like muscle, liver, serum and kidney of the fish were measured after 3, 7, and 21 days of feeding following withdrawal of the toxins from the diets. Maximum concentration of all mycotoxins was found within 3 days in all tissues. Tissue concentration of residues was generally higher in fish administered with high toxin dose. Within 3 days after withdrawal of aflatoxin B, and Ochratoxin A from diets, tissue concentration had decreased to 95 - 99%. Tissue clearance of fumonisin was much slower. Coefficient of net absorption for mycotoxins were determined. Net absorption coefficients of aflatoxin B1, fumonisin B and Ochratoxin A was relatively high and similar: 85.3, 87.8 and 83.7% respectively. Feed tissue ratio was found to be lowest for aflatoxin B, and highest for Ochratoxin A. It was concluded that aflatoxin B1, fumonisin B and Ochratoxin A residue appear in flesh of channel catfish soon after the fish consumes these mycotoxins in their diets. The rate at which they are retained in the tissues varies among mycotoxins and tissues.

3. Materials and Method:

Bioassay was conducted on three Air-breathing teleosts like *Clarias batrachus*, *Heteropeneustus fossilis* and *Anabas testudineus* were taken for bioassay. Healthy fish obtained from a local fisherman were acclimatized for 15 days in laboratory conditions. For each toxin 50 well-acclimatized fish were subdivided in groups of 20 fish along with control group of 10 fish. The test groups of fish designated as AF/Gr. I, AF/GR-II, Och/GR-I, Och/GR-II, Stg/GR-I, Stg/Gr-ll were administered with 0.5 ml of crude toxin extract of each toxin. Test groups AF/Gr I and Il were given crude extract of A. flavus (S/MOC-139 culture capable of elaborating approximately 100 ug/kg aflatoxin B1), Och Gr I and Il with crude extract of A. ochraceous (W/MOC-9, capable of elaborating 50 ug/kg ochratoxin A) and Stg/G I and II with crude extract of A Versicolor (WWF-41, capable of elaborating 10 ug/kg sterigmatocystin) dissolved in 0.5 ml of propylene glycol. The control fish were administered with 0.5 ml. of propylene glycol only. The fishes were incubated for 80 days. Ten fish of each subgroup were scarified on day 40 and 80 respectively for biochemical, hematological and histopathological observations. During experiment fish were closely monitored for behavioural alterations in response to toxin insult. The fish were fed with Gordon's diet (Axelrod, 1969) prepared in the laboratory using rice flour and finely minced chicken liver.

Bioassay was also conducted on the two major carps, *Labeo rohita* and *Catla catla*. The fish having an average weight of 18.53 ± 2.6 g (*L. rohita*) and 13.4 + 1.76 g (*C. catla*) were obtained from local culture pond and brought to the laboratory immediately in aluminium pots avoiding over

crowding. The fish following 1% KMNO. wash were stocked in large aquarium (120x45x45 cms) for acclimatization species-wise. The aquariums were provided with sufficient aeration, filtration device and water was changed frequently. On day five onwards fish were fed with a diet formulated in the laboratory following Jain (2000). The diet contained soyabean flour, mustard oil cake and rice bran in the ratio 28:28:48 added with 200 mg of vitamin E and vitamin C per Kg. The fish were fed twice a day up to satiation and left out amount was removed. Healthy fish were selected for final experiment.

Four groups containing 10 fish of each species (*L. rohita* and *C. catla*) were separated and designated as R-AF (for aflatoxin), R-Och (for ochratoxin), R-Stg (for sterigmatocystin), R-C (control) and C-AF, C-Och, C-Stg and C-C respectively. Groups R-AF and C-AF were administered with 0.2 ml of crude aflatoxin extract in propylene glycol intreperitonially. Similarly, groups R-Och, C-Och and R-Stg, C-Stg were given crude ochratoxin and sterigmatocystin extracts (refer bioassay on air-breathing fish) respectively. The control fish were given 0.2 ml propylene glycol only 20 times and as the fish blood contains much higher count of leucocytes per unit area it becomes difficult to make a proper counting.

4. Observations and Discussion

Behavioural changes first appeared in H. fossilis and almost simultaneous in C. batrachus, In aflatoxin treated fish the body color start darkening on day 15-20 onward with increase in mucus over body surface. The fish become listless and appear lethargic with poor toxic response. The intensity of early symptoms was readily marked in H fossilis than C. batrachus. Behavioural response of fishes receiving ochratoxin A was almost comparable to that of afl toxin group in both early and advanced conditions except swollen belly discussed in major carps) in the later case. With increase in the treatment period the symptoms become more and more pronounced. The fish showing loss in appetite gradually refuse to feed leading to apparent loss in weight in advanced conditions. External respiratory acts like opercular movement and surfacing also increases and the ailing fish were observed to face difficulty even in over to the water surface (approx. 30 cm height). On day 40 onwards water level in ll coming over aquaria was lowered to half enabling the fish to gulp air with minimum possible effort. In contrast, the A testudineus, fairly tolerated all the three toxins. In present experiment aflatoxin appeared to be most toxic followed by ochratoxin A. The three group of fishes treated with sterigmatocystin reflected delayed response. This, primarily, may be due to the fact that the present strain of A versicolor (W/WF-41) was capable of producing 10 mg/kg sterigmatocystin in given laboratory conditions. Thus the amount of toxin in 0.5 ml propylene glycol was too low to cause drastic toxic manifestation as apparent in case of the two other mycotoxins (aflatoxin and ochratoxin). All the three species of air-breathing teleost tested, *H. fossilis* appear to be the most sensitive model for bioassay experiments followed by *C. batrachus. A testudineus* reflects moderate tolerability.

Clinical response of the major carps subjected to bioassay against sublethal concentrations of crude aflatoxin, ochratoxin A and sterigmatocystin extracts have been observed. Early symptoms in all the three groups of fishes were practically indistinguishable. However the group receiving crude sterigmatocystin appeared to be more stable than the others in comparison to control. A defined symptomatology in treated fish appeared on day 22 onwards with darkening of body colour added with loss in appetite and increased mucus over body surface, as observed in case of air-breathing teleosts experimentally exposed to the same toxins. There was an apparent loss in weight indicating depression in growth rate. C. catla to be relatively more resistant than L. rohita as evidenced from the severity of the behavioural manifestations. The characteristic response of the two toxins viz. aflatoxins and ochratoxins could be marked only in advanced conditions on account of swollen belly in the later case. Thus, a listless fish with darkened body colour, increased mucus over body surface, dilated pupils and overall pale appearance including gill may be diagnosed as a case of chronic aflatoxicosis but if the belly is swollen with persistent sign of bowel disorder and added with one or more symptoms indicating chronic aflatoxicosis, the condition specially indicate chronic ochratoxicosis. Characteristic response of sterigmatocystin was not well defined but the condition more or less simulated aflatoxicosis with mild response.

Behavioural response of air-breathing teleosts and major carps exposed to mycotoxins are poorly documented. However, a few earlier reports on rainbow trout exposed to aflatoxins (Bauer et al., 1969) find agreement with the present findings in both groups of fishes, air-breathing ones and major carps. The symptomatology observed in case of C. batrachus, H. fossilis and A. testudienus alsosimulate the conditions described by Verma (1989) in case of C. punctatus administered with pure crystalline aflatoxin B, via intraperitonial route. In case of major carps, C. catla, Murjani and Mukherjee, (1997) and Murjani (1998) recorded similar clinical features in response to aflatoxin insult.

Similarly, there is complete dirth of literature on ochratoxin poisoning in case of fish. Moreover, any Indian fish has probably been investigated for the first time against ochratoxin A. The toxin, like aflatoxins, is also potentially lethal targeting the kidney. However, in general, it significantly alters the normal physiological and metabolic processes making a fish lethargic under toxic influence. Hohler et al. (1999) suggested that ochratoxin A inside the alimentary tract is initially hydrolysed to ochratoxin a. The transformation affects the physiological process adversely and the reflected symptoms are increase in thrust, moderate to severe bowel disorder. Prolonged exposure to the toxin alters the structure of the alimentary canal leading to pronounced clinical symptom of swollen belly, and consequent loss of appetite.

Fading of eye colour and dilated pupils, in general, indicate some sort of hormonal imbalance, probably over production of adrenocortical hormones. Such clinical findings has been described as routine response against many of the toxic insults (Wogan, 1973, Krogh, 1987). Anemic appearance of

present fish may be attributed to variable degree of hemorrhage of internal organs.

Gross anatomical response of the air-breathing teleosts and major carps were observed. On account of similarities between anatomical response of air breathing fish and major carps this part has been discussed commonly. A few added symptoms in case of air-breathing fish becomes more expressive due to prolonged incubation than the carps.

All the treated fish showed moderate to serve anemia. A diffuse pattern of hemorrhage was primarily found on liver and parts of gastrointestinal tract. These symptoms increase with increase in the incubation period. A general patter of swelling found associated with parts of intestine and stomach, was more pronounced in ochratoxin A treated carps. The spleen and head kidney reflected no gross alteration at low incubation but were mostly swollen in response to aflatoxin in advanced condition. Similarly, in case of ochratoxin treated fish the kidney was grossly affected. Hyperamic patches often associated with swelling was observed in descending order of severity on liver, excretory kidney and intestine with minimal and infrequent trace on spleen and head kidney, particularly in case of air-breathing fishes. One added feature, not observed in case of major carp, included appearance of micro-nodular outgrowths (less than 0.5 mm in dia) on liver of H. fossilis characterising hepatocarcinogenic response of aflatoxins.

General anemia as marked in all the treated fish irrespective of the dose and type of mycotoxin may be attributed to hemorrhage ranging from moderate to severe on liver and pats of gastrointestinal tract. According to Bauer et al. (1969) the rainbow trout also exhibit multiple hemorrhage in the viscera and visceral fats. Ashley (1970) reported gross multiple hemorrhage area in liver, viscera and visceral fats. Moribund fish had nearly white gills indicative of severe anemia. Similar observations have been made by Verma (1989) in case of C. punctatus, Das (2002) in C. mrigala. In case of C. punctatus exposed to ochratoxin A (Prasad, 2002) multiple hemorrhage of internal organ have been reported. Gross swelling did not involve any of the organs except spleen and head kidney in aflatoxin treated fish and trunk kidney in case of ochratoxin treated fish. The localised inflammatory reaction on liver and infrequently on other internal organs were often associated with hyperamia. In case of C. punctatus exposed to pure crystalline aflatoxin B: the symptoms have been described to be a preceding stage of tumorous outgrowth (Verma 1989).

Experimental induction of tumorous outgrowths in fish exposed to aflatoxins has been variously described. In rainbow trout, a sensitive species to aflatoxin By, tumorous outgrowths do not appear earlier than 4 months (Halver, 1967). It was further suggested that visual detection of small developing nodules can be estimated after 6-9 months if the carcinogen has been fed orally. Verma (1989) has explained that in C. punctataus incidence of tumorous outgrowths exhibit a linear correlation with increase in dose and treatment period. There are various factors operating simultaneously in experimental condition that affect the event, in particular, if induction of tumors in experimental animals are the target (refer introduction). Manifold increase in sensitivity of a fish to a particular carcinogen has been suggested if the route of administration is opted as intravenous or intraperitonial than the oral (Ueno and Ueno, 1978).

Among the present fish tested against three types of mycotoxins, only H. fossilis developed micronodules following 80 days of treatment with crude aflatoxins administered intraperitonially.

The gross pathology of tumorous outgrowths as evident in case of rainbow trout has earlier been described by Wood and Larson (1967) and Halver (1969). The hepatoma, in response to aflatoxins, shows two general forms. First, characterised by light yellow, well vascularised, protruding lesions of 1-2 mm in size and the other grayish-white, translucent that may develop into large necrotic tumors. As also explained by Verma (1989) in C. punctataus, the present lesion on liver of H. fossilis simulates group one of Wood and Larson (1967). Such outgrowths may be scattered throughout the liver surface indicating multicentric origin. The interaction between biotransformational products of aflatoxins and hepatocytes involves several loci and may cause multicentric origin of the tumorous outgrowths.

5. Conclusion:

Many of the mycotoxins well documented for their ubiquitous occurrence besides being a potent toxin are often a carcinogen, mutagen, teratogen and immunosuppressant too. Considering the two facts: concentration of mycotoxins usually encountered in natural conditions and the concentration of the same to which the fish population is really exposed through dietary intakes, the sub-chronic or even below level of mycotoxin exposure appears to be the most expected condition in case of fish. A condition in which immune system becomes prone to the effects of these mycotoxins. Unfortunately, investigations on this score are still to be taken up seriously but a weak self-defence system will render the population prone to all opportunistic secondary infectants.

References:

[1] Allcroft, R., and Carnaghan, R.B.A. (1963): Groundnut toxicity - An examination for toxin in human food products from animal fed toxic groundnut meal. Vet. Record. 75:259-263.

[2] Ashley, L.M. (1970): Pathology of fish fed aflatoxins and other antimetabolites. Spec. 10. X X.11. Publs. Am. Fish. Soc. No. 5. p. 366-379.

[3] Ashsley, L.M. (1967): Histopathology of rainbow trout aflatoxicosis. Res. Rep. U.S. Wild 1.Serv. No. 70, p. 103-120.

Bauer, D., Lee, D. J. and Sinnhuber, R. C. (1969). Acute taxicity of aflatoxin B and G: in the rainbow trout (Salmo-gairdneri). Toxicol. Appl. Pharmacol; t. XV: 415-419.

[4] Butler, W.H., and Clifford, J.I. (1965): Extraction of aflatoxin from rat liver. Nature 206. p:1045-1046.

[5] Das, M. (2002): Mycobial feed contaminants and its histopathological major carp. Ph.D. Thesis, L.N. Mithila University, Darbhanga.

[6] De Boevre M, Di Mavungu JD, Landschoot S, Audenaert K, Eeckhout M, Maene P, Haesaert G, De Saeger S (2012) Natural occurrence of mycotoxins and their

masked forms in food and feed products. World Mycotoxin J 5(3):207–219.
[7] De-longh, H., Vles, R.O. and Vanpelt, J.G. (1964): Milk of mammals fed on aflatoxin containing diet. Nature 202. p: 466-467.
[8] De-longh, H., Vles, R.O., and de Vogel, P. (1965): The occurrence and detection of aflatoxin in food. In: "Mycotoxins in Foodstuffs". ed. Wogan, G.N. M.I.T. Press, Cambridge, Massachusetts. p: 235-245.
[9] Ezekiel CN, Bandyopadhyay R, Sulyok M, Warth B, Krska R (2012a) Fungal and bacterial metabolites in commercial poultry feed from Nigeria. Food Addit Contamin Part A 29:1288–1299.
[10] Ezekiel CN, Sulyok M, Warth B, Odebode AC, Krska R (2012b) Natural occurrence of mycotoxins in peanut cake from Nigeria. Food Control 27:338–342.
[11] Food and Agriculture Organisation of the United Nations (1977): Report of the joint FAO/WHO/UNEP Conference on mycotoxins. FAO Food and Nutrition Paper p. 1-51.
[12] Frank, H.K. (1966): Aflatoxin in foods. Arch. Lebensmittethys. 17. p: 237-242 (Chem. Abstr. 66. 74-84)
[13] Goldblatt, L.A. (1969): Aflatoxin. Scientific background, control and implications. (ed.) Academic Press, New York. San Francisco. London.
[14] Halver, J.E. (1967): Crystalline aflatoxin and other vectors for trout hepatoma. Res. Rep. U.S. fish. Wildl. Serv. No. 70.p: 78-92.
[15] Halver, J.E. (1969): Aflatoxicosis and trout hepatoma. In: Aflatoxins Scientific Background, Control and Implications. ed. Goldblatt, L.A., Academic Press, New York. p: 265-304.
[16] Hayes, A.W. (1981): Mycotoxin Teratogenicity and Mutagenecity. ed. C.R.C. Press. Inc. Boca Raton, Florida.
[17] Hohler, D., Sudekum, K.H., Wolffarm, S., Frohlich, A.A., Margvardt, R.R. (1999): Metabolism and excretion of ochratoxin A fed of sheep. Journal of Animal Science. 77(5): p: 1217-23.
[18] Holzapfel, C.W., Purchase, I.F.H., Steyn, P.S. and Gouws, L. (1966): The toxicity and chemical assay of sterigmatocystin, a carcinogenic mycotoxin and its isolation from two new fungal sources. S. Africa Med. J. 40: 1100-1101.
[19] Hossain SA, Haque N, Kumar M, Sontakke UB, Tyagi AK (2011) Mycotoxin residues in poultry product: their effect on human health and control. Wayamba J Anim Sci 2011:92–96
[20] Keyl, A.C. and Masri, M.S. (1966): The effects of graded dietary levels of aflatoxins on various physiological parameters in swine. Abstra. 152nd Meeting Am. Chem. Soc. New York A.p: 96.
[21] Krogh, P. (1987): Mycotoxins in Food. ed. Academic Press. London. p: 97.
[22] Labuda R, Parich A, Veikru E, Tancinova D (2005) Incidence of fumonisins, moniliformin and Fusarium species in poultry feed mixtures from Slovakia. Ann Agric Environ Med 12:81–86
[23] Murjani, G. (1998): Aflatoxicosis in Catla catla. Ph.D. Thesis. Central Institute of Freshwater Aquaculture. Kausalyagang, Bhubaneshwar.
[24] Murjani, G. and Mukharjee, S.C. (1997): Acute Aflatoxicosis in Catla catla. An experimental study in National Workshop on Fish and Shellfish. Health Management. p: 30.
[25] Nabney, J., Burbage, M.B., Allcroft, R. and Lewis, G. (1967): Metabolism of aflatoxin in sheep: excretion pattem in the lactating ewe. Food Cosmet. Toxicol. 5.p: 11-17.
[26] Njobeh PB, Dutton MF, Aberg AT, Haggblom P (2012) Estimation of multi-mycotoxin contamination in South African compound feeds. Toxins 4:836–848.
[27] Patterson, D.S.P., Shreeve, B.J. and Roberts, D.A. (1978): Mycotoxin residue in body fluids and tissue of food-producing animals. In: 12 Int. Congr. Microbiology, Munich.
[28] Prasad, S. (2002): Ochratoxin A in fish feeds and its pathophysiological manifestation in fish. Ph.D. Thesis, L.N. Mithila University, Darbhanga
[29] Rodrigues I, Naehrer K (2012) A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feeds. Toxins 4:663–675.
[30] Shareef AM (2010) Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis. Iraqi J Vet Sci 24(1):17–25.
[31] Shephard GS (2008) Determination of mycotoxins in human foods. Chem Soc Rev 37:2468–2477
[32] Stojkovic, R., Hult, K., Gamulin, S. (1984): High affinity binding of ochratoxin A to plasma constituent. Biochem. Int. 9: 33-38.
[33] Stoloff, L. (1982): Analaytical methods for aflatoxins - An overview. In: Environmental carcinogen selected Method of analysis. Int Agency for Research on Cancer. Lyon.
[34] Ueno, Y. and Ueno, I. (1978): Toxicology and Biochemistry of Mycotoxins. In: Toxicology Biochemistry and Pathology of Mycotoxins. ed. Urgachi, K. and Yamazaki, M.A.. Halsted Press Book, p. 107-108.
[35] Valenta, Hana (1998): Chromotographic methods for the determination of ochratoxin A in animal and human tissues and fluids. Journal of Chromatography A. Institute of Animal Nutrition, Federal Agricultural Research Centre, Braunschweig-Volkenrode, Bundesallee, 50. D-38116 Braunschweig. 815. p. 75-92.
[36] Verma, S.K. (1989): Effect of Aflatoxin contaminated feed on the histopathology of different organs of local fish (Teleost). Ph. D. Thesis. L.N. Mithila University, Darbhanga.
[37] Verma, S.K. and Pandey, Sadhana (1990): Effect of aflatoxin B on the haematopoietic tissue of an air-breathing teleost, Channa punctatus. J. Zool. Soc. India, 40(182), 79-87.
[38] Warth B, Parich A, Atehnkeng J, Bandyopadhyay R, Schuhmacher R, Sulyok M, Krska R (2012) Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/ MS multitoxin method. J Agric Food Chem 60(36):9352–9363.
[39] Wogan, G.N. (1973): Aflatoxin carcinogenesis. In: Methods in Cancer Research. ed. Bush M. Academic Press, NEW. York. p. 309-344.
[40] Wood, E.M. and Larsen, C.P. (1967): Hepataic carcinoma in rainbow trout. Arch. Pathol. 71. p. 471-479.

[41] Wu, F. (1999): Retention of Diet-related Mycotoxins in tissue of channel catfish Dissertation Abstracts International, Part B: Science and Engineering. Vol. 59, No. 8. p.3791.
[42] Zinedine A, Manes J (2009) Occurrence and legislation of mycotoxins in food and feed from Morrocco. Food Control 20:334–344.