AFLATOXIN AND OCHRATOXIN CONTAMINATION IN POULTRY -A REVIEW-

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Abstract

Aflatoxin and ochratoxin are the most common mycotoxins in poultry feed. Their presence contributes to significant health disorders and decrease in production performances. This leads to considerable economic losses for the poultry industry by increasing mortality, decrease in body weight, number and quality of eggs, greater feed conversion and immunosuppression. The risk associated with mycotoxin residues in poultry meat and eggs represents a concern in human health.
The present article reviews the most important scientific literature on aflatoxin and ochratoxin contamination in poultry and their relationship with food safety.
Recent studies showed that young poultry are more sensitive to aflatoxin and ochratoxin than adults. Ochratoxin has high affinity for liver and kidney, meanwhile aflatoxin has high carcinogenic potential and hepatotoxicity. Lesions in the liver include hepatomegaly, hydropic degeneration, fatty changes, bile-duct hyperplasia and periportal fibrosis.
In order to prevent and reduce the negative implications of these mycotoxins in poultry production, it is necessary to create both global and national strategies to reduce the amount of mycotoxins in grain, to use advance analytical techniques and to establish new limits concerning the maximum amount of mycotoxins allowed in poultry feed and products from poultry for human consumptions .

Keywords: aflatoxin, food safety, lesions, ochratoxin, poultry feed.

INTRODUCTION

Mycotoxicoses are intoxications of animals caused by toxin ingestion coming from contaminated grains, feed or litter. In general, fungi produce a large variety of complex chemicals. Some are toxic to animals (mycotoxins), some to bacteria (antibiotics) and some to both. The type and the amount of toxin produced depend on the strain of fungus, temperature, moisture, grain substrate and degree of stress on the host plant.
Nowadays, food safety policy is orientated to possible contamination of poultry feed and consecutively processed food from poultry with fungi and the risk of mycotoxin contamination. High levels of mycotoxins in feed contribute to acute mycotoxicoses and high mortality rate. Lower levels

cause chronic mycotoxicoses with or without clinical symptoms, followed by considerable decrease in production performances, immunosuppressive effects and presence of residues in poultry meat and eggs. Primarily, the toxicity of mycotoxins depends on the type, quantity and duration of ingestion of mycotoxins, species, gender and age of the animal, general health, immune and nutritional status, as well as environmental factors.

Aflatoxicosis and ochratoxicosis are the most common mycotoxicoses in commercial poultry (Pattison et al., 2008).

AFLATOXICOSIS

Aflatoxin is the most prevalent and economically significant mycotoxin that can be ingested by poultry. In the United States the total annual loss due to aflatoxins in corn is about $163 million. The annual market loss through corn rejected for food is about $31 million, while the loss through corn rejected for feed and through livestock losses is estimated at $132 million. The cost of research and monitoring activities are between $500 million and $1,5 billion a year to manage mycotoxin-producing fungi.(Abbas, 2005).

The name ''aflatoxin'' derives from the first letter of the word Aspergillus and the first three letters of flavus. Structurally, aflatoxins (AFs) are difurocoumarin derivatives with specific fluorescence under ultraviolet light. Depending on the colour of the fluorescence, AFs are divided into aflatoxin B1 and B2 (AFB1, AFB2) for blue florescence, and G1 and G2 (AFG1, AFG2) for green florescence. Aflatoxin M1 and M2 (AFM1, AFM2), known as milk-AFs, are the metabolites of AFB1 and AFB2. Other metabolites of AFB1 are aflatoxin Q1 (AFQ1) and aflatoxicol. Aflatoxin is the most studied mycotoxin, due to both its toxicity to animals and people and its high carcinogenic potential. Out of AFs group, AFB1 is the most toxic and is classified as human carcinogen (Talebi et al. , 2011).

Etiology

Aflatoxins are secondary metabolites produced by the common moulds of *Aspergillus flavus, A. parasiticus* and *A.nominus*. These fungi are ubiquitous in the environment and produce aflatoxin in warm (30–35°C) and high-humidity conditions.

The occurrence of aflatoxins in agricultural commodities depends on region, season and the conditions under which a particular crop is grown, harvested or stored. Crops grown under warm and moist weather in tropical or subtropical countries are more prone to aflatoxin contamination than those in temperate zones. Aflatoxin production is also stimulated by high zinc concentration in feed (Pattison et al., 2008).

Stressed plants by insect damage, drought, poor nutrition or delayed harvest increase aflatoxin production. Aflatoxin is stable once formed in grain and is not degraded during normal milling and storage (http://www.worldpoultry.net).

Host sensibility

Young poultry are more sensitive to aflatoxin than adults. There are also large species differences: ducks being 10 times more sensitive than chickens. Furthermore, certain poultry breeds are more sensitive than others. Chickens and quails are considered relatively resistant, so acute intoxication occurs relatively rare. Chronic intoxication with aflatoxin is the results of aflatoxin ingestion for several weeks (one week minimum). Aflatoxin in concentration of 0.7 ppm reduces the growth rate of turkey but without any effect in quails and chickens (Arafa et al., 1981). A diet containing 400 ppm AFB1 severely affects body and liver weights in turkeys, with no effect in chickens (Leeson et al., 1995). Dalvi (1986) showed that the lethal dose is different between poultry species (Table 1).

Table 1. Oral LD 50 for different poultry species

Species	Oral LD50 (ppm)
Chick embryo	0,025
Duckling	0,3
Turkey poultry	0,5
Chicken, New Hampshire	2
Chicken, Rhode Island	6,3

Pathogenesis

After absorption, the highest concentration of the toxin is found in the liver (target organ). At liver level, aflatoxin B_1 is metabolized by microsomal enzymes into different metabolites.

In one day-old chicks, AFB_1 reduces the activity of liver UDP glucose-glycogen transglucosylase resulting in depletion of hepatic glycogen stores (Shankaran et al., 1970). Also, lipid accumulation occurs in the liver of chickens and ducklings exposed to aflatoxin (Carnaghan et al., 1966; Shank and Wogan, 1966). Aflatoxin is rapidly excreted in the bile (eliminated in faeces) and urine and does not accumulate or persist in body tissues. This perhaps explains the rapid recovery of egg production and hatchability after cessation of toxin ingestion. Aflatoxin reduces resistance of poultry to infection with *Pasteurella multocida*, *Salmonella spp.*, Marek disease virus, infectious bursal virus, *Coccidia*, and *Candida albicans* (Smith et al., 1969; Hamilton and Harris, 1971).

Clinical sings

Aflatoxicosis does not induce mortality directly, although high levels (>10 ppm) may be lethal. Acute aflatoxin poisoning leads to impaired

coordination of movement, vertigo, and paresis, followed by diarrhea with admixtures of blood, haemorrhages, tumescence, jaundice (icterus), coma and death. The most economically significant effects of aflatoxicosis on poultry are decreased growth, weakness, reduced food consumption and poor feed conversion. Poultry exposed to aflatoxins are pallid, as a consequence of poor pigmentation, which is the result of reduced ingestion, resorption, and transport of carotenoid.

Intoxicated adult hens, with concentration above 2 ppm have decreased egg production and the hatchability of their eggs is reduced. In adult male breeder, testicular weights and sperm counts are reduced.

Dietary exposure of broiler hens to AF (10 ppm) resulted in embryonic mortality and reduced the immunity in the progeny chicks. Embryonic exposure with AFs resulted in long-term depression of the immune function in chickens (Resanovic et al., 2009).

The levels of serum proteins are reduced by aflatoxins. The synthesis of albumin and of the most of the globulins takes place in liver and in chronic hepatic diseases, hypoalbuminaemia occurs. The level of globulins decreases, but not as much as the albumin because not all the globulins are formed in the liver, such as gamma-globulins (Fernandez et al., 1995).

Lesions

Lesions depend on the age of the host and the dose of toxin ingested and can include: hepatosteatosis or fatty liver, kidney hypertrophy, splenomegaly, atrophy of the thymus, testes and bursa of Fabricius. Bruising associated with an increase of capillary fragility and haemorrhagic points (petechial haemorrhages) on the surface of muscles of the leg and breast have been described in aflatoxicosis of broiler chickens (Biro et al., 2002).

Acute toxicity of aflatoxins in chickens may be characterized by liver necrosis with icterus, distended gallbladder and bile ducts, white pinhead-sized lesions and paleness in liver. Histopathological, liver damage is manifested as vacuolation of hepatic cells and bile duct proliferation. Metabolic alterations caused by aflatoxins in chickens result in elevated lipid levels, disruptions in hepatic protein synthesis, which result in several blood coagulation disorders, immunosuppression and decreased plasma amino acid concentrations (Sumit et al., 2010).

In aflatoxicosis, a thickened basement membrane in the glomeruli and associated hyaline droplets in the renal tubules are noted. It is not known if the glomerulus is damaged by toxins or by a leakage of unusual protein from a severely damaged liver. Liver lesions in chicken are characterized by

retrogressive and regenerative parenchymal changes.(Herenda and Franco, 1996).

Acute and chronic toxic effects of aflatoxins, particularly AFB1, in turkeys are not very different from those in chickens: diffuse necrosis of liver parenchyma, proliferation of the bile duct epithelial cells and small haemorrhages. The prominent cellular changes in liver included swelling and vacuolation of the parenchyma cells and enlargement of the nucleus. There has also been observed: hypertrophy and congestion of the kidneys.

In ducklings over 3 weeks subcutaneous haemorrhages of legs and feet are characteristic. Lesions of the liver have been reported to be common in acute and chronic cases of aflatoxicosis in ducks. The duckling has been recommended as a convenient species for experiments because of its rapid response to aflatoxin, manifested by marked bile duct hyperplasia 48 to 72 hours after exposure. Prolonged exposure of the duck to low levels of aflatoxins leads to marked nodular hyperplasia of the liver, bile duct proliferation and fibrosis and hepatocellular carcinoma. Chickens and turkeys have not been shown to develop liver neoplasm after ingestion of aflatoxins. Since metabolites of aflatoxins are implicated in carcinogenesis and ducks are known to metabolize aflatoxins rapidly, they appear to be prone to aflatoxin-induced carcinoma (Resanovic et al., 2009).

OCHRATOXICOSIS

Ochratoxicosis occurs less frequently in poultry than aflatoxicosis but is more lethal because of its acute toxicity. Ochratoxins are a family of toxic compounds consisting of three members, A, B and C, which are structurally related and are produced as secondary metabolites of several species of fungus. Ochratoxin A contaminates agricultural products and due to its accumulation in food, represents a serious threat to human and animal health worldwide (Pattison et al., 2008).

Etiology

The name "ochratoxin" derives from *Aspergillus ochraceus*, the first fungus discovered to produce this toxin. Ochratoxins are mostly produced by *Penicillium verrucosum,* but five other species of *Aspergillus* and six other species of *Penicillium* produce it as well. Ochratoxin A (OTA) is the most commonly detected and most toxic member of the family.

OTA is a common contaminant of cereals (corn, wheat, barley, oats, rye, sorghum) and peanuts, as well as soya, coffee and cocoa beans. Environmental conditions for ochratoxin production are similar to those for

aflatoxin and simultaneous contamination with both is common (Pattison et al., 2008).

Host sensibility

Young poultry are more sensitive to ochratoxin ingestion than adults and ducks are seven times more sensitive than chickens. Quail and turkeys are also more sensitive to ochratoxicosis than chickens. Variations in sensitivity towards OTA exists among avian species, as LD 50 ranges from 0.5 to 16.5 ppm body weight for ducks and Japanese quail, respectively, chickens 2-4 ppm (Pattison et al., 2008).

Pathogenesis

After resorption, the highest quantity of ochratoxin can be found inside kidneys and liver, and a considerably smaller extent in muscle. It is characteristic of poultry to have a more efficient and faster excretion of ochratoxins than other animals, approximately 48 hours.

Ninety percent of the ingested OTA is excreted. OTA in poultry diets leads to reduction in growth rate, feed consumption and feed efficiency and increased mortality. One of the profound effects of OTA is the its ability to alter the function of the immune system in avian species, causing severe leucocytopenia, impaired complement activity, reduction in immunoglobulin and several functional properties of macrophages and heterophils and finally it causes atrophy of the lymphoid organs along with depletion of lymphocytes. OTA causes enlargement of the kidney and subsequently impairing its function, therefore, considered as a nephrotoxic mycotoxin in birds (http://ntp.niehs.nih.gov).

Ochratoxin A inhibits protein synthesis, produces acute proximal tubular epithelial necrosis in the kidneys and inhibits normal renal uric acid secretion. A decrease in the concentration of proteins, triglycerides, cholesterol, calcium, phosphorus and potassium is followed by an increase in the level of uric acid and creatinine and a decrease in glomerular filtration (Elaroussi M.A , 2008).

Some authors explain how OTA inhibits respiration in mitochondria, where it acts as a competitive inhibitor of the carrier's proteins, localized on the inner membrane of mitochondria. Furthermore, it is considered that OTA represents a teratogenic agent for chickens, but not for other domestic animals (Bennett and Klich, 2003).

Clinical sings

The unspecific clinical image of chronic ochratoxicosis in poultry is followed by a decrease in egg production of laying hens, whereas, as far as broilers are concerned, their growth is hindered and conversion of food is

weakened. The egg shell often becomes thin and fragile, with different discoloration appearing on the surface.

Growth inhibition is linked with malabsorption syndrome, as confirmed by the presence of hypocarotenoidemia. The minimum amount of ochratoxin also causes reduced bone firmness and poor pigmentation.

Nephropathies are not clinically manifested, although polydipsia accompanied by a substantial amount of moist excrement appears. Acutely intoxicated birds are depressed, dehydrated and often polyuric and die in acute renal failure. Survivors will be poorly feathered, have delayed sexual maturity, increased clotting times, anaemia and immunosuppression.(Resanovic R, 2009)

Lesions

The enlargement of the liver and kidney in OTA intoxications is caused by the involvement of these organs in detoxification and elimination. Affected kidneys are white to tan, swollen, hard and may have white pinpoint urate crystals. If damage is extensive enough to cause renal failure, dehydration, hyperuricaemia and visceral urate deposition appears at kidney level. Pasty white urates are deposited on pericardial, perihepatic, peritoneal and articular surfaces. These deposits may be mistaken with inflammatory exudates but their true nature can be determined by microscopic examination. More commonly, birds survive in compensated renal failure and kidneys appear enlarged, fibrotic and pale (Biró et al., 2002).

In ochratoxicosis, hypertrophy, hyperplasia, mitosis and individual cell necrosis of proximal tubules are noted. Other lesions include a thickened basement membrane in the glomeruli and lymphoid depletion from the lymphoid organs.

In addition to the renal lesions there is mild to moderate glycogen deposition in hepatocytes, mainly at the periphery of the liver lobes at higher levels of dietary OTA (4 and 8 ppm), resulting in yellow enlarged livers. Signs of liver disease were further supported by the significant decrease in total protein, albumin and globulin. There is also some mild decrease in bursal and thymic size consistent with immunosuppression.(Herenda and Franco, 1996).

Since gross lesions observed in ochratoxicosis seem to be neither characteristic nor consistent in poultry, microscopic and ultrastructure changes in the liver, kidney and lymphoid organs can be considered the best diagnostic features for OTA toxicity. Tubular dilatation and hypertrophy, swelling of tubular epithelial cells, localized necrosis, and desquamation of the tubular basement membrane as signs of tubulonephrosis were reported

by Dwivedi and Burns (1984). The changes were usually confined to proximal convoluted tubules, but distal convoluted tubules could also be affected. Thickening of the capillary walls of glomeruli and the presence of granular eosinophilic casts in the tubular lumen were also reported. (http://en.wikipedia.org/wiki/Ochratoxin_A).

Glomerulonephrosis, tubulonephrosis, focal tubular epithelial cell proliferation and the multiplex adenoma-like proliferation of renal parenchyma are considered to be primarily related to the toxin, while focal intertubular infiltration of lymphocytes and histiocytes can also occur either primarily or secondly as reparation of tubulonephrosis or as a consequence of immune stimulation (Elaroussi et al., 2008).

The reduction seen in the number of lymphocytes in the pulpar region of the spleen has also been reported by Dwivedi and Burns, (1984), who detected a marked degree of lymphocytic depletion and obscure distinction between red and white pulp in some areas of the spleen. The lack of visible damage in heart and muscles indicates a low sensitivity of these tissues to OTA toxicity.

Prevention and control of mycotoxin formation

The best way to control aflatoxin and ochratoxin formation is to prevent the growth of fungi on harvested and stored grains and other susceptible commodities. Crops should be harvested at maturity and pre- or post-harvest mechanical damage should be avoided. Moisture contents of harvested crops should be reduced to a safe level. Moisture build-up in the stored grain should be prevented by measures such as regular aeration. Aflatoxin production can be decreased by storing food in a low-oxygen, high-CO2 environment. In areas of the southern United States, where the preferred conditions for aflatoxin production are common (25-30°C, humidity 85%), refrigeration of food is often necessary to prevent aflatoxin production.(Ritchie, 1994)

In the United States, the Food and Drug Administration, has established a tolerance of 20 ppb of aflatoxin for foods other than milk, but European markets are striving for a lower Codex importation standard of 2 ppb. (Abbas, 2005);

AFB_1 contamination is practically unavoidable, chemoprevention strategies aimed to reduce AFB_1 toxicity in poultry and in other animals have been the subject of numerous studies.(Arafa et al., 1981; Leeson et al,1995).

Several chemopreventives have been evaluated in poultry for reducing symptoms of aflatoxicosis. Because of their sensitivity, poultry have been used as models for discovering AFB1 chemopreventives.

Since 1990s, particular studies (Maciorowski et al., 2007; Wyatt, 1991) have shown the value of non-nutritive clays, such as aluminosilicates, zeolites, bentonites and clinoptilolites on aflatoxicosis prevention. They have high binding capacity against aflatoxin, reduce the absorption from the gastrointestinal tract and are generally inert, nontoxic and economical in use. Antifungal agents such as

gentian violet and propionic acid have been evaluated and appear to be most promising substances in the control of aflatoxin-producing fungi. Similarly, benzoic acid has been found to be quite effective against *A. flavus*. Other feed additives including selenium and carotenes have also been reported to have the some value in reducing the toxicity of AFB_1 in chickens and in turkeys. Also, high protein diet has been found to have protective effect against aflatoxins in chickens (Sumit et al., 2010).

The aflatoxin and ochratoxin content in food can be determined by analytical techniques such as: thin layer, gas or liquid chromatography, spectrofluorometry and spectrophotometry (Talebi , 2011). HPLC (high-performance liquid chromatography) still remains the technique of choice for aflatoxin and ochratoxin analysis. HPLC methods include HPLC with fluorescence detection and HPLC with near-ultraviolet, laser- induced fluorescence detection (near- UV LIF) (Abbas, 2005). ELISA test for poultry are available for identification of total aflatoxin and ochratoxin A. Detection of aflatoxin and ochratoxin residues in tissues requires 100 g of fresh or frozen liver or kidney. Samples for analysis should be placed in sealable plastic bags. Although not ideal, tissues from several dead birds can be pooled for analysis if necessary.(Ritchie, 1994);

 Chemical detoxification of aflatoxins (acid treatment, alkaline treatment with hydroxide, bisulfites, chlorinating compounds and oxidizing agents) in foods and feeds is important as a short-term postharvest solution to the problem. Although there are many chemical methods, ammoniation is still the most utilized and approved method for decontamination. New methods such as ozonation treatment do show promise, but require further testing for safety and scalability. With any chemical method, studies must be done to determine if new toxins are formed as a result of the treatment. It is also important to determine whether the treatment will alter the functional and nutritional characteristics of the products. (Abbas, 2005).

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