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OCHRATOXICOSIS IN MONOGASTRIC ANIMALS – A REVIEW

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin produced by several fungi of the genera *Aspergillus* and *Penicillium*, principally *P. verrucosum* in temperate climate and *A. ochraceous* in warm regions. In poultry feed materials, mycotoxins are found most commonly in cereals and to a lesser degree, in meals. The presence of OTA in animal feed contributes significantly to health disorders and decreased production. In addition to aflatoxins, which is an ubiquitously distributed toxin, OTA is one of the reasons for economic losses in the poultry industry due to poor performance and immunosuppression. Moreover, OTA has also been noted for the carryover effect in meat and tissues. This review provides the information regarding the nephrotoxic and hepatotoxic effects of OTA in monogastric animals. Histopathological studies revealed a depletion of lymphoid tissues, granular degeneration in the epithelial and mononuclear proliferation and activation of capillary endothelium cells in the kidney and liver tissue of monogastric animals. Elevated liver enzymes and blood biochemical parameters related to kidney were also observed. For the first time, this article revealed that the reduced Newcastle Disease (ND), Infectious Bursal Disease (IBD) and Hydropericardium Syndrome (HPS) vaccine titers were noticed in broilers intoxicated with OTA. There are various possible ameliorating strategies that exist; however, deactivation of OTA is more convenient as compared to adsorption techniques. In brief, to overcome the implications of toxins on animal health, there is a need of good management practices to reduce the contamination in cereals, the usage of advanced analytical techniques and establishment of guidelines for OTA in animal feed and products.

Keywords: ochratoxin A, serum chemistry, deactivation, nephrotoxicity, hepatotoxicity.

INTRODUCTION

There is a common perception that ‘human made’ chemicals are more dangerous than ‘natural substances’. Interestingly, the most toxic compounds known are natural i.e., ‘mycotoxins’. Mycotoxins are highly toxic secondary metabolites of various molds, mainly those belonging to the genera *Fusarium, Aspergillus* and *Penicillium*. Although approximately 500 mycotoxins have been described (Tapani *et al*., 2000), it has been estimated that at least 300 of these fungal metabolites are potentially toxic to animals and humans (CAST, 2003). The consumption of mycotoxins contaminated food/feed by human and animals may result in toxicity termed as ‘mycotoxicoses’. Among the various mycotoxins which contaminate agricultural commodities and cause mycotoxicoses, ochratoxin A (OTA) has been increasingly coming into focus these days. There is growing evidence that OTA might be responsible not only for clinical

and subclinical intoxications in livestock, but may also be involved in the etiology of kidney diseases in humans (Fink-Gremmels *et al*., 1995).

Chemistry of Ochratoxin A

Ochratoxins are a group of seven compounds: ochratoxin A (OTA), ochratoxin B (OTB), ochratoxin C (OTC), ochratoxin A ethyl ester, ochratoxin A methyl ester, ochratoxin B ethyl ester, ochratoxin B methyl ester and 4-Hydroxyochratoxin A. Ochratoxin A (OTA), OTB and OTC are the toxic members of the group, with OTA being the most toxic one (Raju and Devegowda, 2000). Ochratoxins are white, odorless and crystalline solids with a molecular formula of $C_{20}H_{18}Cl$. These are derived from isocoumarin and L-β-phenylalanine, and are biosynthetically classified as pentakeptide.

Ochratoxin A producing fungus

Ochratoxin A was discovered as a metabolite of *Aspergillus ochraceous* during the course of a large screening of fungal metabolites that was specifically designed to isolate and identify new mycotoxins (Bennett and Klich, 2003). Ochratoxin A is a mycotoxin produced by *Aspergillus ochraceous* (*A. ochraceous*), the first fungi from which it was isolated and after which it is named. It is also produced by many other *Aspergillus* and *Penicillium* species. Recent studies have shown its production by *A. niger* (Stander *et al*., 2000). Although, all *Aspergillus* species produce OTA, *A. carbonarius* produces the highest quantities of this mycotoxin (Pitt *et al*., 2002).

Preliminary incidence of OTA in Pakistan

In Pakistan, data on the natural occurrence of OTA in food, feed and feed ingredients are scanty. Over the last two decades, isolated attempts were made regarding OTA contamination, but more emphasis was given to aflatoxins. A brief outline of work on aflatoxins in Pakistan is mentioned in the below Table 1.

Table 1: Preliminary studies on Aflatoxin B_1 contamination in food and feed samples in Pakistan

Work Place	Commodity	No. of samples examined	No. of positive sample (%)	Aflatoxin B1 levels (ppb)	Reference
PCSIR-Karachi	Corn	239	68	4-487	Butool *et al.* (1984)
	Fish meal	197	45	4-259	Batool and Mansoor (1987)
	Blood meal	38	2	4-49	
	Meat Meal	67	4	5-38	
	Guar Meal	42	6	8-126	
	Matri	37	7	6-96	
	Red Chilli	176	116	1-79.9	Shamsuddin *et al*. (1995)
	Pistachio	300	97	4-1400	Mansoor *et al*.

	Almonds	250	32	5-148	(1995)
	Peanuts	512	158	4-723	
QAU, Islamabad	Poultry feed	-		10-55	Karim (1993)
	Cattle feed	-	-	22-58	
Rawalpindi/ Austria	Poultry feed	-	-	13.2-291	Anjum & Naseem (2000)
PRI, Rawalpindi	Poultry feed	-	40	Minicolumn/ qualitative tests	Rehman *et al*., 1994
	feed	440	22	112.0	Rehman *et al*., 2003
Romer Labs, Pakistan	Feed	41	51	2-75	Hanif et al., 2006
	Total mixed ration	135	100	0.1-230	Sultana *et al.*, 2013
	Feed	-	37.50	0.1-169	
	Silage	-	77.80	0.1-262	
	Cat food – solid	240	15	0.1 – 18	Tahira *et al*., 2015
	Cat food – semisolid	90	13.13	0.1 – 4	
	Dog food – solid	150	35.33	0.1 – 20	
	Dog food - semisolid	30	26.66	0.1 – 8	

QAU – Quaid-i-Azam University; PRI – Poultry Research Institute

Nephrotoxic potential of OTA

Ochratoxin A is known to alter kidney functions. About 70 years ago, in Denmark, porcine nephropathy was identified for the first time, and known to be associated with exposure to OTA. Ochratoxin A, a nephrotoxin, caused an enlargement and discoloration of the kidneys and a subsequent accumulation of uric acid. Histological examination shows nephropathy is characterized by atrophy and degeneration of proximal and distal tubules, and interstitial fibrosis was detected. Furthermore, Hamilton *et al.* (1982) investigated five independent episodes of ochratoxicosis in about 970,000 turkeys, two episodes in about 70,000 laying hens and two episodes in about 12,000,000 broiler chickens. The main symptoms in turkeys were mortality (up to 59 percent), nephropathy, decreased feed consumption and secondary air sacculitis. The episodes in laying hens were characterized by reduced egg production, poor egg shell quality and nephropathy. However, the episodes in broiler chickens were characterized by poor growth rate, poor feed conversion efficiency, poor pigmentation, nephropathy and increased incidence of air sacculitis.

Several studies were conducted to evaluate the toxic effects of OTA in combination with other nephrotoxic mycotoxins. Manning *et al*. (1985) studied the combined effect of citrinin (CTN) and OTA in broiler chicks fed with 300 ppm CTN and 3 ppm of OTA for three weeks. The analysis of this study revealed that birds fed both CTN and OTA in combination had lower body weights and increased water consumption. In addition, increased histological alterations including tubular casts and tubular hyperplasia in kidneys was also observed. In another study,

Glahn (1993) assessed three nephrotoxic mycotoxins, CTN, OTA and AFB_1, for their ability to alter avian renal functions. At non-lethal doses, CTN appeared to have acute reversible effects on the distal portion of the nephron, possibly acting to inhibit water absorption. Ochratoxin A was found more potent and less acute than CTN but less site-specific in that both proximal and distal tubules are damaged, resulting in a severe loss of both fluids and electrolytes.

Dwivedi and Burns (2005) stated that the target organ of OTA for poultry seems to be the kidney, as reported for other species. Nonetheless, other systems such as the liver, gastrointestinal tract, lymphoid organs, skeletal system, hemopoietic tissues and the reproductive organs can also be affected. In addition, OTA acts essentially in the proximal renal tubules, inhibiting the enzyme phosphoenolpyruvate carboxylase, which is a lipid peroxidase, and it alters the structural and functional renal ability to metabolize calcium. Morphologically, the kidney shows atrophy and sclerosis of the proximal tubules.

Stoev *et al*. (2002a,b) carried out a study to elucidate the etiology of a nephropathy syndrome of chickens encountered in Bulgaria. In this study, a close relationship was observed between the frequency of this nephropathy and the rate of nephrotoxic mycotoxin OTA in the muscles, kidneys and livers of the chicken, but the levels of OTA in corresponding feed samples (0.1-0.3 ppm) were significantly lower than the levels (2-4 ppm) required to reproduce such nephropathy. Shlosberg *et al.* (1997) found that feed from a shipment of imported corn was associated with a severe reduction in growth and increased mortality in geese and broilers. Pathological examinations revealed hepatopathy, visceral gout, and mild nephropathy in geese, while in broilers, hepatopathy, which was often severe, and ascites, was observed.

Humoral and cell mediated immune response to ochratoxicosis

In avian species, the immune system like that of mammals has developed several levels of defense strategies to cope with a wide range of pathogens, including innate immunity and adaptive immunity. Humoral mediated immunity is mediated by antibodies released by B-cells into the bloodstream is effective against extracellular antigens. Cell-mediated immunity (CMI) is based on specific antigen recognition by thymus derived T-lymphocytes and specializes in the elimination of intracellular antigens (Surai and Dvorska, 2005). In birds, precursors of T-cell and B-cell originate in bone marrow, while the actual development of T-cells takes place in the thymus and B-cells in the bursa of Fabricius and in the bone marrow of mammals. Basically, specific immunity develops due to the interactions between T- and B-cells, as well as antigen presenting cells. This specific immunity induced/stimulated defense mechanism is triggered by exposure to foreign antigen/macromolecules and increases in its intensity with each successive exposure to a particular macromolecule (Miles and Calder, 1998).

Avian immune systems (structurally and functionally) are of a unique nature. Therefore, the immune system serves as a sensitive indicator of management production impacts on poultry health, as it is directly influenced by genetic, physiological, nutritional and environmental factors. Immunosuppression is defined as a temporary or permanent state of dysfunction of immune response resulting from damage to the immune system and leading to increased

susceptibility to diseases (Yegani *et al*., 2005). Several immunosuppressive agents are affecting poultry, including infectious (viruses, bacteria, and parasites) and non-infectious (mycotoxins, drugs, nutritional deficiencies) in nature. Ingestion of mycotoxins caused clinical toxicological syndromes which have been well characterized in domestic, including poultry, and laboratory animals ranging from acute mortality to decreased performance and immunosuppression (Surai and Dvorska, 2005). Immunosuppressive potency of various mycotoxins is known to vary substantially.

Immunotoxic potential of Ochratoxin A

Immunotoxicology is a relatively new discipline, although the allergic responses to various chemicals have long been studied and well recognized. Chemicals like mycotoxins can either suppress or stimulate the immune system. Ochratoxin A has been described as an immunosuppressant fungal compound. The immunosuppressive activity of OTA is characterized by the reduction in size of vital immune organs, depression of antibody responses, alterations in the numbers and functions of antibody responses, alterations in number and function of immune cells, and the modulation of cytokines production (Al-Anati and Petzinger, 2006).

Immunotoxic effects of OTA after feeding a diet at 5 ppm for 56 days to broiler chicks have been studied. Reduced contents of *alpha*1-, *alpha*2-, *beta*-, and *gamma* globulins in plasma were observed (Rupic *et al.*, 1978). Similarly, Chang *et al*. (1979) fed broiler chicks OTA (0, 0.5, 1.0, 2.0, 4.0 and 8.0 ppm) from day one to three weeks of age and bled them for total leukocyte and differential count. Ochratoxin A induced significant leukocytopenia, i.e. the reduction in white blood cell count, which they considered primarily a lymphocytopenia, and to a lesser extent, a monocytopenia. In another study, Chang *et al*. (1981) reported the suppression of bone marrow activity and lymphoid depletion from the spleen and *bursa of Fabricius* in young chicks, and the regression of the thymus in turkey poults after an OTA treatment. Dwivedi and Burns (1984a) observed a significant decrease in lymphoid cell population of immune organ. In this study, broiler chicks were fed a diet containing OTA at a concentration of 2-4 ppm for 20 days. Furthermore, Dwivedi and Burns (1984b) observed depressed IgG, IgA and IgM levels in the lymphoid tissues and serum of chicken fed diets containing OTA at a concentration of 2-4 ppm for 20 days. Similar findings were observed by Harvey *et al*. (1987). In this study, 2.5µg of OTA was injected in the *bursa of Fabricius* of chick embryos at day 13. In response, reduced IgG and increased IgM were observed. This did not affect their immunocompetance. However, after challenging the hatched chicken with *E. coli* at 1, 2 and 4 weeks of age, a transient effect on immunoglobulins was noticed. Similarly, the complement activity was slightly affected in birds fed diets containing 2 ppm of OTA for 5-6 weeks (Campbell *et al*., 1983).

In monogastric animals, OTA is known to cause regression and cellular depletion of major lymphoid organs, resulting in a significant effect on cellular immunity (Devegowda and Murthy, 2005). Previously, Singh *et al.* (1990) studied the effects of dietary OTA (0.5-2 ppm) on humoral and cell-mediated immunity in broiler chickens and observed highly significant ($P<0.05$) reduced CMI in terms of diminished skin sensitivity, graft versus host reactions and T-

lymphocyte counts. Total lymphocyte counts, total serum proteins, serum albumin and serum globulin were significantly depressed on the twenty-first day of intoxication. The weights of the thymus, *bursa of Fabricius* and spleen were significantly reduced.

Elissalde *et al*. (1994) studied the pathological effects in young male broilers of *S. typimurium* in the presence or absence of 3 ppm of OTA in the diet. OTA not only altered the serum concentrations of proteins, enzymes, calcium and phosphate salts, but also reduced microcytic and hypochromic erythrocytes; decreased phytohemagglutin and concanavalin A-stimulated blastogenesis was investigated. Decreases in phytohemagglutin and concanavalin A-stimulated blastogenesis is an indication of a decrease in immunity. In another study (Raju and Devegowda, 2002), the dietary effect of AFB_1 (0 and 0.3 ppm) and OTA (0 and 2 ppm), T-2 toxin (0 and 3 ppm) in the presence or absence of esterified glucomannan (0 and 1 percent) on the immunity status of 960 commercial broilers was investigated. The thymus size was reduced by AFB_1 and T-2 toxin and the bursa by AFB_1. The significantly decreased antibody titers were observed against Newcastle Disease (ND) and Infectious Bursal Disease (IBD). Esterified glucomannan significantly improved the antibody titers indicating its ameliorating efficacy against immunosuppression caused by multiple mycotoxins. In another study, the effect of OTA at 0.5 and 1 ppm in the presence and/ or absence of a toxin deactivator on the histology of the bursa of Fabricius, liver and kidney (Hanif et al., 2008) was investigated. This exposure to OTA in the presence or absence of the toxin deactivator reduced their humoral immune response to various vaccines (Hanif and Muhammad, 2015).

Effect of OTA on hematology and serum chemistry

Various studies have been conducted and changes in various hematological and serum biochemical parameters have been observed by some authors. These studies revealed OTA as an inducer of hypochromic-microcytic anemia of iron deficiency type in broilers fed dietary OTA levels of 8 ppm for three weeks. The packed cell volume and hemoglobin concentrations were significantly decreased. Moreover, the influence of OTA at 2 ppm and T-2 toxin at 4 ppm singly and in combination on broiler chicks reported an additive effect of the two toxins resulting in decreased hemoglobin concentration, reduced corpuscular volume, and the activity of serum alkaline phosphatase (ALP), but also increased activity of gamma-glutamyl transferase (GGT) Khan *et al*., 2014). It also decreased the concentrations of serum total protein, as well as the concentration of albumin and cholesterol, and increased the concentration of serum creatinine and uric acid. According to Kubena *et al.* (1994), the effect of feeding 2 ppm OTA and 6 ppm of DAS of the diet singly and in combination in male broiler chicks (1-19 day of age) were characterized by toxicity, as well as antagonism in male broiler chicks from 1 to 19 d of age. There was a significant antagonistic interaction between OTA and DAS for uric acid and cholesterol. When compared with controls, additive toxicity was exhibited by reduced efficiency of feed utilization, increased relative weights of the liver and gizzard, and a decreased concentration of serum total protein, mean corpuscular volume and mean corpuscular

hemoglobin. In another study, OTA caused significant increases in the recalcification time of clotted blood and the prothrombin time in a dose related fashion due to reduction in fibrinogen levels in the blood (Doerr *et al*., 1981). Elissalde *et al*. (1994) investigated the pathological alterations of young male broiler chicks by *S. typhimurium* in the presence (3.0 ppm) or absence of OTA in the diet. OTA altered the serum concentrations of proteins, enzymes, calcium and phosphate salts. Birds fed diets containing OTA had microcytic and hypochromic erythrocytes and a decrease in phytohemagglutin and concanavalin A-stimulated blastogenesis. In a trial, Agawane and Lonkar (2004) investigated the toxicopathological effects of OTA (0.5 ppm) on the hematobiochemical parameters of broiler chickens.

Hematological studies revealed significant decreases in hemoglobin and packed cell volume in the group fed OTA contaminated feed. Biochemical analysis revealed decreased values of the total protein, albumin, globulin and increased levels of serum creatinine and serum (Glutamate Pyruvate Transaminase) GPT as compared to control group. Moura *et al.* (2004) evaluated alterations in the qualitative cellular profiles of leukocytes associated with the administration of a low dose of OTA (0.04 ppm of body weight) in poultry. Differential leukocyte counting demonstrated that OTA reduced the percentage of lymphocytes and eosinophils and significantly increased the number of heterophils and monocytes. Similarly, Janaczyk *et al*. (2006) evaluated the effects of OTA administration on the function and morphology of chicken blood leukocytes for two different levels of OTA (6 ppm for 10 days and 6 ppm for 20 days). After 10 days of administration of OTA, an increase of hematocrit (PCV), characteristics of stress, and a decrease of the lymphocyte percentage with a simultaneous increase of heterophils percentage was observed. Prolonged administration of the OTA to chicks for a further 10 days led to a withdrawal of the mentioned leukogram changes, decrease of PCV value and decrease of hemoglobin level. In a toxicity trial, graded dietary doses of OTA given to young chicks for three weeks from hatching, resulted in a positive correlation between pathohistological changes in kidney and increases in Lactate Dehydrogenase (LDH) activity caused by different doses of OTA (Jelena *et al*., 2005). In later studies, Bhanuprakash *et al*. (2006) investigated the effect of OTA (1 ppm) and AFB_1 (0.5 ppm) on the serum biochemical parameters and found a decrease in total protein and albumin. Elevated serum GGT and ALP values were noticed in birds fed with OTA, as compared to the control birds.

Pathohistological changes associated with ochratoxicosis

A trial was carried out to investigate the ultrastructural changes in experimentally combined OTA and penicillic acid (PA) intoxication in one hundred broiler chickens fed a diet containing 130, 300 and 800 ppb OTA and 1000-2000 ppb PA. The main ultrastructural changes were in the epithelium of the proximal tubules in the kidneys (slight edema and degenerative changes in capillary endothelium), slight changes in hepatocytes, and pronounced mitochondrial damage and loss of the membrane integrity of cell organelles leading to death (Stoev, 2000a). Stoev *et al.* (2002) conducted another trial to study the progression of coccidiosis (inoculation

with *Eimeria tenella*) and the resultant mortality in chicks fed an OTA-contaminated diet (5 ppm). Ochratoxin A induced degenerative changes an increase in the weight of the kidneys, liver, heart and ventriculum. Other significant effects included a depletion of lymphoid tissue and a decrease in the lymphoid organs' weight and the body weight. The intensity of clinical signs, impairment of kidney functions, histopathological changes and deviations in growth depression were greater in chicks infected with *E. tenella* and OTA. Furthermore, Stoev *et al.* (2004) studied the combined effect of OTA and PA on the body mass, weight and pathomorphology of some internal organs in 85 broiler chickens fed a moldy diet containing 130, 300 or 800 ppb OTA and 1000-2000 ppb PA. In this study, the main pathomorphological changes were cloudy swelling and granular degeneration in the epithelium, mononuclear cell proliferation and the activation of capillary endothelium in the kidney and liver.

In several studies, different sequesting agents have been used for neutralizing the toxic effect of OTA in poultry. Nedeljkovic-Trailovic *et al.* (2001) devised a study to examine the protective effect of modified clinoptilolite (0.5%) on the adverse effects of OTA (1 ppm) in 42 day long, 36 day old broiler chicks. The kidneys of birds only exposed to OTA showed that the proximal tubules of the kidneys were prominently affected with microgranulation of cytoplasmic tubulocytes and masked nuclei. Morphological alterations in kidney samples of groups offered OTA in combination with modified clinoptilolite were expressed in intracellular edema. In another study, aluminosilicate as an adsorbent was used to evaluate the effect of dietary OTA in the presence or absence of aluminosilicate on the histology of the bursa of Fabricius, liver and kidney. The exposure of birds to 2 ppm OTA in the presence or absence of aluminosilicate reduced their humoral immune response and number of mitotic cells in the bursa. In the liver, microscopically, there was hepatocytes vacoulation and megalocytosis with accompanying hyperplasia of the biliary epithelium. Kidneys showed hypertrophy of the renal proximal tubular epithelium with thickening of the glomerular basement membrane (Santin *et al*. 2002).

Biro *et al*. (2002) revealed microscopic changes that could primarily be associated with toxin exposure, including glomerulonephrosis, tubulonephrosis, focal tubular epithelial cell proliferation and multiple, adenoma-like structure in the renal parenchyma. Kumar *et al*. (2004) conducted a study to evaluate the effects of OTA on *Escherichia coli*-challenged broiler chickens. The group fed with a mash diet containing 2 ppm OTA, showed changes in the kidneys including swollen proximal convoluted tubules, degeneration of tubular epithelium and interstitial nephritis. Degenerative changes and mononuclear cell infiltration were recorded in the liver, while atrophy of the lymphoid organs, along with the depletion of lymphocytes, was also observed. Balachandran (2006) showed histopathological changes, and observed degeneration and necrosis of hepatocytes and periportal fibrosis in the livers of intoxicated broilers. However, their kidneys showed a degeneration of the tubular epithelium and a thickening of the basement membrane of the glomeruli.

Interaction of OTA with infectious diseases

In the animal industry, the potential for mycotoxins to interfere with the development of immunity is of great interest. Several mycotoxins, including aflatoxins, OTA and mycotoxins of Trichothecenes group, are typically associated with interference of resistance to infectious diseases. However, it is difficult to discern their role, because the casual involvement of mycotoxins is often overshadowed by the infectious diseases, and thus is not considered in the overall syndrome (CAST, 2003). In fact, these mycotoxins are known to inhibit protein synthesis and cause alterations of the blood system including the bone marrow (Wyatt, 2005).

Elissalde *et al*. (1994) studied the effect of OTA (3 ppm) on *Salmonella typhimurium* (1 x 10^6 cfu) challenged broiler chicks. This study revealed that *S. typhimurium* alone had no effect on the variables measured except for the decrease in the body weight. With the exception of an increase in mortality and a decrease in body weight, *Salmonella* in combination with OTA did not alter the values of the remaining variables measured from those measured in the OTA diet alone. Cecal colony count of *S. typhimurium* was not affected by treatment with OTA. In another study, Singh *et al*. (1994) described the effect of feed mycotoxins, i.e. AFB_1 (1.25 ppm for 3 to 38 days of age) and OTA (from 3 to 38 days of age), along with Inclusion Body Hepatitis Virus (IBHV) (at 10 days of age) singly and in combination in broiler chicks. Birds in combined treatment groups showed more changes in activities of phosphatase (AKPase, ATPase, and ACPase) and oxido-reductases in the liver and kidney tissues than their individual treatment groups. Muco-polysaccharides reaction was more marked in both the combined treatment groups, than in the single treatment group. Intensity of lipid reaction was more in the ochratoxin-virus combination group, than in either group alone.

The findings of Fukata *et al*. (1996) revealed that OTA at the level of 3 ppm was observed to be one of numerous factors that affect the susceptibility of chicken to *Salmonellae typhimurium* colonization. The number of *S. typhimurium* in both duodenal and cecal contents of chickens administered high doses of OTA increased significantly when compared with control birds. Stoev *et al*. (2002) studied the clinicomorphological effects in boilers chicks fed an OTA (5 ppm) contaminated diet while simultaneously developing coccidiosis (2 x 10^4 oocytes of *Eimeria tenella* per chick). The intensity of the clinical signs, the impairment of kidney function, macroscopic and histopathological changes, deviations in the weights of some organs and the general depression in growth were greater when chicks infected with *E. tenella* were also given OTA. Similarly, in another study, the mortality and severity of *S. gallinarum* infection in broiler chicks was increased significantly by the presence of OTA in the diet (Gupta *et al*., 2005).

Different types of vaccines are being used to prevent or reduce problems that can occur when a poultry flock is exposed to field infectious diseases. Mycotoxins of various types are a contributing factor in reducing the immunity and thus, are likely to increase the susceptibility of birds to infectious diseases and reduces the responses to vaccines (Hanif and Muhammad, 2015). Stoev *et al.* (2000) studied the combined effect of OTA (at dietary levels of 130, 305 and 790 ppb) and PA in the presence and or absence of water of artichoke extract (5 percent) on humoral immune response against the ND vaccine in 100 broiler chicks. A serological investigation revealed lower hemagglutination inhibiting antibody titers in chicks of groups (receiving 305 and 790 ppb OTA) immunized with the vaccine against Newcastle disease, than in the control group. A significant protective effect of artichoke extract on the humoral immune response and other clinical changes induced by OTA was established. Raju and Devegowda (2002) conducted an experiment of a 5-week duration to study the effects of two dietary levels each of aflatoxin (AFB_1) (0 and 300 ppb), OTA (0 and 2 ppm), T-2 toxin (0 and 3 ppm) and esterified glucomannan (0 and 0.1 percent) on the immune competence of a total of 960 day-old commercial broilers. Reduction in the size of the thymus (by AFB_1 and T-2) and the bursa (by AFB_1), and antibody titers against ND and IBD (by all toxins) were observed. Esterified glucomannan significantly improved antibody titers, indicating its counteracting efficacy against immunosuppression in mycotoxicosis of multiple origins.

Santin *et al.* (2002) aimed to evaluate the effect of ochratoxin (2 ppm) in the presence or absence of aluminosilicate (0.25 percent) on the humoral immune response of broilers immunized against ND. The exposure of birds to OTA, in the presence or absence of aluminosilicate, reduced their humoral immune response and the number of mitotic cells in the bursa. In another trial, Gounalan *et al*. (2006) induced experimental ochratoxicosis in vaccinated layer chickens by feeding them a diet containing 0.25 ppm OTA from 0 to 14 weeks of age for ND interaction studies. Lower humoral immunity was observed in the vaccinated OTA group. The OTA fed birds were immunocompromised, even if adequately and predisposed to ND. Similarly, for assessment of the mycotoxin-NDV interaction, Balachandran (2006) induced mycotoxicosis by feeding a ration containing AFB_1 (0.1ppm), OTA (0.25 ppm) and T-2 toxin (0.5 ppm) from 0 to 14 weeks of age in layer chicks vaccinated against NDV-F, NDV LaSota and NDV-K at 7, 28 and 56 days of age, and challenged with velogenic NDV three weeks after each vaccination. The HI titers to NDV decreased significantly in the vaccinated mycotoxin fed groups. Koynarsky *et al.* (2007) investigated the progression of coccidiosis in turkey poults provoked by *Eimeria adenoeides* (4×10^5 oocytes per turkey) in the presence or absence of OTA (2 ppm) in feed. A rapid progress of coccidiosis occurred in OTA-treated turkey pouts than in those fed an OTA free diet. Coccidiosis in the presence of OTA further induced growth depression, impaired kidney functions, caecal hemorrhages and histopathological changes in certain body organs, as well as causing a depletion of lymphoid tissues. In a recent study, it was noticed that intoxication of OTA in broilers, significantly reduced HI titers of ND, IBD and HPS vaccine with declined weights of the bursa of Fabricius were observed (Hanif and Muhammad, 2015).

Carry-over effect of OTA

Ochratoxin is widely detected as a contaminant of agricultural commodities and that is further transmitted to animals and humans. Several studies determined and estimated OTA levels in the internal organs, blood, muscles, eggs of poultry, kidneys of cows and kidneys, liver, muscle, fat, blood of pigs (Table 2). As can be seen in this table, OTA tends to concentrate in tissues and organs.

Table 2: Transmission of Ochratoxin A from Feed to Tissues & fluids of animals

Species	OTA levels in feed (ng/g)	Tissues	Contamination levels (ng/g)	Reference
Cow	1125	Kidney	5	Hult *et al*. (1976)
Chicken	0.5-5.0	Liver	124	Frye and Chu (1977)
		Kidney	80	
		Breast	8	
		Leg	7	
		Eggs	2.8	
Swine	Porcine nephropathy	Kidney	ND-23	Golinski *et al.* (1984)
		blood	3-270	
***Broiler Chicken**	50-2000	Kidney	0.8	Kuiper-Goodman and Scott (1989)
		Liver	11-59	
		Muscle	3.0-8.5	
		Blood	1.2-4.6	
***Laying Hens**	50-5200	Kidney	3.8-8.0	Kuiper-Goodman and Scott (1989)
		Liver	1.5-18	
		Muscle	≤0.8-2.7	
		Blood	4.0-14	
		Eggs	1.6-4	
***Pigs**	25-1400	Kidney	1.8-67	Kuiper-Goodman and Scott (1989)
		Liver	2-30	
		Muscle	ND-37	
		Fat	ND-11	
		Blood	665	
***Cow**	-	Milk	ND	Valenta and Goll (1996)
Sheep	0-5000	Serum	8.2-111.7	Holer *et al*. (1999)
Cat	General survey (natural contamination)	Kidney	0.35-1.5	Razzazi *et al* (2001)
Rabbit	1000-2000 (body weight/day)	Kidney	1.2g/kg	Ferrufino-Guardia (2000)
		Liver	0.158	
		Mammary gland	0.105	
		Muscle	0.038	
Pig	General survey	Serum	13.4	Curtui e*t al.* (2001)

	(natural contamination)	Kidney	3.18	
		Liver	0.61	
		Muscle	0.53	
Bull	-	Liver	145	Mahmoud *et al.* (2001)
Poultry	-	Liver	ND	
Swine	Porcine nephropathy	Kidney	0.29	Matrella *et al.* (2006)
		Muscle	0.024	
Poultry	0.5 & 1 ppm	Serum	27.80	Hanif et al. (2012)
		Liver	1.98	
		kidney	12.29	

**Pohland et al. (1992)*

Effect of OTA on performance of broiler chicken

Several studies have been undertaken to determine the toxic effect of OTA in different animal species. In several toxicity trials, graded doses of OTA (0 to 4.0 ppm) given to broiler chicks for 6 weeks from hatching, resulted in depressed growth, poor feed conversion ratio, enlargement of the kidneys, liver, proventiculus, regressed spleen and bursa, and mortality. The minimum growth inhibiting level was 2 ppm (Gibson *et al*., 1990).

Gentles *et al*. (1999) investigated the effects of OTA and cyclopiazonic acid (CPA) on the performance of broiler chickens. A group of 20 broiler chickens were fed a diet containing OTA alone at 0 or 2.5 ppm, or in combination with CPA for 3 weeks. A significant ($P<0.05$) reduction in body weight gain was observed by the second week of feeding and continued at the third week (19 percent). The relative weights of the kidneys were increased in groups only fed OTA, and a significant increase in serum uric acid and triglycerides, but decreased total proteins, albumin and cholesterol were also seen. In another study, Verma *et al*. (2004) studied the effects of dietary AFB_1 (0.5, 1.0 and 2.0 ppm), OTA (1.0, 2.0 and 4.0 ppm) or combinations of these on body weight gain, feed efficiency, organ weight and immune response. Significant growth depression, reduced feed consumption and poor feed conversion efficiency were recorded in broilers fed a diet containing the two higher concentrations of AFB_1 (1 and 2 ppm) and OTA (2 and 4 ppm). In a recent study, Elaroussi *et al*. (2006) reported that feeding OTA, even at low levels, as compared to previous studies, (at levels of 400 and 800 ppb) for 1-5 weeks of age resulted in a significantly decreased body weight, thymus weight, feed consumption, feed conversion ratio (FCR) and thyroxine concentration.

Deactivation/ Detoxification of OTA

Since the first report of mycotoxicosis early in the 1960s, scientists from all over the world have been meticulously searching for methods to eliminate or minimize the effects of these inevitable natural contaminants. The simplest strategy is based on the prevention of the

formation in the feed. Even then, in the presence of latest technologies, it is very difficult to predict their occurrence either pre-harvest or during storage and feed processing. Mycotoxin detoxification means to methods by which the toxic properties of mycotoxins are removed.

a. Chemical detoxification methods

Different chemical methods have been tried to minimize the toxic effects of mycotoxins. Activated charcoal (AC) may be considered an efficacious product to decontaminate foodstuffs containing aflatoxins (Galvano *et al*., 2001), except a study in which activated charcoal induced at 0.5% of diet was not effective in reducing toxicity of AFB_1, but rather enhanced the toxic effect. Albeit, the mechanism was not clear, but it was hypothesized that it might be due to the chemical alteration of the sites and the rates of sorption and desorption of AFB_1 (Kubena *et al*., 1990). In other study, AC was used *in vivo* and *in vitro* as an antidote for lethal doses of OTA. The findings revealed AC as an impractical method for reducing OTA toxicity in poultry chronically exposed to OTA (Rotter *et al*., 1989).

Several scientists stated the ameliorating role of vitamin C against OTA. Haazele *et al*., (1993) conducted three experiments to study the beneficial effects of dietary vitamin C supplementation on layer hens subjected to OTA toxicosis under normal and high ambient temperatures. All the negative effects of OTA, apart from body weight changes, reductions in feed intake, and increases in egg shell elasticity at 33°C were either moderated or significantly *($P \leq 0.05$)* reversed by dietary ascorbic acid supplementation. While Toos *et al.* (2003) observed that vitamin C has a lesser pronounced antioxidant effect than those obtained with vitamin E. Better results were obtained in groups receiving a combination of vitamin E and C. *In vitro* mycotoxin adsorption capacity of 8 agents as reviewed by Huwig *et al.* (2001) has been presented in Table 3. Similarly, Table 4 depicts *in vivo* adsorption of OTA by 5 different adsorbents.

Table 3: *In vitro* adsorption of mycotoxins by different adsorbents

Adsorbents	Mycotoxins	Adsorption capacity (mg/g)	Reference
Activated charcoal	OTA	100.0	Bauer (1994)
Activated charcoal	OTA/tri	124/9.9	Galvano et *al.* (1998)
Phylllosilicates	AF/OTA/ZON	0.03-0.44	Schall *et al.* (2000)
Diatomaceous earth	AF/OTA/ZON/tri	0.5-1.5	Natour and Yousaf (1998)
Bentonite	OTA	1.5-9.0	Bauer (1994)
HSCAS	OTA	0-2.2	Bauer (1994)
Yeast (40% sterilized yeast, 60% fermentation residue of beer production)	OTA	1.2-8.6	Grunkemeier (1998); Bauer (1994)

Modified yeast cell walls extracts	AF/OTA/ZON/tri	0.2-1.9	Howes and Newman (2000)
Cholestyramine	OTA	9.6	Bauer (1994)
Modified Clinoptilolite	AF/OTA	0.05-0.3; 35 %	Zlatan and Resanovic (2005)

afla, aflatoxin; OTA, ochratoxin A; ZON, Zearalenone; tri, trichothecenes; HSCAS, hydrated sodium calcium aluminosilicates (Adapted from Huwig *et al*., 2001)

Table 4: *In vivo* adsorption of ochratoxin A by different adsorbents in monogastric animals

Adsorbents	Concentration (%)	Effects Observed	References
Activated charcoal	10.0	OTA - Significant reduction of the och concentration in blood, bile and tissues of pigs	Bauer (1994)
HSCAS	0.5	Afla/OTA - Growth inhibitory effects on chickens diminished by 65%, no effect against toxicity of OTA, little effect against toxicity of combined toxins	Huff *et al*.(1992)
HSCAS	1.0	OTA – No significant effects (pigs)	Bauer (1994)
Bentonite	1.0/10.0	OTA – No significant effects (pigs)	Bauer (1994)
Yeast (40% sterilized yeast, 60% fermentation residue of beer production)	5.0	No reduction of OTA concentration in blood, bile and tissues of pig	Bauer (1994)
Cholestyramine	1.0	No reduction of OTA concentration in blood, bile and tissues of pig	Bauer (1994)

(Adapted from Huwig e*t al*., 2001)

A Hydrated Sodium Calcium Aluminosilicate (HSCAS) from natural zeolite is the most widely studied mycotoxin sequestering agent among the mineral clay. Santin *et al*. (2002) fed broiler chickens ochratoxin A at the level of 2 ppm in the presence or absence of HSCAS (2.5 percent) on the histology of some organs and on the humoral immune response of broilers vaccinated against NDV. Aluminosilicate did not ameliorate the deleterious effects of OTA. Similarly, in another study, Watts *et al*. (2003) evaluated the effects of feeding combinations of mycotoxins (1 ppm DON, 5 ppm moniliformin, 5 mg fumonisin B_1, 100 µg, 1mg ZON, and 0.5 mg OTA of diet) in broiler chicks and turkey poults in the presence or absence of HSCAS (0 or 1 percent) for 21 days. The addition of HSCAS to diets containing multiple mycotoxins did not prevent the negative effects observed in chicks and poults. Furthermore, Bhanuprakash *et al*. (2006) evaluated the alleviating role of HSCAS on the toxic effects of AFB_1, OTA and their combination in feed given to 240 day old broiler chicks. This study revealed no protective effect but reduction in serum enzymes GGT and ALP was noticed.

b. Biological detoxification methods

i. By herbal extracts

Stoev *et al*. (2000) studied the effect of an extract of artichoke on the immunity and health of 100 broilers fed OTA (at dietary levels of 130, 305 and 790 ppb). A statistically significant protective effect of 5% total water extract of an artichoke on humoral immune response (increase of haemagglutination inhibiting antibody titer), relative organ weight, as well as pathomorphological, hematological and biochemical changes induced by OTA was established. Similarly, Pathan *et al*. (2006) undertook a project to study the effect of Bio-Bantox® (@ 5 kg/ ton of feed) in the presence or absence of OTA (1 ppm) from day 1 to 42 of age, on growth and serum biochemical parameters. The findings of this study revealed a significant ($P<0.01$) ameliorating effect of Bio-Bantox® on FCR and serum values of protein, albumin, globulin, albumin/globulin ratio, cholesterol and uric acid. In the latest study, Sakhare and co-workers (2007) conducted a trial to study the protective role of a polyherbal feed additive (Toxiroak® @ 0.75 g/kg of feed) on the performance of broiler chicks during induced mycotoxicosis (AFB_1 @ 0.2 ppm; OTA @ 0.2 ppm) in 240 day-old broilers. Findings of the present study reflected the significant effect of mycotoxins singly or in combination on body weights, reduced serum proteins, cholesterol, triglycerides and raised creatinine and uric acid. The body weight, hemoglobin and total leukocyte counts, percentage changes in organ mass and impaired immune response were protected by Toxiroak®.

ii. *By yeast*

Initially, Stanley *et al*. (1993) observed improved performance of poultry by using a yeast cell culture based on the *Saccharomyces cerevisiae* strain 1026. This study led other researchers to hypothesize that the yeast culture had an ability to detoxify mycotoxins. In later studies, researchers identified that specific cell wall i.e. glucomannan (GM) component in yeast interacted with mycotoxins. Raju and Devegowda (2000) described the individual and combined effects of AFB_1 (0, and 3 ppm of feed), OTA (0 and 2 ppm), T-2 (0 and 3 ppm) and esterified glucomannan (E-GM 0 and 1 g/kg) in 960 broiler chicken from 1 to 35 d of age. The findings revealed possible beneficial effects on mycotoxicosis in broiler chickens by increasing the body weight (2.26 percent) and food intake (1.6 percent), decreasing weights of the liver (32.50 percent), adrenal (18.9 percent) and activity of serum GGT (8.70 percent), and elevating serum protein (14.7 percent), cholesterol (21.9 percent), BUN (20.80 percent) and hemoglobin (3.10 percent) contents. Similarly, in another study, Raju and Devegowda (2002) evaluated the *in vitro* binding efficacy of esterified glucomannan (0.10 percent) on AFB_1 (330 ppb), OTA (2 ppm) and T-2 toxin (3 ppm) when present alone or in combination. Esterified glucomannan showed significantly higher binding with AFB_1 (81.6 percent), whereas those recorded with T-2

(27.80 percent) and OTA (25.6 percent) were moderate. Santin *et al*. (2003) evaluated that OTA in the level used in the diet impaired the feed intake, weight gain and feed conversion of the birds, and the CWSC did not ameliorate these parameters in the presence of OTA. Agawane and Lonkar (2004) investigated the toxicopathological effects of OTA (0.5 ppm) on the hematobiochemical parameters of broilers with efficacy of the dietary concentration of the probiotic yeast culture *Saccharomyces boulardii* (10 mg/kg of feed). Biochemical profiles revealed a significant improvement in the probiotic treated group when compared with decreased values of total protein, albumin, globulin and increased levels of serum creatinine and SGPT in birds fed OTA.

iii. By rumen microflora

Kiessling and co-workers (1984) have studied the effect of rumen microbes on six mycotoxins (AFB_1, OTA, ZON, T-2 toxin, DAS and DON) by incubating mycotoxins with rumen fluid/rumen protozoa and bacteria from sheep and cattle in the presence or absence of feed. The findings of this study revealed that rumen fluid had no effect on AFB_1 and DON, but the remaining four mycotoxins were all metabolized, and protozoa were more active than bacteria. For Trichothecenes, several authors described the de-epoxidation reaction of ruminal or intestinal flora (Kollarczik *et al*.1994), but Binder *et al.* (2000) were the first to isolate a pure bacterial strain (a new strain of *eubacterium*) that is able to biotransform ep-oxide ring of Trichothecenes into diene (Figure 1). This bacterial strain was named after the research team (Binder, Binder, Schatzmayr, Heidler). This bacterial strain, belonging to eubacteria, is a gram positive anaerobic microbe, none motile, non-spore forming, rod shaped irregular bacterium.

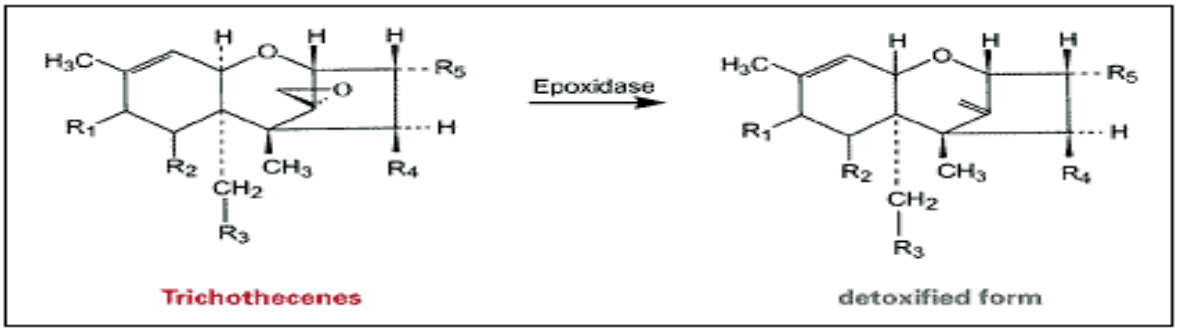

Figure 1: Biotransformation of trichothecenes to non-toxic metabolites.

Binder *et al.* (2000) conducted *in vitro* experiments with pig intestines to optimize the cell count of final product; 3.55 x 10^6 cfu/g of cell counts were found for 100 percent transformation of Trichothecenes into less toxic de-epoxy metabolites. Schatzmayr and coworkers (2002) continued their work and used crude enzyme preparations and pure carboxypepsidase A for degradation of ochratoxin to non-toxic products. Furthermore, Kiessling *et al. (*1984) documented that rumen protozoan are responsible for the detoxification of

OTA in rumen fluid. Schatzmayr *et al*. (2002) clearly demonstrated that bacteria play an important role in detoxification by applying several enrichment methods and isolation procedures resulting in the isolation of two bacterial spp. (related to *Clostridium sporogenes* and *lactobacillus vitulinus*) from rumen fluid, which were capable to cleave OTA into a nontoxic metabolite ochratoxin α (OTα) and phenylalanine (Figure 2). Besides bacterial strains, yeast genera *Trichosporon, Rhodotorula* and *Cryptococcus* were also investigated for their ability/activity to detoxify OTA.

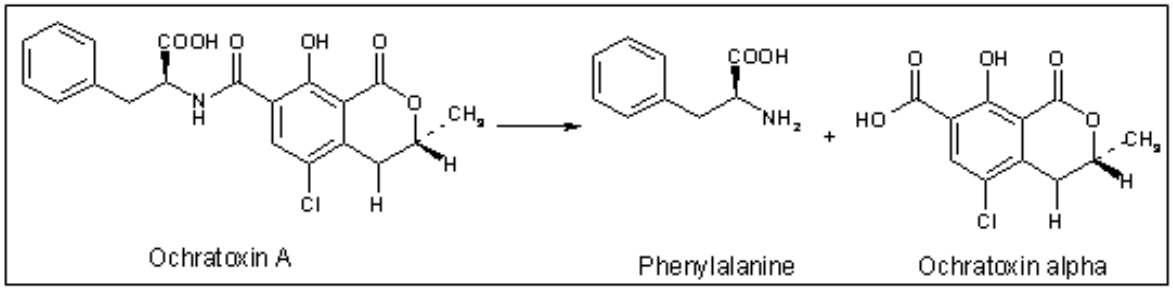

Figure 2: Cleavage of OTA into OTα and Phenylalanine (Courtesy Biomin, Austria)

As a consequence of a very comprehensive selection process, a novel yeast formed the hind gut of the lower termite *Mastotermes darwiniensis* (*Mastotermitidae*), capable of degrading OTA and ZON was isolated and characterized. This yeast was named *Trichosporon mycotoxinivorans (Trichosporon* MTV 115) due to the yeast affiliation to the genus Trichosporon and its property to degrade mycotoxins (Bruinink *et al*. 1999; Schatzmayr *et al*. 2003 & 2004; Molnar *et al*. 2004; Schatzmayr *et al*. 2006).

Legislation for Ochratoxin A

By the end of 2003, on a worldwide basis, at least 99 countries had mycotoxin regulations for food and/or feed representing 87% of the world's inhabitants. All countries with mycotoxin regulations have at least regulatory limits for $AflB_1$ or total afaltoxins (B1+B2+G1+G2). Similarly, for several other mycotoxins (like AfM_1, Trichothecenes, ZON, Patulin, Fumonisins, OTA, etc.) regulatory limits exist as well. Pitt and Hocking (2003) stated that Asia/Oceania cover the most of the globe, with most of the countries in tropical and subtropical regions. Therefore, it is supposed/expected that these regions, except New Zealand (which has a temperate to cool climate and separate mycotoxin problem form Asia and Australia) are at high risk for most mycotoxin problems caused by those fungi which grow at higher temperatures (Figure 3).

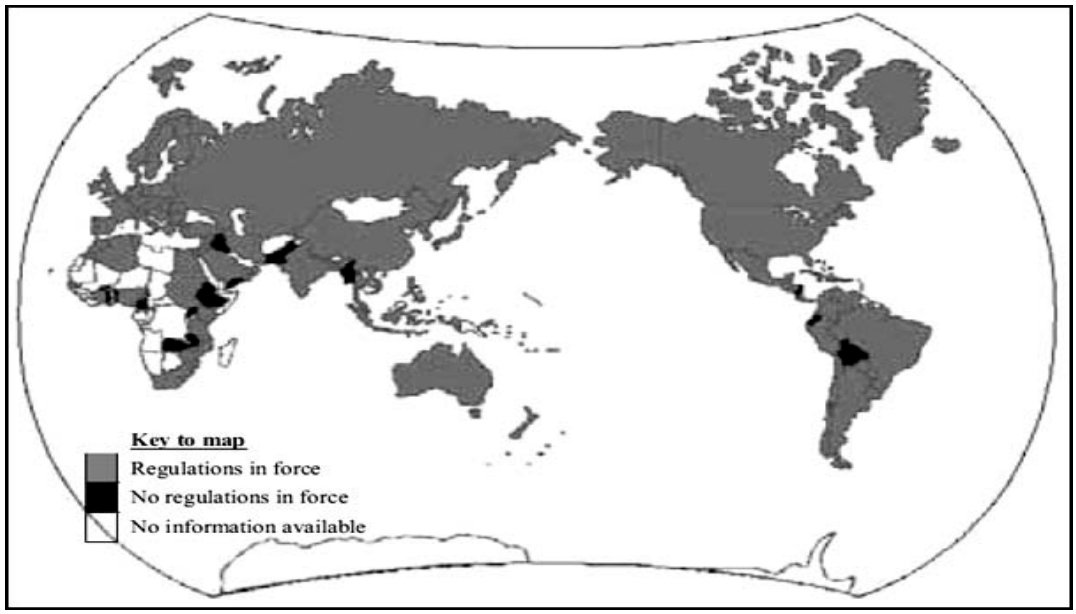

Figure 3: Worldwide Regulations for Mycotoxins (Courtesy FAO, 2004)

The presence of chemical contaminants/undesirable substances in feed is controlled by the EC Directive 2002/32 and endowment for its application is made by the Animal Feed (England) Regulations 2010 and by parallel legislation in Scotland, Wales and Northern Ireland. This Directive sets maximum permitted levels (MPLs) for contaminants that are present in animal feed that pose a potential danger to animal or human health or to the environment, or could harmfully affect livestock production. Presently, aflatoxin B1 is the only toxin for which MPL is set under directive 2002/32. Furthermore, guidance values have been sanctioned for a further five mycotoxins under Commission Recommendation 2006/576/EC, i.e. deoxynivalenol, zearalenone, ochratoxin A and fumonisins B1 and B2. These mycotoxins cause a risk to animal health and can affect livestock production for several species; however, the risk to public health is considered low. Food from animal origin only contributes marginally to the total human exposure to these toxins. (Table 5).

Table 5: Maximum levels of Ochratoxin A as set by Commission Regulation 2006/576/EC in animal feed

Feed Material	Guidance value in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12%
Cereal and cereal products	0.25
Complementary and complete feedingstuff for poultry	0.1

Note: 'Cereals and cereal products' includes other feed materials derived from cereals, in particular cereal forages and roughages.
'Maize and maize products' includes other feed materials derived from maize, in particular maize forages and roughages.

Conclusion

Ochratoxin A has been spotlighted due to its possible carcinogenicity (nephrotoxicity) declared by the International Agency of Research on cancer (IARC). The main source of OTA is cereal grains. Moderate environmental conditions are suitable for the growth of OTA producing fungi, i.e. *Aspergillus and Penicillium*, species. Farm animals are usually fed on the ration that is mainly prepared form cereals and industrial by-products. Therefore, these animals are at risk of exposure to these toxins. Several *in vitro* and *vivo* studies revealed the physiological effects of OTA on monogastric animals, like nephrotoxicity, immunosuppression, etc. Co-administration of OTA with deactivators attenuated the undesirable alterations associated with the feeding of OTA. Moreover, this review provides a strong base for comprehensive research to be carried out to fully assess the virulence factor of OTA, interaction of OTA with other mycotoxins, drugs, nutrition and risks of OTA exposure due to its occurrence in a variety of foods and feeds in local environment. Furthermore, the need of the present time is to develop legislation for mycotoxin-contamination for animal feed, as well as human food, in Pakistan.

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