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Anatomo-Pathological Consequences of Mycotoxins Contamination in Rabbits Feed

Review Article

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ABSTRACT

Mycotoxins are secondary metabolites produced by certain filamentous microscopic fungi, which occur naturally in the environment and may persist in animal feed. A mycotoxin contaminated diet may lead to feed refusal, poor feed conversion, diminished body weight gain and causes pathological effects associated with gross and histological changes, which are responsible for great economical losses. The present review summarizes the pathological lesions caused by the most widespread mycotoxins, aflatoxins, ochratoxins and their interaction, potentially hazardous ingredients of rabbit feed.

KEY WORDS feed, mycotoxins, pathological lesions, rabbits.

INTRODUCTION

Mycotoxins are secondary metabolites produced by certain filamentous microscopic fungi, which occur naturally in the environment and can commonly grow on a variety of crops, including wheat, maize and soybean (Goswami and Kistler, 2004; Murphy *et al.* 2006; Marin *et al.* 2013). They comprise a group of several hundreds of chemically different toxic compounds (Sweeney and Dobson, 1998) and their occurrence in cereal grains and animal feed have been reported worldwide (Placinta *et al.* 1999). Food and feedstuffs prepared using mycotoxin-containing crops deteriorate nutritional content and represent a potential risk for animal and human health (Hussein and Brasel, 2001; Bennett and Klick, 2003).

The mycotoxins are cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes such as protein, RNA and DNA synthesis (Guerre *et al.* 2000). They destroy the tissues by oxidizing proteins, have immunosuppressive effects and increase diseases incidence (Kumar *et al.* 2008). Mycotoxins may cause fever (Cannon *et al.* 1982), gastrointestinal problems, internal bleeding, haemorrhages or bruising, stomach ulcers (Aziz *et al.* 1995), mouth sores, kidney or liver damage (Szilágyi *et al.* 1994), central nervous system problems (Gabal *et al.* 1986), immune-suppression (Richard *et al.* 1991; Kumar *et al.* 2008), tumour-genesis, eye and lung problems, hypertrophy of the adrenal cortex, reproductive organ problems (Szilágyi *et al.* 1994), damage to the heart muscle, tachycardia, skin problems (Fairhurst *et al.* 1987), bone marrow and spleen problems (Niyo *et al.* 1988), blood abnormalities (Mizutani *et al.* 1997), rectal prolapses and increased vascular fragility.

In addition to the toxic effects, a mycotoxin contaminated diet may lead to other consequences like feed refusal, poor feed conversion and diminished body weight gain (Council on Agricultural Science and Technology, 1989; Council on Agricultural Science and Technology, 2003; Kolpin *et al.* 2014) which are responsible for great economical losses. These effects, in both humans and animals, depend on a

number of factors including intake levels, duration of exposure, toxin species, mechanisms of action, metabolism, and defense mechanisms (Galvano *et al.* 2001; Hussein and Brasel, 2001).

The most frequent sources of mycotoxins are the fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* (Bennett and Klich, 2003). While *Fusarium* species are destructive plant pathogens producing mycotoxins before or immediately post harvesting, *Penicillium* and *Aspergillus* species are more commonly found as contaminants of commodities and foods during drying and subsequent storage (Sweeney and Dobson, 1998). Weather conditions, including moisture level and temperature have greatest influence on mold growth and mycotoxin production: while *Aspergillus* prefers warmer tropical areas, *Fusarium* and *Penicillium* also grow in European temperate areas (Bryden, 2012).

Rabbit feed ingredients that constitute complete feed products are derived from different raw materials and the contamination of feed materials would represent an important potential hazard. Contamination of animal feed with mycotoxins still occurs very often, despite great efforts in preventing it. Multitoxin studies on animal feed reported that 75-100% of samples analysed contained more than one mycotoxin (Streit *et al.* 2012; Schatzmayr and Streit, 2013).

Two environmentally important mycotoxins, which have gained an immense importance due to their biological effects and widespread toxicity, are ochratoxin A (OTA) and aflatoxin B_1 (AFB_1). WHO-IARC, (1993) designated AFB_1 as group-1 and OTA as group-2B carcinogen (Hussein and Brasel, 2001) and are most frequently detected in the agricultural commodities (Bennett and Klich, 2003; Gabarty and Abou, 2016; Jan *et al.* 2017).

Aflatoxins are a family of extremely toxic, mutagenic and carcinogenic compounds produced by fungi belonging to genus *Aspergillus flavus* and *Aspergillus parasiticus* (Jacobsen *et al.* 2007; Cortes *et al.* 2010); ochratoxins, potentially as important as the aflatoxins (Bennett and Klich, 2003), are commonly produced by two species of fungi, *Penicillium verrucosum Dierckx* and *Aspergillus ochraceus Wilhelm* (Frisvad and Samson, 1991). The alarming feature of mycotoxins is their occurrence in combination and in low dose levels frequently in cereals / feedstuffs which may exert a greater degree of damage (additive or synergistic) in comparison to the individual effects (Roberts *et al.* 1981; Raina and Singh, 1991; Adebajo, 1993; Sundaram *et al.* 1999; Prabu *et al.* 2013).

The toxicity and clinical signs observed in animals when more than one mycotoxin is present in feed are complex and diverse (Prabu *et al.* 2013). The effects observed during multiple mycotoxin exposure can differ greatly from the effects observed in animals exposed to a single mycotoxin (Huff *et al.* 1988).

Animals are usually exposed to mycotoxins through their diet and depending on different factors such as age, sex, route of administration: this can result in acute, sub-acute or chronic mycotoxicosis. Rabbits are highly susceptible to mycotoxins and they can have very pervasive yet subclinical effects on their health which very often remain unnoticed: when the clinical symptoms of mycotoxin poisoning can be observed, significant damage has already occurred.

The aim of this review is to summarize the toxicopathological effects caused by the most widespread mycotoxins (aflatoxins and ochratoxin and their interactions), potentially hazardous ingredients of rabbit feed.

Aflatoxins

Aflatoxins are a group of naturally occurring carcinogens that are known to contaminate different food stuffs causing serious consequences in human and animal health. Aflatoxins have been reported to affect the various body organs like liver, kidneys, lungs, brain, testes and many endocrine and exocrine organs, the heart, skeletal muscles and the different body systems (Bbosa *et al.* 2013).

They are produced by fungi belonging to genus *Aspergillus flavus* and *Aspergillus parasiticus* (Jacobsen *et al.* 2007; Cortes *et al.* 2010). The four major known aflatoxins include AFB_1, AFB_2, AFG_1, and AFG_2 where the "B" and the "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography platres, while the subscript numbers 1 and 2 indicate major or minor compounds (Thrasher, 2012; Bbosa *et al.* 2013). The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB_1> AFG_1> AFB_2>AFG_2 (Cortes *et al* 2010). AFG_2 occurs in high quantities though less toxic while AFB_1 is the most toxic of all the aflatoxin. The World Health Organization (WHO) classifies AFB_1 as a class 1 carcinogen (WHO-IARC, 2000).

Aflatoxicosis is a condition caused by aflatoxins which can result in acute, sub-acute or chronic mycotoxicosis. The acute primary aflatoxicosisis is produced when moderate to high levels of aflatoxins are consumed; the chronic primary aflatoxicosis results from ingestion of low to moderate levels of aflatoxins (USAID, 2012)

Animals get exposed to aflatoxins by two major routes: (a) by direct ingestion of aflatoxin-contaminated foods (Agag, 2004) and (b) by inhalation of dust particles of aflatoxins, especially AFB_1 in contaminated foods in industries and factories (Coulombe, 1994).

The lethal toxicity of AFB_1 varies in different animals. Rabbits are highly susceptible to aflatoxins having the least median lethal dose (LD_{50}) of any animal species studied (Prabua *et al*. 2013; Cardona *et al*. 1991; Lakkawar *et al.* 2004).

AFB_1 as low as 15 mg/kg feed causes high levels of morbidity and mortality (Makkar and Singh, 2009; Mezes and Balogh, 2009).

Experimental AFB_1 toxicosis is known to cause alterations in enzyme levels along with patho-anatomical changes in vital organs (Clark *et al*. 1980; Abdelhamid *et al.* 1990; Sahoo *et al*. 1993). Short exposure to large doses of aflatoxins produces acute toxicity including fever oedema, vomiting, abdominal pain, inappetance, lethargy ataxia, rough hair coat and pale potentially fatal liver failure (Lakkawar *et al*. 2004).

The prolonged rabbit feeding of AFB_1 in a diet, beyond 40 days, results in cumulative toxicosis which is manifested by altered clinical signs. Symptoms are characterized by anorexia, diarrhoea, decrease in body weight gain, lethargy, fur chewing and dehydration (Clark *et al.* 1980; Abdelhamid *et al*. 1990; Prabu *et al*. 2013; Lakkawar *et al.* 2004). It has been reported that a decrease in body weight is one of the earliest indicators of clinical aflatoxicosis in animals (Clark *et al.* 1980).

The gastrointestinal tract (GIT) is the main route of entry of aflatoxins as a result of consumption of aflatoxin-contaminated foods especially AFB_1. Orally-ingested AFB_1 is most efficiently absorbed from the small intestine at the duodenum (Kumagai, 1989) and the effects of AFB_1 on the GIT are indirect following alterations in the liver's detoxification mechanism and a possible reduction in nutrient uptake (Stetinova *et al.* 1998).

The liver and kidneys are the most affected organs followed by stomach, intestine, lungs, heart, spleen, gonads, thyroid and brain (Lakkawar *et al*. 2004; Prabu *et al.* 2013). AFB_1 is a potent hepatotoxic and hepato-carcinogenic mycotoxin which requires bio-activation to an active AFB_1-8,9-epoxide (Essigmann *et al*. 1982) which binds to DNA resulting in abnormal cellular proliferation, leading to mutagenesis and carcinogenesis (Guengerich, 2001). AFB_1 is metabolized in the liver by the cellular cytochrome-P450 enzyme system to form the reactive intermediate, AFB_1-8, 9- epoxide, which in turn reacts with macromolecules such as lipids and DNA, leading to lipid peroxidation and cellular injury (Stresser *et al.* 1994). The mitochondrial damage following aflatoxicosis can result in a decrease in the oxidation of fats by these organelles, with a concomitant accumulation of lipids, necrotic changes, fibroblastic proliferation, mononuclear cellular infiltration, bile duct hyperplasia (Sahoo *et al.* 1993; Mclean and Dutton, 1995; Lakkawar *et al.* 2004). Icterus observed in terminal stages may be due to an increase in the levels of bilirubin as a sequela to hepatic necrosis and cholestasis resulting from decreased cytochromed P450, increased heme oxygenase and biliverdin reductase activities along with an increase in heme catabolism in the liver (Guerre *et al.* 1997). AFB_1 has been reported to cause pallor discoloration of liver and hepatomagaly, congestion of liver parenchyma, cytoplasmic vacuolation or fatty change of hepatocytes, necrosis of hepatocytes and newly formed bile ducts (Krishna *et al.* 1991; Sahoo *et al.* 1993; Churamani *et al.* 1995; Singh *et al.* 1999; Vinita *et al.* 2003; Lakkawar *et al.* 2004).

Haemolytic anaemia and strong cytotoxic effects have been also observed (Verma and Metha, 1998).

The renal lesions are secondary to those observed in the liver. The target site of action of AFB_1 is the glomerular region causing reduction in the glomerular filtration rate and glucose reabsorption (Glahn *et al.* 1991). Kidneys appear congested, slightly enlarged and show a moderate degree of nephrosis (Ahmed *et al.* 2012). The urinary bladder appears distended with tick yellowish turbid urine (Lakkawar *et al*. 2004).

Aflatoxin have reported to have serious acute effects on the respiratory systems. Besides the liver, the lung and trachea are also capable of activating AFB_1 and rabbit lung and tracheal microsomes show high activity for this reaction (Daniels *et al*. 1990). Bio-activation related toxicity of AFB_1 has also been observed in tracheal mucosa following intra-tracheal instillation of AFB_1 in rabbits (Mezes, 2008; Coulombe *et al*. 1986). The pulmonary inflammation and oedematous changes observed might also be due to the production of eicosanoids stimulated by the AFB_1. This might have also contributed to the manifestation of dyspnoea in the early stages of toxicosis (Massey *et al.* 1995; Lakkawar *et al.* 2004).

Furthermore, aflatoxins have been reported to disrupt the reproductive system in both male and female animals after ingestion of aflatoxin-contaminated foods. Short exposure to large doses of aflatoxins produces acute toxicity; subsymptomatic exposure to aflatoxins is known to produce male reproductive toxic effects with several manifestation.

The principal target organ in causing male reproductive toxicity is the testis and various aspects of spermatogenesis and androgen biosynthesis are affected (Baker and Greene, 1987; Sahoo *et al.* 1993). However, the epididymis and vas deferens also are a target for the action of such reproductive toxicants (Akbarsha *et al.* 2000). The severity of pathological changes in epididymis are aflatoxins (AF) dose dependent and summarized as interstitial oedema and atrophy of epididymal tubules, associated with congestion of the blood vessels and capillaries.

The seminiferous tubules show degeneration of the epithelium and a reduction in the number of mature spermatids in aflatoxin treated rabbits (Lakkawar *et al.* 2004). Salem *et al.* (2001) reported a relative decrease in testes weight and an increase in the number of abnormal/dead sperms following a 9-week administration of sublethal doses of AFB_1 to mature male rabbits.

Aflatoxins have reported to have serious acute effects on cardiovascular systems including vascular fragility, endothelial injury and haemorrhaging in tissues (Harriet, 2003).

Heart can show epicardial congestion (Lakkawar *et al*. 2004).

The coagulation defect and bleeding associated with aflatoxicosis has previously been attributed to either vitamin K antagonism, decreased hepatic protein and coagulation factor synthesis or disseminated intravascular coagulation secondary to hepatocellular degeneration and necrosis (Baker and Greene, 1987).

In the brain or central nervous system, the neurons have a high metabolic rate but little capacity for anaerobic metabolism and subsequently, inadequate oxygen flows to the brain kills the neuronal brain cells within minutes. Aflatoxins and its metabolites and other products such as the reactive oxygen species (ROS) like the AFB-8,9-epoxides may interfere with the normal functioning of the nerve cells by forming DNA adducts, protein adducts, oxidative stress factors, mitochondrial directed apoptosis of the nerve cells as well as inhibiting their synthesis of protein, RNA and DNA (Bbosa *et al.* 2013). AFB_1 is known to alter the distribution of acetylcholine esterase (AChE) in the brain affecting cholinergic transmissions at the nerve endings and thus can result in manifestations of nervousness and behavioural deficiency (Egbunike and Ikegwuonu, 1984; Lakkawar *et al.* 2004). Sahoo *et al.* (1993) reported vascular congestion and focal mononuclear infiltration in the meninges along with perivascular cuffing, mild neuronal degeneration and gliosis following the oral administration of AFB_1 to New Zealand White Rabbits at the rate of 0.0625 mg/day/animal for a period of 30 days.

AFB_1 is recorded to be teratogen in rabbits (El-Nahla *et al.* 2013). The dose of 0.1 mg/kg AFB_1 body weight AFB1is the minimum teratogenic dose that interfere with intrauterine development during 6th-18th days of gestation (Wangikar *et al.* 2005).

Ochratoxins

Ochratoxin, commonly produced by two species of fungi, *Penicillium verrucosum* and *Aspergillus ochraceus* (Frisvad and Samson, 1991; Ostry *et al*. 2013; Saleemi *et al*. 2015; Valtchev *et al*. 2015), is potentially as important as the aflatoxins (Bennett and Klich, 2003). The most abundant and most toxic mycotoxin within the ochratoxins is ochratoxin-A (OT-A) (Marquardt and Frohlich, 1992), which occurs in maize, cereal grains such as wheat and barley and oil seeds such as soybean and peanuts (Manning *et al.* 2003). The OT-A is toxic to numerous animal species, the kidney being the main target organ in birds and mammals, but not adult ruminants (Pfohl-Leszkowicz and Manderville, 2007). The most consistent effect of ochratoxicosis in different species including rodents, guinea pigs, swine and poultry is growth depression (Thacker and Carlton, 1977; Dwivedi and Burns, 1985; Stein *et al.* 1985; Dwivedi and Burns, 1986; Rati *et al.* 1991; Harvey *et al.* 1994).

OT-A is well known nephrotoxic, hepatotoxic, immunosuppressive, mutagenic, cardio-vascular toxic, teratogenic and possible human carcinogen (Ahmed *et al.* 2012; Hussain *et al*. 2016). In addition to these effects, OT-A is also considered as potent myelotoxic agent (Moura *et al*. 2004).

OT-A is also extremely cytotoxic and may cause red blood cell haemolysis in rabbits (Zofair *et al*. 1996). OT-A at low doses influences energy metabolism such as carbohydrate, amino acid, cofactors and vitamins. However, in the high doses pathways influenced by OT-A are associated with the different body systems including circulatory, digestive, endocrine, excretory system (Jan *et al.* 2017).

Rabbits are comparatively more susceptible to OT-A than mice, rats and guinea pigs (Ponnuchamy, 2000). OT-A has been observed to be acutely toxic to young rabbits with LD50 of 10 mg OT-A/kg body weight (Mir *et al*. 1999).

Long term exposures have been observed to favor accumulation in the tissues and cause severe impairment of health, wide spread pathoanatomical alterations, and even death (Mir and Dwivedi, 2000; Mir and Dwiwedi, 2010). OT-A has been known to be actively reabsorbed through the proximal convoluted tubules (Stein *et al.* 1985). OT-A produces significant nephrotoxicity with the pale, soft and enlarged kidneys showing discoloured foci over the surface. The proximal tubule of the kidney is the primary site targeted in OT-A induced nephrotoxicity (Suzuki *et al*. 1975). The reason behind the high sensitivity of kidneys to the toxin might be correlated to the fact that the kidneys constitute the primary excretory pathway for OA and its heavy blood circulation (25% of cardiac output) (Marquardt and Frohlich, 1992).

Testis, being an organ with rapid meiotic and mitotic cell divisions, could be a possible target of OT-A as it inhibits DNA, RNA and protein synthesis (Marquardt and Frohlich, 1992). OT-A (~99%) is bound to plasma proteins, mainly albumin, which can't be excreted by glomerular filtrate. However, unbound portion (~1%) can be found in the urinary filtrate. The remaining OT-A is only execrated via organic anion transporter (OAT) route, which prone the proximal tubular epithelial cells to damage, by virtue of depletion of indigenous dicarboxylic acid (glutarate, ketoglutarate) on expense of OT-A internalization (Sekine *et al*. 2006; Khatoon *et al*. 2016). Several natural and experimental OT-A exposure studies have recorded similar changes in the kidney function (Khan *et al*. 2014). The increase in the serum biomarkers of kidneys damage, and the mechanistic nephrotoxicity associated with the OT-A, has

also been augmented by histological alteration in the tubular cells of nephrons (Jan *et al.* 2017). Microscopically, kidneys reveal degeneration of the proximal convoluted tubules and the testes atrophic (Prabu *et al.* 2013).

OT-A has been reported to cause direct effects on lymphocytes and plasma cells in primary and secondary lymphoid organs (Dwivedi and Burns, 1985).

OT-A has also been reported to be teratogenic in rabbits (Wangikar *et al.* 2005), rats (Still *et al.* 1971; Brown *et al.* 1976; Mayura *et al.* 1982; Abdel-Wahhab *et al.* 1999), mice (Hayes *et al.* 1974; Arora and Fr¨ol, 1981), hamsters (Hood *et al.* 1976). Teratogenic effects were found among the 0.1 mg/kg dose group in the form of a significant increase in the incidence of gross anomalies (wrist drop, rudimentary tail, knuckling of fetlock and agenesis of tail), skeletal anomalies (agenesis of caudal vertebrae, incomplete ossification of skull bones and wavy ribs) and soft tissue anomalies (internal hydrocephalus, microphthalmia and kidney agenesis).

Ochratoxicosis induces also biochemical alterations in the rabbits (Mir and Dwiwedi, 2010).

Aflatoxins and ochratoxins interaction

The alarming feature of mycotoxins is their occurrence in combination and in low dose levels frequently in cereals/feedstuffs which may exert a greater degree of damage to animal health (additive or synergistic) in comparison to the individual effects (Roberts *et al.* 1981; Raina and Singh, 1991; Adebajo, 1993; Sundaram *et al.* 1999; Prabu *et al.* 2013). The effects observed during multiple mycotoxin exposure can differ greatly from the effects observed in animals exposed to a single mycotoxin (Huff *et al.* 1988).

Simultaneous occurrence of these two mycotoxins in feedstuffs under natural conditions was observed during screening of different food and feed stuffs (Mir, 1998).

Although the scientific literature offers a broad variety of information on the effects of individual mycotoxins in various animal species, concurrent exposure to multiple mycotoxins is more likely in the livestock industry (Zaki *et al.* 2012). Occurrence of spontaneous aflatoxicosis and ochratoxicosis, individually and in interaction, the combined presence of low dose levels of AFB_1 ranging from 0.03 to 2.06 ppm and OT-A ranging from 0.01 to 1.23 ppm in cereals/feedstuffs have been frequently reported in different species of animals (Raina and Singh, 1991; Dwivedi *et al.* 2004).

There exists an interaction in terms of synergism in the toxicological effects when both the chemicals are fed together. Heavy mortalities of up to 75% in German Angora rabbits in Kangra district of Himachal Pradesh (India) were reported due to simultaneous spontaneous aflatoxicosis and ochratoxicosis with high levels of AFB_1 and OT-A in the feed (Sharma, 1998). In experimental simultaneous exposure to OT-A e AFB_1 in rabbits, during long term feeding of AFB1 and OT-A in combination, the intensity of clinical symptoms was comparatively more severe as than when either of these mycotoxins was given alone (Doerr and Huff, 1981; Wangikar *et al.* 2005; Prabu *et al.* 2013). The changes were more severe as evidenced by gross, histological, ultra structural, immunological and enzyme antioxidant changes observed both in liver and kidneys, suggesting an additive interaction of AFB_1 and OT-A in rabbits (Prabu *et al.* 2013). Similar presence of changes both in the liver and kidney has been reported by several workers in guinea pigs (Richard *et al.* 1975). OT-A and AFB_1, when administered in combination in pregnant rabbits, resulted in significant increase in the incidence of various fetal anomalies and post implantation losses and decreased fetal weights (Wangikar *et al.* 2005). Testis revealed severe disruption of the normal spermatogonial cell pattern and marked atrophic changes (Prabu *et al.* 2013). Similar results were observed in previous investigation with different combinations of OT-A and AFB_1 in rats (Wangikar *et al.* 2004b; Wangikar *et al.* 2004c), suggesting that in combination, these toxins might have possibly antagonistic interaction.

Mycotoxins comprise a group of several hundreds of chemically different toxic compounds (Moss, 1996; Rotter *et al.* 1996; Sweeney and Dobson, 1998) and their occurrence in cereal grains and animal feed have been reported worldwide (Placinta *et al.* 1999). Food and feedstuffs prepared using mycotoxin-containing crops deteriorate nutritional content and represent a potential risk for animal and human health (Hussein and Brasel, 2001; Bennett and Klick, 2003).

Consumption of a mycotoxin contaminated diet may induce acute and long-term chronic effects resulting in teratogenic, carcinogenic, and oestrogenic or immune-suppressive effects. Direct consequences of consumption of mycotoxin contaminated animal feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (due to immune-suppression), and reduced reproductive capacities (Fink-Gremmels and Malekinejad, 2007; Morgavi and Riley, 2007; Pestka, 2007; Voss *et al.* 2007) which leads to economic losses (Huwig *et al.* 2001; Wu, 2004; Wu, 2006).

It was widely reported that aflatoxins affect the various body organs like liver, kidneys, lungs, brain, testes and many endocrine and exocrine organs, the heart, skeletal muscles and the different body systems (Bbosa *et al.* 2013).

In particular, rabbits are highly susceptible to aflatoxins having the least median lethal dose (LD_{50}) of any animal species studied (Prabua *et al.* 2013; Cardona *et al.* 1991; Lakkawar *et al.* 2004). AFB_1 as low as 15 mg/kg feed causes high levels of morbidity and mortality (Makkar and

Singh, 2009; Mezes and Balogh, 2009). OT-A has been observed to be acutely toxic to young rabbits with LD50 of 10 mg OT-A/kg body weight (Mir *et al.* 1999). Rabbits are comparatively more susceptible to OT-A than mice, rats and guinea pigs (Ponnuchamy, 2000). Long term exposures have been observed to favor accumulation in the tissues and cause severe impairment of health, wide spread patho-anatomical alterations, and even death (Mir and Dwivedi, 2000; Mir and Dwiwedi, 2010). OT-A has been known to be actively reabsorbed through the proximal convoluted tubules (Stein *et al.* 1985).

Multi-toxin occurrence may be one important explanation for divergences in effect levels described in the scientific literature, where defined, mostly purified mycotoxins are used in most studies. In field outbreaks, naturally contaminated feeds may contain multiple mycotoxins and thus apparently lower contamination levels of a single specific mycotoxin can be associated with more severe effects (Zaki *et al.* 2012). The presence of AFB_1 and OT-A, both alone and in interaction at very low dose levels of 0.5-1 ppm in animal feeds and cereals can cause significant toxicity in rabbits with gross and histological changes and might be a potential threat to animal health (Prabu *et al.* 2013).

CONCLUSION

Based on literature review and data available, it has to be pointed out that far more work has to be done on this particular research field, especially in case of mycotoxins sub-acute contamination range as well as with combinations of more than two toxins.

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