Norwegian University of Life Sciences

Master's Thesis 2018 30 ECTS

Department of Animal and Aquacultural Sciences
Main Supervisor: Professor Trond Storebakken

Potential for using aluminosilicates for removal of heavy metals and mycotoxins from feed and water

Ramesh Kandel

Master of Science in Feed Manufacturing Technology
Faculty of Biosciences

Acknowledgement

I would like to express my sincere appreciation and gratitude to my supervisor Professor Trond Storebakken for his creative guidance, intellectual support, stimulating discussions and inspiring words. I am grateful for his excellent hospitality and wonderful attitude.

I would like to thank Department of animal and aquacultural sciences, Norwegian university of life sciences for providing the opportunity for education.

Thanks to all my family members especially my father, mother, sister, and niece for their love, support, and encouragement during my stay here.

Last but not the least, my warmest appreciation to all my colleagues and friends from Norway.

Ås, May 2018

Ramesh Kandel

ABASTRACT

Mycotoxins are naturally occurring secondary fungal metabolites capable of producing toxic effects in humans and animals. The complexion of toxic effects of mycotoxins depend on the chemical structure of the toxin, duration of exposure, and concentration present in food/feed. The most studied mycotoxins are aflatoxins, trichothecenes, zearalenone, and fumonisins. Animals exposed to low levels of mycotoxins show signs of reduced feed intake and weight gain, decreased immunity and overall productivity leading to economic losses. Different physical, chemical, and biological methods to prevent mycotoxicosis in animals have been reported but practical and cost-effective methods for efficiently decontaminating mycotoxins containing feedstuffs are currently not available. The addition of non-nutritive inert adsorbents in feeds contaminated with low levels of toxins have shown to sequester mycotoxins and reduce their gastrointestinal absorption. Aluminosilicates owing to their cation exchange capacity are favorable mycotoxin adsorbent in animal feeds contaminated with low levels of toxins. Most of the aluminosilicates tested *in-vitro* and *in-vivo* have shown high adsorption against aflatoxins. However, aluminosilicates are not good adsorbent of mycotoxin other than aflatoxins, especially trichothecenes and zearalenone. Newer adsorbents derived from cell wall of the yeast *Saccharomyces cerevisiae* and lactic acid bacteria have shown effective binding of *Fusarium* toxins *in-vitro*. Similarly, microorganisms from rumen of cattle, intestine of pigs and chickens, and some soil bacterium have shown effective bio-transformation activities against *Fusarium* toxins. Further animal studies using newer adsorbents or mycotoxin modifiers are needed to demonstrate mycotoxin removal capabilities *in-vivo*.

Table of Contents

1. Silicates and Aluminosilicates

Mother earth is composed of 92 different elements. The inner core is believed to be composed of metallic iron alloyed with elements especially nickel, cobalt, carbon, and sulphur originating from a meteorite. The mantle is composed of silicates and oxides of the common elements like magnesium, aluminum, calcium, iron. The crust is composed of complex patterns of rocks with diverse chemical and mineralogical compositions i.e., igneous, sedimentary, and metamorphic. The crust also consists of compounds of oxygen mainly silicates and aluminosilicates of iron, calcium, magnesium, and alkali metals. Moreover, crust contains a small number of other elements and organic matter which supports flora and fauna.

1.1 Silicates

Silicates are compounds of silicon and oxygen; the most abundant elements found on the earth and constitute approximately 90 % of earth's crust. The basic chemical unit of silicate is SiO_4^{-4} and exists as a tetrahedron shaped anionic group. The central silicon ion has 4 positive charges and each oxygen has 2 negative charges. Thus, each silicon-oxygen bond is equal to ½ (one half) the total bond energy of oxygen.

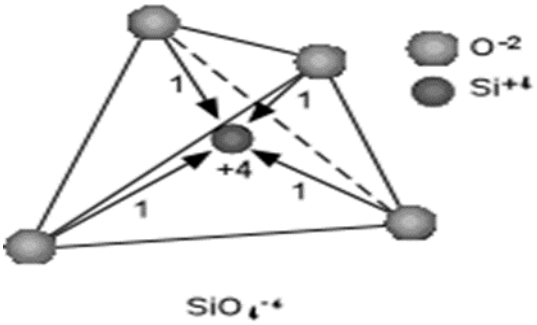

Fig 1: A silicate SiO_4^{-4} tetrahedral structure. Source: (Nelson).

This charge imbalance condition allows the oxygen to bind with another silicate ion and link one SiO_4^{-4} tetrahedron to another and many more allowing to form chains, sheets, rings, and framework

structures. Since silicates are electrically negative, they tend to combine with other elements especially alkali and alkali earth elements (cations) to form electrically neutral species.

1.2 Aluminosilicates

Aluminum is the third most abundant element present in earth's crust after oxygen and silicon. Silicates tend to form an electrically neutral complex by reacting with aluminum and other metals present in earth's crust forming aluminosilicates. Basically, aluminosilicates are compounds consisting oxides of silicon, aluminum and other elements present in earth's crust. Two types are most abundant depending on the different arrangement of atoms and molecules: phyllosilicates and tectosilicates.

1.2.1 Phyllosilicates

Phyllon in Greek means leaf. Phyllosilicates are leaf-like or layered or sheets of aluminosilicates. They are generally soft and have low specific gravity. Their structure is based on interconnected six-member rings of SiO_4^{-4} tetrahedra which extends outwards in infinite sheets. Three oxygen from each tetrahedron is shared with other tetrahedra giving the basic structural unit of $Si_2O_5^{-2}$. Most of the phyllosilicates contain a hydroxyl OH^- ion situated in the center of 6 membered rings. The hydroxyl ion helps to bond with other cations in octahedral coordination. This gives rise to layers of cations- most often iron, magnesium, or aluminum that occur in octahedral coordination with the oxygen and hydroxyl ions of the tetrahedral layer. Several phyllosilicates exist in nature and have the diverse use.

Table 1: Important phyllosilicate minerals.

Mineral group	Chemical formula
Serpentine group Antigorite, Chrysotile, Lizardite	$Mg_3Si_2O_5(OH)_4$
Clay mineral group	
Kaolinite	$Al_2Si_2O_5(OH)_4$

Talc	$Mg_3Si_4O_{10}(OH)_2$
Pyrophyllite	$Al_2Si_4O_{10}(OH)_2$
Mica group	
Muscovite	$KAl_2(AlSi_3O_{10})\ (OH)_2$
Phlogopite	$KMg_3(AlSi_3O_{10})\ (OH)_2$
Biotite	$K\ (Mg, Fe)_3(AlSi_3O_{10})\ (OH)_2$
Lepidolite	$K\ (Li, Al)_{2\text{-}3}(AlSi_3O_{10})\ (OH)_2$
Margarite	$CaAl_2(Al_2Si_2O_{10})\ (OH)_2$
Chlorite group	
Chlorite	$(Mg, Fe)_3\ (Al, Si)_4O_{10}(OH)_2\ .\ (Mg, Fe)_3(OH)_6$

Several other phyllosilicates exist outside the classification given above. They are bentonites, montmorillonites, kaolinites, smectites, illites, and hydrated sodium calcium aluminosilicate. Phyllosilicates have the plastic properties when wet and harden when dried or fired. The small particle size of clay material provides this function. Mankind has been using phyllosilicates belonging to clay mineral groups to make ceramics for centuries. Millions of tons of phyllosilicates are used each year across the globe for various industrial and domestic purposes such as ceramics, pharmaceuticals, cosmetics, foods, beverages, composites, buffer material for long-term nuclear waste disposal, drilling fluids, and catalysis.

Phyllosilicates are widely used in pharmaceutical and cosmeceutical industries as excipients owing to their micrometer size particles, inertness, stability, flow properties, specific reactivity, and adsorption capability. Excipients are inactive substances which provide varied and specialized pharmaceutical and cosmeceutical functions. They are formulation additives used as a solubilizer, suspending, thickening, emulsifying, isotonic and flavoring agents, disintegrants, diluents, binders, pigments, and carriers for active drug substances. Palygorskite, sepiolite, kaolinite, talc, montmorillonite, saponite, and hectorite are used as excipients. Some phyllosilicates are used in geophagy, the practice of eating clay minerals (Wilson 2003). Recently, clay minerals such as Fe-smectite (French green clay) has been found to have antibacterial properties (Williams & Haydel 2010).

1.2.2 Tectosilicates

Tectosilicates are framework silicates that extend through an interlocking 3-dimensional network of covalent bonds and thus are stable and hard. All the 4 oxygens of SiO_4^{-4} tetrahedra are shared with other tetrahedra giving rise to 1:2 ratio between silicon and oxygen. Each individual tetrahedron is linked to four others. Tectosilicates comprise about 64% of earth's crust. Variety of tectosilicate minerals exist in the earth for example quartz, feldspar, feldspathoids, zeolites, and many more.

1.2.3 Zeolites

The term 'Zeolite' (zeo = boil; lithos = stone in greek) was introduced by Swedish mineralogist Alex F. Cronstedt in 1756 for certain silicate minerals. Zeolites are widespread in nature and stilbite, a natural zeolite was first discovered by Cronstedt in 1756. Natural zeolite is known to mankind for more than two thousand years and has been used as a building material since Roman times.

Zeolites have been defined by (Chairman et al. 1998) as '*a crystalline substance with a structure characterized by a framework of linked tetrahedra, each consisting four oxygen atoms surrounding a cation. This framework contains open cavities in the form of channels and cages. These are usually occupied by water molecules and extra-framework cations that are commonly exchangeable. The channels are large enough to allow the passage of guest species. In the hydrated phases, dehydration occurs at temperatures mostly below about 400* ^0C *and is largely reversible. The framework may be interrupted by (OH, F) groups: these occupy a tetrahedron apex that is not shared with adjacent tetrahedra'*.

Zeolites are crystalline, microporous, hydrated aluminosilicates of alkali and alkaline earth cations having an infinite, open 3-dimensional structure (Mumpton 1999). Like other tectosilicates, zeolites are formed by a continuous network of oxygen-sharing SiO_4^{4-} or AlO_4^{5-} tetrahedra. At some places in the tetrahedral Al^{3+} replace Si^{4+} and thus the zeolite framework becomes negatively charged. Loosely held cations (extra framework cations) such as Na^+, K^+, Mg^{++} and Ca^{++} reside within the cavities provide electro neutrality to the zeolite. The framework structure contains large

pores or interconnected channels that are occupied by cations, such as NH_4^+ and water molecules. Some of those cations are open to cation exchange and can interact reversibly with polar molecules. The ion exchange process in the zeolite is reversible, allowing for adsorption of ions and molecules. This property has made zeolites useful as filters for dust, toxin removal and as chemical sieves (Pavelic & Hadzija 2003).

In nature, zeolites are found in volcanogenic sedimentary rocks and are thought to be formed by the dissolution of volcanic ashes and magmas and their precipitation to micrometer size crystals. Zeolites are formed as result of slow to fast cooling of warm to hot magmas, which are basic, oversaturated in silicate and aluminate species and contain alkaline and/or alkali earth cations. Magmas may originate from a volcano or from the interaction of warm to hot fluids with loose volcanic materials, usually fine ashes, deposited in more or less far times as a product of volcanic eruptions (Cejka 2005). The basic character of magma favors the tetrahedral coordination of aluminum, which contributes with silicon to the formation of zeolite framework. Zeolites are concentrated in massive formations, called tuff deposits, from which they can be excavated and after suitable processes, utilized. Tuff deposits are widely spread in numerous locations all over the world and new reserves are being discovered continuously. Zeolite tuffs basically are rocks containing various crystalline and amorphous phases along with zeolites. The main parameter determining nature of the formed zeolites and other phases is the silicon content of the rock. High silicon content give rise to siliceous zeolites such as clinoptilolite, ferrierite, and mordenite, whereas intermediate silica content leads to the formation of less siliceous zeolites like analcime, chabazite, phillipsite.

The general chemical composition of zeolite is:

$M\ n_{x/n}\ Si_{1-x}\ Al_x\ O_2\ .\ yH_2O$

Where, M = Na^+, K^+, Li^+, Ag^+, NH_4^+, H^+, Ca^{++}, Ba^{++}, etc...

Zeolites can be differentiated from other tectosilicates depending on tetrahedral density, i.e. number of Si or Al atoms in 1000 $Å^3$. For zeolites, tetrahedral density is between 12 and 20 whereas for other tectosilicates it is higher than 20 Si or Al / 1000 $Å^3$. The factor that distinguishes zeolite from other porous materials is their variety of pore sizes and shapes.

Table 2: Examples of important natural and synthetic zeolites. Adapted from (Mumpton 1999).

Zeolite	Representative chemical formula	Void volume, %	Cation exchange capacities, CEC $meqg^{-1}$
Analcime	$Na_{10}(Al_{16}Si_{32}O_{96}).\ 16H_2O$	18	4.54
Chabazite	$(Na_2Ca)_6(Al_{12}Si_{24}O_{72}).\ 40H_2O$	47	3.84
Clinoptilolite	$(Na_3K_3)\ (Al_6Si_{30}O_{72}).\ 24H_2O$	34	2.16
Erionite	$(NaCa_{0.5}K)_9(Al_9Si_{27}O_{72}).\ 27H_2O$	35	3.12
Faujasite (Synthetic)	$(Na_{58})\ (Al_{58}Si_{134}O_{384}).\ 240H_2O$	47	3.39
Ferrierite	$(Na_2Mg_2)\ (Al_6Si_{30}O_{72}).\ 18H_2O$	28	2.33
Heulandite	$(Ca_4)\ (Al_8Si_{28}O_{72}).\ 24H_2O$	39	2.91
Laumonitte	$(Ca_4)\ (Al_8Si_{16}O_{48}).\ 16H_2O$	34	4.25
Mordenite	$(Na_8)\ (Al_8Si_{40}O_{96}).\ 24H_2O$	28	2.29
Phillipsite	$(NaK)_5(Al_5Si_{11}O_{32}).\ 20H_2O$	31	3.31
Linde A (Synthetic)	$(Na_{12})\ (Al_{12}Si_{12}O_{48}).\ 27H_2O$	47	5.48
Linde X (Synthetic)	$(Na_{86})\ (Al_{86}Si_{106}O_{384}).\ 264H_2O$	50	4.73

Easy exchange of extra-framework cations at relatively low temperature is a typical feature of zeolites and zeolitic behavior but varies from species to species (Chairman et al. 1998). Natural zeolites contain silicon and aluminum with a molar ratio of Si/Al around 2-5. Because of this, they have superior selectivity and stability characteristics. Their surface is highly selective for water, polar, and polarizable molecules which serve as the basis for their application as adsorbents and ion exchangers (Ribeiro 2012). At least 61 different natural minerals exhibiting zeolitic behavior have been identified and reviewed (Cejka 2005; Chairman et al. 1998). Zeolite synthesis started in the early 1950s. Synthetic zeolites are more expensive than natural ones and also find wide industrial applications (Sherman 1999) and are outside the scope of this review.

2. Mycotoxins contamination of feedstuffs and their biological effects

Mycotoxins are naturally occurring, non-volatile, relatively low molecular weight, and toxic secondary metabolites produced by filamentous fungi or molds as the product of primary metabolic processes (Calvo et al. 2002; Moss 1991). Mycotoxins have no biochemical importance in fungal growth and their development and are thought to be produced by the fungus as a defense mechanism against insects, microorganisms, nematodes, animals, and humans. Food plants are prone to fungi infiltration under stress conditions such as draught or over-irrigation, insect/bird damage, pesticides and fungicides exposure. The spectrum of mycotoxins produced depends on physical factors (moisture, relative humidity, temperature) and chemical factors (oxygen, carbon dioxide, and composition of the substrate). Moisture and temperature are the major factors influencing mould growth and mycotoxin production. More than 400 different mycotoxins produced by 350 species of fungi can be found in literature. However, in animal feeds only small number of toxins produced by three main genera of fungi *Aspergillus*, *Penicillium*, and *Fusarium* are of importance (Kuhn & Ghannoum 2003).

Aquaculture feeds have undergone a huge change in the source of protein over the last decade. Marine-based proteins, fish meal (FM) are replaced with less expensive and abundantly available plant-based proteins which have increased the risk of contamination of feeds with mycotoxin (Binder 2007). Plants ingredients are contaminated by mycotoxins either through fungi growing as pathogens on plants or growing saprophytically on stored grains (Glenn 2007). Mycotoxins are chemically stable and once produced in feedstuffs will continue to contaminate the products manufactured using contaminated feedstuff. Variety of feed ingredients used in aquaculture such as; cottonseed, peanuts, corn, rice, wheat, soybeans, dried fish, shrimp and fish meals have been found to be contaminated frequently.

Approximately more than 25 % of global crop production is contaminated with one or more type of mycotoxins and is main risk factor affecting human and animal health and is responsible for the significant economic loss (Rodrigues et al. 2011). These economic losses are shared by all members of crop producers, crop processors, animal producers, grain handlers and distributors,

and last but not least by the consumers in society (Rodrigues et al. 2011). Major fungi and mycotoxins affecting animal health and economy around the world are presented in Table 3.

Table 3: Fungi and mycotoxins of economic importance. Adapted from (Fokunang et al. 2006).

Fungal species	Mycotoxin
Aspergillus parasiticus	Aflatoxins B1, B2, G1, G2
A. flavus	Aflatoxins B1, B2
Fusarium graminearum, F. roseum	Deoxynivalenol, Zearalelone
F. moniliforme	Fumonisin B1
F. Sporotrichioides	T2-toxin
Penicillium verrucosum, F. poae, F. tricintum	Ochratoxin A
Aspergillus ochraceous, A. paraciticus, *A. niger*	Ochratoxin A
P. rubrum	Rubratoxin
P. islandicum	Yellow rice toxin

Contamination of food chain by mycotoxins result in serious health problems in humans and animals as they are toxic in low concentrations. Mycotoxicosis or diseases caused by mycotoxins may vary from acute toxicity to chronic health-related problems. Transmission of mycotoxin/s to host animal occurs via ingestion of contaminated article but can also occur by inhalation and dermal contact with toxins. Exposure to mycotoxins (humans and animals) results directly via consumption of contaminated cereals. Moreover, humans can also be exposed indirectly via consumption of animal products like meat, milk, and eggs since a significant number of mycotoxins and their metabolites are carried over to animal products (Bennett 1987; Kuiper-Goodman 1991).

Ergotism, a kind of mycotoxicosis caused by consumption of cereals contaminated with fungus *Claviceps purpurea* is known to humanity since historic times. Systemic research on mycotoxins started only after 1960 when around 100000 turkeys died on a farm in England from acute necrosis of liver and hyperplasia of the bile duct after consumption of contaminated peanut meal. Later it was found that peanut meal imported from Brazil was contaminated with aflatoxins from *A. flavus*. In the early 1960s it was reported that rainbow trout fed diet formulated using contaminated peanut and cottonseed meal showed signs of liver cancer (Wolf & Jackson 1963). Subsequently, it was found that mycotoxins can cause diverse nature of illness in humans and animals including acute toxicity (or death), and chronic effects like immunosuppression, carcinogenicity, teratogenicity, mutagenicity, and estrogenicity (Peraica et al. 1999; Pier et al. 1980; Richard 2007; van Egmond et al. 2007).

Fig 2: *Aspergillus* contaminated peanuts. Source: (Kooijmans)

The impact of mycotoxin on health of animals is influenced by the type of mycotoxin; amount and duration of exposure; age, sex, and nutritional condition of individual animal; and presence of other mycotoxins Animals consuming mycotoxin contaminated diets show signs like: reduced feed intake, feed refusal, poor feed conversion, less body weight gain, higher incidence of diseases,

reduced fertility, and increased mortality all of which leads to health problems and economic losses (Fink-Gremmels & Malekinejad 2007; Morgavi & Riley 2007; Pestka 2007; Wu 2006). Mycotoxins belonging to *Aspergillus* and *Fusarium* genus are the most potent, most studied and widespread contaminant of foods and feed around the world and will be focused in this review.

2.1 Aflatoxins

Aflatoxins are colorless to pale-yellow crystals that intensely fluoresce under UV light. They are very slightly soluble in water, insoluble in non-polar solvents, and freely soluble in moderately polar solvents such as chloroform, methanol, and dimethyl sulfoxide. Aflatoxins are a group of nearly 20 related fungal metabolites (polysubstituted bisfuranocoumarins). These toxic secondary metabolites are produced mainly by the fungus *A. flavus* and *A. parasiticus*. *A. flavus* is more widespread in tropical climates whereas *A. parasiticus* is less widely distributed and geographically confined to south-east Asia. *A. flavus* is not so common in cold temperate climates except in foods/feeds imported from tropical countries. Most infected food commodities are maize, peanuts, and cottonseed. Also, different spices and some tree nuts are found to be contaminated. Low levels are found in all of major foods/feeds including, fish and meat meals (IARC ; Pitt 1993).

Fig 3: Aflatoxin infected maize. Source: (Schmidt).

Aflatoxins are produced by the fungus during production, harvest, storage, and food processing and are considered as an unavoidable contaminant of foods. There are four main aflatoxins: B1, B2, G1, and G2, with letters indicating the color produced by individual toxin under ultraviolet light (blue or green) and the numbers indicate their relative distance on a thin layer chromatographic plate (Klich 2007). *A. parasiticus* is reported to produce all of four main toxins whereas *A. flavus* producing only B1 and B2. Among all the aflatoxins, Aflatoxin B1 is the most common form and the most potent of all (Eaton & Groopman 2013). Aflatoxin M1 (fluoresces blue-violet in UV light) and M2, the hydroxylated toxic metabolite of aflatoxin B1 & B2 respectively are secreted in human and animal milk and found in a variety of dairy products all over the world (Galvano et al. 1996). Moreover, low levels of aflatoxins and its metabolites are found in poultry products like eggs and meat of birds consuming contaminated diets (Chen et al. 1984). Apart from above-mentioned metabolites, several others including aflatoxicol are reported in literature (Fernández et al. 1997; McLean & Dutton 1995).

Aflatoxicosis or poisoning caused by aflatoxins may cause a range of clinical problems which largely depend on dose and duration of exposure to toxins. Large doses lead to severe acute intoxication or death resulting from severe liver damage whereas chronic sub-acute doses may have nutritional and immunological consequences. Either in large or small doses, exposure to aflatoxins increases the risk of cancer in humans and food animals (Williams et al. 2004). Aflatoxins are genotoxic. It binds to chromosomes leading to genetic changes in target cells. Aflatoxins can impair protein synthesis and interfere with normal production of cellular regulators. However, the proper mechanism of aflatoxicosis is not understood yet. Aflatoxins are extremely toxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive compounds. Owing to its carcinogenicity, International Agency for Research on Cancer (IARC) has classified aflatoxins as Group 1 human carcinogens. Aflatoxins have shown carcinogenicity in many animal species including, nonhuman primates, rodents, fish, and many food animals (Eaton & Groopman 2013; Santacroce et al. 2008).

In humans, aflatoxicosis is more prevalent in developing and poor countries. Aflatoxins are found to play an important role in modifying risk of liver cancer with individuals previously infected with Hepatitis B or Hepatitis C virus. In a study with human subjects, it was found that aflatoxin exposure increases risk of liver cancer by many folds in individuals previously infected with

hepatitis B virus (IARC ; Qian et al. 1994). It was also found that children suffering from protein-energy malnutrition in poor countries are exposed to high levels of aflatoxins in their life (Ramjee et al. 1992). Similarly, aflatoxins are reported to cause jaundice in neonates and are present in human breast milk, umbilical cord, and variety of body fluids including, semen which may be cause of infertility in males (Hendrickse 1999; Ibeh et al. 1994; Maxwell 1998). Most recent human aflatoxicosis was reported from Kenya in June 2004. 180 people had been hospitalized after consumption of contaminated corn with symptoms of liver failure, yellow eyes, vomiting and bleeding from the nose. Eight of them died from acute poisoning. There are reports claiming that 4.5 billion people living in developing countries are chronically exposed to uncontrolled amount of aflatoxins (Williams et al. 2004).

In food animals: chick embryos, goslings, ducklings, turkeys, and fishes are most sensitive to aflatoxin exposure. Chickens exposed to as low as 0.2-1 ppm of aflatoxins in diets have shown signs like poor growth rate, reduced feed conversion, decrease in egg production, liver damage, bile duct proliferation, and decreased resistance to infectious diseases (Smith & Hamilton 1970). Among fishes, it was found that species of warm-water (channel catfish, tilapia) are more resistant to aflatoxins than species of cold-water (trout) (Manning et al. 2005). Rainbow trout fed a diet containing 0.4 ppb aflatoxin B1 for 15 months had 14 % incidence of liver cancer (Lee et al. 1968). Similarly, rainbow trout fed a diet containing 20 ppb aflatoxin B1 for 8 months showed 58 % incidence of liver cancer whereas when fed for 12 months they showed 83 % incidence (Schoenhard et al. 1981). Salmon species are more resistant towards aflatoxins compared to rainbow trout. When both species were fed diet containing 20 ppb aflatoxin B1, trout showed 96 % incidence of liver cancer whereas 0 % salmon were affected (Coulombe Jr et al. 1984).

Nile tilapia responds to aflatoxins in dose-dependent manner. Tilapias fed a diet containing higher dose i.e. 1.88 mg aflatoxin B1/kg feed showed the reduction in growth rate whereas when fed 0.94 mg aflatoxin B1/kg feed showed no changes (Chavez-Sanchez et al. 1994). Precancerous liver lesions were observed in tilapias consuming higher dose but there were no signs of liver cancer in fishes eating lower dose. Genotoxic, mutagenic, carcinogenic, reproductive and immune effects of aflatoxins in aquatic species has been reviewed (Anater et al. 2016; Jantrarotai & Lovell 1990; Sahoo et al. 2001; Santacroce et al. 2008; Tuan et al. 2002). The increase in understanding and

awareness of aflatoxicosis and their effects on humans and animals has helped the establishment of limits and regulations for aflatoxins in feed.

Table 4: Maximum limit of aflatoxins in animal feeds (EC 2002).

Mycotoxin	Products intended for animal feed	Maximum limit mg/kg (ppm)
Aflatoxin B_1	All feed materials	0.02
	Complete feed for cattle, goat, and sheep	0.02
	Complete feed for diary animals	0.005
	Complete feed for calves and lambs	0.01
	Complete feed for pigs and poultry (except young animals)	0.02
	Complementary feed for cattle, sheep, and goats (except for diary animals, calves, and lambs)	0.02
	Complementary feed for pigs and poultry (except young animals)	0.02
	Other complementary feeds	0.005

2.2 Fusarium mycotoxins

Mycotoxins produced by *Fusarium* genus of fungi are major plant pathogen and cause a huge loss in yields throughout the world. The toxin-producing species cause head blight in wheat and barley, ear rot in maize and to lesser extent contaminate oats, sorghum, soybeans, millet, rye, and rice. The contamination of grains with *Fusarium* toxins has the significant impact on human and animal health. The most prevalent and toxic *Fusarium* toxins are: trichothecenes (TCTs), zearalenone (ZEN), fumonisins (FB_1 and FB_2), moniliformin.

2.2.1 Trichothecenes

Trichothecenes are a family of structurally related more than 180 compounds (tetracyclic sesquiterpenoid) produced by *Fusarium*, *Stachybotrys*, and other moulds growing in grains. TCTs are colourless, optically active, non-volatile compounds and are resistant to degradation by light and temperature and are deactivated under strongly acidic or alkaline conditions. All trichothecenes contain an epoxide group responsible for toxicological activity at the $C_{12,13}$ position (Sudakin 2003). According to the functional group, four classes of trichothecenes exists. But only toxins of type A and B are produced by *Fusarium* species. The type A consists of T-2 toxin, HT-2 toxin, neosolaniol (NEO), and deacetoxyscirpenol (DAS), while type B includes deoxynivalenol (DON or vomitoxin), its 3-acetyl and 15-acetyl derivatives, nivalenol (NIV), and fusarenon-X (Placinta et al. 1999). Type A toxins are primarily produced (post-harvest) by *F. sporotrichioides* and *F. poae,* whereas Type B toxins are produced (pre-harvest) by *F. graminearum* and *F. culmorum*. Environmental conditions such as temperature, humidity, and growth substrate influence the production of toxins.

Dietary ingestion is the most common route of human and animal exposure to trichothecenes. TCTs are routinely detected in agricultural commodities such as corn, wheat, rice, rye, barley, oats, other cereals, vegetables, and forages. Moreover, they are detected at low levels in certain commercial foods/beverages such as beer, fermented beverages, breakfast cereals, bread, and related products. Cereals are the most common feed stuff and farm animals are likely to consume the high amount of toxins and are worst affected. Trichothecenes are rapidly absorbed, metabolized and excreted from the animals with little or no accumulation in any specific organs. So, the risk of transfer of toxins to animal tissues and ultimately to humans via animal-derived products is of less importance (Beasley & Lambert 1990; Eriksen 1998).

2.2.2 T-2 and HT-2 Toxin

T-2 and the closely related HT-2 toxins are most acutely toxic members of trichothecenes family. These toxins can cause nausea, vomiting, diarrhea, lethargy, weight loss, hemorrhage, immunosuppression, necrosis, damage of cartilaginous tissues, apoptosis, and death (Fairhurst et al. 1987). T-2 toxin is considered the main cause of alimentary toxic aleukia (ATA), a human disease characterized by leukopenia, agranulocytosis, necrotic angina, a haemorrhagic rash, sepsis, exhaustion of bone marrow and bleeding from the nose, throat, gums and intestinal epithelium (Lutsky & Mor 1981).

Table 5: Indicative levels for the sum of T-2 and HT-2 toxin in cereals and cereal products (EC 2006).

	Maximum limit T-2 + HT-2 ug/kg or ppb
Unprocessed cereals	
Barley and maize	200
Oats with husk	1000
Wheat, rye and other cereals	100
Cereal products for feed and complete feed	
Oat milling products with husks	2000
Other cereal products	500
Compound feed, except cats fed	250

T-2 and HT-2 toxins occur together in infected cereals. HT-2 is a major metabolite of T-2 toxin and is formed rapidly after exposing an animal to the T-2 toxin. The intensity of T-2 toxicity is related to animal species, age, exposure route, dosage, and young animals are more sensitive than adults. The main toxic effects of the T-2 toxin is inhibition of protein synthesis possibly by inhibiting polypeptide chain initiation or elongation. The T-2 toxin binds to the 60S subunit of a eukaryotic ribosome and interferes with the activity of peptidyl transferase, thus inhibiting protein synthesis. T-2 toxins are also shown to inhibit DNA and RNA synthesis at much higher concentrations. They affect actively dividing cells of GI tract, skin, lymphoid and erythroid cells. The toxicity of T-2 toxin results in necrosis of oral mucosa and skin in contact with the toxin, acute effect on the digestive tract and decreased bone marrow and immune functions. In addition, T-2 toxin is known to alter serotonin activity in the central and peripheral nervous system resulting in feed refusal in farm animals. T-2 toxin is toxic to many animal species, but the sensitivity differs greatly between species and between the different toxins. Monogastric animals like pigs and rodents are the most sensitive to T-2 toxin whereas poultry and ruminants are less sensitive.

Pigs fed T-2 toxin (5-10 mg/kg feed) show signs of reduced feed intake or complete feed refusal and reduced weight gain (Harvey et al. 1990). Pigs (7 weeks old) fed as little as 0.5 mg T-2 toxin/kg feed shows sign of feed refusal (Rafai et al. 1995). High levels of T-2 toxins in pigs are known to

be abortifacient (Weaver et al. 1978) but such high levels are not encountered in feed not visibly infected by moulds. Calves fed 20 mg T-2 toxin/kg feed shows sign of feed refusal, diarrhoea, reduced weight gain, decreased thymus and adrenal weights, and decreased plasma antibody (I_gA and I_gM). Cows exposed to high experimental levels of T-2 toxin express low levels of T-2 toxin and/or its metabolites in milk. Chickens fed as low as 2-6 mg T-2 toxin/kg feed shows sign of reduced feed intake, weight gain, and feed efficiency. Histopathological changes in heart, liver, duodenum, and kidney are observed in chickens fed 0.5 mg pure T-2 toxin/kg feed. The decrease in egg production was observed in hens fed 8 mg T-2 toxin/kg feed. Moreover, low levels of T-2 toxins in the feed are associated with oral lesions and decreased immunity in chickens.

Some fish species are known to be affected by the T-2 toxin. Rainbow trout fed 2.5 mg/kg or higher levels of T-2 toxins shows signs of reduced feed intake, growth, lowered hematocrit and blood haemoglobin. Similarly, channel catfish showed reduced growth with low levels of T-2 toxins and high mortality was seen when the levels were higher than 2.5 ppm (Manning et al. 2003). Adult trout fed 15 mg T-2 toxin/kg feed had presented with focal intestinal haemorrhage and enlarged spleen and gall bladders.

2.2.3 Deoxynivalenol (DON)

Deoxynivalenol (DON) is commonly known as vomitoxin because of its strong emetic effects. DON is one of the most common contaminants of wheat, corn, rye, and barley worldwide. DON is heat and uv light stable compound and doesn't degrade during storage and cooking/processing of grains. DON is soluble in polar organic solvents such as aqueous methanol, ethanol, chloroform, acetonitrile, and ethyl acetate. DON is produced in grains infected with *F. graminearum* and *F. culmorum*. DON co-occur with low concentrations of the related metabolite, 15-acetyl DON in North American continent. While in Asia, DON producing fungi coproduce 3-acetyl DON and nivalenol. DON is primarily produced pre-harvest especially during periods of low temperatures and high humidity. However, it can also be produced during storage when the moisture content of stored grains is not controlled.

DON exerts similar toxicity pattern as the T-2 toxin. Many animal species are sensitive to DON and sensitivity follows the order: swine > mice > rats > poultry > ruminants (Rotter 1996). Animals

exposed to dietary DON for prolonged time show signs of decreased weight gain, feed refusal, altered nutritional efficiency, and decreased immunity and thus increased susceptibility to infections.

Fig 4: *Fusarium culmorum* infected wheat. Source: (CORMA)

Pigs are worst affected by dietary DON. Pigs fed low levels of DON (0.6-2 mg/kg feed) shows partial feed refusal, decreased feed consumption and weight gain, whereas 12 mg DON/kg feed cause complete feed refusal and 20 mg/kg resulted in vomiting after minutes of ingestion (Young et al. 1983). Epithelial lesions in the esophageal region of the stomach of pigs are observed when fed diets containing 3-6 mg DON/kg feed (Foster et al. 1986). In a pig feeding trial with 3.5 mg/kg DON, (Bergsjø et al. 1993) reported an increase in liver weight, decrease in serum protein and albumin, and decrease in packed cell volume, serum calcium and phosphorous.

Cattle and poultry are more tolerant towards DON compared to pigs. Poultry is reported to tolerate DON up to concentrations of 8 mg/kg feed without affecting productivity (Hamilton et al. 1985). However, rapidly growing broilers are found more sensitive to feed refusal than laying hens (Huff

et al. 1986). Chickens show reduced feed intake and weight gain when fed high levels of DON 16-20 mg DON/kg feed (Kubena et al. 1985).

Among cultured fish species, warm water fish like channel catfish and carps and cold-water fish like trout are adversely affected by dietary DON. Rainbow trout fed diets containing 0.3- 2.6 mg/kg DON showed a linear or quadratic decrease in feed intake, weight gain, growth rate, feed efficiency, and retained nitrogen and energy (Hooft et al. 2011; Hooft & Bureau 2017). Rainbow trout fed diets containing 2 mg/kg DON for 23 days developed significant effects on the immune system (Matejova et al. 2015).

Table 6: Maximum limit of DON in animal feeds (EFSA 2004).

Mycotoxin	Products intended for animal feed	Maximum limit mg/kg (ppm)
Deoxynivalenol (DON)	Cereals and cereal products except for maize by-products	8
	Maize by-products	12
	Complementary and complete feeds	5
	Complementary and complete feed for pigs	0.9
	Complementary and complete feed for calves (<4 months), lambs and kids	2

2.2.4 Zearalenone (ZEA)

Zearalenone, a non-steroidal estrogen mycotoxin biosynthesized by numerous species of *Fusarium* fungi in warm and temperate climates is the common contaminant of cereal crops and derived products worldwide. Zearalenone is a white, crystalline, and stable compound both during milling/storage and cooking/processing of foods. ZEA is rapidly absorbed after oral dosage with 80-85 % bioavailability of ingested dose (Kuiper-Goodman et al. 1987). ZEA is rapidly metabolized in the gastrointestinal tract to its major metabolite alpha- and beta- zearalenol.

Zearalenone as well as some of its metabolites have been shown to competitively bind to estrogen receptors (Shier et al. 2001) resulting in hyper estrogenic effects in humans, many laboratory, and domestic animals.

Table 7: Maximum limit of Zearalenone in animal feeds (EC 2006).

Mycotoxin	Products intended for animal feed	Maximum limit mg/kg (ppm)
Zearalenone	Cereals and cereal products except maize by-products	2
	Maize by-products	3
	Complementary and complete feed for piglets and gilts	0.1
	Complementary and complete feed for sows and fattening pigs	0.25
	Complementary and complete feed for calves, dairy cattle, sheep, and goats including lambs and kids	0.5

Estrogenic effects such as; alterations in the reproductive tract, decreased fertility, increased embryolethal resorptions, reduced litter size, changed the weight of adrenal, thyroid, and pituitary glands and change in serum concentrations of progesterone and estradiol are observed in animals (Kuiper-Goodman et al. 1987; Maaroufi et al. 1996). Pigs and rodents are found to be more sensitive than other species. Gilts exposed to ZEA develop swelling and thickening of the vulva and enlarged uterus. ZEA shows acute toxicity in mice, rats, and guinea pigs after oral administration. Zearalenone is rapidly bio-transformed and excreted from food animals and the risk of dietary intake by humans from meat and products is negligible. However, ZEA is found to be excreted in the milk of lactating cows if fed in high levels i.e., 12 mg/kg body weight (Prelusky et al. 1990).

Zearalenone is found in surface and ground waters which is an environmental threat to aquatic animals. Fish species are also susceptible to hyper estrogenic effects of zearalenone. *In-vivo* studies with fish using ZEA and its metabolites have disclosed effects on immune system and growth impairments, decreased sperm production and motility, decreased fertilization and dose-dependent

induction of estrogen responsive genes like vitellogenin and zona radiata proteins (Arukwe et al. 1999; Celius et al. 2000). Zebra fish exposed to dietary ZEA for short-term showed reduced spawning frequency whereas long-term exposure resulted in transgenerational effects like increase in the size of female fish and shift in sex of offspring towards the female (Schwartz et al. 2010).

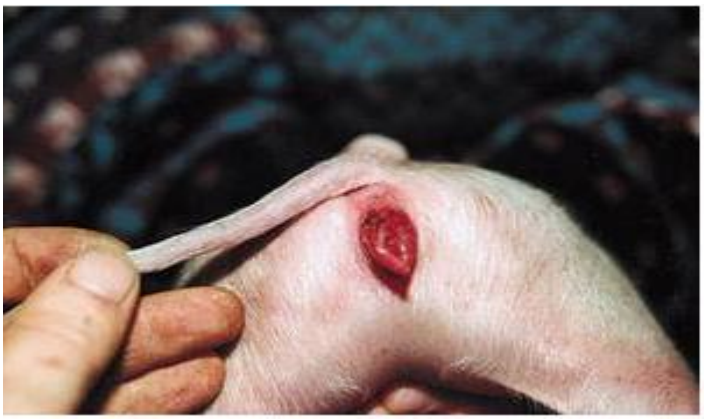

Fig 5: Hyper estrogenic effect of ZEA on gilts (swelling and thickening of vulva). Source: (Biomin).

2.2.5 Fumonisins

Fumonisins are produced in significant quantities by *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. monoliforme* (Sheldon)) and related *F. proliferatum* (Matsushima) Nirenberg. Fumonisins are diesters of different polyhydric alcohols and tricarballylic acid. *F. monoliforme* is related with the disease of maize plant development infecting the roots, stalk, and kernels. Fumonisins are polar, water-soluble compounds with long chain structure of polyhydroxy alkylamines esterified with two tricarballylic acids. Fumonisins are of four types: Fumonisins B_1 (FB_1), Fumonisins B_2 (FB_2), FB_3 and FB_4. However, FB_1 and FB_2 are most toxic and thoroughly studied. Fumonisins are found frequently in foodstuffs like maize and infrequently in sorghum, asparagus, rice, beer, and mung beans. The human and animal health problems associated with Fumonisins are virtually linked with the consumption of contaminated maize or products made from maize (Marasas 2001).

Fumonisins are specific inhibitors of sphinganine (sphingosine) *N- acyltransferase*, an enzyme responsible for de novo sphingolipid biosynthesis and reacylation of sphinganine acquired from dietary sources or complex sphingolipid turnover, hence changing the ratio of free sphinganine to

free sphingosine in the tissues (Riley, R. et al. 1993). Interference with sphingolipid metabolism produces a cascade of biochemical and cellular changes that lead to toxic effects of fumonisins (Riley, R. T. et al. 1993).

Fumonisins are known to cause leukoencephalomalacia (LEM) in horses, liver cancer in rats, pulmonary edema in pigs and are highly toxic to experimental animals (Gelderblom et al. 1991; Kriek et al. 1981). Fumonisins are also thought to induce esophageal and liver cancers, neural-tube defects and cardiovascular problems in human populations consuming large amounts of food made from contaminated maize (Fincham et al. 1992; Gelineau-van Waes et al. 2005; Ueno et al. 1997). IARC has classified Fumonisins as class B, possibly carcinogenic to humans. Rats chronically exposed to more than 50 ppm dietary FB_1 develop liver and kidney cancer (Howard et al. 2001).

Fig 6: Fumonisin contaminated maize. Source: (Laurain).

Ruminants and poultry are found to be less sensitive to fumonisins compared to horses, pigs, rabbits, and rodents. Fumonisins are poorly absorbed from the gastrointestinal tracts of animals, have a rapid elimination, and low tissues accumulation (Prelusky et al. 1996). Channel catfish fed FB_1 shows increase in free sphingolipid ratios as seen in higher animals like horses, pigs, and rats suggesting fumonisins toxicity (Goel et al. 1994). Both channel catfish and Nile tilapia fingerlings

show a reduction in weight gain, poor feed conversion when fed diets containing low levels (10 ppm) of fumonisins (Manning & Abbas 2012). Rainbow trout fed diets containing fumonisins show changes in sphinganine to sphingosine ratios, suggesting fumonisins toxicity.

Table 8: Maximum limit of Fumonisin B_1 + B_2 in animal feeds (EC 2006).

Mycotoxin	Products intended for animal feed	Maximum limit mg/kg (ppm)
Fumonisin B_1 + B_2	Maize and maize products	60
	Complementary and complete feed for:	
	Pigs, horses, rabbits, and pet animals	5
	Fish	10
	Poultry, calves (<4 months), lambs, and kids	20
	Adult ruminants (>4 months) and mink	50

3. Co-occurrence of mycotoxins

The contamination of feedstuffs with only one type of mycotoxin is scared and mycotoxins occur together ubiquitous, presenting the greater risk to animals. Fungal growth and mycotoxin production are largely determined by water activity and temperature during growth and storage. Two or more different fungus can grow on the same substrate if proper conditions are met. Naturally contaminated grains might contain multiple mycotoxins and presence of multiple toxins can be associated with severe effects. The co-occurrence of two or more toxins provide additive and/or synergistic effects in the progression of mycotoxicosis in animals.

Simultaneous occurrence of mycotoxins in grains is reported worldwide. There are reports of co-occurrence of aflatoxins with trichothecenes and ZEA (Wang et al. 1995). Fumonisins and orchatoxin A were found in same samples of maize (Jurjevic et al. 1999). Many species of *Fusarium* fungi can produce trichothecenes along with Zearalenone. High levels of zearalenone and fumonisins have been found in maize for animal feed (Doko et al. 1996; Scudamore et al. 1997). Similarly, corn and corn-based products around the world are found to be contaminated with aflatoxins and *Fusarium* toxins (Abbas et al. 2006; Ali et al. 1998; Chamberlain et al. 1993).

Co-occurrence of fumonisins with ZEA, DON, and T-2 toxin in naturally contaminated wheat have been reported (Stanković et al. 2012).

4. Decontamination of mycotoxins

Chronic exposure to mycotoxins not only significantly affect animal health and productivity but direct exposure to mycotoxin contaminated foodstuff pose a great risk to the consumer. Since mycotoxins are unseen poison in food/feed commodities, decontamination is the must before contaminated materials enter the food chain. Adoption of Good Agricultural Practices (GAP) like improvement in farm management, rapid drying, and controlled storage are helpful in reducing naturally occurring mycotoxins in foods and feeds. Development of resistant crop varieties may help mitigate mycotoxins production and several types of research are going on around the world to identify genes responsible for toxin production. However, due to diverse nature of fungus involved and wide geographical region to be covered, resistant crop production doesn't seem feasible. Also, it is difficult to control moisture content in grains as it is directly influenced by changing environmental conditions. Under these conditions, it is necessary to implement decontamination methods to reduce toxin risk by bringing down the level of mycotoxin to permissible level.

A variety of methods have been developed for removal of contaminated commodities or inactivation of toxin present in commodities. Techniques including physical methods such as removal of contaminated parts from the produce, filtration/flotation, extraction, milling, heating and adsorption on inert adsorbent; chemical methods such as oxidation, alkalization, ammonization, and irradiation are found on literature (Jemmali 1980; Kabak et al. 2006; Rustom 1997). Biological detoxification of mycotoxins using microorganisms and enzymes have recently got much attention and will be discussed in another chapter. Regardless of the method used, it must be technically and economically feasible. Also, the chosen method must: destroy or inactivate mycotoxin; not produce/leave carcinogenic residue in the final product; ensure the safety of human and animal; destroy if possible all fungal spores and mycelia; preserve nutritive quality and acceptability of final product; not alter important technological properties. To date, not any single method possessing all above-mentioned qualities are available.

Binding of mycotoxins to adsorbent materials like natural clays (mineral adsorbents or inorganic adsorbents) provides a promising and cost-effective approach for decontamination of contaminated feed stuff and is considered most promising dietary approach (Galvano et al. 2001). At present, the use of adsorbents is the most applied method of protecting animals against detrimental effects of contaminated feed and feed stuff. These mineral binders are often called the first generation of mycotoxin binders.

5. Adsorption of mycotoxins using natural clays- Aluminosilicates

Adsorbent materials are nutritionally inert additives in diet that are thought to adsorb/sequester mycotoxins in the gastrointestinal tract of animals and reduce and/or prevent their absorption to the systemic circulation, thus avoiding toxic effects for food animals and carry-over of toxins to animal products. This approach of reducing the adverse effect of toxins in animals is the most economical way and is considered prophylactic rather than therapeutic. Aluminosilicates such as natural zeolites, clinoptilolite, bentonites, montmorillonite, and hydrated sodium calcium aluminosilicate (HSCAS) possess favorable adsorbing characteristics (Grant & Phillips 1998; Papaioannou et al. 2005).

Adsorption of mycotoxins on clays depends on physical properties of clay such as total charge and charge distribution, size of pores and accessible surface area. At the same time, it also depends on properties of adsorbate molecules/toxins like polarity, solubility, size, and shape. The adsorption process is pretty much dependent on the type of exchangeable cations present on the adsorbent material (Huwig et al. 2001). Moreover, since mycotoxins are complex organic compounds containing different functional groups, their adsorption efficacy depends on the p^H of the solution.

5.1 Adsorption of aflatoxins

The approach of reducing the bioavailability of aflatoxins in animals by the addition of dietary clay started after (Phillips et al. 1988) demonstrated high affinity of HSCAS for aflatoxin B_1 *in-vivo* in Leghorn and broiler chickens. Since then many different aluminosilicates have been tested

for adsorption of aflatoxins in different food animals. The early studies showed that HSCAS can reduce the toxic effects of aflatoxins in young animals such as; rats, chickens, turkeys, lambs, and pigs (Harvey et al. 1994; Kubena et al. 1990). HSCAS was reported to decrease the concentration of aflatoxin M_1 in milk from lactating cows and goats (Harvey et al. 1991; Smith et al. 1994). Moreover, clinoptilolite administered (200 g per animal per day) significantly reduced AFM_1 concentrations in milk of dairy cattle (Katsoulos et al. 2016).

(Schell et al. 1993) fed 1 % Na bentonite with corn-soybean meal-based contaminated with 922 ppb aflatoxin B_1 to nursery pigs and observed the decrease in gain/feed ratio and improvement in liver functions compared to pigs fed same diets without Na bentonite. Similarly, addition of 0.5 % HSCAS to growing pigs diet formulated with aflatoxin contaminated corn showed improved weight gain and feed intake compared to control diets without HSCAS (Colvin et al. 1989). Moreover, hepatocellular changes associated with aflatoxin consumption were not observed in the histopathology of liver sections from pigs fed a contaminated diet with the adsorbent.

(SCHEIDELER 1993) observed that different aluminosilicates were able to adsorb up to 60 % of AFB_1 in-vitro and alleviate growth depression in 2-3 weeks old chickens caused by the addition of 2.5 ppm AFB_1 to diets compared to control. Montmorillonite clay 0.5 % fed along with 4000 ppb aflatoxin to commercial broiler chicken was able to provide some degree of protection against the effects of aflatoxin compared to chickens fed diets without the adsorbent (Bailey et al. 2006). Clinoptilolite (15 g/kg diet) added to diets containing either 50 or 100 ppb aflatoxin and fed to broilers for 42 days had moderately reduced the adverse effects of aflatoxins on the performance of chicks compared to control with no aflatoxin in diets (Oguz et al. 2000). Similarly, addition of 50 g/kg clinoptilolite to diets contaminated with 20 mg aflatoxin effectively reduced adverse effects of aflatoxin on 10-45 days old Japanese quail chicks (Parlat et al. 1999).

Addition of 0.25-0-75 % HSCAS in the diet of Nile tilapia showed improve in growth, pepsin activity, and nutrient digestibility (Liu et al. 2009). Calcium bentonite 0.5 and 1 % have shown to improve growth performance and reduce tissue residues of aflatoxin in Nile tilapia fed either 2 or 4 ppm AFB_1 (Hussain et al. 2017). Rainbow trout fed diets containing 20 ppb aflatoxin and 2 % Na bentonite showed the reduction of absorption of toxin from the intestinal tract and decrease in kidney and liver metabolites of aflatoxin compared to trout fed the same diet without adsorbent (Ellis et al. 2000). Supplementation of 5 g/kg bentonite or montmorillonite to Nile tilapia (two-

month old) with diets containing 1.5 ppm aflatoxin showed that sorbents can bind aflatoxin in intestinal tract thus decreasing the bioavailability of toxins and protect fish from aflatoxin hazards (Hassan et al. 2010).

5.2 Mechanism of adsorption of aflatoxins on aluminosilicates

Aluminosilicates contain three types of binding sites for aflatoxins: cations located within interlayer channels, cations located on the surface, and the uncoordinated metal ions located at the edges. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) analysis of aflatoxin-HSCAS complex shows the chelation between the uncoordinated ions and the beta-dicarbonyl moiety in aflatoxin (Phillips et al. 1995). Infrared analysis of aflatoxin-smectite complex shows that aflatoxins were chelated to smectite by the formation of hydrogen bonding between carbonyl oxygens and hydration-shell water at high humidity (Deng et al. 2010). Under dry conditions ion-dipole interactions was found to be involved in chelation as shown in figure 8 (Deng et al. 2010). The dicarbonyl moiety of aflatoxin is electron dense and thus is strongly attracted towards cations of aluminosilicates.

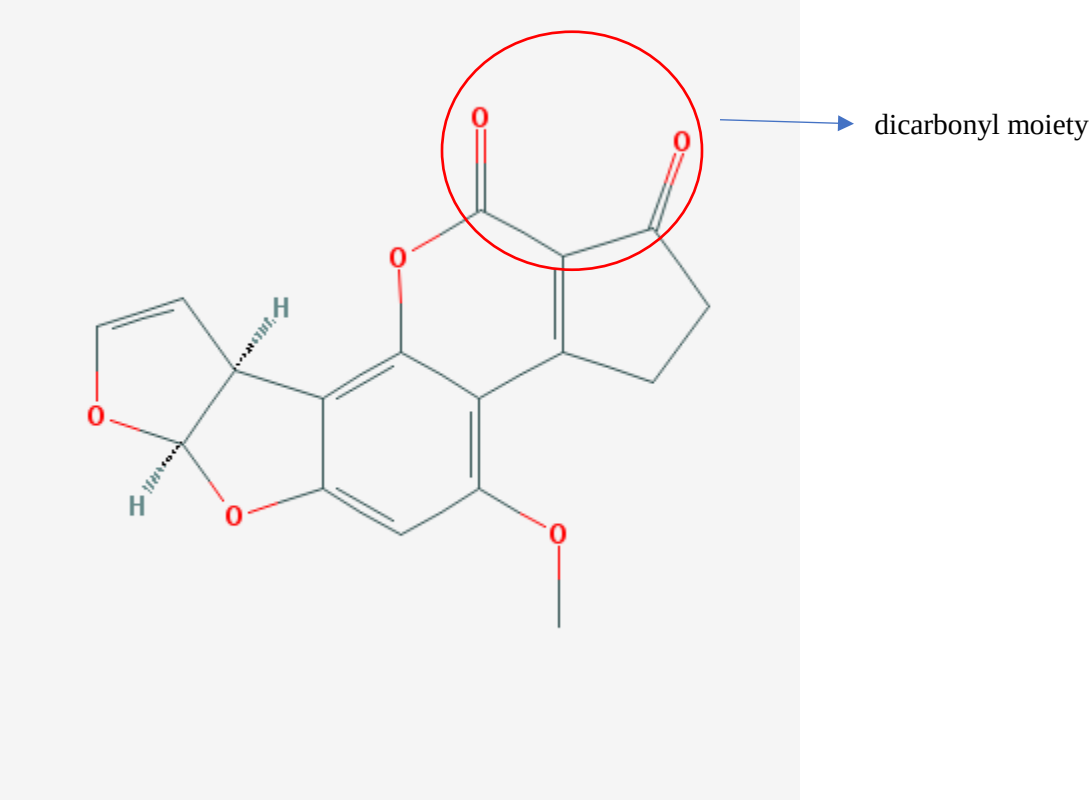

Fig 7: Chemical structure of aflatoxin B_1. Source: (PubChem).

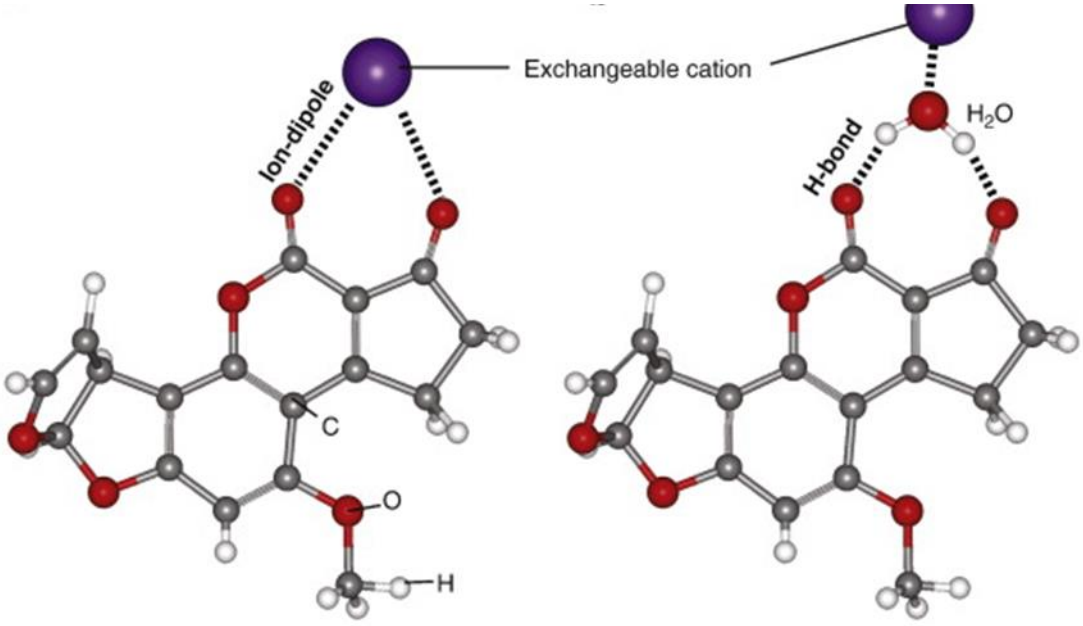

Fig 8: Proposed binding mechanisms of aflatoxins with smectite clay. Source: (Deng et al. 2010)

5.3 Adsorption of *Fusarium* toxins

Fusarium toxins, especially DON and ZEA are of great importance in animal feeding since they might be present in concentrations affecting the health and performance of food animals. Treatment of contaminated feedstuffs before feeding is labour and cost intensive and thus are not feasible. Therefore, supplementation of adsorbents with contaminated diets/feedstuffs seems promising dietary approach to counteract *Fusarium* toxicosis in farm animals. However, *Fusarium* toxins are less polar molecules and are not easily adsorbed into adsorbent compounds.

Several aluminosilicates have shown some degree of affinity for the adsorption of ZEA while the adsorption of trichothecene, especially DON is practically insignificant. Absence of dicarbonly moiety in structure of DON (figure 8) can be the reason for inability to bind with aluminosilicates. Moreover, DON contains a bulky epoxy group which doesn't favour adsorption on plane surfaces. *In-vitro* adsorption studies with ZEA revealed that cholestyramine and polyvinylpyrrolidone are

better adsorbents of ZEA compared to aluminosilicates (Ramos et al. 1996). The adsorption capacity of HSCAS is limited against ZEA and are ineffective against trichothecenes like T-2 toxin and DON. Similarly, Na bentonite was found to be effective in reducing toxic effects of T-2 toxin in rats whereas it was ineffective against ZEA and nivalenol in pigs (Huwig et al. 2001).

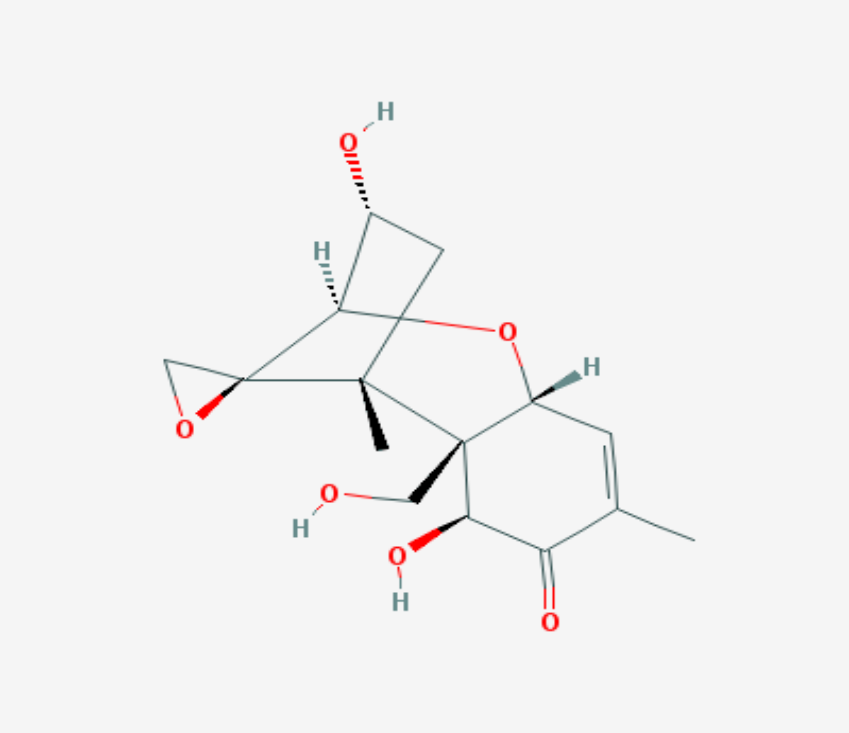

Fig 8: Chemical structure of Deoxynivalenol (DON). Source: (PubChem).

Surfactant modified montmorillonite have higher adsorption against ZEA *in-vitro* (Lemke et al. 1998). Similarly, surfactant modified zeolites have shown effective adsorption of ZEA *in-vitro* (Tomašević-Čanović et al. 2003). The reason for increased adsorption is due to the increased hydrophobicity of the clay surface thus high affinity for hydrophobic ZEA. However, increased toxicity of ZEA was reported in the mouse when surfactant modified clays were used to adsorb mycotoxins *in-vivo* (Lemke et al. 2001). Moreover, activated carbon and cholestyramine significantly reduce intestinal absorption of ZEA in a dynamic *in-vitro* model of pig's gastrointestinal tract (Avantaggiato et al. 2003).

The possibility of adsorption of nutrients (vitamins, amino acids, and minerals in feed) and potential risks of complexing chemicals to mineral adsorbents limits the use of first-generation

binders. Moreover, aluminosilicate adsorbents are not efficient binder of mycotoxins other than aflatoxins.

6. Organic mycotoxin binders

Owing to the inefficacy of aluminosilicate adsorbents against mycotoxins other than aflatoxins especially trichothecenes, natural organic binders (cell wall components of microorganisms) are gaining attention. These organic binders are often named as second-generation adsorbents/binders. Cell wall components (beta-D-glucans and mannan-oligosaccharides) of yeast *Saccharomyces cerevisiae* have shown to bind DON, T-2 toxin, ZEA, ochratoxin A, and aflatoxin B_1 *in-vitro* (Bejaoui et al. 2004; Freimund et al. 2003; Yiannikouris et al. 2004). Adsorption of mycotoxins to cell wall components of yeast is due to the formation of hydrogen and van der Waals bonds (Jouany et al. 2005).

Cell wall derivative of *Saccharomyces cerevisiae*, Esterified glucomannans (1 g/kg) when added to feed contaminated with 0.3 mg/kg AFB_1, 2 mg/kg ochratoxin A, and 3 mg/kg T-2 toxin produced beneficial effects on mycotoxicosis in broilers (Raju & Devegowda 2000). The beneficial effects observed increase in body weight and feed intake, decreased liver weight, and improved serum biochemical and hematological parameters which were negatively influenced by mycotoxins in feed. (Kamalzadeh et al. 2009) reported that addition of yeast glucomannan to diets contaminated with 184 ppb aflatoxin reduce the adverse effects of aflatoxin on performance, liver weight, and mortality on broiler chickens. Similarly, the addition of glucomannan (2 g/kg) to diets contaminated with 3 mg/kg DON counteracted the plasma biochemical parameters alteration caused by DON in chickens (Faixová et al. 2006). Yeast glucomannan (1 g/kg) added to 2 ppm aflatoxin containing diet diminished the adverse effects of aflatoxins on the pathological changes in growing broiler chickens (Karaman et al. 2005).

Addition of glucomannans to contaminated diets have demonstrated some positive effects in pigs as well. Addition of 0.2 % glucomannans was shown to counteract the changes in serum biochemical parameters produced by 5.5 mg/kg DON in sows (Díaz-Llano & Smith 2007). Improved performance in pigs fed 0.1 % esterified glucomannan with diets contaminated with

3.84 and 5.12 mg/kg ZEA was reported (Nešić et al. 2008). (Raymond et al. 2003) demonstrated the reduction of toxic effects of *Fusarium* toxins in horses fed 0.2 % glucomannan polymer with contaminated diets.

Lactic acid bacteria (LAB) used for fermentation and food preservation for ages have shown to bind mycotoxins as well (Dalié et al. 2010). The most widely investigated mycotoxin binding strain of LAB is *Lactobacillus rhamnosus*. Cell wall components of LAB, exopolysaccharides (glucans and mannans) and peptidoglycans are responsible for binding toxins (Haskard et al. 2000; Lahtinen § et al. 2004). The binding of mycotoxins with cell wall components of the LAB is similar to binding with glucomannans of yeast (El-Nezami et al. 2004; Lahtinen § et al. 2004). *Lactobacillus rhamnosus* strains have shown the *in-vitro* binding capability of DON, ZEA, T-2 toxin, FB_1, AFB_1, and ochratoxin A (El-Nezami et al. 1998; El-Nezami, HS et al. 2002; El-Nezami, Hani et al. 2002; Niderkorn et al. 2006; Piotrowska & Zakowska 2005). However, no *in-vivo* trials have been conducted to demonstrate mycotoxin binding potential of the LAB to date.

7. Biological degradation of mycotoxins (Mycotoxin modifiers)

The application of microorganisms, called mycotoxins bio-transforming agents is most recent and promising approach for controlling mycotoxicosis in animals. A variety of microorganisms such as; bacteria and fungi have exhibited mycotoxins bio-degrading capacity. Such microbes biodegrade or bio-transform mycotoxins to less toxic metabolites and act in the intestinal tract of animals prior to the absorption of mycotoxins. Moreover, the contaminated feedstuff can be fermented with microbes for few days before feeding. These bio-transforming agents must fulfill certain prerequisites such as; safety of use, rapid degradation into non-toxic or far less toxic metabolites under different oxygen conditions in a complex environment of the gastrointestinal tract, preserve the organoleptic and nutritive properties of feed, and stability along the intestinal tract. Moreover, the bio-degradation approach should be practical and economically feasible.

Certain bacteria such as; *Nocardia corynebacterioides, N. asteroids, Corynebacterium rubrum, Rhodococcus erythropolis, Mycobacterium fluoranthenivorans,* and *M. smegmatis* have shown to degrade aflatoxins (Kong et al. 2012; Mann & Rehm 1976; Taylor et al. 2010). *Lactobacillus* sp.

LA2 have shown to inhibit the mutagenic potential of aflatoxins. Similarly, some species of fungi *Aspergillus* including *A. paraciticus*, *A. flavus*, *A. niger* are reported to degrade aflatoxins (Mishra & Das 2003; Wu et al. 2009; Zhang et al. 2014). Other fungal strains such as *Trichoderma* sp. 639, *Rhizopus* sp. 668 and 720, *Sporotrichum* sp. ADA, *Sporotrichum* sp. SF and *Alternaria* sp. have demonstrated aflatoxin B_1 degradation (Kabak et al. 2006).

Microbes originating from rumen fluid of cattle (Kiessling et al. 1984; King et al. 1984) and sheep (Westlake et al. 1989) and intestines of chickens (Young et al. 2007) and pigs (Kollarczik et al. 1994) have shown to degrade various *Fusarium* toxins especially DON and ZEA. (Zhou et al. 2014) reported that a gram-positive bacterium from large intestine of white leghorn hen exposed to mycotoxin through feed showed high bio-transformation of DON to less toxic metabolite de-epoxy deoxynivalenol (DOM-1). Some microbes originating from soil are also found to degrade some *Fusarium* toxins (Shima et al. 1997). *Eubacterium* strain BBSH 797 isolated from cow rumen fluid have shown to bio-transform DON both *in-vitro* and *in-vivo* (Schatzmayr et al. 2006). This bacterium is the first microbe to be authorized in EU as mycotoxin modifier in feeds for fattening poultry, turkeys, pigs, and laying hens under the name Biomin BBSH 797 (Additives & Feed 2013; Rychen et al. 2017). Animal trials with the strain BBSH 797 indicated alleviation of adverse effects of DON on pigs, dairy cows, and T-2 toxin on growing broilers. Another bacterium obtained from soil named DX100 have shown high de-epoxidation of DON under aerobic and anaerobic conditions to less toxic metabolite *in-vitro* (Ahad et al. 2017). A bacterial strain *Sphingomonas* S 3-4 isolated from wheat fields in Wuhan, China, have shown to transform DON to 3-oxo-DON and 3-epi-DON (He et al. 2017).

8. Contamination of natural ecosystems with heavy metals, radio-isotopes and organic pollutants and adsorption of those from water and environment using natural zeolites

Human activities such as mining, industrial processing, defence industries and nuclear power plants have contaminated water ecosystems with heavy metals (cations and anions) and radio-isotopes. Such pollutants are not bio-degradable and persist in the environment for a long time causing poisonous and toxic effects on ecosystems and organisms living there. Heavy metals such

as mercury, lead, silver, copper, cadmium, chromium, zinc, nickel, cobalt, manganese, and radioisotopes can produce extreme health problems and damage to plants and animals.

Zeolites can exchange ions with the external medium, which is the characteristic feature. Zeolites owing to their ion exchange capabilities are able to adsorb above-mentioned pollutants from ecosystems rendering them less toxic These characteristic behavior of natural zeolites is dependent on the framework structure, ion size and shape, charge density of anionic framework, ionic charge, and concentration of electrolyte in external medium (Kalló 2001). Depending on site of formation, natural zeolites have a differing chemical composition and cation-exchange capacities. Natural zeolites are often modified by acid/base treatment or surfactant impregnation to increase adsorption capacity or exchange of various ions and organics (Benkli et al. 2005; Cortés-Martínez et al. 2004; Hernandez-Beltran et al. 2008; Karadag et al. 2007; Kurama et al. 2002; Panayotova & Velikov 2003). This property of zeolites has made them suitable for removal of undesirable heavy metal ions from industrial effluent water.

Majority of radioactive wastes are generated from nuclear power plants, nuclear test sites, and nuclear power accidents like the Chernobyl and the Fukushima disasters. The contamination of ecosystems by radioactive Caesium and Strontium has raised concerns on the safety of drinking water, agricultural soil, irrigation water, and crops (Lee et al. 2013; Zhu & Smolders 2000). Presently, radioactive Cs and Sr are removed from the environment by various methods such as precipitation, adsorption on cation exchangers, vacuum evaporation, reverse osmosis, filtration and solvent extraction, phytoextraction, and electrodialysis (Rahman et al. 2011). Among all, adsorption on zeolites is promising because of its simplicity, specific cation selectivity, high efficiency, low cost, chemical stability, and thermal resistance (Abusafa & Yücel 2002; Tiwari et al. 2006; Wang & Peng 2010). Clinoptilolite, a natural zeolite is used since 1985 in Sellafield nuclear power plant as ion-exchanger in the effluent plant to decrease discharge of radioactive Cs and Sr to the Irish sea. Natural zeolites were used after the Chernobyl accident to limit the spread of radioactive isotopes.

Moreover, low to intermediate levels of radioactive wastes (Cs and Sr) are produced from use of radionuclides in medicine and radioisotope production facilities. Treatment of these radioactive wastes from industries is crucial and is subject to international regulations for the protection of humans and animals' health and the environment from the adverse effects of radiation. Zeolites

can adsorb radioisotopes of Sr, Cs, Co, Pu, Np, and U from contaminated ecosystems (Comans & Hockley 1992; Munthali et al. 2015; Triay et al. 1996). Among all-natural zeolites, clinoptilolite has shown high efficacy for heavy metals and radioisotopes adsorption.

9. Contamination of fish ponds with heavy metals and ammonia and removal of those using zeolites

As mentioned earlier, various human activities have polluted ecosystems with heavy metals and such metals are contaminating fish ponds and aquaculture systems throughout the world. Rapid development in agriculture and industries and lack of strict regulations have led heavy metals to be emitted to the environment through solid waste emissions, sludge applications, and waste water irrigation. Fish and vegetables grown in such polluted environments are at high risk of accumulating pollutants. Heavy metals in aquatic ecosystems enter fish tissues through the food chain and ultimately to humans. The presence of such metals in ecosystems have detrimental effects on species diversity and high levels of contamination in human foods.

Intensive aquaculture systems throughout the world are facing problems with high concentrations of total ammonia nitrogen (TAN) and dissolved nutrients because of excretion from reared species and high feeding rate. Excessive nitrogen and dissolved nutrients in water decrease fish growth and immunity and release of nutrient-rich waste water and sediments from such aquaculture systems result in eutrophication, oxygen depletion in natural ecosystems and environmental degradation. Various novel techniques have been developed in last decades for the control of TAN and other pollutants in aquaculture systems (Abeysinghe et al. 1996; Kim et al. 2000; Nora'aini et al. 2005; Timmons et al. 2006; Travieso et al. 1996). All such techniques have varying rates of nitrogen removal, high capital costs, and high overall maintenance and operating costs. In addition, application of such sophisticated treatment systems is not feasible for low-cost aquaculture systems as commonly found in developing countries.

The availability of inexpensive natural zeolites and growing scientific awareness on the use of zeolites in agriculture and aquaculture has promoted the concept of 'zeo-agriculture'(Mumpton & Fishman 1977). In recirculating aquaculture systems (RAS), natural zeolites are used to remove

and control ammonia and been found more effective in removal of nitrogen compared to biological nitrification (Johnson & Sieburth 1974). Furthermore, zeolites are used during transport of live fish as they adsorb the produced ammonia and help maintain high oxygen concentrations. Clinoptilolite, a natural zeolite has shown high affinity for removal of ammonium (NH_4^+) from aquaculture wastewaters (Beler-Baykal et al. 1996; Bergero et al. 1994; Booker et al. 1996; Nguyen & Tanner 1998). The removal of ammonia is possible by zeolites because ammonia nitrogen exists as ammonium (NH_4^+) and ammonia (NH_3) in equilibrium as follows:

$NH_3 + H_2O = NH_4^+ + OH^-$

The extra framework cations (Na^+, K^+, Ca^{++} and Mg^{++}) in zeolites are easily exchanged with NH_4^+ in solution thereby lowering TAN concentration and decrease the concentration of ammonia in equilibrium.

10. Conclusion

Cereal grains all over the world are contaminated with one or more kinds of mycotoxins both in the field as well as during storage. Mycotoxins are unseen poison in food and feed. They are present in feed and feed ingredients around the world. Mycotoxins are stable compounds and survive heat treatment and processing during feed production. Mycotoxins produce adverse effects on health and performance of animals leading to economic losses. One or more mycotoxins might be present in contaminated grains. The co-occurrence of different mycotoxins increases the spectrum of mycotoxicosis. Moreover, there is a risk of transfer of mycotoxins to humans via contaminated animal products. Mycotoxins are a serious threat to global food and feed safety and thus it necessitates decontamination before toxins enter the food chain.

The study on adsorption of mycotoxins in aluminosilicates started in the late 1980s and *in-vitro* experiments demonstrated that some of the mycotoxins were strongly bound by at least one adsorbent. Depending on *in-vitro* binding capacities, adsorbents were tested in animals and it was found that some aluminosilicates can alleviate the toxic effects of specific mycotoxins. Aluminosilicates such as HSCAS, bentonites, montmorillonite, and zeolites provided some degree of protection against aflatoxicosis. However, the efficacy of these adsorbents against *Fusarium*

toxins is practically insignificant. No single adsorbent was found to be effective against the most type of mycotoxins but the addition of different adsorbents to animal feeds provide a versatile tool for preventing mycotoxicosis. Addition of adsorbents in the feed for reduction of toxic and economic impacts of mycotoxins is the most promising approach considering its efficacy, safety, and cost-effectiveness.

Cell wall components of yeast *Saccharomyces cerevisiae* have shown to bind *Fusarium* toxins and help reduce the toxic effects in animals. Moreover, microbes originating from the rumen of cattle and intestine of chickens and pigs have shown to degrade *Fusarium* toxins. Similarly, some bacterium isolated from soil have demonstrated degradation of DON. Further animal studies using microorganisms are required to demonstrate mycotoxin removal capabilities *in-vivo*.

Moreover, aluminosilicates especially zeolites find diverse use in agriculture and pollution control in natural ecosystems and industries. Zeolites are capable of adsorbing ammonia, heavy metals and radioisotopes in the same way they adsorb mycotoxins. Owing to its adsorption capacity, zeolites will find a way into various industrial sectors in future.

References

Abbas, H. K., Cartwright, R. D., Xie, W. & Shier, W. T. (2006). Aflatoxin and fumonisin contamination of corn (maize, Zea mays) hybrids in Arkansas. *Crop Protection*, 25 (1): 1-9.

Abeysinghe, D., Shanableh, A. & Rigden, B. (1996). Biofilters for water reuse in aquaculture. *Water science and technology*, 34 (11): 253-260.

Abusafa, A. & Yücel, H. (2002). Removal of 137Cs from aqueous solutions using different cationic forms of a natural zeolite: clinoptilolite. *Separation and Purification Technology*, 28 (2): 103-116.

Additives, E. P. o. & Feed, P. o. S. u. i. A. (2013). Scientific Opinion on the safety and efficacy of micro-organism DSM 11798 when used as a technological feed additive for pigs. *EFSA Journal*, 11 (5): 3203.

Ahad, R., Zhou, T., Lepp, D. & Pauls, K. P. (2017). Microbial detoxification of eleven food and feed contaminating trichothecene mycotoxins. *BMC biotechnology*, 17 (1): 30.

Ali, N., Sardjono, Yamashita, A. & Yoshizawa, T. (1998). Natural co-occurrence of aflatoxins and Fusavium mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Additives & Contaminants*, 15 (4): 377-384.

Anater, A., Manyes, L., Meca, G., Ferrer, E., Luciano, F. B., Pimpão, C. T. & Font, G. (2016). Mycotoxins and their consequences in aquaculture: A review. *Aquaculture*, 451: 1-10.

Arukwe, A., Grotmol, T., Haugen, T. B., Knudsen, F. R. & Goksøyr, A. (1999). Fish model for assessing the in vivo estrogenic potency of the mycotoxin zearalenone and its metabolites. *Science of the total environment*, 236 (1-3): 153-161.

Avantaggiato, G., Havenaar, R. & Visconti, A. (2003). Assessing the zearalenone-binding activity of adsorbent materials during passage through a dynamic in vitro gastrointestinal model. *Food and Chemical Toxicology*, 41 (10): 1283-1290.

Bailey, C., Latimer, G., Barr, A., Wigle, W., Haq, A., Balthrop, J. & Kubena, L. (2006). Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. *Journal of applied poultry research*, 15 (2): 198-206.

Beasley, V. & Lambert, R. (1990). The apparently minimal hazard posed to human consumers of products from animals fed trichothecene-contaminated grains. *Veterinary and human toxicology*, 32 (Suppl.): 27-39.

Bejaoui, H., Mathieu, F., Taillandier, P. & Lebrihi, A. (2004). Ochratoxin A removal in synthetic and natural grape juices by selected oenological Saccharomyces strains. *Journal of applied microbiology*, 97 (5): 1038-1044.

Beler-Baykal, B., Oldenburg, M. & Sekoulov, I. (1996). The use of ion exchange in ammonia removal under constant and variable loads. *Environmental technology*, 17 (7): 717-726.

Benkli, Y., Can, M., Turan, M. & Celik, M. (2005). Modification of organo-zeolite surface for the removal of reactive azo dyes in fixed-bed reactors. *Water research*, 39 (2-3): 487-493.

Bennett, J. (1987). *Mycotoxins, mycotoxicoses, mycotoxicology andMycopathologia*: Springer.

Bergero, D., Boccignone, M., NATALE, F., Forneris, G., Palmegiano, G., Roagna, L. & Sicuro, B. (1994). Ammonia removal capacity of European natural zeolite tuffs: application to aquaculture waste water. *Aquaculture Research*, 25 (8): 813-821.

Bergsjø, B., Langseth, W., Nafstad, I., Jansen, J. H. & Larsen, H. (1993). The effects of naturally deoxynivalenol-contaminated oats on the clinical condition, blood parameters, performance and carcass composition of growing pigs. *Veterinary research communications*, 17 (4): 283-294.

Binder, E. M. (2007). Managing the risk of mycotoxins in modern feed production. *Animal feed science and technology*, 133 (1-2): 149-166.

Biomin. *Dealing with Effects of Mycotoxins in Pig Husbandry*. Available at: http://temp.biomin.net/knowledge-center/articles/articles-details/article/dealing-with-effects-of-mycotoxins-in-pig-husbandry/ (accessed: 20.04.2018).

Booker, N., Cooney, E. & Priestley, A. (1996). Ammonia removal from sewage using natural Australian zeolite. *Water science and technology*, 34 (9): 17-24.

Calvo, A. M., Wilson, R. A., Bok, J. W. & Keller, N. P. (2002). Relationship between secondary metabolism and fungal development. *Microbiology and molecular biology reviews*, 66 (3): 447-459.

Cejka, J. (2005). *Zeolites and ordered mesoporous materials: progress and prospects: the 1st FEZA School on Zeolites, Prague, Czech Republic, August 20-21, 2005*, vol. 157: Gulf Professional Publishing.

Celius, T., Matthews, J. B., Giesy, J. P. & Zacharewski, T. R. (2000). Quantification of rainbow trout (Oncorhynchus mykiss) zona radiata and vitellogenin mRNA levels using real-time PCR after in vivo treatment with estradiol-17β or α-zearalenol. *The Journal of steroid biochemistry and molecular biology*, 75 (2-3): 109-119.

Chairman, D. S. C., Alberti, A., Armbruster, T., Artioli, G., Colella, C., Galli, E., Grice, J. D., Liebau, F., Mandarino, J. A. & Minato, H. (1998). Recommended nomenclature for zeolite minerals: Report of the subcommittee on zeolites of the International Mineralogical Association, Commission on New Minerals and Mineral Names. *Mineralogical Magazine*, 62 (4): 533-571.

Chamberlain, W., Bacon, C., Norred, W. & Voss, K. (1993). Levels of fumonisin B1 in corn naturally contaminated with aflatoxins. *Food and Chemical Toxicology*, 31 (12): 995-998.

Chavez-Sanchez, M. C., Palacios, C. M. & Moreno, I. O. (1994). Pathological effects of feeding young Oreochromis niloticus diets supplemented with different levels of aflatoxin B1. *Aquaculture*, 127 (1): 49-60.

Chen, C., Pearson, A., Coleman, T., Gray, J., Pestka, J. & Aust, S. (1984). Tissue deposition and clearance of aflatoxins from broiler chickens fed a contaminated diet. *Food and chemical toxicology*, 22 (6): 447-451.

Colvin, B., Sangster, L., Haydon, K., Beaver, R. & Wilson, D. (1989). Effect of a high affinity aluminosilicate sorbent on prevention of aflatoxicosis in growing pigs. *Veterinary and human toxicology*, 31 (1): 46-48.

Comans, R. N. & Hockley, D. E. (1992). Kinetics of cesium sorption on illite. *Geochimica et cosmochimica acta*, 56 (3): 1157-1164.

CORMA. Available at: https://www.flickr.com/photos/cormaplus/6743724159/in/photostream/ (accessed: 19.04.2018).

Cortés-Martínez, R., Martínez-Miranda, V., Solache-Ríos, M. & García-Sosa, I. (2004). Evaluation of natural and surfactant-modified zeolites in the removal of cadmium from aqueous solutions. *Separation Science and Technology*, 39 (11): 2711-2730.

Coulombe Jr, R. A., Bailey, G. S. & Nixon, J. E. (1984). Comparative activation of aflatoxin B 1 to mutagens by isolated hepatocytes from rainbow trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch). *Carcinogenesis*, 5 (1): 29-33.

Dalié, D., Deschamps, A. & Richard-Forget, F. (2010). Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. *Food control*, 21 (4): 370-380.

Deng, Y., Velázquez, A. L. B., Billes, F. & Dixon, J. B. (2010). Bonding mechanisms between aflatoxin B1 and smectite. *Applied Clay Science*, 50 (1): 92-98.

Díaz-Llano, G. & Smith, T. (2007). The effects of feeding grains naturally contaminated with Fusarium mycotoxins with and without a polymeric glucomannan adsorbent on lactation, serum chemistry, and reproductive performance after weaning of first-parity lactating sows. *Journal of animal science*, 85 (6): 1412.

Doko, M. B., Canet, C., Brown, N., Sydenham, E. W., Mpuchane, S. & Siame, B. A. (1996). Natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from Eastern and Southern Africa. *Journal of Agricultural and Food Chemistry*, 44 (10): 3240-3243.

Eaton, D. L. & Groopman, J. D. (2013). *The toxicology of aflatoxins: human health, veterinary, and agricultural significance*: Elsevier.

EC. (2002). *DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL*

of 7 May 2002

on undesirable substances in animal feed. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20061020:EN:PDF (accessed: 27.04.2018).

EC. (2006). *Commission Recommendation 576/2006/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.* Available at: http://www.ansvsa.ro/download/legislatie/nutritie/subst-nedorite-576-din-2006.pdf (accessed: 27.04.2018).

EFSA, S. (2004). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol (DON) as undesirable substance in animal feed. *EFSA J*, 73: 1-42.

El-Nezami, H., Kankaanpaa, P., Salminen, S. & Ahokas, J. (1998). Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. *Food and Chemical Toxicology*, 36 (4): 321-326.

El-Nezami, H., Chrevatidis, A., Auriola, S., Salminen, S. & Mykkänen, H. (2002). Removal of common Fusarium toxins in vitro by strains of Lactobacillus and Propionibacterium. *Food Additives & Contaminants*, 19 (7): 680-686.

El-Nezami, H., Polychronaki, N., Salminen, S. & Mykkänen, H. (2002). Binding Rather Than Metabolism May Explain the Interaction of Two Food-Grade Lactobacillus Strains with Zearalenone and Its Derivative ά-Zearalenol. *Applied and Environmental Microbiology*, 68 (7): 3545-3549.

El-Nezami, H., Polychronaki, N., Lee, Y. K., Haskard, C., Juvonen, R., Salminen, S. & Mykkänen, H. (2004). Chemical moieties and interactions involved in the binding of zearalenone to the surface of Lactobacillus rhamnosus strains GG. *Journal of agricultural and food chemistry*, 52 (14): 4577-4581.

Ellis, R., Clements, M., Tibbetts, A. & Winfree, R. (2000). Reduction of the bioavailability of 20 µg/kg aflatoxin in trout feed containing clay. *Aquaculture*, 183 (1-2): 179-188.

Eriksen, G. S. (1998). *Fusarium toxins in cereals: a risk assessment*: Nordic Council of Ministers.

Fairhurst, S., Marrs, T., Parker, H., Scawin, J. & Swanston, D. (1987). Acute toxicity of T2 toxin in rats, mice, guinea pigs, and pigeons. *Toxicology*, 43 (1): 31-49.

Faixová, Z., Faix, Š., Leng, L., Vaczi, P., Renáta, S. & Maková, Z. (2006). Effects of feeding diet contaminated with deoxynivalenol on plasma chemistry in growing broiler chickens and the efficacy of glucomannan mycotoxin adsorbent. *Acta veterinaria*, 56 (5-6): 479-487.

Fernández, A., Belío, R., Ramos, J. J., Sanz, M. C. & Sáez, T. (1997). Aflatoxins and their metabolites in the tissues, faeces and urine from lambs feeding on an aflatoxin-contaminated diet. *Journal of the Science of Food and Agriculture*, 74 (2): 161-168.

Fincham, J., Marasas, W., Taljaard, J., Kriek, N., Badenhorst, C., Gelderblom, W., Seier, J., Smuts, C., Faber, M. & Weight, M. (1992). Atherogenic effects in a non-human primate of Fusarium moniliforme cultures added to a carbohydrate diet. *Atherosclerosis*, 94 (1): 13-25.

Fink-Gremmels, J. & Malekinejad, H. (2007). Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone. *Animal Feed Science and Technology*, 137 (3-4): 326-341.

Fokunang, C., Tembe-Fokunang, E., Tomkins, P. & Barkwan, S. (2006). Global impact of mycotoxins on human and animal health management. *Outlook on Agriculture*, 35 (4): 247-253.
Foster, B., Trenholm, H., Friend, D., Hartin, K. & THOMPSON, B. (1986). Evaluation of different sources of deoxynivalenol (vomitoxin) fed to swine. *Canadian journal of animal science*, 66 (4): 1149-1154.
Freimund, S., Sauter, M. & Rys, P. (2003). Efficient Adsorption of the Mycotoxins Zearalenone and T-2 Toxin on a Modified Yeast Glucan. *Journal of Environmental Science and Health, Part B*, 38 (3): 243-255.
Galvano, F., Galofaro, V. & Galvano, G. (1996). Occurrence and stability of aflatoxin M1 in milk and milk products: a worldwide review. *Journal of Food protection*, 59 (10): 1079-1090.
Galvano, F., Piva, A., Ritieni, A. & Galvano, G. (2001). Dietary strategies to counteract the effects of mycotoxins: a review. *Journal of food protection*, 64 (1): 120-131.
Gelderblom, W. C., Kriek, N., Marasas, W. & Thiel, P. (1991). Toxicity and carcinogenicity of the Fusanum monilzforine metabolite, fumonisin B1, in rats. *Carcinogenesis*, 12 (7): 1247-1251.
Gelineau-van Waes, J., Starr, L., Maddox, J., Aleman, F., Voss, K. A., Wilberding, J. & Riley, R. T. (2005). Maternal fumonisin exposure and risk for neural tube defects: mechanisms in an in vivo mouse model. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 73 (7): 487-497.
Glenn, A. (2007). Mycotoxigenic Fusarium species in animal feed. *Animal Feed Science and Technology*, 137 (3-4): 213-240.
Goel, S., Lenz, S., Lumlertdacha, S., Lovell, R., Shelby, R., Li, M., Riley, R. & Kemppainen, B. (1994). Sphingolipid levels in catfish consuming Fusarium moniliforme corn culture material containing fumonisins. *Aquatic toxicology*, 30 (4): 285-294.
Grant, P. G. & Phillips, T. D. (1998). Isothermal adsorption of aflatoxin B1 on HSCAS clay. *Journal of agricultural and food chemistry*, 46 (2): 599-605.
Hamilton, R., Trenholm, H., Thompson, B. K. & Greenhalgh, R. (1985). The tolerance of White Leghorn and broiler chicks, and turkey poults to diets that contained deoxynivalenol (vomitoxin)-contaminated wheat. *Poultry science*, 64 (2): 273-286.
Harvey, R., Kubena, L., Huff, W., Corrier, D., Rottinghaus, G. & Phillips, T. (1990). Effects of treatment of growing swine with aflatoxin and T-2 toxin. *American journal of veterinary research*, 51 (10): 1688-1693.
Harvey, R., Phillips, T., Ellis, J., Kubena, L., Huff, W. & Petersen, H. (1991). Effects on aflatoxin M1 residues in milk by addition of hydrated sodium calcium aluminosilicate to aflatoxin-contaminated diets of dairy cows. *American journal of veterinary research*, 52 (9): 1556-1559.
Harvey, R. B., Kubena, L. F., Elissalde, M. H., Corrier, D. E. & Phillips, T. D. (1994). Comparison of two hydrated sodium calcium aluminosilicate compounds to experimentally protect growing barrows from aflatoxicosis. *Journal of Veterinary Diagnostic Investigation*, 6 (1): 88-92.
Haskard, C., Binnion, C. & Ahokas, J. (2000). Factors affecting the sequestration of aflatoxin by Lactobacillusrhamnosus strain GG. *Chemico-biological interactions*, 128 (1): 39-49.
Hassan, A. M., Kenawy, A. M., Abbas, W. T. & Abdel-Wahhab, M. A. (2010). Prevention of cytogenetic, histochemical and biochemical alterations in Oreochromis niloticus by dietary supplement of sorbent materials. *Ecotoxicology and environmental safety*, 73 (8): 1890-1895.
He, W.-J., Zhang, L., Yi, S.-Y., Tang, X.-L., Yuan, Q.-S., Guo, M.-W., Wu, A.-B., Qu, B., Li, H.-P. & Liao, Y.-C. (2017). An aldo-keto reductase is responsible for Fusarium toxin-degrading activity in a soil Sphingomonas strain. *Scientific reports*, 7 (1): 9549.
Hendrickse, R. (1999). Of sick turkeys, kwashiorkor, malaria, perinatal mortality, heroin addicts and food poisoning: research on the influence of aflatoxins on child health in the tropics. *Annals of tropical paediatrics*, 19 (3): 229-235.

Hernandez-Beltran, N., Olguin, M. & Rosas-Aburto, A. (2008). Effect of acid phosphate media on the stability of clinoptilolite-rich tuff. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 61 (1-2): 93-100.

Hooft, J. M., Elmor, A. E. H. I., Encarnação, P. & Bureau, D. P. (2011). Rainbow trout (Oncorhynchus mykiss) is extremely sensitive to the feed-borne Fusarium mycotoxin deoxynivalenol (DON). *Aquaculture*, 311 (1-4): 224-232.

Hooft, J. M. & Bureau, D. P. (2017). Evaluation of the efficacy of a commercial feed additive against the adverse effects of feed-borne deoxynivalenol (DON) on the performance of rainbow trout (Oncorhynchus mykiss). *Aquaculture*, 473: 237-245.

Howard, P. C., Eppley, R. M., Stack, M. E., Warbritton, A., Voss, K. A., Lorentzen, R. J., Kovach, R. M. & Bucci, T. J. (2001). Fumonisin b1 carcinogenicity in a two-year feeding study using F344 rats and B6C3F1 mice. *Environmental Health Perspectives*, 109 (Suppl 2): 277.

Huff, W., Kubena, L., Harvey, R., Hagler Jr, W., Swanson, S., Phillips, T. & Creger, C. (1986). Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. *Poultry Science*, 65 (7): 1291-1298.

Hussain, D., Mateen, A. & Gatlin III, D. M. (2017). Alleviation of aflatoxin B1 (AFB1) toxicity by calcium bentonite clay: Effects on growth performance, condition indices and bioaccumulation of AFB1 residues in Nile tilapia (Oreochromis niloticus). *Aquaculture*, 475: 8-15.

Huwig, A., Freimund, S., Käppeli, O. & Dutler, H. (2001). Mycotoxin detoxication of animal feed by different adsorbents. *Toxicology letters*, 122 (2): 179-188.

IARC. *Aflatoxins*. Available at: https://monographs.iarc.fr/ENG/Monographs/vol82/mono82-7A.pdf (accessed: 27.03.2018).

Ibeh, I., Uraih, N. & Ogonar, J. (1994). Dietary exposure to aflatoxin in human male infertility in Benin City, Nigeria. *International journal of fertility and menopausal studies*, 39 (4): 208-214.

Jantrarotai, W. & Lovell, R. T. (1990). Subchronic toxicity of dietary aflatoxin B1 to channel catfish. *Journal of Aquatic Animal Health*, 2 (4): 248-254.

Jemmali, M. (1980). Decontamination and detoxification of mycotoxins. *Pure and Applied Chemistry*, 52 (1): 175-181.

Johnson, P. W. & Sieburth, J. M. (1974). Ammonia removal by selective ion exchange, a backup system for microbiological filters in closed-system aquaculture. *Aquaculture*, 4: 61-68.

Jouany, J., Yiannikouris, A. & Bertin, G. (2005). The chemical bonds between mycotoxins and cell wall components of Saccharomyces cerevisiae have been identified. *Archiva Zootechnica*, 8: 26-50.

Jurjevic, Z., Solfrizzo, M., Cvjetkovic, B., Avantaggiato, G. & Visconti, A. (1999). Ochratoxin A and fumonisins (B 1 and B 2) in maize from Balkan nephropathy endemic and non endemic areas of Croatia. *Mycotoxin Research*, 15 (2): 67-80.

Kabak, B., Dobson, A. D. & Var, I. l. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: a review. *Critical reviews in food science and nutrition*, 46 (8): 593-619.

Kalló, D. (2001). Applications of natural zeolites in water and wastewater treatment. *Reviews in mineralogy and geochemistry*, 45 (1): 519-550.

Kamalzadeh, A., Hosseini, A. & Moradi, S. (2009). Effects of yeast glucomannan on performance of broiler chickens. *Int J Agric Biol*, 11: 49-53.

Karadag, D., Akgul, E., Tok, S., Erturk, F., Kaya, M. A. & Turan, M. (2007). Basic and reactive dye removal using natural and modified zeolites. *Journal of Chemical & Engineering Data*, 52 (6): 2436-2441.

Karaman, M., Basmacioglu, H., Ortatatli, M. & Oguz, H. (2005). Evaluation of the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers as assessed by gross examination and histopathology. *British Poultry Science*, 46 (3): 394-400.

Katsoulos, P. D., Karatzia, M. A., Boscos, C., Wolf, P. & Karatzias, H. (2016). In-field evaluation of clinoptilolite feeding efficacy on the reduction of milk aflatoxin M1 concentration in dairy cattle. *Journal of animal science and technology*, 58 (1): 24.

Kiessling, K.-H., Pettersson, H., Sandholm, K. & Olsen, M. (1984). Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. *Applied and Environmental Microbiology*, 47 (5): 1070-1073.

Kim, S.-K., Kong, I., Lee, B.-H., Kang, L., Lee, M.-G. & Suh, K. H. (2000). Removal of ammonium-N from a recirculation aquacultural system using an immobilized nitrifier. *Aquacultural engineering*, 21 (3): 139-150.

King, R. R., McQueen, R. E., Levesque, D. & Greenhalgh, R. (1984). Transformation of deoxynivalenol (vomitoxin) by rumen microorganisms. *Journal of Agricultural and Food Chemistry*, 32 (5): 1181-1183.

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular plant pathology*, 8 (6): 713-722.

Kollarczik, B., Gareis, M. & Hanelt, M. (1994). In vitro transformation of the Fusarium mycotoxins deoxynivalenol and zearalenone by the normal gut microflora of pigs. *Natural toxins*, 2 (3): 105-110.

Kong, Q., Zhai, C., Guan, B., Li, C., Shan, S. & Yu, J. (2012). Mathematic modeling for optimum conditions on aflatoxin B1 degradation by the aerobic bacterium Rhodococcus erythropolis. *Toxins*, 4 (11): 1181-1195.

Kooijmans, K. A. R. *Aflaxotins – a widely underestimated toxin in food*. Available at: https://www.foodsafety-experts.com/food-safety/aflatoxin-detection/ (accessed: 20.04.2018).

Kriek, N., Kellerman, T. S. & Marasas, W. (1981). A comparative study of the toxicity of Fusarium verticillioides (= F. moniliforme) to horses, primates, pigs, sheep and rats. *The Onderstepoort journal of veterinary research*, 48 (2): 129-131.

Kubena, L., Swanson, S., Harvey, R., Fletcher, O., Rowe, L. & Phillips, T. (1985). Effects of feeding deoxynivalenol (vomitoxin)-contaminated wheat to growing chicks. *Poultry Science*, 64 (9): 1649-1655.

Kubena, L., Harvey, R., Phillips, T., Corrier, D. & Huff, W. (1990). Diminution of aflatoxicosis in growing chickens by the dietary addition of a hydrated, sodium calcium aluminosilicate. *Poultry science*, 69 (5): 727-735.

Kuhn, D. M. & Ghannoum, M. A. (2003). Indoor mold, toxigenic fungi, and Stachybotrys chartarum: infectious disease perspective. *Clinical microbiology reviews*, 16 (1): 144-172.

Kuiper-Goodman, T., Scott, P. & Watanabe, H. (1987). Risk assessment of the mycotoxin zearalenone. *Regulatory toxicology and pharmacology*, 7 (3): 253-306.

Kuiper-Goodman, T. (1991). Risk assessment to humans of mycotoxins in animal-derived food products. *Veterinary and Human toxicology*, 33 (4): 325-32; discussion 332-3.

Kurama, H., Zimmer, A. & Reschetilowski, W. (2002). Chemical modification effect on the sorption capacities of natural clinoptilolite. *Chemical engineering & technology*, 25 (3): 301-305.

Lahtinen §, S., Haskard¶, C., Ouwehand §, A., Salminen §, S. & Ahokas, J. (2004). Binding of aflatoxin B1 to cell wall components of Lactobacillus rhamnosus strain GG. *Food additives and contaminants*, 21 (2): 158-164.

Laurain, J. *Combatting fumonisins thanks to algae*. Available at: https://www.allaboutfeed.net/Mycotoxins/Articles/2018/3/Combatting-fumonisins-thanks-to-algae-255076E/?dossier=25646&widgetid=0&cmpid=NLC|dairy_global|2018-03-06|Combatting_fumonisins_thanks_to_algae (accessed: 19.04.2018).

Lee, D., Wales, J., Ayres, J. & Sinnhuber, R. (1968). Synergism between cyclopropenoid fatty acids and chemical carcinogens in rainbow trout (Salmo gairdneri). *Cancer research*, 28 (11): 2312-2318.

Lee, H. S., Lee, J., Yoon, B., Yim, Y., Choi, I., Cho, H., Lee, S., Baik, K., Park, J. H. & Huh, Y. J. (2013). Removal characterization of 133Cs and 127I in a water treatment process using a laboratory scale experiment. *Water Science and Technology: Water Supply*, 13 (5): 1289-1294.

Lemke, S. L., Grant, P. G. & Phillips, T. D. (1998). Adsorption of zearalenone by organophilic montmorillonite clay. *Journal of Agricultural and Food Chemistry*, 46 (9): 3789-3796.

Lemke, S. L., Mayura, K., Reeves, W. R., Wang, N., Fickey, C. & Phillips, T. D. (2001). Investigation of organophilic montmorillonite clay inclusion in zearalenone-contaminated diets using the mouse uterine weight bioassay. *Journal of Toxicology and Environmental Health Part A*, 62 (4): 243-258.

Liu, A., Leng, X., Li, X., Wang, L., Luo, Y. & Zhu, R. (2009). Effects of azomite on growth, intestinal structure and non-specific immunity of tilapia (Oreochromis niloticus× O. aureus). *Chinese Journal of Animal Nutrition*, 21 (6): 1006-1011.

Lutsky, I. & Mor, N. (1981). Alimentary toxic aleukia (septic angina, endemic panmyelotoxicosis, alimentary hemorrhagic aleukia): t-2 toxin-induced intoxication of cats. *The American Journal of Pathology*, 104 (2): 189.

Maaroufi, K., Chekir, L., Creppy, E. E., Ellouz, F. & Bacha, H. (1996). Zearalenone induces modifications of haematological and biochemical parameters in rats. *Toxicon*, 34 (5): 535-540.

Mann, R. & Rehm, H.-J. (1976). Degradation products from aflatoxin B 1 byCorynebacterium rubrum, Aspergillus niger, Trichoderma viride andMucor ambiguus. *European journal of applied microbiology and biotechnology*, 2 (4): 297-306.

Manning, B. B., Li, M. H., Robinson, E. H., Gaunt, P. S., Camus, A. C. & Rottinghaus, G. E. (2003). Response of Channel Catfish to Diets Containing T-2 Toxin. *Journal of Aquatic Animal Health*, 15 (3): 229-238.

Manning, B. B., Li, M. H. & Robinson, E. H. (2005). Aflatoxins from moldy corn cause no reductions in channel catfish Ictalurus punctatus performance. *Journal of the World Aquaculture Society*, 36 (1): 59-67.

Manning, B. B. & Abbas, H. K. (2012). The effect of Fusarium mycotoxins deoxynivalenol, fumonisin, and moniliformin from contaminated moldy grains on aquaculture fish. *Toxin Reviews*, 31 (1-2): 11-15.

Marasas, W. (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environmental Health Perspectives*, 109 (Suppl 2): 239.

Matejova, I., Vicenova, M., Vojtek, L., Kudlackova, H., Nedbalcova, K., Faldyna, M., Sisperova, E., Modra, H. & Svobodova, Z. (2015). Effect of the mycotoxin deoxynivalenol on the immune responses of rainbow trout (Oncorhynchus mykiss). *Veterinarni Medicina*, 60 (9).

Maxwell, S. M. (1998). Investigations into the presence of aflatoxins in human body fluids and tissues in relation to child health in the tropics. *Annals of tropical paediatrics*, 18 (sup1): S41-S46.

McLean, M. & Dutton, M. F. (1995). Cellular interactions and metabolism of aflatoxin: an update. *Pharmacology & therapeutics*, 65 (2): 163-192.

Mishra, H. & Das, C. (2003). A review on biological control and metabolism of aflatoxin.

Morgavi, D. & Riley, R. (2007). An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with Fusarium toxins. *Animal feed science and technology*, 137 (3-4): 201-212.

Moss, M. (1991). The environmental factors controlling mycotoxin formation. *Mycotoxins and animal foods*: 37-56.

Mumpton, F. & Fishman, P. (1977). The application of natural zeolites in animal science and aquaculture. *Journal of Animal Science*, 45 (5): 1188-1203.

Mumpton, F. A. (1999). La roca magica: uses of natural zeolites in agriculture and industry. *Proceedings of the National Academy of Sciences*, 96 (7): 3463-3470.

Munthali, M., Johan, E., Aono, H. & Matsue, N. (2015). Cs+ and Sr2+ adsorption selectivity of zeolites in relation to radioactive decontamination. *Journal of Asian Ceramic Societies*, 3 (3): 245-250.
Nelson, P. S. A. Available at: https://www.tulane.edu/~sanelson/eens211/silicate_structures08.htm (accessed: 08.03.2018).
Nešić, K., Resanović, R., Nešić, V. & Sinovec, Z. (2008). Efficacy of mineral and organic adsorbent in alleviating harmful effects of zearalenone on pigs performance and health. *Acta veterinaria*, 58 (2-3): 211-219.
Nguyen, M. & Tanner, C. (1998). Ammonium removal from wastewaters using natural New Zealand zeolites. *New Zealand Journal of Agricultural Research*, 41 (3): 427-446.
Niderkorn, V., Boudra, H. & Morgavi, D. (2006). Binding of Fusarium mycotoxins by fermentative bacteria in vitro. *Journal of Applied Microbiology*, 101 (4): 849-856.
Nora'aini, A., Mohammad, A. W., Jusoh, A., Hasan, M., Ghazali, N. & Kamaruzaman, K. (2005). Treatment of aquaculture wastewater using ultra-low pressure asymmetric polyethersulfone (PES) membrane. *Desalination*, 185 (1-3): 317-326.
Oguz, H., Kurtoglu, V. & Coskun, B. (2000). Preventive efficacy of clinoptilolite in broilers during chronic aflatoxin (50 and 100 ppb) exposure. *Research in Veterinary Science*, 69 (2): 197-201.
Panayotova, M. & Velikov, B. (2003). Influence of zeolite transformation in a homoionic form on the removal of some heavy metal ions from wastewater. *Journal of Environmental Science and Health, Part A*, 38 (3): 545-554.
Papaioannou, D., Katsoulos, P., Panousis, N. & Karatzias, H. (2005). The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: a review. *Microporous and mesoporous materials*, 84 (1-3): 161-170.
Parlat, S., Yildiz, A. & Oguz, H. (1999). Effect of clinoptilolite on performance of Japanese quail (Coturnix coturnix japonica) during experimental aflatoxicosis. *British poultry science*, 40 (4): 495-500.
Pavelic, K. & Hadzija, M. (2003). Medical applications of zeolites. *Handbook of Zeolite Science and Technology. New York: Dekker*: 1143-1174.
Peraica, M., Radić, B., Lucić, A. & Pavlović, M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, 77 (9): 754-766.
Pestka, J. J. (2007). Deoxynivalenol: toxicity, mechanisms and animal health risks. *Animal feed science and technology*, 137 (3-4): 283-298.
Phillips, T. D., KUBENA, L. F., HARVEY, R. B., TAYLOR, D. R. & HEIDELBAUGH, N. D. (1988). Hydrated sodium calcium aluminosilicate: a high affinity sorbent for aflatoxin. *Poultry science*, 67 (2): 243-247.
Phillips, T. D., Sarr, A. B. & Grant, P. G. (1995). Selective chemisorption and detoxification of aflatoxins by phyllosilicate clay. *Natural toxins*, 3 (4): 204-213.
Pier, A., Richard, J. & Cysewski, S. (1980). Implications of mycotoxins in animal disease. *Journal of the american veterinary medical association*, 176 (8): 719-724.
Piotrowska, M. & Zakowska, Z. (2005). The elimination of ochratoxin A by lactic acid bacteria strains. *Polish Journal of Microbiology*, 54 (4): 279-286.
Pitt, J. I. (1993). Corrections to species names in physiological studies on Aspergillus flavus and Aspergillus parasiticus. *Journal of food protection*, 56 (3): 265-269.
Placinta, C., D'mello, J. & Macdonald, A. (1999). A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Animal feed science and technology*, 78 (1-2): 21-37.
Prelusky, D., Scott, P., Trenholm, H. & Lawrence, G. (1990). Minimal transmission of zearalenone to milk of dairy cows. *Journal of Environmental Science & Health Part B*, 25 (1): 87-103.
Prelusky, D., Trenholm, H., Rotter, B., Miller, J., Savard, M., Yeung, J. & Scott, P. (1996). Biological fate of fumonisin B 1 in food-producing animals. In *Fumonisins in Food*, pp. 265-278: Springer.

PubChem. *Aflatoxin B1*. Available at: https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=186907 (accessed: 25.04.2018).
PubChem. *Deoxynivalenol*. Available at: https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=40024 (accessed: 26.04.2018).
Qian, G.-S., Ross, R. K., Yu, M. C., Yuan, J.-M., Gao, Y.-T., Henderson, B. E., Wogan, G. N. & Groopman, J. D. (1994). A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiology and Prevention Biomarkers*, 3 (1): 3-10.
Rafai, P., Bata, A., Vanyi, A., Papp, Z., Brydl, E., Jakab, L., Tuboly, S. & Tury, E. (1995). Effect of various levels of T-2 toxin on the clinical status, performance and metabolism of growing pigs. *The Veterinary Record*, 136 (19): 485-489.
Rahman, R., Ibrahium, H. A. & Hung, Y.-T. (2011). Liquid radioactive wastes treatment: a review. *Water*, 3 (2): 551-565.
Raju, M. & Devegowda, G. (2000). Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). *British poultry science*, 41 (5): 640-650.
Ramjee, G., Berjak, P., Adhikari, M. & Dutton, M. (1992). Aflatoxins and kwashiorkor in Durban, South Africa. *Annals of tropical paediatrics*, 12 (3): 241-247.
Ramos, A., Hernandez, E., Pla-Delfina, J. & Merino, M. (1996). Intestinal absorption of zearalenone and in vitro study of non-nutritive sorbent materials. *International Journal of Pharmaceutics*, 128 (1-2): 129-137.
Raymond, S., Smith, T. & Swamy, H. (2003). Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *Journal of animal science*, 81 (9): 2123-2130.
Ribeiro, F. R. (2012). *Zeolites: science and technology*, vol. 80: Springer Science & Business Media.
Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—An overview. *International journal of food microbiology*, 119 (1-2): 3-10.
Riley, R., An, N.-H., Showker, J., Yoo, H.-S., Norred, W., Chamberlain, W., Wang, E., Merrill, A., Motelin, G. & Beasley, V. (1993). Alteration of tissue and serum sphinganine to sphingosine ratio: an early biomarker of exposure to fumonisin-containing feeds in pigs. *Toxicology and applied pharmacology*, 118 (1): 105-112.
Riley, R. T., Norred, W. P. & Bacon, C. W. (1993). Fungal toxins in foods: recent concerns. *Annual review of nutrition*, 13 (1): 167-189.
Rodrigues, I., Handl, J. & Binder, E. (2011). Mycotoxin occurrence in commodities, feeds and feed ingredients sourced in the Middle East and Africa. *Food Additives and Contaminants: Part B*, 4 (3): 168-179.
Rotter, B. A. (1996). Invited review: Toxicology of deoxynivalenol (vomitoxin). *Journal of Toxicology and Environmental Health Part A*, 48 (1): 1-34.
Rustom, I. Y. (1997). Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. *Food chemistry*, 59 (1): 57-67.
Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Lourdes Bastos, M., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G. & Gropp, J. (2017). Safety and efficacy of microorganism DSM 11798 as a technological additive for all avian species. *EFSA Journal*, 15 (1).
Sahoo, P., Mukherjee, S., Nayak, S. & Dey, S. (2001). Acute and subchronic toxicity of aflatoxin B 1 to rohu, Labeo rohita (Hamilton).

Santacroce, M. P., Conversano, M., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G. & Crescenzo, G. (2008). Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Reviews in Fish Biology and Fisheries*, 18 (1): 99-130.

Schatzmayr, G., Zehner, F., Täubel, M., Schatzmayr, D., Klimitsch, A., Loibner, A. P. & Binder, E. M. (2006). Microbiologicals for deactivating mycotoxins. *Molecular nutrition & food research*, 50 (6): 543-551.

SCHEIDELER, S. E. (1993). Effects of various types of aluminosilicates and aflatoxin B1 on aflatoxin toxicity, chick performance, and mineral status. *Poultry Science*, 72 (2): 282-288.

Schell, T., Lindemann, M., Kornegay, E. & Blodgett, D. (1993). Effects of feeding aflatoxin-contaminated diets with and without clay to weanling and growing pigs on performance, liver function, and mineral metabolism. *Journal of animal science*, 71 (5): 1209.

Schmidt, M. *A breakthrough against aflatoxin*. Available at: https://www.allaboutfeed.net/Mycotoxins/Articles/2018/3/A-breakthrough-against-aflatoxin-255884E/?dossier=25646&widgetid=1&cmpid=NLC|allaboutfeed|2018-03-06|A_breakthrough_against_aflatoxin (accessed: 19.04.2018).

Schoenhard, G. L., Hendricks, J. D., Nixon, J. E., Lee, D. J., Wales, J. H., Sinnhuber, R. O. & Pawlowski, N. E. (1981). Aflatoxicol-induced hepatocellular carcinoma in rainbow trout (Salmo gairdneri) and the synergistic effects of cyclopropenoid fatty acids. *Cancer research*, 41 (3): 1011-1014.

Schwartz, P., Thorpe, K. L., Bucheli, T. D., Wettstein, F. E. & Burkhardt-Holm, P. (2010). Short-term exposure to the environmentally relevant estrogenic mycotoxin zearalenone impairs reproduction in fish. *Science of the total environment*, 409 (2): 326-333.

Scudamore, K., Hetmanski, M., Chan, H. & Collins, S. (1997). Occurrence of mycotoxins in raw ingredients used for animal feeding stuffs in the United Kingdom in 1992. *Food Additives & Contaminants*, 14 (2): 157-173.

Sherman, J. D. (1999). Synthetic zeolites and other microporous oxide molecular sieves. *Proceedings of the National Academy of Sciences*, 96 (7): 3471-3478.

Shier, W., Shier, A., Xie, W. & Mirocha, C. (2001). Structure-activity relationships for human estrogenic activity in zearalenone mycotoxins. *Toxicon*, 39 (9): 1435-1438.

Shima, J., Takase, S., Takahashi, Y., Iwai, Y., Fujimoto, H., Yamazaki, M. & Ochi, K. (1997). Novel detoxification of the trichothecene mycotoxin deoxynivalenol by a soil bacterium isolated by enrichment culture. *Applied and environmental microbiology*, 63 (10): 3825-3830.

Smith, E., Phillips, T., Ellis, J., Harvey, R., Kubena, L., Thompson, J. & Newton, G. (1994). Dietary hydrated sodium calcium aluminosilicate reduction of aflatoxin M1 residue in dairy goat milk and effects on milk production and components. *Journal of animal science*, 72 (3): 677.

Smith, J. & Hamilton, P. (1970). Aflatoxicosis in the broiler chicken. *Poultry Science*, 49 (1): 207-215.

Stanković, S., Lević, J., Ivanović, D., Krnjaja, V., Stanković, G. & Tančić, S. (2012). Fumonisin B1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. *Food Control*, 23 (2): 384-388.

Sudakin, D. L. (2003). Trichothecenes in the environment: relevance to human health. *Toxicology letters*, 143 (2): 97-107.

Taylor, M. C., Jackson, C. J., Tattersall, D. B., French, N., Peat, T. S., Newman, J., Briggs, L. J., Lapalikar, G. V., Campbell, P. M. & Scott, C. (2010). Identification and characterization of two families of F420H2-dependent reductases from Mycobacteria that catalyse aflatoxin degradation. *Molecular microbiology*, 78 (3): 561-575.

Timmons, M. B., Holder, J. L. & Ebeling, J. M. (2006). Application of microbead biological filters. *Aquacultural engineering*, 34 (3): 332-343.

Tiwari, D., Prasad, S., Yang, J.-K., Choi, B.-J. & Lee, S.-M. (2006). Inorganic and bio-materials in the removal/speciation of radiocesium and radiostrontium: an overview. *Environmental Engineering Research*, 11 (2): 106-125.

Tomašević-Čanović, M., Daković, A., Rottinghaus, G., Matijašević, S. & Đuričić, M. (2003). Surfactant modified zeolites—new efficient adsorbents for mycotoxins. *Microporous and mesoporous materials*, 61 (1-3): 173-180.

Travieso, L., Benitez, F., Weiland, P., Sanchez, E., Dupeyron, R. & Dominguez, A. (1996). Experiments on immobilization of microalgae for nutrient removal in wastewater treatments. *Bioresource Technology*, 55 (3): 181-186.

Triay, I. R., Cotter, C. R., Kraus, S. M. & Huddleston, M. H. (1996). Radionuclide sorption in Yucca Mountain tuffs with J-13 well water: Neptunium, uranium, and plutonium. Yucca Mountain site characterization program milestone 3338: Los Alamos National Lab., NM (United States).

Tuan, N. A., Grizzle, J. M., Lovell, R. T., Manning, B. B. & Rottinghaus, G. E. (2002). Growth and hepatic lesions of Nile tilapia (Oreochromis niloticus) fed diets containing aflatoxin B1. *Aquaculture*, 212 (1-4): 311-319.

Ueno, Y., Iijima, K., Wang, S.-D., Sugiura, Y., Sekijima, M., Tanaka, T., Chen, C. & Yu, S.-Z. (1997). Fumonisins as a possible contributory risk factor for primary liver cancer: a 3-year study of corn harvested in Haimen, China, by HPLC and ELISA. *Food and chemical toxicology*, 35 (12): 1143-1150.

van Egmond, H. P., Schothorst, R. C. & Jonker, M. A. (2007). Regulations relating to mycotoxins in food. *Analytical and bioanalytical chemistry*, 389 (1): 147-157.

Wang, D. S., Liang, Y. X., Chau, N. T., Dien, L. D., Tanaka, T. & Ueno, Y. (1995). Natural co-occurrence of Fusarium toxins and aflatoxin B1 in com for feed in North Vietnam. *Natural Toxins*, 3 (6): 445-449.

Wang, S. & Peng, Y. (2010). Natural zeolites as effective adsorbents in water and wastewater treatment. *Chemical Engineering Journal*, 156 (1): 11-24.

Weaver, G., Kurtz, H., Mirocha, C., Bates, F., Behrens, J., Robinson, T. & Gipp, W. (1978). Mycotoxin-induced abortions in swine. *The Canadian Veterinary Journal*, 19 (3): 72.

Westlake, K., Mackie, R. & Dutton, M. (1989). In vitro metabolism of mycotoxins by bacterial, protozoal and ovine ruminal fluid preparations. *Animal Feed Science and Technology*, 25 (1-2): 169-178.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M. & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American journal of clinical nutrition*, 80 (5): 1106-1122.

Williams, L. B. & Haydel, S. E. (2010). Evaluation of the medicinal use of clay minerals as antibacterial agents. *International geology review*, 52 (7-8): 745-770.

Wilson, M. (2003). Clay mineralogical and related characteristics of geophagic materials. *Journal of chemical ecology*, 29 (7): 1525-1547.

Wolf, H. & Jackson, E. (1963). Hepatomas in rainbow trout: Descriptive and experimental epidemiology. *Science*, 142 (3593): 676-678.

Wu, F. (2006). Economic impact of fumonisin and aflatoxin regulations on global corn and peanut markets. *The Mycotoxin Factbook*: 83-93.

Wu, Q., Jezkova, A., Yuan, Z., Pavlikova, L., Dohnal, V. & Kuca, K. (2009). Biological degradation of aflatoxins. *Drug Metabolism Reviews*, 41 (1): 1-7.

Yiannikouris, A., Francois, J., Poughon, L., Dussap, C.-G., Bertin, G., Jeminet, G. & Jouany, J.-P. (2004). Adsorption of zearalenone by β-D-glucans in the Saccharomyces cerevisiae cell wall. *Journal of Food Protection*, 67 (6): 1195-1200.

Young, J. C., Zhou, T., Yu, H., Zhu, H. & Gong, J. (2007). Degradation of trichothecene mycotoxins by chicken intestinal microbes. *Food and Chemical Toxicology*, 45 (1): 136-143.

Young, L., McGirr, L., Valli, V., Lumsden, J. & Lun, A. (1983). Vomitoxin in corn fed to young pigs. *Journal of animal science*, 57 (3): 655-664.

Zhang, W., Xue, B., Li, M., Mu, Y., Chen, Z., Li, J. & Shan, A. (2014). Screening a strain of Aspergillus niger and optimization of fermentation conditions for degradation of aflatoxin B1. *Toxins*, 6 (11): 3157-3172.

Zhou, T., Gong, J., Yu, H. & Li, X.-Z. (2014). *Bacterial isolate and methods for detoxification of trichothecene mycotoxins*: Google Patents.

Zhu, Y. G. & Smolders, E. (2000). Plant uptake of radiocaesium: a review of mechanisms, regulation and application. *Journal of Experimental Botany*, 51 (351): 1635-1645.

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway