*Int.J.Curr.Microbiol.App.Sci* (2018) *7*(1): 3410-3426

EXCELLENT PUBLISHERS

*International Journal of Current Microbiology and Applied Sciences*
*ISSN: 2319-7706* **Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com

IJCMAS
www.ijcmas.com

**Review Article** https://doi.org/10.20546/ijcmas.2018.701.403

# Myco-toxins Monitoring Device and their Management Strategies through Detoxifying Agents in Feed

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## A B S T R A C T

**Keywords**

Mycotoxin, Biosensors, Detoxifying agents, Adsorbing agents, Bio-transforming agents

**Article Info**

***Accepted:***
26 December 2017
***Available Online:***
10 January 2018

The naturally toxigenic fungal flora, existing in conjunction with food production is most dominated genera namely, *Aspergillus, Fusarium* and *Penicillium* and to lesser extent the *Alternaria, Claviceps* and *Stachybotrys*. More than 300 mycotoxins are known to exist in nature. The economically important species of fungi producing significant mycotoxins as: aflatoxins (AFs), citrinin (CIT), cyclopiazonic acid (CPA), fumonisins (FBs), moniliformin (MON), ochratoxin A (OTA), deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), patulin (PAT) and zearalenone (ZEA). Several impacts on consumers, such as loss of human and animal lives; health care and veterinary care costs; contaminated foods and feeds disposal costs; and investment in research and management of the myco-toxin problem. Myco-toxins are able to induce powerful and diverse biological effects. Diverse actions of myco-toxins have been characterized on animals and humans to include cytotoxic, carcinogenic, immunosuppressive, nephrotoxic, neurotoxic, mutagenic and oestrogenic effects. Pre and post-harvest management strategies are most important for management of toxicogenic fungi in food materials. Mycotoxin-detoxifying agents for reduction of the contamination of feed by mycotoxins that substances can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action. These feed additives transform mycotoxins into less toxic metabolites either by reducing their bioavailability or by degrading them. Therefore, we can define at least two main categories; including first various mycotoxin adsorbing agents and second bio-transforming agents leads to degradation of mycotoxins into non-toxic metabolites. And also advanced detection techniques are help in ultra-trace amount of myco-toxin in food products as like; bio-sensors (1) electrochemical biosensors, 2) optical biosensors 3)electronic biosensors, 4)piezoelectric biosensors, 5) gravimetric biosensors, 6) pyro-electric biosensors.

## Introduction

Mycotoxins have a wide range of chemical structures and their different physiological activities range from neutral to toxic potentials (Richard, 2007; Njobeh *et al.,* 2010b). Approximately 300-400 mycotoxins are known to exist in nature (Huff *et al.,* 1992) in Table 3. St Anthony's fire was one of the first well-known recognized disease caused by mycotoxins in Europe. This was ergot alkaloids produced by the mold *Claviceps purpurea* in rye (Bove, 1970). In African continent, a cumulative effect between mycotoxin and some important diseases such as tuberculosis, malaria, kwashiorkor and AIDS/ HIV have been suggested (Turner *et al.,* 2003; Gong *et al.,* 2003, 2004). Nowadays, mycotoxins are recognized as causal factors for primary liver cancer, ergotism and alimentary toxic aleukia (Shephard *et al.,* 2007). Most storage fungi grow at $a_W$ below 0.75 generally, the required $a_W$ for fungal growth is between 0.61 and 0.91 (Robert and Raymond, 1994). *Leland C. Clark* introduced the principle of the first enzyme electrode with immobilized glucose oxidase at the New York Academy of Sciences Symposium in 1962 (Heineman and Jensen, 2006). The first commercially produced biosensor Springs Instruments (Yellow Springs, OH, USA) placed on the market in 1975 (Pohanka and Skladal, 2008). Aflatoxin has inhibitory effect on acetylcholinesterase (AchE) and its detection is coupled with the decrease in the activity of AchE which is measured using a choline oxidase amperometric biosensor. (Ben Rejeb *et al.,* 2009). Early studies found that Aflatoxin production by *Aspergillus flavus* is reduced when it is cultivated with certain other fungi and bacteria (Ashworth *et al.,* 1965; Weckbach and Marth, 1977; Wicklow *et al.,* 1980, 1988; Horn and Wicklow, 1983; Ehrlich *et al.,* 1985). These organisms produce certain myco-toxin degrading enzymes. *Flavobacterium auranticum* first micro-organism with myco-toxin degradation activity was isolated in 1966 (Ciegler *et al.,* 1966). Detoxifying agents (adsorbing agents and bio-transforming agents) is one of the well-known strategies for the management of aflatoxins in foods and feeds in Table 4.

### Biosensor

A biosensor is an analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector (Turner *et al.,* 1987, Bănică, 2012).

### Components of biosensor

***Sensitive biological element:*** A biologically derived material or biomimetic component that interacts the analyte under study e.g. tissue, microorganisms, organelles, cell receptors, artificial receptors, enzymes, antibodies, nucleic acids, etc. (URL1).

***Transducer/ detector element:*** is transform the signal resulting from the interaction of the analyte with the biological element into another signal that can be more easily measured and quantified. Transducer works in a physicochemical way; Electro-chemical, optical, electronic, piezoelectric, gravimetric and pyro-electric (Li *et al.,* 2011).

***Biosensor reader device:*** is associated electronics or signal processors that are primarily responsible for the display of the results in a user friendly way. Known manufacturers of biosensor electronic readers include Palm Sens, Gwent Biotechnology Systems and Rapid Labs (Cavalcanti *et al.,* 2008).

### Classification of biosensors based on type of bio-transducer

Biosensors can be classified by their bio-transducer type (URL1) in Table 2. The most common types of bio-transducers used in

biosensors are1) Electrochemical biosensors, 2) Optical biosensors, 3) Electronic biosensors, 4) Piezoelectric biosensors, 5) Gravimetric biosensors, 6) Pyro-electric biosensors

**Electrochemical biosensors:** are normally based on enzymatic catalysis of a reaction that produces or consumes electrons. The sensor substrate usually contains three electrodes; a reference electrode, a working electrode and a counter electrode. Measures rate of flow of electrons that is proportional to the analyte concentration at a fixed potential. Potential of the working electrode is space charge sensitive and this is often used. Further, the label free and direct electrical detection of small peptides and proteins is possible by their intrinsic charges using bio-functionalized ion sensitive field effect transistors (Lud *et al.,* 2006).

**Principle:** Based on their operating principle, the electrochemical biosensors can employ potentiometric, amperometric and impedimetric transducers converting the chemical information into a measurable amperometric signal. In electrochemistry, the reaction under investigation generate a measurable current (amperometric), a measurable potential or charge accumulation (potentiometric) or measurably alter the conductive properties of a medium (conductimetric) between electrodes (Tothill and Turner, 2003). Cottrell equation can indicate this situation better here-

$$I=nFAc_o\sqrt{\frac{D}{\pi t}}$$

If, *I:* current is dependent on, *F:* Faraday's constant, *n:* The number of transferred electrons for each molecule,*A:* The electrode area, $c_0$: The analyte concentration, *D:* The diffusion coefficient and *t:* Time (Koyun *et al.,* 2012).

**Cryptogram:** ***Measured Parameter> Applied Voltage> Sensitivity> Governing Equation> Fabrication***

**Amperometric:** Current> Constant potential (DC)> High> Cottrell Equation> FET+ Enzyme 2 electrodes
**Conductimetric:** Conductance/ Resistance> Sinusoidal (AC)> Low> Incremental resistance> FET+ Enzyme

**Potentiometric:** Potential/ Voltage> Ramp Voltage> High> Nernst Equation> FET+ Enzyme Oxide electrode

**Optical biosensors:** An optical biosensor, the transduction process induces a change in the phase, amplitude, polarization and frequency of input light in response to the physical or chemical change produced by the bio-recognition process (Lading et. al., 2002; Bosch et. al., 2007). The sensor works by sending light through an optical fiber to the bio sample. The amount of light absorbed by the analyte is determined by measuring the light coupled out via second optical fiber. Catalytic and affinity reactions can be measured (Biran and Walt, 2004). The main components of an optical biosensor are light source, optical transmission medium (fiber, waveguide etc.), immobilized biological recognition elements (enzymes, antibodies, or microbes) and optical detection system (Ligler and Taitt, 2008; Borisov and Wolfbeis (2008). Pharmacia Biosensors AB (Sweden) marketed a device called BIAcore, which measures antibody-antigen interactions for the study of drug-receptor interactions (Tothill, 2011).

**Principle:** The reaction causes a change in fluorescence or absorbance resulting due to change in the refractive index of the surface between two media which differ in density. Based on the Lambert–Beer's law stated that "absorbance is proportional to the concentrations of the attenuating species in the material sample" in 1852 (Valeur, 2001).

Lambert-Beer's law is used for the absorption here-

$$A = \log \left( {}^{Io}/_{I} \right) = \varepsilon . [C] . l$$

If, A=Optical absorbance, I0= incident light intensity, I= transmitted light intensity, l=effective path length, ε=Molar absorption coefficient (Koyun *et al.,* 2012).

There are two type detection protocols-

**Fluorescence-based detection:**

**Fluorescence protein (FP)-based biosensors:** The sensing element consists of one or more polypeptide chains. The polypeptide chain acts as the molecular recognition element (MRE) that under goes conformational changes upon binding with analyte, producing a change in fluorescence properties (Nasir and Jolley, 2002). There are three type of FP- based biosensor based on their structure-

**Fluorescence resonance energy transfer (FRET)- based biosensor:** FRET describe the energy transfer between two chromophores. A doner chromophore, in a higher energy state, may transfer energy to an acceptor chromophore through nonradiative dipole-dipole coupling. In FRET- based biosensors, two fluorescent proteins are genetically linked either to each end of a polypeptide chain i.e molecular recognition element (MRE) or two seprate polypeptides, the MRE and analyte protein. Change in the fluorescence intensities of the donor and acceptor FPs, which is measured in FRET efficiency (Chandran *et al.,* 2015; Piatkevich and Verkhusha, 2010; Jares-Erijman and Jovin, 2003).

**Bio-molecular fluorescence complementation (BiFC) - based biosensor:** Used to visualize a variety of protein- protein interactions in live cells. FP is split up and MRE are linked to one portion and the analyte protein is linked to other portion. Two proteins fuse together, refolding properly into three dimensional structures and producing a fluorescence signal (Kerppola, 2008a, b, Kodama and Wada, 2009, Chandran *et al.,* 2015).

**Single FP-based biosensor:** A single fluorescent protein coupled with an MRE causes conformational changes of the fluorescent protein consequently altering its fluorescent properties (Su *et al.,* 2012**,** Ibraheem and Campbell, 2010).

**Chemiluminescence-based biosensors**

Reaction between analyte and immobilized biomolecule that has been marked with Chemiluminescence species will end in generating light as result of biochemical reaction. Emitted light can be detected using a photo multiplier tube (PMT) (Aboul-Enein *et al.,* 2000; Gübitz *et al.,* 2001).

**Label- free detection**

**SPR-based biosensor:** Use surface plasmon wave (electromagnetic wave) to detect changes when the target analyte interacts with the bio-recognition element on the sensor. SPR first observed by Wood (Wood, 1902, 1912) but SPR-based sensors first demonstrated by Liedberg *et al.,* (1983).

**Electronic biosensors:** Ions or electrons are produced during the course of biochemical reaction, the overall conductivity or resistivity of the solution is changing. Transducer measured the electrical conductance/resistance of the solution. Conductance measurements have relatively low sensitivity. Using a sinusoidal voltage (AC) the electric field is generated which finally minimize undesirable effects such as Faradaic processes, double layer charging and concentration polarization (Mohanty and Kougianos, 2006). The inverse value of resistance is called conductance and thus the name conductometric has been used.

Novel trends in case of impedimetric biosensors were reviewed by Guan *et al.,* (2004) and the use of conductometric biosensors for biosecurity by Muhammad-Tahir and Alocilja (2003).

**Principle:** The Faradaic current is the current generated by the reduction or oxidation of some chemical substance at an electrode. The net faradaic current is the algebraic sum of all the faradaic currents flowing through an indicator electrode or working electrode. These biosensors are operated by field-effect modulation of carriers in a semiconductor due to nearby charged (Dzyadevych. *et al.,* 2006, Rastislav *et al.,* 2012).

**Piezoelectric/ Acoustic biosensors:** The first scientific experiment of piezoelectricity was conducted in 1880 by Pierre and Paul Jacques curie, who found that compression of various crystals leads to voltage production on their surface (Manbachi and Cobbold, 2011). These type of biosensors have two components; a receptor and a detector (transducers). The receptor is responsible for the selectivity of the sensor. Examples include enzymes, antibodies, and lipid layers. The detector, which plays the role of the transducer, translates the physical or chemical change by recognizing the analyte and relaying it through an electrical signal (Milstein and Das, 1977, Li, 2008.). There are two types of piezoelectric sensors-

**Bulk acoustic wave piezoelectric sensors:** Transmit an acoustic wave from one crystal face to another (Kim *et al.,* 2007; Fohlerová *et al.,* 2007).

**Surface acoustic wave piezoelectric sensors:** Transmit an acoustic wave along a single crystal face from one location to another (Bowers *et al.,* 1991, Guntupalli *et al.,* 2007).
The resonance frequency of these acoustic waves depends on physical properties of piezoelectric materials and these physical properties changes i e. mass, elasticity, conductivity and dielectric properties from mechanical or electrical variations. When antibodies are coated or when antigens bind to these antibodies. Antibodies lead to increase in mass (Liliana *et al.,* 2011).

**Principle:** The basic equations describing the relationship between the resonance frequency of an oscillating piezoelectric crystal and the mass deposited on the crystal surface have been derived by Sauerbrey (Lostis, 1958; Sauerbrey, 1959, Stockbridge, 1996). In 1959, Sauerbrey developed an empirical equation for AT-cut quartz crystals vibrating in the thickness shear mode that describes the relationship between the mass of thin metal films deposited on quartz crystals and the corresponding change in resonant frequency of the crystal:

$$\Delta F = -2.3x10^6 F^2 = \frac{\Delta M}{A}$$

Where, $\Delta F$ = frequency change in oscillating crystal in Hz, F = frequency of piezoelectric quartz crystal in MHz, $\Delta M$ = mass of deposited film in g, and A = area of electrode surface in $cm^2$.

**Gravimetric biosensors:** are based on mass variation induced by an enzymatic reaction. Enzyme are proteic nature often fragile and instable results in a decrease in the enzyme activity and consequently in a decrease in the biosensor performances (Patolsky *et al.,* 1999) in Table 1.

**Principle:** Gravimetric sensing is based on the fact that when the device mass increases, the resonant frequency of the device decreases. When mass attaches to the sensing surface, the frequency change of the resonant peak is detected. A higher quality factor allows for better resolution of detection (Shuchen *et al.,* 2013; Walton *et al.,* 1993).

**Pyro-electric biosensors/ calorimetric**

**biosensors:** Calorimetric biosensors measure the change in temperature of the solution containing the analyte, following enzyme action and interpret it in terms of the analyte concentration in the solution (Maskow *et al.,* 2012; Kirchner *et al.,* 2012). The sensitivity ($10^{-4}$M) and the range ($10^{-4}$– $10^{-2}$M) of such biosensors are quite low for most applications. Calorimetric technique is capable of rapidly detecting the DNA hybridization.

**Principle:** Calorimetrics measured the changes in temperature in the reaction between bio-recognition element and a suitable analyte. Change in temperature can be correlated to amount of reactants consumed or products formed (Xie *et al.,* 1999).

## Current biosensor research

1. Identification of volatile marker compounds for biosensors.
3. Microsensor array for mycotoxin analysis.
4. Food pathogen biosensor scientists are inventing a new ways to protect the food supply from potentially deadly food pathogens.
5. Phosphate ion biosensor.
6. Biosensor has been developed for detection of insecticide, herbicide and their influence on the environment.
7. Wheat flour quality sensor to determine the starch damage and diacritic activity of wheat flour by the use of FIA biosensor (Hideaki *et al.,* 2003).

## Application of bio-sensors

- Measurement of product quality such as, aroma, taste, nutritional value, functional properties, compliance with specifications (Turner, 1989).
- Screening for product safety include, chemical contaminations, residues, toxins taints, microbial contamination, total load, pathogens, indicators of activity (Rogers, 1995).
- Assessing product stability towards, water activity, solute concentration, pH value, preservative concentration, protective atmosphere composition, chemical reaction and microbial growth (Lowe, 1985).
- Biosensors that use whole cells or enzymes have been used for the detection of alcohol (Valach at al., 2009).

**Table.1** Surface area to mass ratio comparison for various gravimetric sensors

| Device | Mass (g) | S.A. ($mm^2$ ) | Thickness (µm) | Operating Frequency(Hz) | S.A. to mass ratio ($cm^2$/g) |
|---|---|---|---|---|---|
| **SAW (Bowers, et al. 1991)** | – | 10 | – | 200 x $10^6$ | 452 |
| **QCM (Heineman and Jensen, 2006)** | 0.14 g | 154 | 300 | 5 x $10^6$ | 11 |
| **FPW(Clark et al. 2002)** | 15 x $10^{-3}$ | 600 | 25 | 2-30 | 400 |
| **MEMS membrane (Bartkovsky et al, 2004)** | 94.4 x $10^{-9}$ | 0.02 | 1.65 | 200 x $10^3$ | 2120 |

**Table.2** Classification of biosensors based on type of bio-transducer

| Mycotoxin | Matrix | Biosensor principle | Limit of Detection | Reference |
|---|---|---|---|---|
| **Aflatoxin B1** | Maize, sorghum, nuts | Optical | 5 µg $kg^{-1}$ | Nasir & Jolley (2002) |
| | Barley<br>Corn | Electrochemical<br>QCM | 0.03 µg $kg^{-1}$<br>0.03 µg $L^{-1}$ | Ammida *et al.,*(2004)<br>Piermarini *et al.,*(2007) |
| **Aflatoxin M1** | Milk<br>Milk<br>Milk<br>Milk<br>Milk | Electrochemical | 0.02 µg $L^{-1}$<br>0.05 µg $L^{-1}$<br>0.039 µg $L^{-1}$<br>0.008 µg $L^{-1}$<br>0.01 µg $L^{-1}$ | Badea *et al.,*(2004)<br>Micheli at al., (2005)<br>Parker & Tothill (2009)<br>Parker *et al.,*(2009)<br>Paniel *et al.,*(2010) |
| **Fumonisin B1** | Corn<br>Corn<br>Corn | Optical<br>Electrochemical | 50 µg $kg^{-1}$<br>10 µg $L^{-1}$<br>5 µg $L^{-1}$ | Mullett *et al.,*(1998)<br>Thompson & Maragos (1996), Abdul Kadir & Tothill (2010) |
| **Ochratoxin A** | Cereals<br>Wheat<br>Wine<br>Wine | Optical<br>Electrochemical<br><br>QCM | 3.8 to 100 µg $kg^{-1}$<br>0.06 µg $L^{-1}$<br>0.01 µg $L^{-1}$<br>16.1 µg $L^{-1}$ | Ngundi *et al.,*(2005)<br>Alarcon *et al.,*(2006)<br>Heurich *et al.,*(2011)<br>Tsai and Hsieh (2007) |
| **Deoxynivalenol** | Wheat<br>Wheat<br>Oats | Optical | 2.5 µg L-1<br>2.5 µg L-1<br>50 µg kg-1 | Schnerr *et al.,*(2002)<br>Tudos *et al.,*(2003)<br>Ngundi *et al.,*(2006) |
| **α-zearalenol**<br>**α-zearalanol**<br>**Zearalanone**<br>**Zearalenone**<br>**β-zearalanol**<br>**β-zearalenol** | Milk<br>Milk<br>Milk<br>Milk<br>Milk<br>Milk | Electrochemical | 0.001 µg $L^{-1}$<br>0.001 µg $L^{-1}$<br>0.002 µg $L^{-1}$<br>0.009 µg $L^{-1}$<br>0.015 µg $L^{-1}$<br>0.071 µg $L^{-1}$ | Välimaa *et al.,*(2010) |

**Table.3** Myco-toxins: Types, classification, food commodity, action level, mode of action and pathological effects

| S No. | Mycotoxins | Type(IARC Classification) | Fungal Species | Food commodity | Action level | Mode of action | Pathological Effects |
|---|---|---|---|---|---|---|---|
| **1.** | *Aflatoxins* (6T) (Post-harvest) | $AFB_1$(1) $C_{17}H_{12}O_6$, MW= 312, $AFB_2$(1) $C_{17}H_{14}O_6$, MW= 314, $AFG_1$(1) $C_{17}H_{12}O_7$, MW= 328, $AFG_2$(1) $C_{17}H_{14}O_7$, MW= 330, $AFM_1$(2B) & $AFM_2$(2B) | *Aspergillus flavus, A. nomius, A. parasiticus, A. arachidicola, A. bombycis, A. pseudotamarii, A. minisclerotigenes, A. rambellii, A. ochraceoroseus, Emericell astellata, E. venezuelensis, E. olivicola* (CAST, 2003). | Maize, wheat, rice, spices, sorghum, groundnut, treenut, almonds, milk, oilseeds, dried fruit, cheese, eggs and meat (D'Mello, 2003). | $AFB_1$, $AFB_2$=<20 ppb. $AFM_1$ & $AFM_2$ <0.5 ppb (Christopher and Gong, 2010). | Cytochrome P450 enzymes convert aflatoxins to the reactive 8, 9-epoxide form which is capable of binding to both DNA and proteins. Aflatoxin B1-DNA adducts can result in GC to TA transversions (Raj *et al.*,1986) | Carcinogenic, mutagenic, teratogenic, heptotoxic, nephrotoxic, immunosuppressive, hemorrhage of intestinal tract and kidney, liver disease (Christopher and Gong, 2010). |
| 2 | Fumonisins (6T) (Pre-harvest) | $FB_1$(2B), $FB_2$(2B), $FB_3$(2B), $FB_4$(2B), $FA_1$(2B)& $FA_2$(2B) | *Alternaria alternate, Fusarium anthophilum, F. moniliforme, F.dlamini, F. napiforme, F. proliferatum, F. nygamai, F. verticillioides* (Łukasz, 2014, Picot *et al.* 2010) | Maize, maize based products, corn based products, sorghum, asparagus, rice and milk (D''Mello, 2003). | <2000 ppb (Christopher and Gong, 2010). | Interfering with sphingolipid metabolism (Merrill *et al.*, 2001) | Hepatotoxic, cerebral edema, necrosis, immunotoxic (Christopher and Gong, 2010). |
| 3 | Ochratoxin (Post-harvest) | OTA(2B) $C_{20}H_{18}O_6NCl$, OTB(2B), OTC(2B) | *A. alutaceus, A. alliaceus, A. auricomus, A. glaucus, A. niger, A. carbonarius, A. melleus, A. albertensis, A. citricus, A. flocculosus, A. fonsecaeus, A. lanosus, A. ochraceous, A. ostianus, A. petrakii, A. sulphureus, A. pseudoelengans, A. roseoglobulosus, A. sclerotiarum, A. steynii, A. westerdijkiae, Neopetromyces muricatus, Penicillium viridicatum, P. verrucosum, P.cyclopium* and *P. carbonarius* (D'Mello, 2003). | Cereals, dried vine fruit, wine coffee, oats, spices, rye, raisins and grape juice Cereals, dried vine fruit, wine coffee, oats, spices, rye, raisins and grape juice (Halasz *et al.*,2009) | OTA =<5 ppb (Murphy, *et al.* 2006) | Inhibits the enzyme involved in the synthesis of the phenylalanine tRNA complex (Bunge *et al.*,1979) (Marquardt and Frohlich, 1992). In addition, it inhibits mitochondrial ATP production (Meisner and Meisner, 1981). | Carcinogenic, mutagenic, teratogenic, heptotoxic, nephrotoxic, immunodeppressants and inhibition of protein synthesis (Thrasher and Crawley, 2009). |
| 4 | *Trichothecenes* (Pre-harvest) | T-2(3) and HT-2 toxin(3), diacetoxyscirpenol, Neosolaniol, nivalenol(NIV) (3), deoxynevalenol, 3-acetyl DON(3), 1,5-acetyl DON, fusarenon X(3) | *Fusarium sporotrichioides, F. poae, F. acuminatum, F. culmorum, F. equiseti, F. graminearum, F. cerealis, F.moniliforme, F. myrothecium, F. lunulosporum, Cephalosporium* sp. (D'Mello, 2003). | Cerial and cerial based products (D'Mello, 2003). | T-2 =<100 ppb, DON=<1000 ppb(Murphy *et al.*,2006) NIV =<0.2 ppm | Acting both on the cell immune system and on the number of macrophages, lymphocytes and erythrocytes. T-2 and deoxynivalenol (DON) are known to inhibit protein synthesis and cause cell death in various parts of the body. Upadhaya *et al.*, (2010) | Immuno-depressants, mutagenic, gastrointestinal haemorrhaging and neurotoxic (Thrasher and Crawley, 2009). |
| 5 | Zearalenone (Pre-harvest) | α-ZON(3)& β-ZON(3) | *Fusarium graminearum, F.culmorum, F. crookwellense, F. equiseti, F. sporotrichioides Fusarium graminearum, F.culmorum, F. crookwellense, F. equiseti, F. sporotrichioides* (D'Mello, 2003). | Barley, oats, wheat, rice sorghum, sesame, soybeans and cereal based products (D'Mello, 2003). | <1ppm (Abdelhamid, 1990). | Binds to oestrogen receptors binds to oestrogen receptors (Zinedine *et al.*, 2007). Decrease in the amounts of luteinizing hormone (LH) and progesterone produced affecting the morphology of uterine tissues, decrease in milk production, feminization of young males due to decreased testosterone production( Guerre *et al.*,2000) | Estrogenic activity (infertily, vulval oedema, vaginal prolapsed, mammary hypertrophy in females, feminisation of males (Thrasher and Crawley, 2009). |

**Note:** 1 µg/kg = 1 ppb, 1ppm = 1000ppb, 1 mg/kg = 1 ppm

**Table.4** Detoxifying agents: absorbing and Bio-transforming agents and their product, dose, target myco-toxin and company based on available literature

| S. No. | Product | Dose | Targeted myco-toxin | Company |
|---|---|---|---|---|
| A. | **Mycotoxin-adsorbing agents** | | | |
| **1.** | Astra Ben 20® (sodium bentonite), Red Crown® (calcium bentonite) | 227 g/cow daily | AFB1, AFM1 | Prince Agri-products (Diaz *et al.*,2004) (Kissell et al. 2013) |
| **2.** | Flow Guard® (sodium bentonite) | 1.1% of DM | AFB1, AFM1 | Laporte Biochem, Inc. (Diaz *et al.*,2004) |
| **3.** | Microsorb® (sodium bentonite) | 1.1% of DM | AFB1, AFM1 | American Colloid Co. (Diaz *et al.*,2004) |
| **4.** | Milbond-TX®: inert montmorillonite clay-based adsorbing agent | 2.0% of DM | AFB1 | Milwhite Inc. (Marroquin-Cardona *et al.*,2009) |
| **5.** | NovaSil™ | 1 to 5 kg/t of feed | AFB1, AFM1 | Engelhard Chemical corporation (Galvano *et al.*,1996), (Jaynes *et al.*,2007), (Moschini *et al.*,2008), (Wang *et al.*,2008) |
| **6.** | Myco-Ad® Zeolex® | 2.5 kg/ t of feed or 3.0 kg/t of feed | T-2 toxin | Special Nutrients (Diaz *et al.*,2005) |
| **7.** | Activated carbon (AC) | 2% | ZEA, FB1, FB2, OTA, DON, AFB1, AFM1 | Sigma F.I.S. (Galvano *et al.*,1998), (Nageswara and Chopra, 2001) (Avantaggiato *et al.*,2007), |
| **8.** | Filtrasorb 400 | 2% | Aflatoxin | Calgon carbon corporation (Di Natale *et al.*,2009) |
| **9.** | Nuchar® SA-20 | 2% | AFB1, AFM1 | Westvaco (Diaz *et al.*,2004) |
| **10** | Mycosorb™, MTB-100® (polymeric glucomannan adsorbing agent extracted from the cell wall of yeast) | 0.05-0.2% | OTA, FB1, Moniliformin, ZEA, AFB1, AFM1, T-2 toxin, DAS, DON, NIV, | Alltech (Bursian, 2004), (Diaz *et al.*,2004), (Chowdhury and Smith, 2005), (Kogan and Kocher, 2007), (Dvorska, 2007), (Smith *et al.*,2008), (Moschini *et al.*,2008), |

| | | | | |
|---|---|---|---|---|
| | | | fusaric acid | (Meissonnier *et al.*,2009) |
| **11** | *Lactobacillus rhamnosus strain GG Lactobacillus helveticus 46 and 72 Lactobacillus jugurti 63 Lactobacillus lactis 170 Lactobacillus casei spp. Casei C3 Streptococcus thermophilus NG40Z and C5 Lactobacillus paraplantarum* | 1.7 x $10^8$ to 2.2 x $10^9$ cfu kg-$^1$ of complete feedstuff | DON, FB1, FB2, ZEA | Promochem INRA Thiverval-Grignon ( Niderkorn *et al.*,2006), (Niderkorn *et al.*,2007), (Niderkorn *et al.*,2008), (Niderkorn *et al.*,2009) |
| **12** | Adfimax® 82 (oat fibers)<br>Adfimax® 75(apple fibers)<br>Adfimax® 59 (grape pulp fibers)<br>Adfimax® 90 (pea seed fibers)<br>Adfimax® 84(lupine seed fibers)<br>Adfimax® 80(lupine seed fibers)<br>( Micronized fibers) | 20g/l | OTA | REALDYME (Tangni 2003, (Tangni *et al.*,2005), (Aoudia *et al.*,2008), (Aoudia *et al.*,2009) |
| **13** | Antitox Vana (Polyvinylpolypyrrodilone, $C_6H_9NO$) | 20g/l | DON | Qualitech Products Inc. (Friend, 1984) |
| **2. Bio-transforming agents** | | | | |
| **1.** | Biomin BBSH 797 (*Eubacterium* s.p. BBSH 797) | 1.7 x $10^8$ to 2.2 x $10^9$ cfu kg-$^1$ of complete feedstuff | T-2 toxin, HT-2 toxin, T-2 tetraol, T-2 triol, scirpentriol | Biomin (Fuchs *et al.*,2002) |

| | | | | |
|---|---|---|---|---|
| **2.** | *Aspergillus niger*, *Eurotium herbariorum*, *Rhizopus* sp., and non-aflatoxin (AF)-producing *A. flavus* | 2g/ kg of feed | AFB1, Aflatoxiol | (Nakazato *et al.*,1990) |
| **3.** | Mycofix® Plus | 2g/ kg of feed | FB1, ZEA, DON, NIV, DAS, T-2 toxin, OTA | Biomin (Avantaggiato *et al.*,2005), (Dänicke *et al.*,2002), (Dänicke *et al.*,2002), (Dänicke, 2002), (Dänicke *et al.*,2003), (Diaz *et al.*,2002), (Diaz *et al.*,2005), (Hanif *et al.*,2008), (Politis |
| **4.** | Combination of *Eubacterium* BBSH 797 and *Trichosporon mycotoxinivorans* | 2g+1g/ kg of feed (Karaman *et al.*, 2005) | OTA, ZEA | Biomin (Hofstetter *et al.*,2006) |
| **5.** | Amano" 2 SD (Protease A - *Aspergillus Niger*) | 2000 units per kg (Xu *et al.*, 2014) | OTA | Amano Inc. (Abrunhosa *et al.*,2006) |
| **6.** | Lipomod™ 801MDP Pancreatin (Porcine pancreas) | 300-1000 U for 1 kg of the main ingredient (Xu *et al.*, 2014). | OTA | Biocatalysts (Abrunhosa *et al.*,2006) |

## Management of myco-toxin through detoxifying agents in feed

Mycotoxin-detoxifying agents for reduction of the contamination: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action". Depending on their mode of action, these feed additives may act by reducing the bioavailability of the mycotoxins or by degrading them or transforming them into less toxic metabolites Table 4. Therefore, we can define at least two main categories; including first various mycotoxin adsorbing agents leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs. Adsorbing agents are also called binding agents, adsorbents and second bio-transforming agents leads to degradation of mycotoxins into non-toxic metabolites; such as bacteria/fungi or enzymes (EFSA, 2002). Absorbing agents (AAs), on the basis of the toxicokinetic principle, since they can reduce mycotoxin absorption by the gastro-intestinal tract, they can reduce the further steps such as toxin distribution and metabolism in organs and tissues: for example, the more mycotoxins such as AFB1 and OTA are sequestered, the less residues could be found in liver and kidneys. The consequence is also the decrease of residue and/or metabolite excretion in eggs for poultry and in milk for mammals and in particular mycotoxins: for example the more AFB1 is sequestered, the less AFM1 could be found in milk. Some AAs, have shown also other health benefits than those of counteracting the direct and indirect effects due to mycotoxins. The cell wall of *Saccharomyces cerevisiae* is composed of beta-D-glucans which have, besides their high binding properties towards ZEA and AFB1 and to a lesser extent to FB1 and DON, a well-known ability to stimulate the immune system (Yiannikouris *et al.,* 2004). Regarding the ability of kaolin when given to animals in their diet, to bind, in addition to aflatoxins, heavy metals, plant metabolites, poisons, diarrhea-causing enterotoxins, and pathogenic microorganisms. Bentonites have been also found to be excellent adsorptive materials of heavy metals and bacteria. Bentonites including in particular montmorillonites, when used in animal diets, act as gut protectants (Trckova *et al.,* 2004; Phillips *et al.,* 2008). Since biotransforming agents are capable of modifying the molecular structure of some mycotoxins, the new conpound is supposed to be less toxic than the parent mycotoxin. If molecular structure(s) of new compound(s) is not modified during the toxicokinetic steps, the minimized toxicity is respected. Consequently, the action of biotransforming agents can improve the sanitary safety of some animal products.

In conclusions, there is a worldwide interest in innovation of the sensing receptor, the transducer and the accompanying electronics and analysis software and microfluidics. A single analyte testing to multianalyte analysis, miniaturization and novel materials such as nanoparticles and micro/nano based transducers are playing a major part in producing highly sensitive and cost effective devices. Current trends to produce chip-based micro/ nano-arrays for multi myco-toxins analysis are challenging. Nanoparticles of such as gold, silver, metal oxides and quantum dots assay developments will enhance the capability of the biosensor technology for myco-toxins analysis. In early, sensitive detection will aid in eliminating these toxins from entering the food chain and preventing ill health and protecting life. The chemical complexity of mycotoxins means that the effectiveness of an absorbing compound does not equalize ability to sequester one mycotoxin to other. Mycotoxin-adsorbing agents can interact with vitamin or minerals other nutrients of feed. In this case, health benefits of these nutriments could be

reduced. Biotransforming agents to be used in practice as animal feed additives must rapidly degrade mycotoxins into non-toxic metabolites, under different oxygen conditions and in a complex environment. They must be safe for animals and stable in the gastrointestinal tract. Therefore these innovative technologies need to be appropriate funding to move the technology from research to commercial products.

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**How to cite this article:**

Shrvan Kumar, Asha Sinha, Shweta Meshram, Mukesh Kumar Singh, Vimla Singh and K.S. Hooda. 2018. Myco-toxins Monitoring Device and their Management Strategies through Detoxifying Agents in Feed. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 3410-3426.
doi: https://doi.org/10.20546/ijcmas.2018.701.403