TOXINDEGRADE

A PRACTICAL STABLE RATIONAL BIOSOLUTION TO DESTROY TOXINS

And continues to perform in the G I tract and at the Hepatic Level.

Enhances nutrient digestion and absorption
Halts growth of pathogenic microbes and moulds
Improves feed intake
Reduces damages to Liver and G I Tract membrane
Simulates Immune System

Starts functioning when once comes into contact with the feeding stuff

WHAT IS FUNGI?

Fungi (*Myces*) are plant-similar micro organisms, some of them are large sized (as mushrooms) and the others are microscopic, therefore they are poly-or-mono-cellular. Some of the fungi are useful for man, since they could be eaten or used in producing drugs, dairy products, bread…. etc., and used in fungal biocontrol. Yet, the others are harmful for man, animals and plants, since they cause diseases (mycoses) and / or intoxications (mycotoxicoses). Therefore, fungi are responsible for crops damage (25% of the yearly production), whether in the field, during transportation, and / or during storage. Toxic fungi can also invade various feed - and foodstuffs and hence affect agricultural animals (Abdelhamid and Saleh, 1996) and humans (Abdelhamid *et al.*, 1999). Moreover, these toxigenic fungi occur also in and / or on moist houses, libraries, air conditioners, feed mills, dust, air, insects, temples, banknotes, and computer disks and compact disks (Abdelhamid, 1998, 1999-b, and 2000-b).

WHAT IS MYCOTOXIN?

It is a fungal toxin, i.e. it is a secondary toxic - metabolite which produced from a toxigenic fungus. Any mycotoxin could be produced from many fungal species, and any fungal strain can produce many mycotoxins. Therefore, any moldy sample may contain numerous fungal genera and species (multi-infection), hence and consequently it may be contaminated with different mycotoxins.
For instance, zearalenone (F-2) is produced by *Fusarium roseum*, *F. tricinctum*, *F. oxysporum,* and *F. moniliforme.* Also, diacetoxyscirpenol (DAS) producing fungi are *F. equiseti, F. sambucinum, F. tricinctum, F. scirpi, F. solani, F. rigidiusculum, F. culmorum* and *F. avenaceum*. On the other hand, *A. ochracious* produces aflatoxins, ochratoxins, penicillic acid, cycalonic acid, viomllin….. etc., and *A. flavus* produces, aflatrime, aflatoxins, aspergillic acid, aspertoxin, cyclopiazonic acid, kojic acid, penetrimes, rubratoxins, sterigmatocystin, tremorgns etc. So, when one mycotoxin is detected, man should suspect that others are also present in a contaminated feed (Abdelhamid, 2000-b). However, the story of mycotoxins is very new comparing with the old known story of fungi. It began with the detection of ergot, trichothecines, aflatoxins…. and recently fumonisins. Nowadays, more than one thousand different chemically identified

mycotoxins are isolated. They are of low molecular weights. Some of them acts with each other synergistically as fumonisin-B1 and aflatoxin-B1, ochratoxin-A and aflatoxin, T2 toxin and aflatoxin. Mycotoxins cause a wide variety of adverse clinical signs depending on the nature and concentration of mycotoxins present, duration ofexposure, the animal species, its age and nutritional and health status at the time of exposure to contaminated feed (Horvath, 1998).

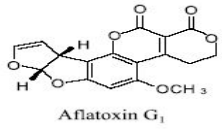
CHEMICAL STRUCTURES OF THE MYCOTOXINS

They are peptide derivatives (Cyclochoritme, Ergot, Gliotoxin, Sporidismine), quinone derivatives (Lotuskirin, Rogulosin), peron derivatives (Aflatoxin, Citrinin, Kojic acid, Sterigmatocystin), terpine derivatives (Fusarinone, Satratoxin, Trichothecines, Vomitoxin), nonadrid (Rubratoxin), alkaloid (Lesergic acid, Slaframin), xanthine (Sterigmatocystin), lacton (Patulin, Penicillic acid, Rubratoxin, Zearalenone), botnolid (Patulin), phynol (Zearalenone), glucose (Kojic acid), qumarin (Aflatoxin, Ochratoxin, Sterigmatocystin) as citd by Abdelhamid (2000-b).

WHAT ARE THE IMPORTANT TOXINS AND WHAT ARE THEIR EFFECTS?

Aflatoxin B1 from *A. flavous*

Increases Embryonic Mortality.
Decreases feed efficiency.
Decreases hatchability.
Poor Growth.
Reduced RBC.
Anemia.
Impaired Blood Clotting.
Damage to Liver
Causes Liver Tumors
Decreased Immune responsiveness
Causes Necrosis, basophilia of hepatocytes
Enlarges blood sinusoids in the kidney
Necrosis of gastric glands
Causes liver cancer, hepatoma.
Reduces Survival Rate
Elevates internal organs indices
Causes chromosomal aberrations
Lowers mitotic index of gill cells.
Reduces muscles area
Causes accumulation of iron in intestinal mucosa epithelium
cellulartoxic – free-radical and active oxygen producing
Damages the tissues of gills, intestine, liver, subcutaneous tissue and muscle, spleen, kidneys, and brain.

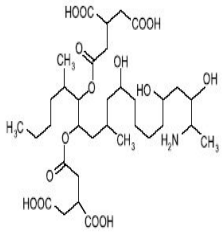


Aflatoxin G_1

Aflatoxin G1 and G2
Affects circulatory system
regurgitation of stomach contents

Aspergillic acid
neurotoxic
Citrinin from *P.Citrinum*
Carcinogenic
nephrotoxic
Damages Kidney and liver
heapatotoxic
Citriovuridine
Affects circulatory system
Cyclopiazonic acid
Affects circulatory system
necrosis of gastric glands
Neuro toxin.
Suppresses Growth
causes accumulations of proteinaceous granules in renal tubular epithelium
Cyclosorine
Affects circulatory system
Deoxynivalenol
Decreases production
Induces Liver toxicity
Leads to Death
Carcinogenic
Destruxin-A
chromatide plaster
Ferrocarin E

carcinogenic



Neurotoxic
Hepatotoxic
Nephrotoxic Depresses Growth
Lowered Hematocrit
increased liver glycogen
increased vacuolation in nerve fibers Reduces red and white blood cell counts Perivascular lymphohistiocytic investment in the brain

Fumonisins

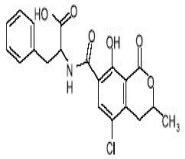
Causes Imbalances in reproduction system Results in poorer egg shell quality

Gliotoxin

Immunotoxic Respirotoxic

Lotuskerine

Affects digestive system



Ochratoxin A from *A. ochracius*

Decreases body weight *Causes deformities of the head, tail and eyes.*
Reduces feed Intake *Necrosis of Liver, Kidney*
Inhibition of DNA, RNA and protein synthesis Nephrotoxic
Increased incidence and severity of melanomacrophage centers in hepatopancreatic tissue and posterior kidney
Reduction of number of Exocrine pancreatic cells

Oosporein

Nephrotoxic

Rubratoxin

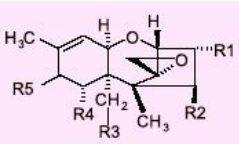
Affects digestive system

Sterigmatocystine

Carcinogenic hepatotoxic
digestive system toxins Reduces Survival Rate
decreases growth rate as well as muscular protein content

T-2 Toxin

Leads to oral lesions, Gizzard lesions *Decreases chick weight*
Causes dermatitis *Decreases Hatchability*
estrogenic, sexual disorders Affects circulatory system
Damages the intestinal tracts Causes severe oedema
Causes fluid accumulation in the body cavity and behind the eyes.
loss of the intestinal mucosa Reduced Growth Rate
poor F C R



Trichothecines

Carcinogenic dermal toxic

Vomitoxin
Immunotoxic Neurotoxic
Zeralenone
Lessens feed intake *Decreases Growth rate*
Results in Fatty Liver Genotoxic

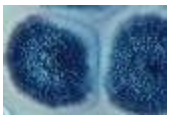
Biochemical effects of various mycotoxins

System	Mycotoxins
Metabolic systems	
Carbohydrate metabolism	Aflatoxins, ochratoxin A, Phomopsin A
Lipid metabolism	Aflatoxins, ochratoxin A, T-2, Toxin, citrinin, rubratoxin B
Vitamin assimilation	Aflatoxins, dicoumarol
Protein synthesis	Aflatoxins, trichothecenes toxins
Mitochondrial respiration	Aflatoxins, ochratoxin A, rubratoxin B, patulin
Endocrine system	Aflatoxins, zearalenone, ergot alkaloids
Skeletal system	Aflatoxins, ochratoxin A

OCCURRENCE OF FUNGI IN FEEDS

A Survey for microorganisms associated with the aquatic and terrestrial animals revealed that more than 20 fungal isolates belonging to different genera and species including *Saprolegnia, Trichoderma, Alternaria* spp., *Penicillium, Fusarium* sp., *Fusarium semitectum* (= *F. incarnatum*), *Cladosporium*, *Phoma, Nigrospora*, *Aspergillus niger* and *Aspergillus flavus* were isolated from naturally diseased animals.

OCCURRENCE OF MYCOTOXINS IN FEEDS



The most widely found in nature and grow and produce toxins under the proper conditions are fungal genera *Aspergillus, Penicillium* and *Fusarium*. The latest genus requires high moisture content, so outspreads in fields and attacks vegetative substances and known as “field fungi”. Whereas, both former genera require low humidity, so are outspreading in store houses and known as “storage fungi”.
However, moisture content greater than 14% and relative humidity greater than 70% are required for fungal growth and toxin production. Fungal invasion negatively affects physical (texture, color, odor, flavor) and chemical (mineralization) properties as well as feeding value of the infected feed (Abdelhamid, 1993b; 1995b&c; 1999a; 2000a and 2001 and Abdelhamid *et al.,* 1985).
So, it is economically important to avoid buying damaged (mechanically or moldy) feed stuffs, maintain good conditions in store houses and do not store finished feeds for long periods (Abdelhamid, 1985; 1989 & 1990 and Noonpugdee *et al*., 1986).
Toxigenic fungi and their toxins are found often in various feeds of plant and animal origins including *Aspergillus flavus, A. niger, Mucor,* and *Pencillium* .
The following Table illustrates some feeding stuff and their mycotoxins content (Abdelhamid, 1980, 1983a - e, 1985, 1990, 2000b & 2005 and Abdelhamid *et al., 1996*)**:**

Feeds	**Mycotoxins**
Bone meal	Vomitoxin and Zearalenone

Cottonseed, Rice bran	Aflatoxin-B1, Citrinin, Ochratoxin-A, Vomitoxin, and Zearalenone
Grains	Aflatoxin-B1 & G1, Citrinin and Ochratoxin-A
Maize	Aflatoxin-B1, Fumonisins, Ochratoxin-A and Vomitoxin
Maize flour, beans	Aflatoxins, Cyclopiazonic acid, Patulin and Griseofulvin
Maize, pea/Groundnut meal	Aflatoxins, Cyclopiazonic acid, Ochratoxin-A, and Zearlenone
Sunflower meal, sorghum, wheat	Aflatoxins, Cyclopiazonic acid, Ochratoxin-A and Zearlenone
Maize, Peanut oil	Aflatoxin-B1
Milk products	Aflatoxins-B1, B2. M1 and Patulin

A. flavus producing for aflatoxins was found in dried Jawla, Prawn Head and Shell . Also, *A. ochracious, A. flavus, A. tamari* and *A. niger* were found in smoked fish, so smoked fish contain aflatoxins and ochratoxin-A.
Fish meal contained aflatoxin-B1 and ochratoxin-A; hence, sea foods were contaminated with aflatoxin-B1 residues, therefore caused human mycotoxic food poisoning. However, feedstuff samples were tested for the presence of some mycotoxins and found to be contaminated, particularly with vomitoxin, aflatoxin, citrinin, zearalenone and ochratoxin, in descending order concerning the percentage of rejected (highly contaminated than the tolerable level) samples. Feeds were heavily contaminated with aflatoxin up to 3388 ppb (Abdlhamid *et al*., 1997). However, co-occurrence of cyclopiazonic acid was found in the aflatoxin-contaminating feed samples (Balachandran and Parthasarathy, 1996). Generally, mold toxins are more toxic to the juveniles of any species (Lim and Webster, 2001).

FACTORS AFFECTING MYCOTOXINS PRODUCTION

Each fungus requires special conditions (substrate, moisture, temperature….) for its growth and other conditions for its toxin(s) production which are different than those of the other fungi and toxins. However, the main affecting factors on toxin production are genetic factors (related to the fungus, its strain and its genetic capability) and environmental factors including:

1. The substrate (on which the fungus will grow) and its nutritious content.
2. Water content {water activity (aw)} of the substrate and ambient relative humidity.
3. Ambient temperature (dry growing season).
4. Ambient oxygen content (is required for fungal growth).
5. Ambient carbon dioxide (not required for fungal growth).
6. Mechanical damage (enable fungal invasion and mycotoxin production).
7. Insects invasion (enable fungal invasion and mycotoxin production).
8. Increased count of fungal spores accumulates the produced mycotoxin.
9. The growth of non-toxic fungal strains inhibits the production from the toxigenic fungi.
10. Presence of specific biota inhibit growth of fungi and mycotoxin production.
11. Time of fungal growth (after the plateau , the capability of producing toxins decreases).
12. Cultivation operations [plants density/area unit (micro clime), agricultural rotation,
13. fertilization, wet harvest, mechanization, storage period….. etc.].
14. Low layer thickness of a crop (< 50 cm) during drying strongly decreases mycotoxin production

Most of AFB1 and AFB2 ingested by mammals is eliminated through urine and faeces, however a fraction is biotransformed in the liver and excreted together with milk in the form of aflatoxins AFM1 and AFM2, respectively.

Fungi belonging to the genus *Fusarium* are associated with the production of fumonisins. Among the fumonisins, fumonisin B1 (FB1) in particular is of international, agroeconomic, and food safety concern.

Ochratoxin A (OTA) produced by *Aspergillus* and *Penicillium* spp. is a natural contaminant in cereals and beverages

Based on the experimental results available, it has been concluded that patulin produced by *Penicillium* spp. is genotoxic, although no adequate evidence of carcinogenity in experimental animals exists

MYCOTOXINS DETECTION

The method of mycotoxin analysis depends mainly on the mycotoxin it self (or its metabolites) and the contaminated tissue or substance will be tested. Therefore, there are many detection methods for each mycotoxin, and there are screening methods for detecting more than one mycotoxin simultaneously in the same sample. However, each method has specific accuracy, sensitivity, recovery and reproducibility within a specific range of the mycotoxin levels (Abdelhamid, 1981, 1995a and 1996).

The principles of analysis consist of precise sampling, sample preparation and toxin extraction, purification, derivation, elution, concentration, qualitative detection, confirmation, and quantitative detection.
Methods of mycotoxins examination include biological methods (e.g. cells, tissues, eggs, shrimp, fish, chicks...etc)**,** physical methods (e.g. UV-light)**,** physico-chemical methods {e.g. spectrophotometer and chromatography (Paper, Column, TLC, HPTLC, LC, HPLC, GLC – MS)} and immuno-enzyme methods, e.g. ELISA
(Schweighardt *et al*., 1980-a & b and Abdelhamid, 1985, 1996 & 2000-b).

FACTORS AFFECTING SEVERITY OF A MYCOTOXIN

It may be affected by many factors including the mycotoxin it self, level of contamination (chronic, sub acute, acute), time of exposure, route of application, presence of other mycotoxins, the organism exposes to a mycotoxin (genetic effect on the enzyme system), sex and age of the exposed organism (hormonal effect), and clinical status of the exposed organism (hepatic enzymes status) (Abdelhamid, 2000-b).

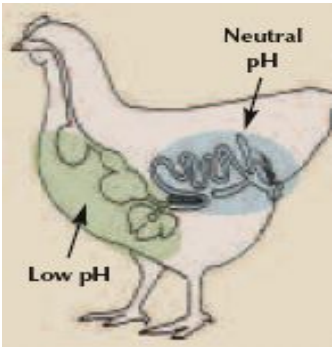
Toxin	**LD50**
Aflatoxin-B1	**0.5**
Aflatoxin-B1	**0.5 (mg/Kg body weight)**
Aflatoxin-B1	**0.08 (mg/Kg body weight)**
Aspertoxin	**6.6**
Grusiofolvin	**0.28**
Ochratoxin-A	**1.7**
Ochratoxin-A	**3.0 (mg/Kg body weight)**
Ochratoxin-B	**13.0 (mg/Kg body weight)**
Patulin	**18**
Stemfon	**1.2**
Sterigmatocystin	**0.24**
Sterigmatocystin	**137 (ppb in diet)**
T-2 toxin	**6.5 (mg/Kg body weight)**

Feed	**AFB1 (µg kg-1)**
Feed (exceptions below)	**50**
Complete feedstuff for pigs and poultry	**20**
Groundnuts, copra, palm kernel, cottonseed, babasu, maize and products derived from processing thereof	**20**
Other complete feedstuffs	**10**
Complete dairy feed	**5**

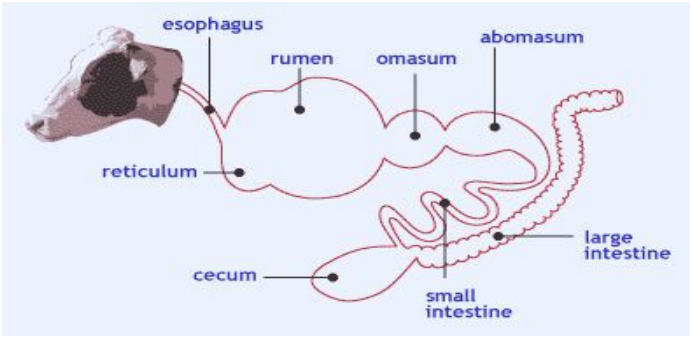
Complementary feedstuffs
for cattle, sheep, goats
(except dairy, calves and lambs) 50
Complete feed for lambs and calves 10
Complementary feedstuffs for pigs
and poultry (except for young animals) 30
European Union regulations for aflatoxins in feeds (µg kg-1).

PROPHYLAXIS AND TREATING

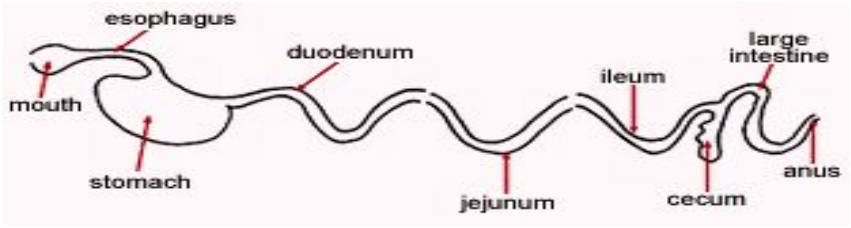
Prophylaxis is more better, easier, cheaper and realizable than treating (curing) mycotoxin. Therefore, preventing fungal invasion is a must because there are no effective means for overcoming mycotoxins and their negative effects (Lee, *et al.,* 1969; Wellford, *et al.,* 1978; Abdehamid, 1993a; Abdelhamid and Mahmoud, 1996; Horvath, 1998; Abdelhamid *et al.,* 2002a; Heiler and Schatzmayr, 2003 and Shehata *et al.,*2003a & b). However, it could be beneficial to alleviate these effects through one or more of the following steps:
separation, screening, washing, heating, roasting, microwave,



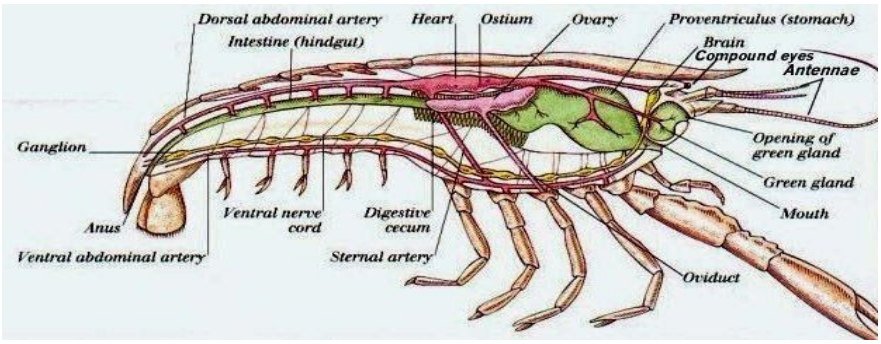
Poultry



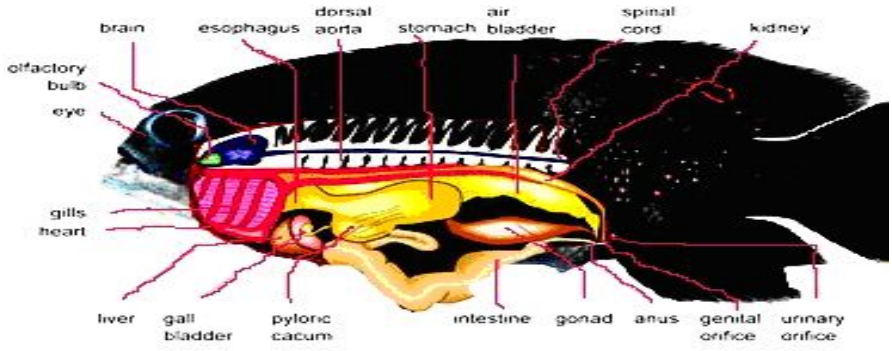
Cattle



Swine



Shrimp



Fish

CITATIONS USEFUL Regarding degradation of Toxins.

1. ROLE OF DFM

Aflatoxins are cancerogenic compounds produced predominantly by certain strains of the Aspergillus genus. The ideal solution for minimization of health risk that aflatoxins pose is the prevention of foods and feeds contamination. Unfortunately, these contaminants can never be completely removed, and on that account, many studies have been carried out to explore an effective process of their detoxification to a threshold level. Biological decontamination seems to be attractive because it works under mild, environmentally friendly conditions. This review is focused on the biological detoxification of aflatoxins, especially aflatoxin B1, by microorganisms. There are briefly mentioned aflatoxin metabolic pathways in the human and animal body. Microorganisms such as soil or water bacteria, fungi, and protozoa and specific enzymes isolated from microbial systems can degrade aflatoxin group members with varied efficiency to less- or nontoxic products. Some aflatoxin-producing fungi from Aspergillus species have the capability to degrade their own synthesized mycotoxins. Yeasts and lactic acid bacteria work as biological adsorbents that prevent aflatoxin's transfer to the intestinal tract of humans and animals. Aflatoxin B1 absorbed into the organism could be metabolized by significantly different

pathways. They lead to the production of the relatively nontoxic compounds, on the one hand, or to highly toxic active forms on the other hand.
(https://www.ncbi.nlm.nih.gov/pubmed/19514968)

Antioxidants could be promising agents for management of oxidative stress-related diseases. New biologically active compounds, belonging to a rare class of natural lignans with antiangiogenic, antitumoral and DNA intercalating properties, have been recently synthesized. These compounds are benzo[kl]xanthene lignans (1,2) and dihydrobenzofuran neolignans (3,4). The radical scavenging and chain-breaking antioxidant activities of compounds 1-4 were studied by applying different methods: radical scavenging activity by DPPH rapid test, chain-breaking antioxidant activity and quantum chemical calculations. All studied compounds were found to be active as DPPH scavengers but reaction time with DPPH and compounds' concentrations influenced deeply the evaluation. The highest values of radical scavenging activity (%RSAmax) and largest rate constants for reaction with DPPH were obtained for compounds 2 and 3. Comparison of %RSAmax with that of standard antioxidants DL-α-tocopherol (TOH), caffeic acid (CA) and butylated hydroxyl toluene (BHT) give the following new order of %RSA max: TOH (61.1%) > CA (58.6%) > 3 (36.3%) > 2 (28.1%) > 4 (6.7%) > 1 (3.6%) = BHT (3.6%). Chain-breaking antioxidant activities of individual compounds (0.1-1.0 mM) and of their equimolar binary mixtures (0.1 mM) with TOH were determined from the kinetic curves of lipid autoxidation at 80 °C. On the basis of a comparable kinetic analysis with standard antioxidants a new order of the antioxidant efficiency (i.e., protection factor, PF) of compounds 1-4 were obtained: 2 (7.2) ≥ TOH (7.0) ≥ CA (6.7) > 1 (3.1) > 3 (2.2) > ferulic acid FA (1.5) > 4 (0.6); and of the antioxidant reactivity (i.e. inhibition degree, ID): 2 (44.0) >> TOH (18.7) >> CA (9.3) >> 1 (8.4) > 3 (2.8) > FA (1.0) > 4 (0.9). The important role of the catecholic structure in these compounds, which is responsible for the high chain-breaking antioxidant activity, is discussed and a reaction mechanism is proposed. Higher oxidation stability of the lipid substrate was found in the presence of equimolar binary mixtures 2 + TOH, 3 + TOH and 4 + TOH. However, an actual synergism was only obtained for the binary mixtures with compounds 3 and 4. The geometries of compounds and all possible phenoxyl radicals were optimized using density functional theory. For description of the scavenging activity bond dissociation enthalpies (BDE), HOMO energies and spin densities were employed. The best correlation between theoretical and experimental data was obtained for compound 2, with the highest activity, and for compound 4 with the lowest activity. The BDE is the most important theoretical descriptor, which correlates with the experimentally obtained antioxidant activity of the studied benzo[kl]xanthene lignans and dihydrobenzofuran neolignans
(http://www.ncbi.nlm.nih.gov/pubmed/21884748)

Moreover, huminic acids are important ingredients of the preparation. These are reactive high-molecular organic compounds with polyelectrolytic character, which are capable of participating in a number of chemical reactions including: ionic exchange, forming complexes with metals, oxide reduction and other. They play very important role in maintaining the state of acid-base equilibrium in propermineral nutrition for animals. Bacteriological examinations of huminic acids have proven that they have bacteriostatic and bactericidal effect, which probably slows down growth of pathogenic microorganisms in digestive tract of animals, and this directly affects their health state and proper growth. The tests carried out for calves have proven stimulating effect of huminic acids on the development of immune system, resulting in increasing level of immunity substances, mainly gamma-globulin.

(http://www.randstadnieuws.nl/406-BusinessPortHolland/612-animal-health-feed-additives-toxin-binder-diarrhea/2726-animal-feed-additives-anti-diarrhoea/)

Phytogenic are a relatively young class of feed additives and in recent years this feed additives have gained considerable attention in the feed industry. They are a wide variety of herbs, spices and products derived thereof and are mainly essential oils. Although, numerous reports have demonstrated antioxidative and antimicrobial and immune stimulation efficacy in vitro, respective experimental in vivo evidence is still quite limited.
(http://www.medwelljournals.com/fulltext/?doi=javaa.2010.2295.2304)

Roy, Batish, Grover, and Neelakantan (1996)isolated 2100 colonies of LAB and screened them using several types of moulds and an agar well diffusion assay on potato dextrose agar containing 0.1% Triton X-100. Six colonies were identified for their antifungal activity against Aspergillus flavus IARI, and one of them showed a broad spectrum of antifungal activity against A. flavus IARI,A. flavus NCIM 555,Aspergillus parasiticus NCM 898 and Fusarium spp. This isolate was identified as **Lc. lactis subsp.lactis** CHD 28.3. Aspergillus IARI was the most sensitive indicator of the antifungal metabolite produced by this lactic strain. Some other Lactococcus strains identified as Lc. lactis (Coallier- Ascah & Idziak, 1985; Luchese & Harrigan, 1990; Wiseman & Marth, 1981), Lc. lactis subsp.diacetylactis DRCI (Batish, Lal, & Gro- ver, 1989) and Lc. subsp.cremoris (Florianowicz, 2001) were reported to control mycotoxinogenic mould growth.

Lactobacillus plantarum 21B isolated from sourdough and grown in wheat flour hydrolysate was shown to possess an efficient antifungal activity against Penicillium corylo-philum,Penicilliumroqueforti, Penicilliumexpansum, Aspergilus niger, A. flavus, and Fusarium graminearum (Lavermicocca et al., 2000). These authors demonstrated that part of the antifungal activity ofLb. plantarum 21B was ascribed to the production of phenyllactic and 4-hydroxy-phenyllactic acids. Less than 7.5mg/ml of phenyl- lactic acid was required to obtain full inhibition of mould growth (Lavermicocca, Valerio, & Visconti, 2003). Earlier,Niku-Paavola, Laitila, Mattila-Sandholm, and Haikara (1999)described the ability ofLb. plantarum VTTE-78076 to suppress the growth ofFusarium VTTD-80147. The antifungal activity of this strain was detected in low molecular fractions eluted from a chromatography column loaded with culture supernatant. Antifungal activity was ascribed to the occurrence of benzoic acid, an imidazolidinedione derivative and a piperazinedione derivative.

Lb. plantarum strains VTTE-78076 and VTTE-79098 have also been described as being active against different plant pathogenic, toxigenic and gushing-activeFusarium fungi (Laitila, Alakomi, Raaska, Mattila-Sandholm, & Haikara, 2002). Using automated tubidimetry as well as direct and indirect impedimetric methods, theprevious authors showed thatLb. plantarum strains VTTE-78076and VTTE-79098 were effective against Fusarium species such as Fusarium avenaceum, Fusarium culmorum, F. graminearum and Fusarium oxyporum with efficiency depending on the target organism.

Lactobacillus coryniformis subsp. Coryniformis Si3, isolated from grass silage, was able to inhibit the growth of a great number of mycotoxinogenic moulds including Aspergillus

fumigatus and Aspergillus nidulans, Penicillium roqueforti, Fusarium poae, F. graminearum, F. culmorum and Fusarium sporotrichioides (Magnusson &
Schnürer, 2001). In liquid medium, the production of antifungal metabolites byLb. coryniformis subsp.coryniformis Si3 was shownto be a growth phase-dependent process. Ethanol has been reported to enhance the antifungal activity of this metabolite, whichwas irreversibly lost after treatment with proteolytic enzymes including proteinase K, trypsin and pepsin (Magnusson &Schnürer, 2001). After a partial purification, the molecular mass of the potent antifungal compound produced byLb. coryniformis subsp.coryniformisSi3 was estimated to be close to 3 Kda, to be heat stable, sensitive to proteolytic enzymes and active within a narrow pH range.These Activated Carbonacteristics are in accordance with those of bacteriocinsof subclass II (Klaenhammer, 1993).

Other species of Lactobacillus, including, **Lactobacillus casei** (Florianowicz, 2001; Gourama, 1997; Mäyrä-Mäkinen, Kristianinkatu, & Suomalainen, 1994; Suzuki, Nomura, & Morichi, 1991), **Lactobacillus sanfranciscoCB1** (Corsetti et al., 1998) and **Lactobacillus rhamnosus**(Stiles, Penkar, Plockova, Chumchalova, & Bullerman, 2002), have also been described as being able to inhibit toxinogenic mould growth. Moreover, several papers have reported the ability of the genus **Pediococcus** to control mycotoxinogenic mould growth.
(Effat, Ibrahim, Tawfik, & Sharaf, 2001; Mandal, Sen, & Mandal, 2007;Rouse,Harnett, Vaughan, & van Sinderen, 2008).

Using vacuum-packed fermented meat, Mandal et al. (2007) isolated an antifungal lactic strain, identified as **Pediococcus acidilactici** LAB 5, that exhibited varying degrees of antifungal activity against A. fumigatus,A. parasiticus, Fusarium oxysporum and Penicilliumsp.

Certain bacteria, particularly strains of **lactic acid bacteria, propionibacteria** and **bifidobacteria**, appear to have the capacity to bind mycotoxins, including aflatoxin and some Fusarium produced mycotoxins (El-Nezami *et al.*, 2000, 2002a, 2002b; Haskard *et al.*, 2001 and Oatley *et al.*, 2000; Yoon *et al.*, 1999). The binding appears to be physical with deoxynivalenol, diacetoxyscerpenol, nivalenol, and other mycotoxins associated with hydrophobic pockets on the bacterial surface. Research reports on the subject are limited.

Stanley et al. (1993) reported that **Sac. Activated Carbonomyces cerevisiae** was helpful in the case of aflatoxin contamination, and their conclusion was that the cell wall was binding with the mycotoxins. Santin et al. (2003) studied the effects of yeast cell wall against ochratoxin in broilers. Their results indicate that ochratoxin impaired the feed intake, weight gain and feed conversion of the birds. The yeast cell wall could not improve these parameters. Yiannikouris et al. (2004) studied the interaction of yeast cell wall with zearalenone in vitro.
Their conclusion was that weak non-covalent bonds are involved in the complex-forming mechanisms, and that the chemical interactions are therefore more of an adsorption type than a binding type.

Shima et al. (1997) have for example reported the case where a bacterium belonging to the Agrobacterium-Rhizobium group was able to transform deoxynivalenol into a less toxic compound called 3-ketodeoxynivalenol, and suggested that the biotransformation was caused by an extracellular enzyme excreted by the organism. Similarly, Völkl et al. (2004) observed that a mixed culture of micro-organisms was able to transform deoxynivalenol into two

chromatographically separable products, the main one being identified as 3-keto-deoxynivalenol. Again, they stated that an extracellular enzyme was involved. Other trichothecenes such as 15-acetyl- deoxynivalenol, 3-acetyl- deoxynivalenol and fusarenon-X were also transformed.

Zearalenone (ZON) is a potent estrogenic mycotoxin produced by several *Fusarium* species most frequently on maize and therefore can be found in food and animal feed. Since animal production performance is negatively affected by the presence of ZON, its detoxification in contaminated plant material or by-products of bioethanol production would be advantageous. Microbial biotransformation into nontoxic metabolites is one promising approach.

El-Sharkawy and Abul-Hajj (9) were the first to report inactivation of ZON after opening of the lactone ring by *Gliocladium roseum*. This filamentous fungus was capable of metabolizing ZON in yields of 80 to 90%. Also Takahashi-Ando et al. (31) described the degradation reaction of ZON with *Clonostachys rosea* (synonym of *G. roseum*). A hydrolase (encoded by a gene designated *ZHD101*) cleaves the lactone ring, and as recently proved (37; unpublished data) by subsequent decarboxylation of the intermediate acid, the compound 1-(3,5-dihydroxyphenyl)-10′ -hydroxy-1′ *E*-undecene-6′ -one is formed. In contrast to ZON and 17 β -estradiol, which showed potent estrogenic activity, this cleavage product did not show any estrogenic activity in the human breast cancer MCF-7 cell proliferation assay (16). Further details, e.g., on the conditions of the maximum activity of *ZHD101* and its exploitation in genetically modified grains, can be found in later published work of this research group (32, 33).

Only a few authors reported the loss of estrogenicity in microbial metabolites of ZON, which are based on reactions other than cleavage of the lactone undecyl ring system. El-Sharkawy and Abul-Hajj demonstrated (10) that binding to rat uterine estrogen receptors requires a free 4-OH phenolic group (devoid of methylation or glycosylation). Loss of estrogenicity was, for instance, observed with 2,4-dimethoxy-ZON, one of the metabolites produced by *Cunninghamella bainieri* ATCC 9244B. Nevertheless, this rule cannot be generalized, as 8′ -hydroxyzearalenone formed by *Streptomyces rimosus* NRRL 2234, despite having a free 4-phenolic hydroxyl group, did not bind to the estrogen receptor. Also, other authors reported that 8′ -hydroxyzearalenone and 8′ -epi-hydroxyzearalenone are nonestrogenic (13). However, so far, no practical application in feed or food detoxification has been found for the microorganisms producing these compounds.

It has been shown previously that the yeast *Trichosporon mycotoxinivorans* has a very high capability to degrade both ochratoxin A (OTA) and ZON (22, 26, 27). When *T. mycotoxinivorans* is used as a feed additive preparation, microbial degradation of the mycotoxins is assumed to take place in the gastrointestinal tract of the animal after consumption of contaminated feed. The protective effect of *T. mycotoxinivorans* against OTA toxicity has already been shown with broiler chicken (24).
(http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2849244/)

Zearalenone can be converted into a far less oestrogenic product, called 1-(3,5-dihydroxyphenyl)-10'-hydroxy- 1'-undecen-6'-one (Takahashi-Ando et al., 2002). The enzyme responsible for the detoxification appears to be a hydrolase that cleaves the lactone ring. Zearalenone affects the reproduction cycle of animals when it interferes with oestrogen

receptors. Since the structure of the mycotoxin is modified by the enzymatic reaction, it loses its toxic effect.
The application of such enzymatic transformations to the feed sector gives new opportunities. Indeed, enzymes can have a specific action and their reaction, compared to binding, is not reversible. With this new approach, we can talk about “mycotoxin eliminators” in contrast to “mycotoxin binders”.

It was also found that ***Streptococcus*** and ***Enterococcus*** strains have the ability to bind deoxynivalenol, zearalenone, and fumonisins. It has been shown, that the strain ***Lactobacillus rhamnosus*** can bind aflatoxin B1 *in vitro* (Haskard et al., 2001; Lahtinen et al., 2004) and *in vivo* (Gratz et al., 2006). El-Nezami et al., (2002) identified that some strains of *Lactobacillus* and ***Propionibacterium*** bind trichothecenes *in vitro*. Schatzmayr et al., (2006) demonstrated that a species of ***Eubacterium*** has the ability to deactivate trichothecenes.

Curcumin induces drug metabolizing enzymes like gluthathione-s-transferase and induction of enzymes results in efficient detoxification of cytotoxic or carcinogenic compounds (Shalini and Srinivas, 1987; Soni et al., 1992)

Palumbo et al. reported that a number of ***Bacillus***, ***Pseudomonas***, ***Ralstonia*** and ***Burkholderia*** strains could completely inhibit *A. flavus* growth. ***B. subtilis*** and ***P. solanacearum*** strains isolated from maize soil were also able to inhibit aflatoxin accumulation.

L. rhamnosus *at a concentration of* 2 x 109 CFU/mL removes about 80% of the AFB1 toxin at 37^0C.

Peltonen et al. found that between 5 and 60% of the aflatoxin in solution was bound by the bacteria, with ***L. amylovorus*** and ***L. rhamnosus***.

Saprophytic yeast species such as ***Candida krusei*** and ***Pichia anomala*** have shown promise as biocontrol agents for decontamination of aflatoxins . Similar to bacterial agents, these yeast strains were able to significantly inhibit *Aspergillus* growth and resultant toxins under laboratory conditions . Shetty et al. found that the ability of *S. cerevisiae* to bind AFB1 was strain specific with 7 strains binding 10-20%, 8 strains binding 20-40% and 3 strains binding more than 40% of the added AFB1.

There are many reports on the use of physically separated yeast cell walls obtained from brewery as feed additive in poultry diet resulting in amelioration of toxic effects of aflatoxins. When dried yeast and yeast cell walls were added to rat-ration along with AFB1, a significant reduction in the toxicity was observed. In an *in vitro* study with the cell wall material, there was a dose dependent binding of as much as 77% (w/w) and modified mannan-oligosacActivated Carbonides derived from the ***S. cerevisiae*** cell resulted in as much as 95% (w/w) binding.

Stiles and Bullerman studied the effect of ***L. rhamnosus*** on growth and mycotoxin production by *Fusarium* species, including *F. proliferatum, F. verticillioides* and *F. graminearum*. The results showed that production of FB1 was reduced up to 63.2%, FB2 up to 43.4% and deoxynivalenol and zearalenone up to 92% and 87.5%, respectively.

L. rhamnosus was evaluated for its potential to remove or degrade zearalenone and α-zearalenol and both viable and non-viable cells were able to remove about 50% of the toxin from solution, indicating that binding rather than metabolism was the mechanism in action.

L. rhamnosus removed up to 55% of deoxynivalenol, while *Leuconostoc mesenteroides* removed about 82% of FB1 and *L. lactis* removed 100% of FB2. ***B. amyloliquefaciens*** and ***Microbacterium oleovorans*** isolates were shown to effectively reduce *F. verticillioides* propagules and fumonisin content in maize kernels at harvest when applied as seed coatings.

Several bacterial and fungal strains belonging to ***Streptococcus, Bifidobacterium, Lactobacillus, Butyribrio, Phenylobacterium, Pleurotus, SacActivated Carbonomyces, Bacillus*** and ***Acinetobacter*** genera and certain fungi belonging to ***Aspergillus*** **(*A. fumigatus, A. niger, A. carbonarius, A. japonicus, A. versicolor, A. wentii*** **and *A. ochraceus*)**, ***Alternaria, Botrytis, Cladosporium, Phaffia, Penicillum*** **and *Rhizopus*** **(*R. stolonifer*** **and *R. oryzae*)** genera, are able to degrade OTA *in vitro* up to more than 95%.

2. ROLE OF ENZYMES

Aflatoxin AFB1 and Ochratoxin OA can be degraded by Enzymes like **REDUCTASE** and **DEHYDROGENASE**.
Trichothecenes T2, Vomitoxin are degraded by **EPOXIDASE**
Zearalenone is degraded by **LACTONASE**
Esterase degrades zearalenone and ochratoxin A

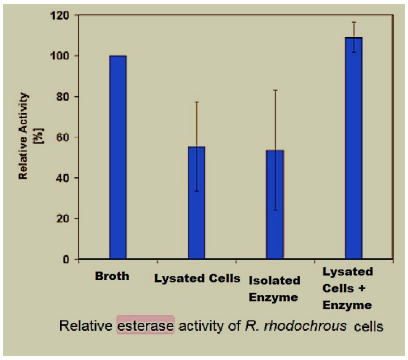
Dioxygenase and Dehydrogenase
2,3-dihydroxybiphenyl 1,2-dioxygenase (2,3-DHBD) and dihydrodiol dehydrogenase (DHD) enzymes were produced through extracellular expression of respectively the bphC1 and bphB genes of R. erythropolis.

Esterase and Epoxidase

Esterase is an α- carboxylesterase.

Biodegradation of di-ester plasticizers like Butyl butyrate, o-nitrophenyl acetate, di-methyl phthalate; involve the hydrolysis of the ester bonds by esterase or lipase enzymes

Cells of *Rhodococcus rhodochrous* contain this esterase enzyme.



T-2 toxin was also found to be non-toxic to *Selenomonas ruminantium a*nd Anaerovibrio lipolytica and both organisms were capable of breaking down T-2 Toxin to HT-2 toxin and T-2 triol but not neosolaniol.
Anaerovibrio Lipolytica was slightly more active than *S.ruminantium* with only 59% of added T-2 toxin remaining at the end of the growth phase compared to 62% for *S.ruminantium.*
Generally, however, the amount of T-2 toxin left at the end of the growth phase was approximately the same for all bacteria.

For the elimination of the toxic effects of trichothecenes - a large family of more than 200 structurally similar mycotoxins, from which vomitoxin (deoxynivalenol or DON) and T-2 toxin are the most well known members - *Eubacterium* BBSH 797 was isolated. The enzymes produced by this organism within the gastro-intestinal tract (for example epoxidases) play an important role by enabling the specific disruption of the toxic epoxy ring possessed by this group of mycotoxins (Figure 1).

Some years later, the non-pathogenic yeast *T. mycotoxinivorans MTV* was isolated, described and patented for its ability to degrade zearalenone and ochratoxin A. For the elimination of zearalenone's negative effects it is vital that the lactone ring within the molecule is destroyed. This reaction is once again mediated by enzymes (e.g. esterases). In doing so, zearalenone's resemblance with the sexual female hormone estradiol is lost and therefore impairment of the reproduction system is avoided. In the case of Ochratoxin A, cleavage of the phenylalanine moiety results in the derivate ochratoxin alpha (Figure 3), considerably less toxic than the original molecule.

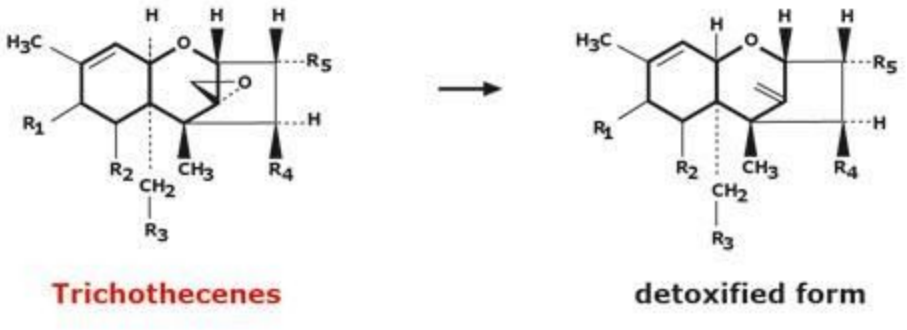


Figure 1 – Biotransformation of trichothecenes into the detoxified forms (de-epoxy structures)

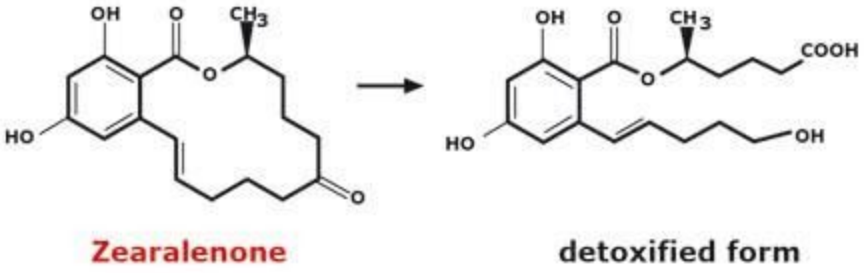


Figure 2 – Biotransformation of zearalenone into a detoxified form (ZOM-1)

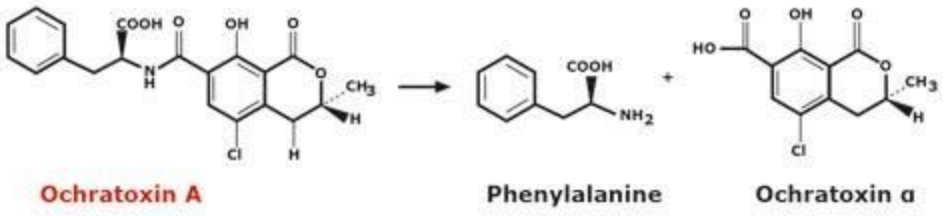


Figure 3 - Biotransformation of ochratoxin A into a detoxified form (ochratoxin alpha)

Production of the enzymes that mediate these reactions occurs within the gastrointestinal tract of the animal and, therefore, their production and activity cannot be quantified, as is the case of purified enzymes.

Their activity is not simple. Not only must they show a rapid degradation of the mycotoxin into less- or non-toxic metabolites, but they must also remain active at

various pH values and in complex environments, with the presence of various metabolites and nutrients. Furthermore, the non-toxicity of these biotransforming microorganisms must be assured and the possibility of being applied as lyophilisates should be granted for a practical use in animal diets.

As a conclusion, mycotoxin risk management strategies must comprise several components. The elimination of adsorbable mycotoxins, such as aflatoxins and ergot alkaloids is possible to be done through adsorption; however, for the elimination of the toxicity of non-adsorbable mycotoxins, such as zearalenone, ochratoxins and trichothecenes biotransformation is crucial. Biotransformation, which is enabled by enzyme-producing microorganisms, allows the conversion of the toxic structure of mycotoxins into non-toxic, harmless metabolites.

(http://temp.biomin.net/knowledge-center/articles/articles-details/article/enzyme-producing-microorganisms-and-mycotoxin-risk-management/)

Mycotoxins are secondary fungal metabolites that may have mutagenic, carcinogenic, cytotoxic and endocrine disrupting effects. These substances frequently contaminate agricultural commodities despite efforts to prevent them, so successful detoxification tools are needed. The application of microorganisms to biodegrade mycotoxins is a novel strategy that shows potential for application in food and feed processing. In this study we investigated the mycotoxin degradation ability of thirty-two *Rhodococcus* strains on economically important mycotoxins: aflatoxin B_1, zearalenone, fumonisin B_1, T2 toxin and ochratoxin A, and monitored the safety of aflatoxin B_1 and zearalenone degradation processes and degradation products using previously developed toxicity profiling methods. Moreover, experiments were performed to analyse multi-mycotoxin-degrading ability of the best toxin degrader/detoxifier strains on aflatoxin B_1, zearalenone and T2 toxin mixtures. This enabled the safest and the most effective *Rhodococcus* strains to be selected, even for multi-mycotoxin degradation. We concluded that several *Rhodococcus* species are effective in the degradation of aromatic mycotoxins and their application in mycotoxin biodetoxification processes is a promising field of biotechnology.
(http://www.sciencedirect.com/science/article/pii/S0168160513002894)

B. subtilis producing esterase enzyme had the following characteristics: Gram- positive, rod-shaped cells, cylindrical spore in subterminal position with no bulging. The strain was positive for the following tests: casein, dextran, gelatin, olive oil, pullulan, starch and Tween 20; catalase assay, dihydroxyacetone production, methyl red test, nitrate reduction and Voges- Proskauer reaction. It grew between pH 4.5-7.0 and at temperatures between 37-50 Degrees C and 2 to 5% NaC1. Substrates utilization includes: adonitol, esculin, amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, galactose, fl-gentiobiose, cr D-glucose, glycerol, inositol, inulin, lactose, maltose, mannitol, D-mannose, D-raffmose, rhamnose, ribose, saccharose, salicin, sorbitol, trehalose, D-turanose and L-xylose.

It was negative for the following tests: Tween 80 and urea, anaerobic growth, citrate utilization, gas production from glucose, indole production and phenylalanine. No growth was observed in 7% NaC1, 60 Degrees C or on Mac- Conkey agar

B. subtilis that produce esterase enzyme
T. mycotoxinivorans that produce esterase enzyme
Eubacterium that produce epoxidase enzyme
Rhodococcus erythropolis that produce dioxygenase dehydrogenase enzymes

Mycotoxins are secondary metabolites of fungi that contaminate food and feed, and are involved in a series of foodborne illnesses and disorders in humans and animals. The mitigation of mycotoxin content via enzymatic degradation is a strategy to ensure safer food and feed, and to addresstheforthcomingissuesinviewoftheglobaltradeandsustainability. Nevertheless, the search for active enzymes is still challenging and time-consuming. The in silico analysis may strongly support the research by providing the evidence-based hierarchization of enzymes for a rational design of more effective experimental trials. The present work dealt with the degradation of aflatoxin B1 and M1 by laccase enzymes from Trametes versicolor. The enzymes–substrate interaction for various enzyme iso-forms was investigated through 3D molecular modeling techniques. Structural differences among the isoforms have been pinpointed, which may cause different patterns of interaction between aflatoxin B1 and M1. The possible formation of different products of degradation can be argued accordingly. Moreover, the laccase gamma isoform was identified as the most suitable for protein engineering aimed at ameliorating the substrate specificity. Overall, 3D modeling proved to be an effective analytical tool to assess the enzyme–substrate interaction and provided a solid foothold for supporting the search of degrading enzyme at the early stage.
(https://www.google.co.in/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&cad=rja&uact=8&ved=0ahUKEwjc0NygotvTAhUmSI8KHfvlAtkQFgg4MAM&url=http%3A%2F%2Fwww.mdpi.com%2F2072-6651%2F9%2F1%2F17%2Fpdf&usg=AFQjCNHEgwkbX86NcvrnRRCXoeKehvKnlQ**)**

Laccases (LCs) are multicopper oxidases that find application as versatile biocatalysts for the green bioremediation of environmental pollutants and xenobiotics. In this study we elucidate the degrading activity of Lac2 pure enzyme form Pleurotus pulmonarius towards aflatoxin B1 (AFB1) and M1 (AFM1). LC enzyme was purified using three chromatographic steps and identified as Lac2 through zymogram and LC-MS/MS. The degradation assays were performed in vitro at 25 ◦C for 72 h in buffer solution. AFB1 degradation by Lac2 direct oxidation was 23%. Toxin degradation was also investigated in the presence of three redox mediators, (2,20-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid]) (ABTS) and two naturally-occurring phenols, acetosyringone (AS) and syringaldehyde (SA). The direct effect of the enzyme and the mediated action of Lac2 with redox mediators univocally proved the correlation between Lac2 activity and aflatoxins degradation. The degradation of AFB1 was enhanced by the addition of all mediators at 10 mM, with AS being the most effective (90% of degradation). AFM1 was completely degraded by Lac2 with all mediators at 10 mM. The novelty of this study relies on the identification of a pure enzyme as capable of degrading AFB1 and, for the first time, AFM1, and on the evidence that the mechanism of an effective degradation occurs via the mediation of

natural phenolic compounds. These results opened new perspective for Lac2 application in the food and feed supply chains as a biotransforming agent of AFB1 and AFM1.
(https://www.google.co.in/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&cad=rja&uact=8&ved=0ahUKEwjc0NygotvTAhUmSI8KHfvlAtkQFgg-MAQ&url=http%3A%2F%2Fwww.mdpi.com%2F2072-6651%2F8%2F9%2F245%2Fpdf&usg=AFQjCNESV35XVf_E6jy5imQYg-IOJvWkOQ**)**

Nineteen fungi were tested for their ability to degrade aflatoxin B_1 (AFB_1). An extracellular enzyme from the edible mushroom *Pleurotus ostreatus* showed afaltoxin-degradation activity detected by thin-layer chromatography (TLC). An enzyme with this activity was purified by two chromatographies on DEAE-Sepharose and Phenyl-Sepharose. The apparent molecular mass of the purified enzyme was estimated to be 90 kDa by SDS-PAGE. Optimum activities were found in the pH range between 4.0 and 5.0 and at 25°C. Also, degradation activity of several dyes in the presence of H_2O_2 was tested, resulting in the detection of bromophenol blue-decolorizing activity. Based on these data, we suggest this enzyme is a novel enzyme with aflatoxin-degradation activity. Fluorescence measurements suggest that the enzyme cleaves the lactone ring of aflatoxin.
(http://www.sciencedirect.com/science/article/pii/S094450130470124X)

Oxidation of the mycotoxin aflatoxin (AF) B_1 yields the 8,9-epoxide, which nonenzymatically hydrolyzes rapidly to a dihydrodiol that in turn undergoes slow, base-catalyzed ring opening to a dialdehyde [Johnson, W. W., Harris, T. M., and Guengerich F. P. (1996) *J. Am. Chem. Soc.* **118**, 8213−8220]. AFB_1 dialdehyde does not bind to DNA but can react with protein lysine groups. One enzyme induced by cancer chemopreventive agents is AFB_1 aldehyde reductase (AFAR), which catalyzes the NADPH-dependent reduction of the dialdehyde to a dialcohol. AFB_1 dialdehyde is known to convert nonenzymatically to AFB_1 dihydrodiol at neutral pH, and we reinvestigated the enzymatic reaction by preparing AFB_1 dialdehyde at pH 10 and then used this to initiate reactions (at neutral pH) with rat and human AFAR isozymes. Two monoalcohols were identified as products, and their identities were established by NaB^2H_4 reduction, chemical cleavage, and mass spectrometry. The monoalcohol corresponding to reduction at C-8 formed first in reactions catalyzed by either the rat or the human AFAR. This C-8 monoalcohol was further reduced to AFB_1 dialcohol by AFAR. The other monoalcohol (C-6a) was formed but not reduced to the dialcohol rapidly. Steady-state kinetic parameters were estimated for the reduction of AFB_1 dialdehyde by rat and human AFAR to the monoalcohols. The apparent k_{cat} and K_m values were not adequate to rationalize the observed ΔA_{340} spectral changes in a kinetic model. Simulation fitting was done and yielded parameters indicative of greater enzyme efficiency. A survey of 12 human liver cytosol samples showed a variation of 2.3-fold in AFAR activity. Rats treated with AFB_1 excreted the dialcohol and a monoalcohol in urine. The results of these studies are consistent with a role of (rat and human) AFAR in protection against AFB_1 toxicity.
(http://pubs.acs.org/doi/abs/10.1021/tx010005p)

When 10 strains of lactic acid bacteria were incubated with 50-hydroxyaverantin (HAVN), a precursor of aflatoxins, seven of them converted HAVN to averufin; the same reaction is found in aflatoxin biosynthesis of aflatoxigenic fungi. These bacteria had a dehydrogenase that catalyzed the reaction from HAVN to 50-oxoaverantin (OAVN), which was so unstable that it was easily converted to averufin. The enzyme was purified from Lactobacillus brevis IFO 12005. The molecular mass of the enzyme was 100 kDa on gel filtration chromatography and 33 kDa on SDS polyacrylamide gel electrophoresis (SDS–PAGE). The gene encoding the enzyme was cloned and sequenced. The deduced protein consisted of 249 amino acids, and its

estimated molecular mass was 25,873, in agreement with that by time of flight mass spectrometry (TOF MS) analysis. Although the deduced amino acid sequence showed about 50% identity to those reported for alcohol dehydrogenases from L. brevis or L. kefir, the commercially available alcohol dehydrogenase from L. kefir did not convert HAVN to OAVN. Aspergillus parasiticus HAVN dehydrogenase showed about 25% identity in amino acid sequence with the dehydrogenase and also with these two alcohol dehydrogenases. (http://www.tandfonline.com/doi/pdf/10.1271/bbb.70597)

3. ROLE OF HERBAL EXTRACTS

ANTIBACTERIAL ACTIVITY OF PHYTOBIOTICS

Herbs and spices are well known to exert antimicrobial actions in vitro against important pathogens including fungi (Windisch et al., 2008). A common feature of phytobiotics is that they are a very complex mixture of bioactive components. For example, hawthorn fruit, a common growth-enhancing and digestion modifier has been shown to contain >70 kinds of organic chemicals along with some unidentified factors and active bio-active compounds (Wang et al., 1998).

Growth enhancement through the use of phytobiotics is probably the result of the synergistic effects among complex active molecules existing in phytobiotics. Phytochemicals in phytobiotics are well known to have antimicrobial ability (Cowan, 1999). Phytochemicals exert their antimicrobial activity through different mechanisms, tannins for example act by iron deprivation, hydrogen bounding or non specific interactions with vital proteins such as enzymes (Scalbert, 1991).

Chung et al. (1993) showed that tannic acid inhibits the growth of intestinal bacteria such as Bacteroides fragilis, Clostridium perfringens, E. coli and Enterobacter cloacae. Alkaloid is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Karou et al., 2006). The main mechanism by which saponins display an antimicrobial activity is based on their ability to form complexes with sterols present in the membrane of microorganisms.

This causes damages in the membrane and the consequent collapse of cells (Morrissey and Osbourn, 1999). Essential oils have long been recognized for their anti-microbial activity (Lee et al., 2004a) and they have gained much attention for their potential as alternatives to antibiotics in broiler chickens. Some studies with broilers demonstrated in vivo antimicrobial efficacy of

essential oils against Escherichia coli and Clostridium perfringens (Jamroz et al., 2003; Mitsch et al., 2004).

The exact anti-microbial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property (Conner, 1993) and chemical structure (Farag et al., 1989a, b) can play a role. It was suggested that terpenoids and phenylpropanoids can penetrate the membranes of the bacteria and reach the inner part of the cell because of their lipophilicity (Helander et al., 1998). Moreover, structural properties, such as the presence of the functional groups (Farag et al., 1989c) and aromaticity (Bowles and Miller, 1993) are also responsible for the antibacterial activity of essential oils (http://www.medwelljournals.com/fulltext/?doi=javaa.2010.2295.2304)

Effect of phytobiotic feed additives on production performance in poultry1

Phytobiotic feed additive	Dietary dose (g kg^{-1})	Treatment effect, percentage of difference from untreated control				Reference
		FI	BW	ADG	FCR	
Broilers						
Plant extracts						
Oregano	0.150	-6	-	-2	-4	Basmacioglu *et al*., 2004
Oregano	0.300	-3	-	+1	-2	Basmacioglu *et al*., 2004
Rosemary	0.150	0	-	-1	-1	Basmacioglu *et al*., 2004
Rosemary	0.300	-2	-	+1	-4	Basmacioglu *et al*., 2004
Thymol	0.100	+1	-	+1	-1	Lee *et al*., 2003
Cinnamaldehyde	0.100	-2	-	-3	0	Lee *et al*., 2003
Thymol	0.200	-5	-	-3	-3	Lee *et al*., 2003
Carvacol	0.200	+2	-	+2	-1	Lee *et al*., 2003
Yucca extract	2.000	-1	-	+1	-6	Yeo and Kim, 1997
Essential oil blend	0.024	-4	0	-	-4	Cabuk *et al*., 2006
Essential oil blend	0.048	-5	0	-	-6	Cabuk *et al*., 2006
Plant extracts[2]	0.200	-	-2	0	-2	Hernandez *et al*., 2004
Plant extracts[2]	5.000	-	+2	+3	-4	Hernandez *et al*., 2004
Plant extracts[2]	0.500	0	-2	-2	+2	Botsoglou *et al*., 2004a
Plant extracts[2]	1.000	+2	-1	0	+2	Botsoglou *et al*., 2004a
Essential oil blend	0.075	-7	-	-3	-4	Basmacioglu *et al*., 2004
Essential oil blend	0.150	-7	-	-1	-1	Basmacioglu *et al*., 2004
Essential oil blend	0.036	+3	-8	-	-5	Alcicek *et al*., 2004
Essential oil blend	0.048	+2	-8	-	-4	Alcicek *et al*., 2004
Plant extracts[1]	0.100	+1	-	+1	0	Lee *et al*., 2003
Essential oil blend	0.024	-2	0	-	-2	Alcicek *et al*., 2003
Essential oil blend	0.048	0	+14	-	-12	Alcicek *et al*., 2003
Essential oil blend	0.072	-2	+8	-	-9	Alcicek *et al*., 2003
Herbs and spices						
Oregano	5.000	+5	-	+7	-2	Florou-Paneri *et al*., 2006
Thyme	1.000	+1	+2	-	-1	Sarica *et al*., 2005
Garlic	1.000	-5	-5	-	0	Sarica *et al*., 2005
Herb mix	0.250	0	-	+2	-2	Guo *et al*., 2004a
Herb mix	0. 50	+5	-	+2	+3	Guo *et al*., 2004a
Herb mix	1.000	+2	-	+1	+1	Guo *et al*., 2004b
Herb mix	2.000	+1	-	+1	0	Guo *et al*., 2004b
Turkeys						
Herbs and spices						
Oregano	1.250	-5	+2	-	-	Bampidis *et al*., 2005
Oregano	2.500	-6	+1	-	-	Bampidis *et al*., 2005
Oregano	3.750	-9	+1	-	-	Bampidis *et al*., 2005
Quail						
Essential oils						
Thyme	0.060	0	-	+6	-	Denli *et al*., 2004
Black seed	0.060	+1	-	+2	-	Denli *et al*., 2004
Herbs and spices						
Coriander	5.000	+3	-	+1	+1	Guler *et al*., 2005
Coriander	10.000	+3	-	+5	-1	Guler *et al*., 2005
Coriander	20.000	+4	-	+8	-4	Guler *et al*., 2005
Coriander	40.000	+5	-	+4	+1	Guler *et al*., 2005

Windisch *et al*., 2008 [2]Entire product

Effect of phytobiotic feed additives on production performance in poultry[1]

Phytobiotic feed additive	Dietary dose (g kg^{-1})	Treatment effect, percentage of difference from untreated control				Reference
		FI	BW	ADG	FCR	
Broilers						
Plant extracts						
Oregano	0.150	-6	-	-2	-4	Basmacioglu *et al*., 2004
Oregano	0.300	-3	-	+1	-2	Basmacioglu *et al*., 2004
Rosemary	0.150	0	-	-1	-1	Basmacioglu *et al*., 2004
Rosemary	0.300	-2	-	+1	-4	Basmacioglu *et al*., 2004
Thymol	0.100	+1	-	+1	-1	Lee *et al*., 2003
Cinnamaldehyde	0.100	-2	-	-3	0	Lee *et al*., 2003
Thymol	0.200	-5	-	-3	-3	Lee *et al*., 2003
Carvacol	0.200	+2	-	+2	-1	Lee *et al*., 2003
Yucca extract	2.000	-1	-	+1	-6	Yeo and Kim, 1997
Essential oil blend	0.024	-4	0	-	-4	Cabuk *et al*., 2006
Essential oil blend	0.048	-5	0	-	-6	Cabuk *et al*., 2006
Plant extracts[2]	0.200	-	-2	0	-2	Hernandez *et al*., 2004
Plant extracts[2]	5.000	-	+2	+3	-4	Hernandez *et al*., 2004
Plant extracts[2]	0.500	0	-2	-2	+2	Botsoglou *et al*., 2004a
Plant extracts[2]	1.000	+2	-1	0	+2	Botsoglou *et al*., 2004a
Essential oil blend	0.075	-7	-	-3	-4	Basmacioglu *et al*., 2004
Essential oil blend	0.150	-7	-	-1	-1	Basmacioglu *et al*., 2004
Essential oil blend	0.036	+3	-8	-	-5	Alcicek *et al*., 2004
Essential oil blend	0.048	+2	-8	-	-4	Alcicek *et al*., 2004
Plant extracts[1]	0.100	+1	-	+1	0	Lee *et al*., 2003
Essential oil blend	0.024	-2	0	-	-2	Alcicek *et al*., 2003
Essential oil blend	0.048	0	+14	-	-12	Alcicek *et al*., 2003
Essential oil blend	0.072	-2	+8	-	-9	Alcicek *et al*., 2003
Herbs and spices						
Oregano	5.000	+5	-	+7	-2	Florou-Paneri *et al*., 2006
Thyme	1.000	+1	+2	-	-1	Sarica *et al*., 2005
Garlic	1.000	-5	-5	-	0	Sarica *et al*., 2005
Herb mix	0.250	0	-	+2	-2	Guo *et al*., 2004a
Herb mix	0. 50	+5	-	+2	+3	Guo *et al*., 2004a
Herb mix	1.000	+2	-	+1	+1	Guo *et al*., 2004b
Herb mix	2.000	+1	-	+1	0	Guo *et al*., 2004b
Turkeys						
Herbs and spices						
Oregano	1.250	-5	+2	-	-	Bampidis *et al*., 2005
Oregano	2.500	-6	+1	-	-	Bampidis *et al*., 2005
Oregano	3.750	-9	+1	-	-	Bampidis *et al*., 2005
Quail						
Essential oils						
Thyme	0.060	0	-	+6	-	Denli *et al*., 2004
Black seed	0.060	+1	-	+2	-	Denli *et al*., 2004
Herbs and spices						
Coriander	5.000	+3	-	+1	+1	Guler *et al*., 2005
Coriander	10.000	+3	-	+5	-1	Guler *et al*., 2005
Coriander	20.000	+4	-	+8	-4	Guler *et al*., 2005
Coriander	40.000	+5	-	+4	+1	Guler *et al*., 2005

Windisch *et al*., 2008 [2]Entire product

CITATIONS USEFUL Regarding binding of Toxins.

Effects of Activated Carbon, Hypo, Alum, Clay, Auto claved egg shells, Auto Claved shrimp shells and betaine are significant in overcoming the aflatoxic symptoms (on growth, mortality, feed utilization, organs indices, carcass composition and blood enzymes).

Effects accompanying the addition of montmorillonite included increased growth rate and body weight of the chickens and reduced mortality rate. Dietary additions of zeolites (Smith, J. Animal Science, 1980 Vol. 50(2), pp. 278-285), bentonite (Carson, M.S. Thesis University of Guelph, Canada 1982) and spent bleaching clay from canola oil refining (Smith, Can. J. Animal Science, 1984, Vol. 64, pp. 725-732), have been shown to diminish the adverse effects of T-2 toxin and zearalenone in rats and immature swine. The adsorption of aflatoxin B1 from various liquid media by various clay minerals, including montmorillonites, has been reported.
(Masimanco et al., Ann. de Nutrition et Alimentation, 1973 Vol. 23, pp. 137-147).

WHAT TOXIGRADE CONTAINS?

DFM, ENZYMES, PLANT EXTRACTS AND TOXIN BINDERS

Acetobacter Xylinum,
Activated Carbon
Alum pulvis
Bacillus Subtilis
Benzoic acid
Black pepper
Dhaniya Crude Pulvis
Citric acid
Chitin isolated from crustacean shells
Clove oil
Dextrose
Eclipta alba
Garlic powder
Honey
Tulsi crude pulvis
L acidophilus
Lactobacillus casei
L.delbrueckii subsp. Bulgaricus
L plantarum
L. reuteri
L. rhamnosus
Lactococcus lactis
Hydrated Sodium alluminium Silicate
Nigella sativa
plumbago indica
S. boulardi
Sodium bicarbonate
Sodium Hydroxide
Spirulina
T. Viride
Mannon Oligo SaActivated Carbonides,
1,3/ 1,6 beta glucans
Swertia chirraita
Thio Urea
Thymol,
cinnamon containing trans-cinnamic acid, trans-cinnamaldehyde, and ferulic acid (phydroxy-3-methyl cinnamic acid)

WHAT IS THE MODE OF ACTION OF TOXIBIND BIO?

A. Physical means of adsorption of the toxins is achieved by the Activated Carbon and Hydrated Sodium Aluminum Silicate, Aluminum sulphate.

For some hundred years, research into activated carbon has been showing effective ways of adsorbing pathogenic clostridial toxins such as C. tetani und C. botulinum (Kranich 1920, Luder 1947, Starkenstein 1915). Wang et al (2010) have shown that Activated Carbon has good sorption qualities with regard to the hydrophobic herbicide terbuthylazine and underline the important role it can play in protecting ground water. Graber et al. (2011) studied the binding qualities of the model herbicides S-Metolachlor and Sulfentraton on Activated Carbons with different surface sizes. Graber (2012) confirmed that Activated Carbon can adsorb glyphosate. The use of carbon gained from pyrolysis for feeding purposes has been known for a long time and is recommended in Germany. Mangold (1936) presented a comprehensive study on the effects of Activated carbon in feeding animals, concluding that "the prophylactic and therapeutic effect of Activated Carbon against diarrhoeal symptoms attributable to infections or the type of feeding is known. In this sense, adding Activated Carbon to the feed of young animals would seem a good preventive measure."

Of particular importance is the specific colonisation of the Activated Carbon with gram-negative germs with increased metabolic activity. This results on the one hand in a decrease in endotoxins needing to be resorbed and on the other hand in the adsorption of the toxins in the Activated Carbon.
Ariens and Lambrecht (1985) describe the advantages of activated carbon, stating that it is non-toxic, quickly available, has an unlimited shelf-life, is effective in the gastrointestinal tract, and is effective against already absorbed toxins and mineral oil products.

One major advantage in the use of Activated Carbon is to be found in its "enteral dialysis" property, i.e. already absorbed lipophilic toxins can be removed from blood plasma by the Activated Carbon, as the adsorption power of the huge surface area of Activated Carbon interacts with the beneficial permeability properties of the intestine. Adsorption applies to both lipophilic and hydrophilic substances. The speed at which adsorption takes place is dependent on the size of the activated carbon's pores. What we are thus seeing is the emergence of a genuine alternative to the established medical therapies – peritoneal dialysis, haemodialysis or haemoperfusion.
Via manure and slurry, the Activated Carbon mixed with the feed is returned to the soil, closing the organic cycle. The fact that Activated Carbon returned to the soil this way can be of interest for agriculture was already described by Perotti back in 1935. For him, the presence of Activated Carbon in the soil meant an improvement in its microbiological properties and a better supply of chlorophyll for the plants.
In his view, the benefits of Activated Carbon were as follows:
1. Moisture retention
2. Increased adsorption of ammonium salts
3. Decreased dispersion of nitrates
4. Adsorption of microbial metabolites

Activated Carbon can effectively alleviate lesions of AFB_1 (Mohamed and Mokhbatly, 1997)

ALUMINUM SULPHATE significantly reduces the amount of AFB_1 absorbed from the digestive system following ingestion. (Ellies et al. (2000))

B. Chemical means of Sodium Hydroxide, Sodium Perborate, Calcium Peroxide; Potassium Bi sulfate, Propionic acid, Benzoic acid are also employed to act on contact.

C. Biological means (biotransformation) (by fungi, yeasts and bacteria) are also employed to work at the gut level and hepatic level..

Trichoderma viride is a promising biocontrol agent for the pathogens, *Saprolegnia* sp. and *Aspergillus ochraceus*. It can significantly reduce saprolegniasis severity. It is safe and is also used for biological control purposes against pathogens. S boulardi by secreting H2O2 will oxidize and destroys the toxins.

B. pumilus has been associated with inhibition of aflatoxin, cyclopiazonic acid, ochratoxin A and patulin production.
Munimbazi and Bullerman reported that more than 98% inhibition in aflatoxin production by *A. parasiticus* was caused by *B. pumilus*.

D. Dietary means like vitamins (A, E) and minerals (Se) help in fighting the toxins and bring down the severity of the problem.

E. Addition of Thiourea and Organic Acids capable of destroying the fungi and impairing their ability to produce toxins; in TOXINBIND BIO is made with the sole purpose of providing a synergetic effect and to provide consistent results.

F. Medicinal Herbs like garlic, Cinnamon, Tulsi, Thymol, Menthol are well known Fungicides and they are well documented to combat toxicosis.

Pepaver acts on the CNS of the flukes and makes them to loose their grip and fall into water medium unconscious.
Coriander Seed, Methi inhibit the fungal metabolism.
Nigella sativa significantly ameliorated the adverse effects of dietary AFB_1
(Hussein *et al*. (2000))

G. Immuno stimulants.
Feeding of 1,3/1,6 Beta glucan significantly raised the degree of resistsnce against *A. hydrophila* challenge and the non-specific immunity level. (Sahoo and Mukherjee, 2001a)
Mannon Oligo Sachharides, Activated Carbonides binds the mycotoxins.

H. Anti Oxidants present in TOXIBIND BIO minimizes the resynthesis of mycotoxins at the Hepatic level.

Enzymes produced by the Beneficial Microbes in TOXIBIND BIO degrade the toxins.

Thus stopping of secretion of toxins by pathogens, destroying of the pathogens, degrading the toxins, removal of mycotoxins from the contaminated feeding stuff besides the resulting changes in physical and nutritional properties of these feeding stuffs are well taken care in TOXIBIND BIO.

HOW DOES TOXBIND BIO IS SUPERIOR WHEN COMPARED TO OTHER TOXIN BINDERS AVAILABLE IN THE MARKET?

- ACIDIFIES GUT
- CONSUMES THE TOXINS AND CONVERTS THE SAME INTO TDN
- CONTROLS PESTICIDE AND OTHER CHEMICAL TOXICITY.
- DESTROYS AND DEGRADES THE TOXINS IN A UNIQUE EFFICIENT MANNER.
- DETOXIFIES FASTER IN A COMPLETE AND EFFICIENT WAY
- Dominates And Controls All Pathogens Like *Aspergillus, Fusarium, Claviceps Spp., P. Citrinum, P. Viridicatum, Salmonella, E Coli, Pasteurella.*
- IMPROVES DAILY BODY WEIGHT GAIN
- PRODUCES SEVERAL USEFUL ENZYMES TO IMPROVE F C R
- REDUCES MORTALITY RATE

- TRANSFORMS THE MYCOTOXINS IN THE DESIRED PATHWAY.
- Withstands Pelletisation Temperatures

DOSAGE:

Preventive: 250 gms/ Ton Feed for every 0.5% Moisture in excess of 7% Moisture in the end product.

Curative: 500 gms/ Ton Feed for every 0.5% Moisture in excess of 7% Moisture in the end product for five days.

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