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(54) Title: USES OF MYCOTOXIN-DETOXIFYING ANTIBODIES

(57) Abstract: The present invention discloses uses of mycotoxin-binding antibodies, including antibodies which bind to mycotoxins in feed and feed ingredients resulting in the reduction or elimination of toxic and carcinogenic effects of mycotoxins. The invention also discloses a composition comprising a mycotoxin-binding antibody, an adsorbing agent, a biotransforming agent and an antioxidant for detoxifying mycotoxins in feeds. In addition, the invention teaches the methods of preparing said mycotoxin-binding antibodies, a composition and also the methods of using it as a feed additive. Furthermore, the invention relates to the use of said mycotoxin-binding antibodies alone or a composition comprising said antibodies and other mycotoxin-detoxifying agents in feeds and feed ingredients for detoxifying the major mycotoxins.

USES OF MYCOTOXIN-DETOXIFYING ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application No. 62/255,709, filed November 16, 2015.

FIELD OF THE INVENTION

[0002] The present invention relates to mycotoxin-binding antibodies, processes for the preparation of said antibodies, and the use of said mycotoxin-binding antibodies in feeds and feed ingredients for detoxifying mycotoxins.

BACKGROUND OF THE INVENTION

[0003] Mycotoxins are toxic secondary metabolites produced by fungi. Mycotoxin formation may occur when the causative fungi grow on crops in the field, at harvest, in storage, or during feed processing; essentially whenever favorable conditions for their formation prevail. There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis that are commercially available. The primary classes of mycotoxins are Aflatoxins (B1, B2, G1, G2) of which aflatoxin B1 (AFB1) is the most prevalent, zearalenone (ZEA), trichothecenes such as deoxynivalenol (DON) and T-2 toxin (T-2), fumonisins (FUM: FB1, FB2, FB3) and ochratoxin A (OTA). The major mycotoxin-producing fugal genera are *Aspergillus, Fusarium* and *Penicillium*. Many species of these fungi produce mycotoxins in commodities, feeds and feed ingredients. Mycotoxin contamination in animal feed and human food is a worldwide problem. Rodrigues and Naehrer (Phytopathologia Mediterranea. 51: 175-192, 2012) reviewed mycotoxin contamination of diverse feedstuffs samples from throughout the world for five toxins (AFB1, DON, ZEA, FUM and OTA).

[0004] Mycotoxins are toxic when contaminated feeds or feed ingredients are consumed by animals. Mycotoxicoses are diseases caused by exposure to feeds contaminated with mycotoxins (Nelson et al., 1993. Ann. Rev. Phytopath. 31: 233-249). Mycotoxins exhibit a variety of biological effects in animals, which include liver and kidney toxicity, neurological, estrogenic and teratogenic effects, to name a few. Some mycotoxins such as AFB1, OTA and FB1 are carcinogenic. Additionally, the mycotoxin-contaminated feed consumption in animals can cause loss of appetite, decreased feed efficiency, feed refusal, poor weight gain, immunosuppression, and mortality. Each mycotoxin has its own particular effect, and all can be devastating. Co-contamination by one or more types of mycotoxin occurs naturally, and exerts a greater negative impact on health and productivity of livestock than contamination by individual mycotoxins.

[0005] The mycotoxin contamination of feed results in billions of dollars of economic losses to animal husbandry world-wide and in some cases in health

damage to human consumers due to transfer of contamination via dairy products, eggs and meats. The estimates of the costs of mycotoxins in the US vary and while one report estimated to average $1.4 billion and another estimated $5 billion per year for the US and Canada. Economic losses are due to effects on livestock productivity, crop losses and the costs of regulatory programs directed toward mycotoxins.

[0006] Numerous approaches to reduction of mycotoxin levels in agricultural commodities used as animal feed ingredients have been assessed. These include mixing and dilution with mycotoxin-free grains in order to obtain a level within regulatory guidelines, i.e. 20 ppb or less; physical methods of separation such as cleaning, density segregation and preferential fragmentation; solvent extraction; biological inactivation; thermal inactivation; and chemical inactivation with a variety of acids, aldehydes, oxidizing agents and alkalis'. These approaches have been relatively unsuccessful on a commercial scale due to lack of efficacy, economic constraints of the protocols, unacceptable alteration of feed quality, or the introduction of potentially deleterious substances. Consequently, simple, cost effective, practical and safe processes by which animal feeds can be decontaminated or detoxified are needed. A more viable method of dealing with mycotoxin-contaminated feeds is to blend in a substance capable of binding mycotoxins, thus preventing absorption of the mycotoxins into the animal's bloodstream. These feed additives may act by reducing the bioavailability of the mycotoxins or by degrading them or transforming them into less toxic metabolites.

[0007] There are two subcategories of mycotoxin-detoxifying agents: Adsorbing Agents and Biotransforming Agents (European Food Safety Agency/EFSA Scientific Report, 2009; pp. 1-192). Adsorbing agents are also called binding agents, adsorbents and binders. Adsorbing agents reduce the exposure to mycotoxins by decreasing their bioavailability, including various mycotoxin adsorbing/absorbing agents in the feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs. These adsorbents include aluminosilicates, bentonites, montmorillonites, zeolites, HSCAS (Hydrated Sodium Calcium Aluminosilicate), activated carbons, yeast cell walls, micronized fibers, and polymers (cholestyramine, polyvinylpyrrolidone). Biotransforming agents such as bacteria, yeast/fungi or enzymes degrade mycotoxins into non-toxic metabolites. Among adsorbents, the use of mineral clays as binders is common. For example, U.S. Pat. No. 5,149,549 teaches the use of a montmorillonite clay, particularly a bentonite clay, admixed with animal feeds as a mycotoxin binder. U.S. Pat. No. 5,165,946 discloses the use of a montmorillonite clay in combination with a suitable sequestrant, particularly phosphate and polyphosphate salts, as mycotoxin binders. U.S. Pat. No. 5,639,492 further refines the art, describing the use of an acid-activated calcium bentonite clay admixed with animal feeds to reduce effects of mycotoxin contamination. U.S. Pat. No. 6,045,834 proposes the combination of

modified yeast cells and of inorganic minerals such as zeolite, bentonite or aluminum silicate to deactivate mycotoxins present in feeds and, thus, prevent the absorption of the mycotoxins into animal blood.

[0008] However, a drawback of mineral adsorbent such as zeolite, bentonite, and aluminosilicates, is that they are usually included at concentrations of 1-2% by weight, which reduces the nutritional value of the feed. Furthermore, their activity is not specific and they adsorb only a narrow range of mycotoxins. Also, they can reduce the adsorption of nutrients such as vitamins, minerals and amino acids. It is therefore important to find new products, which can adsorb or inactivate a broad spectrum of mycotoxins without limiting the bioavailability of nutrients and micronutrients in animals.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIG.1 shows the binding of aflatoxin B1(AFB1) to AFB1-specific antibody.

[0010] FIG.2 shows the neutralizing effect of aflatoxin B1 (AFB1)-specific antibodies on AFB1 toxicity induced lethality in zebrafish.

SUMMARY OF THE INVENTION

[0011] An objective of the present invention is to detoxify animal feeds by using mycotoxin-binding antibodies, which bind to mycotoxins such as AFB1, DON, ZEA, FUM and OTA, and detoxify said mycotoxins present in livestock and other animal feeds and feed ingredients.

[0012] A further objective of the present invention is to provide methods of producing and using mycotoxin-binding antibodies, such as mycotoxin-binding chicken egg-yolk antibodies (IgYs), for detoxifying mycotoxins present in animal feeds and feed ingredients.

[0013] Yet a further objective of the present invention is to provide a composition comprising a combination of a mycotoxin-binding antibody and one or more mycotoxin-adsorbing agents selected from the group consisting of zeolites, bentonites, aluminosilicates, montmorillonites, hydrated sodium calcium aluminosilicate (HSCAS), diatomaceous earth, humic substances, yeast cell walls, micronized fibers, cholestyramine, polyvinylpyrrolidone and mineral oil.

[0014] Another objective of the present invention is to provide a composition comprising a mycotoxin-binding antibody and one or more mycotoxin-biotransforming agents selected from the group consisting of bacteria, fungi, yeasts and enzymes.

[0015] Yet another objective of the present invention is to provide a composition comprising a mycotoxin-binding antibody, one or more adsorbing agents and one or more biotransforming agents, and one or more antioxidants selected from the group consisting of phenolic compounds including flavonoids, vitamins, provitamins, sulfur containing compounds, trace minerals, and various plant extracts.

[0016] Still another objective of the present invention is to provide a composition as described above, which has an unexpected additive or synergistic binding or adsorbing effect for reducing or removing mycotoxin contamination in animal feeds and feed ingredients.

[0017] An additional objective of the present, as described above, is to provide a composition, which may be admixed with animal feeds at lower inclusion rates than that required for current commercially available mycotoxin binders suitable for inclusion in animal feeds and feed ingredients. The mycotoxin-binding antibodies alone, or in combination with one or more adsorbing agents and biotransforming agents in the present invention may be added to mycotoxin-contaminated animal feed in amounts from about 0.01% to 2% by weight of feed. In a preferred embodiment, the composition is added to mycotoxin-contaminated animal feed in amounts from about 0.03% to 0.6% by weight of feed. In an especially preferred embodiment, the invention is added to mycotoxin-contaminated animal feed in amounts from about 0.1% to 0.3% by weight of feed.

[0018] The competitive advantages and novel features of the present invention will be set forth in part in the detailed description of the invention that follows and in part will become apparent to those skilled in the art upon examination of the following or may be learned with the practice of the invention. The advantages of the invention can also be realized and obtained by means of the instrumentalities and combinations.

[0019] To achieve the foregoing and other objectives, and in accordance with the purposes of the present invention as described herein, a novel method is described for adsorbing mycotoxins present in animal feeds. In particular, in a preferred embodiment, the invention provides a method and a composition for binding mycotoxins present in animal feeds containing specific mycotoxin-binding antibodies alone or in combination with one or more adsorbing and biotransforming agents. Furthermore, a variety of recombinant antibody production systems, ranging from Gram-negative and -positive bacteria, yeasts, transgenic plants and transgenic or non-transgenic animals can be used for the production of mycotoxin-binding monoclonal antibodies (mAbs), single-chain variable fragment (scFv) of antibodies or single-domain antibodies (sdAb).

[0020] The mycotoxin-binding antibodies alone or compositions, as described above, can be fed to any animal including, but not limited to, avian, bovine,

porcine, equine, ovine, caprine, canine, and feline species. When admixed with feed or fed as a supplement, the mycotoxin-binding antibodies alone or compositions with their increased mycotoxin-binding capacity, decreased absorption or uptake of the mycotoxins by the affected animals, will improve animal performance and health, and reduce the incidence of mycotoxin-associated syndromes or diseases.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The present invention provides an alternative mycotoxin adsorbent or binder, suitable for feeds, which effectively inactivates mycotoxins in a selective manner but does not have unwanted interaction with nutrients. It also provides a simplified process for the preparation of a binder that can be produced at a low cost.

[0022] Monoclonal antibodies are in increasing demand as therapeutics and diagnostics. They are also used in many standard assays such as immunoblot, flow cytometry, or immunohistochemistry. In addition, the emerging field of proteome research has a huge need for binders against different protein antigens and splice variants. Moreover, recombinant antibodies are used for diagnosis of different pathogens and toxins including mycotoxins (Pansri et al., 2009. BMC Biotechnol. 9: 1-16; Fodey et al., 2011. Trends Anal. Chem. 30: 254-269; Frenzel et al., 2013. Frontiers in Immunology 4: 8-20; Muynck et al., Plant Biotechnol. J. 8: 529-563). Antibody generation has been dramatically accelerated by *in vitro* selection systems, particularly phage display. An increasing variety of recombinant production systems have been developed, ranging from Gram-negative and -positive bacteria, yeasts and filamentous fungi, insect cell lines, mammalian cells to transgenic plants and animals (Frenzel et al., 2013. Frontiers in Immunology, 4: 8-20). Although a vast number of recombinant antibody structures have been proposed, the single-chain variable fragment (scFv) and the single-domain (VHH, VH, and NAR V) formats are most widely used for both research and industrial applications (de Marco, 2011. Microbial Cell Factories, 10: 1-14; Marconi, P. and M.A. Alvarez. 2014. J. Immunol. Tech. Infect. Dis. 3: 1-6).

[0023] Many methods of antibody production are known in the art and may be utilized. See, for example, Basic Methods in Antibody Production and Characterization, 2001, Howard, C. and Bethell, D. eds, CRC Press. For example, expression vector(s) encoding the antibody or fragment is transfected into a cell line (see, e.g., U.S. Pat. Nos. 7,531,327; 7,537,930; 7,608,425; and 7,785,880; the Examples section of each of which is incorporated herein by reference). Alternatively, both transfection and antibody expression occur in serum-free medium to decrease the expense of production and remove a source of

contaminating proteins. The antibody is produced into the cell culture medium for further purification.

[0024] In other embodiments, the antibody may be purified from cell culture medium by sequential chromatography, for example by affinity and ion exchange column chromatography. Non-limiting examples include affinity chromatography on Protein A, anion-exchange chromatography on Q-SEPHAROSE and cation-exchange chromatography on SP-SEPHAROSE.

[0025] Recently, considerable research has focussed on the use of egg-yolk antibody (also called immunoglobulin Y or IgY) as an alternative to mammalian antibodies for several applications, including immunotherapeutic applications, especially for the oral passive immunization against various bacteria and viruses (Kovacs-Nolan, J. and Y. Mine., 2004. Avian and Poult. Biol. Rev. 15: 25-46; Shade, R. et al., 2005. ATLA. 33: 1-26)). Much of research has been carried out on the use of chicken egg-yolk antibodies as a replacement for IgG in various immunodiagnostic and immune-affinity purification purposes. The use of egg-yolk antibody offers several advantages over polyclonal antibodies produced in mammals, including the ability to provide a much more hygienic, cost efficient, convenient, humane and plentiful source of antigen-specific antibodies. In particular, mycotoxin binder egg-yolk antibodies with superior mycotoxin binding property and safety will provide an alternative to existing commercial mycotoxin binders (adsorbents).

[0026] For example, a method for the preparation and purification of egg-yolk antibodies is similar to that described by Kim et al. (J. Sci. Food Agric. 79: 1513-1518, 1999). Twenty-week-old white Leghorn laying hens are initially injected intramuscularly with 500 µg of mycotoxin conjugate (for example, DON-BSA conjugate) in 0.5 ml of PBS that is emulsified with an equal volume of complete Freund's adjuvant. The hens are then injected five times at biweekly intervals using the same antigen preparation except that it is emulsified with incomplete Freund's adjuvant and the dosage of mycotoxin-conjugate is 250 mg. Three weeks after the original injection, eggs are collected daily for four months for antibody preparation.

[0027] Egg-yolk antibodies are prepared in at least two ways. In the first procedure, the yolks after removal of the shell are carefully separated manually from the egg white and are dried by lyophilisation or spray-drying. Large quantities of dried whole-egg powder or dried egg-yolk powder are prepared in a commercial egg breaking, separation and drying (spray drying) facility. The egg-yolks, when a semi-purified lipid free preparation is required, are separated and diluted (1: 9, v/v) with double-distilled water that is acidified with concentrated HCl to obtain a final pH of 5.0. The suspension is frozen at -20°C overnight, thawed and

centrifuged at 10,000 g for 30 min at 15°C. The clear, water-soluble supernatant is collected and filtered twice through filter paper (Whatman No. 1). Dried egg-yolk antibody powder is obtained by freeze-drying the water-soluble fraction. The antibody titers of the egg products are estimated using conventional methods.

[0028] The above mentioned variety of recombinant production systems, ranging from Gram-negative and -positive bacteria, yeasts, transgenic plants and transgenic or non-transgenic animals can be used for the production of recombinant antibodies.

[0029] Furthermore, the recent advancements in recombinant monoclonal antibody technology, including the use of single-chain variable fragment (scFv) of antibody and single domain antibody (sdAb), will have potential applications in the development of new generation of antibody-based mycotoxin binders. All these antibodies can be produced in a variety of production/expression systems and are currently used exclusively in applications such as diagnostics and therapeutics. The current invention, related to use of anti-mycotoxin antibodies as a binder to detoxify mycotoxins, is a unique and novel approach to decontaminate human foods and animal feeds containing toxic and carcinogenic mycotoxins.

[0030] Many methods are known for the creation and production of monoclonal antibodies, for example, the hybridoma method as first described by Koehler et al. (1975) Nature 256:495-497 or other methods described in the literature (see Goding, J W (1980) J. Immunol. Methods 34:285-308; Harlow E and Lane D (1988) in Antibodies: A Laboratory Manual, Chapter 6; Kennett R H et al. (1980) Monoclonal Antibodies, Plenum Press; Zola H (1987) Monoclonal Antibodies: A Manual of Techniques, CRC Press).

[0031] In one embodiment, the method of creating hybridomas begins with immunizing a host animal, such as a mouse, to elicit the production of lymphocytes that produce antibodies targeted to the peptide or protein(s) of interest. Lymphocytes may also be immunized in vitro. The antigen used may be a peptide, a protein or a cell displaying the antigen on the cell surface. Lymphocytes are collected then fused by chemical (e.g., with PEG) or electrical (e.g., by electrofusion) methods with myeloma cells to form hybridoma cells, typically under conditions that prevent the growth and/or survival of the parent myeloma cells. Fused cells are allowed to grow because they contain enzymes that facilitate survival in the culture medium. In a preferred embodiment, the culture medium contains hypoxanthine, aminopterin and thymidine (HAT medium), which prevents the growth of cells lacking hypoxanthine quinine phosphoribosyl transferase (HPRT). The HPRT is supplied to the fused cell by the lymphocyte partner, allowing

survival of the hybridoma but preventing survival of the parent myeloma cells, which lack HPRT.

[0032] Culture media in which hybridomas are grown (i.e., conditioned media) are typically assayed for the production of monoclonal antibodies directed against the antigen using a variety of techniques (see Voller, et al. (1978) J. Clin. Pathol. 31:507-520), including but not limited to, immunoprecipitation or an in vitro binding assay such as enzyme-linked immunosorbant assay (ELISA; see Engvall E (1977) in Biomedical Applications of Immobilized Enzymes and Proteins, edited by TMS Chang, 2:87-96, Plenum Press), radioimmunoassay (MA; see Sonksen P H (1974) Brit. Med. Bull. 30:1-103), Western blots or flow cytometry. Conditioned media from the hybridomas were profiled in a series of assays including ELISA (FIG. 1), Western blot (FIG. 2) and flow cytometry (FIG. 3). In preferred embodiments, studies using both native and permeabilized and fixed cells are performed to identify antibodies that may perform well in applications that use fixed cells or tissues, such as immunohistochemistry (IHC). Clones of interest may be subcloned by limiting dilution or single cell flow cytometry.

[0033] As will be known to those skilled in the art, monoclonal antibodies secreted by hybridoma clones (or subclones) can be purified using conventional purification procedures such as, but not limited to, dialysis, affinity chromatography, gel electrophoresis or protein A-sepharose (or protein L-agarose) chromatography.

[0034] The term "mycotoxin" means a secondary metabolite produced by fungi (mold).

[0035] The term "mycotoxicosis" means disease caused by exposure to foods and feeds contaminated with mycotoxins.

[0036] The term "binding agent" means a binding agent, which adsorbs or absorbs mycotoxins present in foods and feeds, and thus reversing the adverse effects of mycotoxins.

[0037] The term "biotransforming agent" means enzyme or bacteria or yeast or fungus, which deactivates (inactivates) mycotoxins present in foods and feeds, and thus reversing the adverse effects of mycotoxins.

[0038] The term "egg-yolk antibody" or "IgY" means immunoglobulin Y (abbreviated as IgY), which is a type of immunoglobulin produced by birds and reptiles. It is found in high concentration in chicken egg yolk.

[0039] The term "plantibody" means an antibody that is produced by plants that have been genetically engineered (also called "transgenic plants") with gene (DNA fragment) encoding the desired antibody.

[0040] The term "transgenic animal" means animal whose genome has been changed to carry genes from other species (e.g., transgenic chicken).

[0041] The term "non-transgenic (nontransgenic)" simply means not transgenic or not genetically modified.

[0042] The term "recombinant microorganism" means a microorganism whose genetic makeup has been altered by deliberate introduction of new genetic elements (e.g., genes). The offspring of these altered bacteria also contain these new genetic elements.

[0043] The term "animal" includes all animals, including human beings. Examples of animals are cattle, (including but not limited to cows and calves); mono-gastric animals, e.g., pigs or swine (including, but not limited to, piglets, growing pigs, and sows); poultry such as turkeys and chicken (including but not limited to broiler chicks, layers); and fish.

[0044] The term "feed" or "feedstuff" or "feed ingredient" or "feed product" means any compound, grain, nut, forage, silage, preparation, mixture, or composition suitable for, or intended for intake by an animal.

[0045] The term "forage" means plant material (mainly plant leaves and stems) eaten by grazing animals.

[0046] The term "silage" means a fermented, high-moisture stored fodder which can be fed to ruminants.

[0047] Preferably the feed is a grain and forage or silage based product. More preferably the feed ingredients comprise cereal(s), e.g., one or more of corn (maize), wheat, barley, rye, rice, sorghum and millet.

[0048] In one embodiment, the feed ingredient may be derived solely from cereal(s), and in another embodiment partly from legumes (e.g., soybean), partly from cereals and partly from animal products. The grain based feed may comprise whole or milled grain (e.g., wet or dry milled grain), including grain based product comprising fractions of wet or dry milled grain (e.g., gluten, protein, starch, and/or oil fractions). Also included are feed ingredients comprising by-product from brewing and/or fermentation processes (e.g., spent grains). Spent grains are the by-products from the production of alcoholic beverages and ethanol fuels. Brewers' spent grain (BSG) is the residue of beer making in breweries, which use malted barley as the major raw material. Distiller's' spent grain (DSG) is the product left in distilleries after alcohol is removed by distillation from the fermented grains such as corn, wheat, barley, rice, and rye. Distiller's' spent grain is also known as distiller's grain. Wet distiller's grain (WDG) is dried to produce dried distiller's grain (DDG) which is used primarily as animal feed.

[0049] In another embodiment, the present invention provides mycotoxin-binding antibodies, which bind to mycotoxins and detoxify mycotoxins present in human food and animal feed. The mycotoxin-binding antibodies and mycotoxin deactivating/inactivating enzymes are produced in chickens, transgenic animals (e.g., chickens, pigs, goats, sheep, cows, etc.), transgenic plants (e.g. corn/maize, soybean, rice, alfalfa, tobacco plant, etc.), recombinant bacteria (e.g., *Escherichia coli, Proteus mirabilis, Pseudomonas putidas, Bacillus subtilis, Bacillus megaterium,* and *Lactobacillus paracasei*) or yeasts (e.g., *Pichia pastoris, Saccharomyces cerevisiae* and *Kluyveromyces lactis*). Furthermore, the mycotoxin-binding monoclonal antibodies (mAbs) produced in recombinant microorganisms or transgenic plants and animals may also include single-chain variable fragment (scFv) of antibody or single-domain antibody (sdAb). In one embodiment, the present invention can provide mycotoxin-binding chicken egg-yolk antibodies produced in chickens.

[0050] In yet another embodiment, the present invention provides a composition comprising mycotoxin-binding antibodies and one or more mycotoxin-adsorbing or absorbing (binder) agents including, but not limited to, sodium bentonite, calcium bentonite, smectite sepiolite, sodium calcium montmorillonite, organophil modified montmorillonite, modified montmorillonite nanocomposite, wyoming sodium montmorillonite, zeolites (clinoptilolite, calcium/potassium/sodium hydrated aluminosilicate), hydrated sodium calcium aluminosilicate (HSCAS), activated carbon, superactivated charcoal, yeast cell wall extracts/fractions (e.g., polymeric glucomannan, esterified glucomannan, beta-glucans fraction or dry yeast cell wall fraction), micronized fibers, polymers (cholestyramine & polyvinylpolypyrrolidone), activated diatomaceous earth, plant fibres, complexed hydrated aluminium silicate, kaolinite, feldspartz, quartz, carbonaceous material, and hydrated sodium-potassium-calcium aluminosilicate.

[0051] In other embodiments, the present invention provides a composition comprising mycotoxin-binding antibodies and one or more mycotoxin adsorbing or detoxifying biotransforming agents including, but not limited to, *Eubacterium* sp. BBSH 797, *Nocardia asteroids, Mycobacterium fluoranthenivorans sp., Rhodococcus erythropolis, Alcaligenes* species., *Bacillus* species., *Achromobacter* species., *Flavobacterium* species, *Pseudomonas* species, *Lactobacillus rhamnosus train* GG, *Lactobacillus helveticus 46 & 72, Lactobacillus casei, Lactobacillus lactis, Streptococcus thermophilus C5 and NG40Z, Lactobacillus paraplantarum, Stenotrophomonas maltophila, Saccharomyces cerevisiae, Cupriavidus basilensis* OR16 *Aspergillus niger, Eurotium herbariorum, Rhizopus species., Trichosporon mycotoxinivorans, Phaffia rhodozyma*, and *Xanthophyllomyces dendrorhous*.

[0052] In a further embodiment, the present invention provides a composition comprising a mycotoxin-binding antibodies and one or more mycotoxin degrading

and detoxifying enzymes including, but not limited to, protease A, pancreatin, carboxypeptidase A, epoxidase from *Eubacterium*, lactonohydrolase, catalase, dehydrogenase, laccase, xylanase, esterase, lipase, oxidase, amino acid oxidase, peroxidase, lactoperoxidase, manganese peroxidase, polysaccharase and dehydrogenase.

[0053] In yet a further embodiment, the present invention provides a composition comprising a mycotoxin-binding antibodies and one or more mycotoxin toxicity-reducing antioxidants in humans and animals. These antioxidants include, but not limited to, rutin, quercetin, lutein, lecithin, melatonin, mannitol, curcumin, curcuminoids, lycopene, allyl sulfides, fructose, chlorophyll and derivatives, sodium thiosulfate, glutathione, methionine, aspartame, trace elements (selenium, zinc, magnesium), catechin (epigallocatechin gallate, epicatechin gallate), morin, kaempferol, fisetin, naringin, vitamins (vitamins E, C, A, B), coenzyme Q10, provitamins (carotene and carotenoids), eugenol, vanillin, caffeic acid, and cholinergic acid.

[0054] In still another embodiment, the present invention provides the mycotoxins of significant importance to food and feed safety, human healthcare, and to livestock industry. The mycotoxins against which antibodies can be developed include, but not limited to, aflatoxins (AFB1, AFB2, AFG1, AFG1, AFG2, AFM1), ochratoxin A, citrinin, citreoviridin, cyclopiazonic acid, moniliformin, penitrem A, PR toxin, sterigmatocystin, rubratoxin, deoxynivalenol, nivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxy nivalenol, diacetoxyscirpenol, Fuserenon-X, T-2, HT-2, T-2 tetraol, neosolaniol, fumonisins, (FB1, FB2, FB3), ergot alkaloids (e.g., ergotamine), tremorgens, zearalenone, and alterneriol

[0055] To achieve the foregoing and in accordance with the purposes of the present invention as described herein, a novel method is described for adsorbing mycotoxins present in animal feeds. In particular, in a preferred embodiment, the invention provides a method and a composition for binding mycotoxins present in animal diets containing specific mycotoxin binder antibodies alone or in combination with one or more adsorbing and biotransforming agents. The mycotoxin-binding antibodies are obtained from the eggs of chickens injected with the selected mycotoxin-protein conjugate antigen and a suitable adjuvant selected from the group consisting of Freund's complete and incomplete adjuvants (FCA & FIA), Ribi Adjuvant System®, TiterMax®, CpG ODNs (ODN 1826, ODN 2006) and toll-like receptor adjuvants, or other adjuvants.

[0056] The mycotoxin-binding antibodies alone or compositions comprising mycotoxin-binding antibodies, mycotoxin-adsorbing agents and mycotoxin-biotransforming agents and mycotoxin toxicity-reducing agents provided by the present invention can be added to any commercially available feed or feedstuffs for

livestock or companion animals including, but not limited to, premixes, concentrates and pelleted concentrates. The said antibodies alone or a composition provided by the present invention may be incorporated directly into commercially available mashed and pelleted feeds or fed supplementally to commercially available feeds. When incorporated directly into animal feeds, the present invention may be added to such feeds in amounts ranging from 0.1 to about 10 kilograms (kgs) per tonne of feed. Preferably, when incorporated directly into animal feeds, the present invention may be added to such feeds in amounts ranging from 0.3 to about 6 kgs per tonne of feed. In an especially preferred mycotoxin-binding antibodies alone or a composition comprising mycotoxin-binding antibodies, -adsorbing agents and -biotransforming agents of the invention is added to feeds in amounts ranging from about 1 to 3 kgs per tonne of feed. The antibodies alone or a composition contained in the present invention may be fed to any animal, including but not limited to, avian, bovine, porcine, equine, ovine, caprine, canine, and feline species. Furthermore, the proposed methods of binding of an extended range of mycotoxins are especially useful for alleviating the effect of mycotoxin concentration while fermenting grains during ethanol and beer fermentations. The resulting wet distiller's grain and dried distiller's grain, including DDGS, has on average a 3-fold increase in mycotoxin content compared to initial materials.

[0057] The composition contained in the present invention may be added to mycotoxin-contaminated animal feed in amounts from about 0.01% to 2% by weight of feed. In a preferred embodiment, the composition is added to mycotoxin-contaminated animal feed in amounts from about 0.03% to 0.5% by weight of feed. In an especially preferred embodiment, the invention is added to mycotoxin-contaminated animal feed in amounts from about 0.1% to 0.3% by weight of feed. Alternatively, the antibodies alone or a composition contained in the present invention may be directly fed to animals as a supplement in amounts ranging from 0.01 to 200 grams per animal per day. An especially preferred embodiment comprises feeding the composition contained in the present invention to animals in amounts ranging from 0.1 to 30 grams per animal per day, depending on the animal species, size and feed intake of the animal, and the type of feed to which the composition is to be added. The mycotoxin antibody binds with its increased mycotoxin binding capacity and its ability to decrease absorption or uptake of mycotoxin will improve performance and health, and reduce the incidence of mycotoxin-associated syndromes or diseases in affected animals when fed alone or in combination with other binders.

EXAMPLE 1: **Indirect Enzyme-Linked Immunosorbent Assays for Mycotoxins**

[0058] Indirect competitive ELISAs were developed for quantitation of the mycotoxins following the procedure outlined by Xiao et al. (J. Agric. Food Chem.

43: 2092-2097, 1995) and Li et al. (J. Food Prot. 11:952-1037, 1994). An example of one of the assays used for aflatoxin B1 (AFB1) is as follows. The procedure for coating and blocking the microtiter plate with the aminodex AFB1 conjugate was similar to that used for the antibody titer assay. The following were then added to the appropriate microtiter plate wells: 75 µl of pH 7.2 PBS-T, 10 µl of mycotoxin or mycotoxin standard diluted with methanol, and 65 µl of anti-AFB1 antibodies diluted in pH 7.0 PBS-T. The plates were incubated at 37°C for 1 h. The remaining procedure is the same as for antibody titer assay described by Harlow, E. and D. Lane (A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA, pp 563-566, 1988) and Xiao, et al. (J. Agric. Food Chem. 43: 2092-2097, 1995). The antibody titer assays for the other anti-mycotoxin antibodies are similar to those for the anti-AFB1 antibody.

EXAMPLE 2: **Preparation of Aflatoxin B1 (AFB1)-Protein Conjugate and AFB1-Specific Antibody Production**

[0059] Materials: All inorganic and organic chemicals used were of reagent grade or better. Most of the reagents were purchased from either Thermo Fisher Scientific Company (Waltham, MA, USA) or Sigma Aldrich Corporation (St. Louis, MO, USA). AFB1 was purchased from Cayman Chemical Company (Ann Arbor, MI, USA), the myeloma cell line was from The American Type Culture Collection (Manassas, Virginia, USA) and mice were from Charles River Laboratories (Wilmington, MA, USA).

[0060] Preparation of Aflatoxin B1 (AFB1)-Protein Conjugate: The Bovine Serum Albumin (BSA) and aminated dextran (aminodex) conjugate of aflatoxin B1 (AFB1) was prepared using the carbodiimide technique described by Maragos and McCormick (Food Agric. Immunol. 12:181-192, 2000) and Xiao et al. (J. Agric. Food Chem. 43: 2092-2097, 1995). The C-3 carbonyl moiety in the cyclopentone group of AFB1 was converted to a carboxyl functionality by refluxing AFB1 with O-carboxymethylhydroxylamine (AFB1-oxime) in a mixture of pyridine/methanol/water (Chu et al., J Assoc. Off Anal Chem. 60: 791-794,1977)). The carboxymethyloxime modified AFB1 was then conjugated to protein using the carbonyldiimidazole (CDI)-based reaction. In brief, AFB1 (1mg) was dissolved in 100 uL of dimethyl sulfoxide and reacted with 1.5 mg of CDI at 25 C for 12 hours. Larger amounts of the conjugate can be prepared using multiple of the amounts of reagents that were used. The bovine serum albumin-AFB1 conjugate was purified using a cold acetone precipitation procedure. The conjugate upon completion of the reaction was transferred into a centrifuge tube and precipitated with excess volumes of cold acetone. The ratio of acetone to conjugate solution was 2:1 (V/V), with the maximum of acetone being 80% (V/V). The mixture was centrifuged for 10 min at 1500g and the supernatant discarded. The pellet was re-dissolved with

phosphate-buffered saline (pH 7.2) and re- precipitated with acetone. The conjugate, following cleanup, was diluted with pH 7.2 phosphate-buffered saline (Harlow and Lane, 1988) to a concentration of 1 mg/ml and stored at -20 C or AFB 1-BSA conjugate was lyophilized and stored at -80 C. The molar ratio of AFB1 conjugated to BSA was determined spectrophotometrically, using an absorption maxima of 360 nm and an extinction coefficient of 21800. For each mole of BSA, 1 mole of AFB1 was conjugated.

[0061] Immunization Protocol: Three BALB/c female mice, 8 to 10 weeks of age were injected three times with AFB1-BSA conjugate. One week following the last injection, sera was collected from each mouse and a titration of antibody was performed using an indirect ELISA. The spleen from the mouse with serum showing optimal relative inhibition was used for fusion. Four days prior to fusion, the mouse was injected with the conjugate antigen.

[0062] Titration of Antiserum by a Competitive Indirect ELISA: This procedure was as outlined by Harlow and Lane (1988). CI-ELISA was used to: (i) determine the sensitivity of anti-AFB1 antibodies in mouse serum produced during the coarse of immunization, (ii) to identify culture wells containing hybridomas secreting the desired antibody following fusion and cloning, and (iii) determine the sensitivity of the mAb (i.e. AFB1-specific monoclonal antibody) secreted by the stabilized cell line. The CI-ELISA and the method used to identify AFB1-specific antibody in fusion and cloning wells were determined using procedures outlined by Harlow and Lane (1988).

[0063] AFB1-Specific Monoclonal Antibody Production: Spleen cells and myeloma cells were fused with polyethylene glycol and assayed using the CL-ELISA. The culture with the highest percentage inhibition was cloned. The cell line which secreted the most sensitive antibody was identified using CI-ELISA. The subclass of secreted antibody was identified (refer to Harlow and Lane for detailed procedures).

[0064] Ascites Fluid Production: Mice were primed intraperitoneally with pristane 1 to 2 weeks prior to hybridoma injection. Fluid was tapped about 17 days after hybridoma injection. The immunoglobulin fraction was precipitated with 100% saturated ammonium sulfate to a final concentration of 50%. The pellet was re-suspended in phosphate buffered saline, pH 2 and dialyzed extensively against 20 mM Tris-HCl, NaCl, pH 8.0. The antibody was lyophilized and stored at -80 C. Further detail on procedures can be obtained from Harlow and Lane (1988).

EXAMPLE 3: **Evaluation of Neutralizing Effect of Aflatoxin B1 (AFB1)-Specific Antibody on AFB1 Toxicity Induced Lethality in Zebrafish.**

[0065] The goal of this study was to assess the neutralizing effect of aflatoxin B1 (AFB1)-specific antibody on AFB1 toxicity induced lethality in zebrafish.

[0066] **Materials & Methods:** Zebrafish embryos were generated by using a Mass Embryo Production System. Approximately 50 embryos were generated per female zebrafish. Embryos were cleaned by removing dead embryos and sorted by developmental stage. As embryos receive nourishment from an attached yolk sac, no feeding was required for 6-days post fertilization (dpf). Aflatoxin B1 was supplied by Cayman Chemical Company (Ann Arbor, MI, USA). 1 mg of AFB1 powder was dissolved in 3.18 ml of DMSO to prepare a 1.0 mM Master Stock (MS) solution. A 0.25 mM sub-MS was then prepared. Aflatoxin B1-specific antibody was purchased from Antibodies-Online, Inc. (Atlanta, GA, USA) for this study. Aflatoxin B1 specific-antibody Master Stock (MS) solution was prepared by dissolving 2 mg of powder in 12.2 ml fish water to generate a 1.0 µM AFB1-specific antibody MS. 4µl of DMSO was added to 2 ml AFB1-specific antibody solution (1.0 µM) in fish water, pre-incubated at room temperature (20-22°C) for 3 hrs. To ensure 4 ml final volume, after pre-incubation, 2 ml fish water containing 2 dpf self-hatched zebrafish (N = 30) was added; final DMSO conc. was 0.1% and final conc. of AFB1-specific antibody was 0.5 µM, i.e. 2 fold higher than final AFB1 conc). 2 dpf zebrafish were treated continuously with each condition for 96 hr. Final treatment conditions for 4 ml fish water are shown in **Table 1.** Final DMSO concentration was 0. 1% for each condition.

Table 1: Final Treatment Conditions for AFB1Toxicity Induced Lethality Test in Zebrafish[1]

Condition	Final Conc. of AFB1 (µM)	Final Conc. of AFB1- Specific Antibody (µM)
0.1% DMSO	0	0
AFB1-Specific Antibody + 0.1% DMSO	0	0.5
AFB1 + 0.1% DMSO	0.25	0
AFB1+AFB1-Specific Antibody + 0.1% DMSO	0.25	0.5

[1]Dead zebrafish were counted daily and removed. After treatment for 96 hr, total lethality was calculated. To obtain mean and Standard Deviation (SD) for each condition, experiments were performed 3 times.

[0067] **Results:** After treatment for 96 hrs, 0% lethality was observed when treated with 0.1% DMSO alone, validating the assay. 0% lethality was also

observed after treatment with AFB1-specific antibody alone + 0.1% DMSO confirming that AFB1-specific antibody did not induce lethality in zebrafish. However, 80.0 ± 3.3% lethality was observed in zebrafish after treatment with AFB1 alone + 0.1%. In contrast, 0% lethality was observed after treatment with AFB1 and AFB1-specific antibody combination (AFB1+AFB1-Specific Antibody; **FIG. 1**) + 0.1% DMSO, indicating that AFB1-specific antibody neutralized 100% AFB1 toxicity induced lethality in zebrafish (**Table 2, FIG. 2)**

Table 2: Results of Testing the Neutralizing Effect of Aflatoxin B1 (AFB1)-Specific Antibody on AFB1 Toxicity Induced Lethality in Zebrafish[1]

% Lethality					
Final Conc. (µM)	**Exp. 1**	**Exp. 2**	**Exp. 3**	**Mean**	**SD**
0.1% DMSO	0 (0/30)	0 (0/30)	0 (0/30)	0	0
AFB1-Specific Antibody + 0.1% DMSO	0 (0/30)	0 (0/30)	0 (0/30)	0	0
AFB1 + 0.1% DMSO	80 (24/30)	76.7 (23/30)	83.3 (25/30)	80	3.3
AFB1 + AFB1-Specific Antibody + 0.1% DMSO	0 (0/30)	0 (0/30)	0 (0/30)	0	0

[1]Numbers in Parentheses: Number of dead zebrafish divided by number of zebrafish per well.

We Claim:

1. Use of a mycotoxin-binding polyclonal or monoclonal antibody which binds to and detoxifies a mycotoxin present in feed, comprising admixing said mycotoxin-binding antibody with a feed or feed ingredient..

2. The use of claim 1, wherein said mycotoxin-binding antibody is produced using recombinant *Escherichia coli, Bacillus subtilis,* or *Pichia pastoris*, hybridoma technology, transgenic plants, or transgenic and non-transgenic animals including avian species.

3. The use of claim 2, wherein the transgenic plants are corn, soybean, rice, alfalfa or tobacco.

4. The use of claim 2, wherein the transgenic or non-transgenic animals are chicken, pig, sheep, goat or dairy cattle.

5. A composition comprising a mycotoxin-binding antibody and one or more of a mycotoxin adsorbing agent, a mycotoxin-biotransforming agent, a mycotoxin-inactivating enzyme and a mycotoxin toxicity-reducing antioxidant.

6. The composition of claim 5, wherein the adsorbing agents are selected from the group consisting of sodium bentonite, calcium bentonite, sodium-calcium montmorillonite, clinoptilolite, calcium/potassium/sodium hydrated aluminosilicate, hydrated sodium calcium aluminosilicate (HSCAS), activated carbon, dry yeast (*Saccharomyces cerevisiae*) cells, polymeric glucomannan, esterified glucomannan and beta-glucan fractions of dry yeast cell wall, cholestyramine, polyvinylpolypyrrolidone, activated diatomaceous earth, plant fibres, kaolinite, and hydrated sodium-potassium-calcium aluminosilicate.

7. The composition of claim 5 or 6, wherein the biotransforming agents are selected from the group consisting of *Eubacterium* sp. BBSH 797, *Nocardia asteroids, Mycobacterium fluoranthenivorans sp., Rhodococcus erythropolis, Alcaligenes* species., *Bacillus* species., *Achromobacter* species., *Flavobacterium* species, *Pseudomonas* species, *Lactobacillus rhamnosus strain* GG, *Lactobacillus helveticus 46 & 72, Lactobacillus casei, Lactobacillus lactis, Streptococcus thermophilus C5 and NG40Z, Lactobacillus paraplantarum*, *Stenotrophomonas maltophila, Saccharomyces cerevisiae, Cupriavidus basilensis* OR16 *Aspergillus niger, Eurotium herbariorum, Rhizopus species., Trichosporon mycotoxinivorans, Phaffia rhodozyma*, and *Xanthophyllomyces dendrorhous*.

8. The composition of claims 5 to 7, wherein the mycotoxin-inactivating enzymes are selected from the group consisting of protease A, pancreatin, carboxypeptidase, epoxidase from *Eubacterium*, lactonohydrolase, catalase, laccase, xylanase, esterase, lipase, oxidase, amino acid oxidase, peroxidase, lactanase,

lactoperoxidase, lactonase, manganese peroxidase, polysaccharase and dehydrogenase.

9. The composition of claims 5 to 8, wherein the mycotoxin toxicity- reducing antioxidants are selected from the group consisting of rutin, quercetin, lutein, lecithin, melatonin, curcumin, curcuminoids, allyl sulfides, chlorophyll and derivatives, sodium thiosulfate, glutathione, methionine, aspartame, selenium, zinc, catechin, vitamin E, vitamin C, vitamin A, vitamin B, coenzyme Q10, carotene and carotenoids.

10. The composition of claims 5 to 9, wherein the composition is a combination of egg-yolk antibodies, hydrated sodium calcium aluminosilicate (HSCAS), activated carbon, cholestyramine, *Saccharomyces cerevisiae*, *Lactobacillus* species, *Eubacterium* sp. BBSH 797, epoxidase and rutin.

11. The use of claims 1 to 4, further comprising use of the composition of claims 5 to 10.

12. The use of claims 1 to 4 or 11 for detoxifying a wide spectrum of mycotoxins, including those selected from the group consisting of aflatoxins (AFB1, AFB2, AFG1, AFG2), deoxynivalenol, nivalenol, T-2 toxin, zearalenone, fumonisins (FB1, FB2, FB3), ochratoxin A, citrinin and ergotamine.

13. The use of claims 1 to 4, 11 or 12, wherein the antibody is specific to a mycotoxin selected from the group consisting of aflatoxin B1, deoxynivalenol, zearalenone, fumonisin B1 and ochratoxin A.

14. A feed blend comprising the mycotoxin-binding antibody which binds to and detoxifies a mycotoxin present in feed, or the composition of claims 5 to 10, and a feed.

15. The feed blend of claim 14, wherein the feed is selected from the group consisting of corn (maize), wheat, barley, rye, rice, sorghum, soybean, peanut, millet, brewers spent grain, distiller's spent grain, distiller's wet grain, and/or distiller's dried grain, forage and silage.

16. The feed blend of claim 14 or 15, wherein the composition is added to feed at a concentration ranging from about 0.1% to about 0.3% by weight of the feed.

17. The feed blend of claim 14 or 15, wherein the mycotoxin-binding antibody is added to feed at a concentration ranging from about 0.01% to about 2% by weight of the feed.

18. The feed blend of claim 14 or 15, wherein the mycotoxin-binding antibody is added to feed at a concentration ranging from about 0.03% to about 0.6% by weight of the feed.

19. A composition of claims 5 to 10, wherein the process of producing the mycotoxin-binding antibody comprises immunization of chickens with mycotoxin - BSA conjugate plus an appropriate adjuvant to produce antibodies in egg yolk.

20. A composition of claims 5 to 10, wherein the process of producing the mycotoxin-binding antibody comprises immunization of mice with mycotoxin -BSA conjugate to elicit the production of lymphocytes in spleen that produce antibodies and recovering antibodies using one of the conventional purification procedures..

21. A composition of claims 5 to 10, wherein the process of producing the mycotoxin-binding antibody comprises use of expression vector(s) encoding the antibody or fragment transfected into a cell line.

22. A composition of claims 5 to 10, wherein the process of producing the mycotoxin-binding antibody comprises production of lymphocytes that produce antibodies, wherein said lymphocytes are fused with myeloma cells to create hybridoma cell lines that produce mycotoxin-specific monoclonal antibodies.

23. A composition of claims 5 to 10, wherein the process of producing the mycotoxin-binding antibody comprises use of recombinant antibody production systems for producing mycotoxin-binding a recombinant monoclonal antibody, a single-chain variable fragment (scFv) of antibody or a single domain antibody (sdAb).

24. A composition of claims 5 to 10, wherein the process of producing the mycotoxin-binding antibody, is accelerated using *in vitro* selection systems, particularly phage display.

25. The method of preparing a feed blend comprising mixing:

(a) a mycotoxin-binding antibody

(b) one or more of a mycotoxin-adsorbing agent, a mycotoxin-biotransforming agent, a mycotoxin-detoxifying enzyme and a mycotoxin toxicity-reducing antioxidant; and

(c) a feed or feedstuffs/feed products.

26. The method of preparing a composition of claims 5 or 10, wherein one or more mycotoxin-adsorbing agents are selected from the group consisting of hydrated sodium calcium aluminosilicate (HSCAS), dry yeast (*Saccharomyces cerevisiae*) or polymeric glucomannan from yeast cell wall, cholestyramine or polyvinylpolypyrrolidone.

27. The method of preparing a composition of claims 5 to 10, wherein one or more mycotoxin-biotransforming agents are selected from the group consisting of

Eubacterium sp. BBSH 797, *Flavobacterium* species, *Cupriavidus basilensis* OR16 and *Lactobacillus* species.

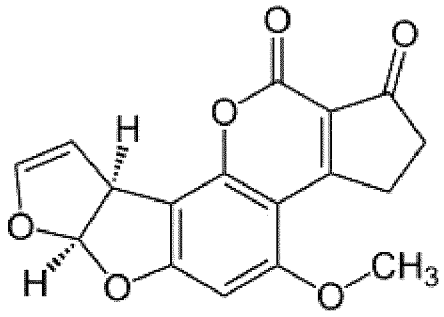
28. The method of preparing a composition of claims 5 to 10, wherein one or more mycotoxin-inactivating enzymes are selected from the group consisting of lactonohydrolase, protease A, epoxidase from *Eubacterium* strain, carboxypeptidase A, pancreatin, xylanase, lactanase and dehydrogenase.

29. The method of preparing a composition of claims 5 to 10, wherein one or more mycotoxin toxicity-reducing antioxidants are selected from the group consisting of rutin, quercetin, lutein, lecithin, curcumin, methionine, aspartame, selenium, vitamin C, vitamin B and carotene.

30. A method of treating or preventing mycotoxin toxicity-associated disease (mycotoxicosis) in a human or non-human animal comprising oral administration of a mycotoxin-binding antibody which binds to and detoxifies a mycotoxin present in food, or oral administration of the composition of claims 5 to 10.

31. The method of claim 30, wherein the composition is provided in a feed for non-human animals selected from one or more of avian, bovine, porcine, equine, ovine, caprine, canine, and feline species.

32. The method of claim 30, wherein the composition is provided in a feed for non-human animals selected from one or more of pigs, dairy cattle, beef cattle, sheep, goats, chickens, and turkeys.

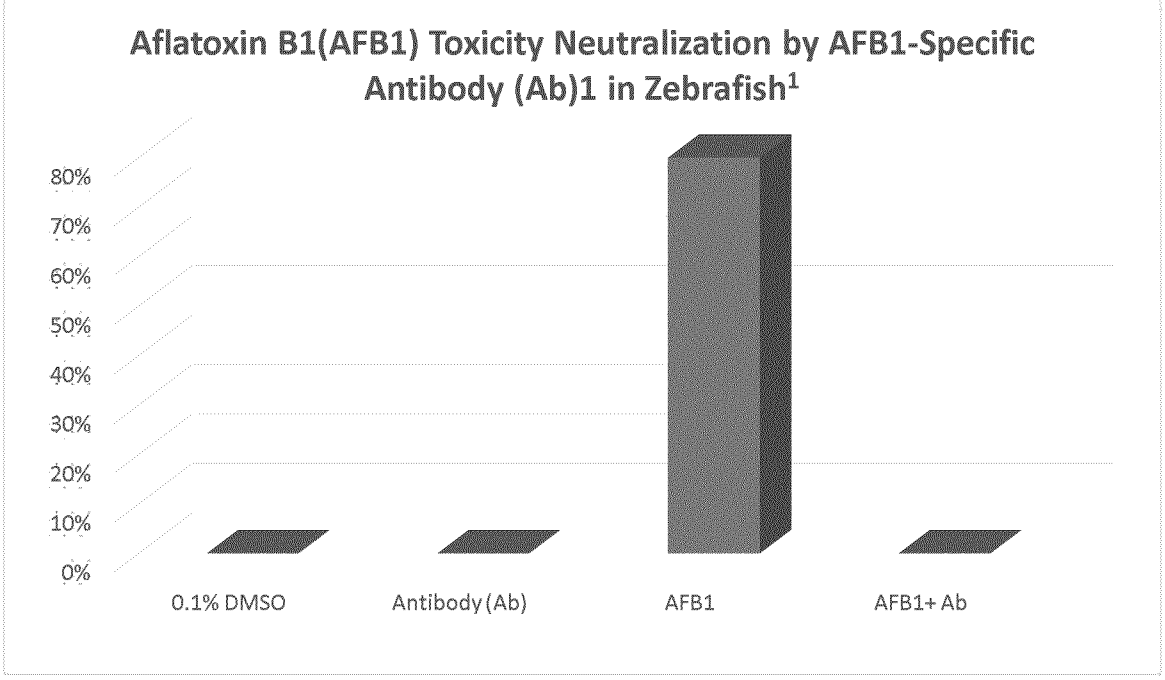


Aflatoxin B1 (AFB1)

+

Aflatoxin B1-Specific Antibody

FIG. 1



[1]Y-Axis: % Lethality in Zebrafish; X-Axis: 4 Treatments, (i) 0.1% DMSO (Control), (ii) Antibody Alone, (iii) AFB1 Alone, and (iv) AFB1+ Antibody Combination

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/051327

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A23K 20/147*** (2016.01) , ***A23K 10/16*** (2016.01) , ***A23K 10/30*** (2016.01) , ***A23K 20/10*** (2016.01) , ***A23K 20/189*** (2016.01) , ***A23K 20/20*** (2016.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: **ALL**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: questel-orbit, scopus, google scholar; Keywords: mycotoxin, aflatoxin, antibody, plantibody, feed, agent, detox, additive, chicken antibody, yolk, IgY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CN104693304A, (SHENG B et al.) 10 June 2015 (10-06-2015) *see whole document, machine translation via patent translate accessed through https://worldwide.espacenet.com/?locale=en_EP *	1-2, 12-18, 30-32 3-32
X	KIM, H, et al. "A Novel Mycotoxin Purification System Using Magnetic Nanoparticles For The Recovery Of Aflatoxin B1 And Zearalenone From Feed", **JOURNAL OF VETERINARY SCIENCE**, vol. 13(4): 363-369; December 2012 (12-2012), ISSN: 1976-555X *whole document*	1-2, 12-15

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 11 January 2017 (11-01-2017)	Date of mailing of the international search report 09 February 2017 (09-02-2017)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Nicole Harris (819) 639-7734

A61K 31/7048 (2006.01) , ***A61K 31/785*** (2006.01) , ***A61K 33/00*** (2006.01) , ***A61K 33/06*** (2006.01) , ***A61K 33/44*** (2006.01) , ***A61K 35/74*** (2015.01) , ***A61K 35/747*** (2015.01) , ***A61K 36/064*** (2006.01) , ***A61K 38/43*** (2006.01) , ***A61K 38/44*** (2006.01) , ***A61K 39/395*** (2006.01) , ***A61P 39/00*** (2006.01) , ***C07K 16/14*** (2006.01) , ***C07K 16/44*** (2006.01) , ***C12N 1/20*** (2006.01) , ***C12N 15/13*** (2006.01) , ***C12N 5/16*** (2006.01) , ***C12P 21/00*** (2006.01) , ***C12P 21/08*** (2006.01)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO2008053232A2, (MANN SP et al.) 08 May 2008 (08-05-2008) *whole document*	3-32
Y	BOUDERGUE C et al. "Review Of Mycotoxin-Detoxifying Agents Used As Feed Additives: Mode Of Action, Efficacy And Feed/Food Safety", **REVIEW OF MYCOTOXIN-DETOXIFYING AGENTS USED AS FEED ADDITIVES: MODE OF ACTION, EFFICACY AND FEED/FOOD SAFETY (2009)**, 2009; Available from: AGRIS, Ipswich, MA. [retrieved on 10 January 2017 (10-01-2017)] Retrieved from the internet: <URL: http://www.efsa.europa.eu/en/supporting/pub/22e *whole document*	3-32
Y	CN101434655 A, (SUQING Z et al.), 20 May 2009 (20-05-2009) *see whole document, machine translation via patent translate accessed through https://worldwide.espacenet.com/?locale=en_EP*	3-29
A	SCHADE, R; et al. "Chicken Egg Yolk Antibodies (IgY-Technology): A Review Of Progress In Production And Use In Research And Human And Veterinary Medicine", ***ALTERNATIVES TO LABORATORY ANIMALS : ATLA***, vol. 2, 2005. ISSN: 0261-1929	1-32
A	CHALGHOUMI, R; et al. "Effects Of Feed Supplementation With Specific Hen Egg Yolk Antibody (Immunoglobin Y) On Salmonella Species Cecal Colonization And Growth Performances Of Challenged Broiler Chickens", ***POULTRY SCIENCE***, vol 88(10), 2081-2092; 1 October 2009 (01-10-2009). ISSN: 00325791	1-32
P, Y	CN105597097A, (SHENG B et al.) 25 May 2016 (25-05-2016) *see whole document, machine translation via patent translate accessed through https://worldwide.espacenet.com/?locale=en_EP*	1-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/051327

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 30-32
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 30-32 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 30-32.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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		CN101434655B	09 November 2011 (09-11-2011)
CN105597097A	25 May 2016 (25-05-2016)	None	