Journal of Food Protection, Vol. 64, No. 1, 2001, Pages 120–131
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Review

Dietary Strategies to Counteract the Effects of Mycotoxins: A Review

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MS 99-346: Received 17 November 1999/Accepted 17 February 2000

ABSTRACT

We reviewed various dietary strategies to contain the toxic effects of mycotoxins using antioxidant compounds (selenium, vitamins, provitamins), food components (phenolic compounds, coumarin, chlorophyll and its derivatives, fructose, aspartame), medicinal herbs and plant extracts, and mineral and biological binding agents (hydrated sodium calcium aluminosilicate, bentonites, zeolites, activated carbons, bacteria, and yeast). Available data are primarily from in vitro studies and mainly focus on aflatoxin B_1, whereas much less information is available about other mycotoxins. Compounds with antioxidant properties are potentially very efficacious because of their ability to act as superoxide anion scavengers. Interesting results have been obtained by food components contained in coffee, strawberries, tea, pepper, grapes, turmeric, *Fava tonka,* garlic, cabbage, and onions. Additionally, some medicinal herbs and plant extracts could potentially provide protection against aflatoxin B_1 and fumonisin B_1. Activated carbons, hydrated sodium calcium aluminosilicate, and bacteria seem to effectively act as binders. We conclude that dietary strategies are the most promising approach to the problem, considering their limited or nil interference in the food production process. Nevertheless, a great research effort is necessary to verify the in vivo detoxification ability of the purposed agents, their mode of action, possible long-term drawbacks of these detoxification-decontamination procedures, and their economical and technical feasibility.

Mycotoxins are highly toxic secondary products of the metabolism of some fungi mainly belonging to *Aspergillus, Penicillium,* and *Fusarium* spp. Toxic syndromes caused by mycotoxin ingestion by humans and animals are indicated as mycotoxicosis. It has been estimated that at least 300 fungal metabolites are potentially toxic for humans and animal, and it can be realistically assumed that other mycotoxins are likely to be discovered. Devegowda et al. *(28)* reported that as much as 25% of the world's cereals are contaminated with known mycotoxins. Mycotoxins can enter the food chain through contaminated cereals and foods (e.g., milk, meat, and eggs) obtained from animals fed mycotoxin-contaminated feeds.

The most notorious and extensively investigated mycotoxins are aflatoxin B_1 (AFB_1), ochratoxin A (OTA), fumonisin B_1 (FB_1), zearalenon (ZEN), deoxynivalenol (DON), and T2 toxin. However, recently the interest of researchers in other toxins, such as citrinin, sterigmatocistyn, diacetoxyscirpenol, and the group of recently discovered *Fusarium* toxins, including beauvericin, fusaproliferin, moniliformin, and fusaric acid, has been growing.

Mycotoxins' chemical, biological, and toxicological properties are diverse. Hence, their toxic effects are extremely variable, depending on the intake level, duration of exposure, animal species, age, sex, physiological status, and eventual synergism between mycotoxins simultaneously present in feed or foods. However, the main toxic effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders, and immunosuppression. Some mycotoxins are specifically indicated or strongly suspected as the cause of severe human and animal diseases, such as Reye's disease (caused by AFB_1) *(12)*, equine leukoencephalomalacia and porcine pulmonary edema (caused by FB_1), human alimentary toxic aleukia (caused by T2 toxin), and Balkan endemic nephropathy (caused by OTA). The positive correlation between the consumption of AFB_1-contaminated foods and the increased incidence of liver cancer in several Asian and African populations has led to the classification of AFB_1 as a group 1A carcinogen by the International Agency for Research on Cancer *(56)*. In 1997, the Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives provided qualitative and quantitative information on aflatoxins and concluded that aflatoxins should be treated as carcinogenic food contaminants, the intake of which should be reduced to levels as low as reasonably achievable *(60)*. Although human health risk assessment involves complete knowledge of toxicological, epidemiological, and exposure data, in the risk management

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of mycotoxins it is necessary to take action before all this information is available *(66)*. However, the lack of this information actually determines some uncertainties in assessing effective human exposure and health risk and in establishing causal relationships between incidence of mycotoxins in foods and human diseases *(100)*.

From a regulatory standpoint, different countries have enforced different thresholds to limit the passage of mycotoxins along the food chain. In the United States, it is required by law that aflatoxin M_1 in milk should be less that 0.5 ppb, whereas in western Europe the regulations are more stringent, and maximum levels are set at 0.05 ppb *(15)*. Currently, the U.S. Food and Drug Administration regulates only aflatoxins among the mycotoxins, and it established an action level ranging from 20 to 300 ppb of AFB_1 in animal feeds. Denmark, on the other hand, has decided that a level of 15 ppb of OTA in the liver or kidney of pigs results in their confiscation and that levels exceeding 25 ppb would result in the confiscation of the entire carcass.

No doubt exists concerning the economic impact of mycotoxins. Recent studies *(20, 39)* evidenced that economic losses occur at all levels of food and feed production, including crop and animal production, processing, and distribution. Even during favorable climatic periods, millions of dollars are lost as a consequence of crop contamination. For all these reasons, prevention, decontamination, and detoxification of mycotoxins are issues of great importance.

Generally, any approach aimed to reduce the toxic and economic impact of mycotoxins should be respondent to as much as possible of the following requisites: (i) prevent, destroy, remove, or detoxify mycotoxins in foods and feeds; (ii) not produce or leave toxic and/or carcinogenic or mutagenic residues in the final products; (iii) not significantly alter the important technologic and nutritional properties of the food or feed; and (iv) be technically and economically feasible *(86)*.

A wide range of chemical, physical, and biological approaches has been experienced in the attempt to reduce the toxicity of mycotoxins. Although some chemical detoxification methods (i.e., ammonia, sodium bisulfite, and calcium hydroxide treatments) are effective, they do not fulfill all the requirements, especially those concerning the safety of reaction products and the safeguarding of the nutritional characteristics of the treated foods and feeds *(29, 86)*.

For these reasons, nutritional approaches, such as supplementation of nutrients, food components, or additives with protective properties against mycotoxin toxicity and addition of nonnutritive sorbents or bacteria, yeast, and modified yeast cells capable of reducing the bioavailability of mycotoxins, are assuming increasing interest and are critically reviewed in this paper.

ANTIOXIDANT SUBSTANCES

Since some mycotoxins (i.e., AFB_1, FB_1, OTA, and T2 toxin) are known to produce membrane damage through increased lipid peroxidation *(8, 9, 25, 52, 53)*, the protective properties of antioxidant substances have been extensively investigated. Selenium, some vitamins (A, C, and E), and their precursors have marked antioxidant properties that act as superoxide anion scavengers. For these reasons, these substances have been investigated as protecting agents against toxic effects of mycotoxins.

Selenium. In a controlled study, Lin et al. *(72)* observed that selenium is able to reduce in vitro toxic effects of T2 toxin on cultured chicken embryonic chondrocytes. When Na_2SeO_3 was added in the culture in the presence of T2 toxin, there was no decrease in collagen microfibril, intramembrane particle numbers, and enzymatic (cytochrome *c*, oxidase, and H+-ATPase) activities.

In an in vivo study on rats, Shi et al. *(96)* demonstrated that selenium inhibits AFB_1-DNA binding and adduct formation. The same authors *(97)* in an in vitro study on cultured hamster ovary cells found that sodium selenite and selenium-enriched yeast extract protect cells from AFB_1 cytotoxicity but not from mutagenicity.

However, McLeod et al. *(80)* reported that rats fed a selenium-deficient diet were more resistant to AFB_1 than those fed a selenium-sufficient diet. According to the authors, the protection conferred by selenium deficiency against AFB_1 is associated with the hepatic expression of an aldo-keto reductase and a glutathione S-transferase subunit that efficiently metabolizes the mycotoxin.

Based on a study on rats, Atroshi et al. *(8)* concluded that selenium, vitamin E, and vitamin C act as an antioxidant system and free radical scavenger that protects spleen and brain against membrane damage caused by T2 toxin and DON.

Vitamins. Further evidence of protective effects of some vitamins and/or their precursors against mycotoxin-induced damages arises from numerous in vivo and in vitro studies.

Grosse et al. *(46)* observed that vitamins A, C, and E reduced DNA adducts in kidney and liver of mice exposed to OTA and ZEN from 70 to 90%.

Vitamin C is also able to protect guinea pigs from AFB_1 hepatotoxicity *(83)*.

In mice exposed to OTA, vitamin C reduced abnormalities in both mitotic and meiotic chromosomes and morphologies of the sperm head *(14)*. Analogous protective actions have also been attributed to vitamin E *(54)* and vitamin A toward exposure both to OTA *(67)* and AFB_1 *(98)*.

Supplementary vitamin E administered to chickens partially counteracts the formation of lipid peroxides due to single and combined exposure to OTA and T2 toxin *(52)*.

As demonstrated by Webster et al. *(108)*, the different vitamin A status is strongly related to the hepatocarcinogenicity of AFB_1 in rats. In fact, the authors observed an enhancement of AFB_1-induced DNA damages during vitamin A deficiency, whereas damages were reversed on vitamin A supplementation.

Coelho *(22)* demonstrated that vitamin supplementations exceeding up to 25% of the standard requirements can reduce the negative effects of AFB_1 and environmental stress on turkeys.

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Also, riboflavin has a potential chemopreventive action against AFB_1-induced DNA damages in rats *(109)*.

Carotenoids (carotene and xanthophylls) are excellent antioxidants with antimutagenic and anticarcinogenic properties. They occur naturally in some foods, such as carrots, red tomatoes, butter, cheese, paprika, palm oil, corn kernels, and red salmon. Dietary carotenoids inhibit AFB_1-induced liver DNA damage in rats, as demonstrated by Gradelet et al. *(44, 45)*. They concluded that beta-apo-8′carotenal, canthaxanthin, and astaxanthin exert their protective effect through the deviation of AFB_1 metabolism toward detoxification pathways, leading to the formation of aflatoxin M_1, a less toxic metabolite. Regarding beta-carotene, since it does not alter metabolism, its protective action should be mediated by other mechanisms, whereas no protective effects were observed by administration of supplementary lycopene and excess of vitamin A.

Okotie Eboh et al. *(85)* conducted a study on broilers to confirm in vivo the protective properties of beta-carotene and canthaxanthin against AFB_1. The authors concluded that beta-carotene was not effective in ameliorating aflatoxicosis in broiler chickens, whereas canthaxanthin was shown to be somewhat effective with respect to certain blood chemistry indicators.

An overall inhibition of biochemical and cellular events thought to be precursors of AFB_1-induced hepatocarcinogenesis in rats was observed by He et al. *(49)* as a consequence of dietary administration of carotenoid-reach extracts. Another study on solvent-extracted carotenoids (i.e., alpha-carotene, beta-carotene, and lycopene) and xanthophylls (beta-cryptoxanthin) from carotenoids-rich foods showed both in vitro and in vivo ability to reduce mutagenic effects of AFB_1 *(92)*.

In a study on antimutagenic activity of natural xanthophylls against AFB_1 in *Salmonella* Typhimurium, González de Mejía et al. *(43)* reported that xanthophylls inhibited the mutagenicity of AFB_1 in a dose-dependent manner.

Yu et al. *(111)*, using woodchuck hepatocytes as a model to investigate the effects of vitamin A, C, and E and beta-carotene on AFB_1-DNA adducts, reported contrasting results. In fact, they showed that vitamin C and, particularly, vitamin A were effective in reducing AFB_1-DNA binding, whereas vitamin E and beta-carotene enhanced it. The authors concluded that additional studies are needed to understand the mechanism of enhanced adduct formation.

Two vitamin A2 compounds (3-dehydroretinol and 3-dehydroretinyl palmitate) mainly present in freshwater fish have been demonstrated to be very effective in inhibiting the microsome-catalyzed formation of DNA-AFB_1 adduct *(3)*. The inhibition should be due to modulation of microsomal enzymes, which activate the carcinogen, hence suggesting a potential chemopreventive role of these compounds against carcinogenesis induced by AFB_1.

In a study conducted on *Bacillus subtilis* cells, vitamin E was able to prevent the genotoxicity of ZEN *(40)*. The authors attributed the specificity of the prevention to the structural similarity of vitamin E and ZEN.

FOOD COMPONENTS AND ADDITIVES

Numerous food components, ingredients, or additives, with or without antioxidant properties, have been investigated with the aim of verifying their chemoprotective properties.

Lu et al. *(76)* indicated that the toxic effect of FB_1 can be eliminated by a reaction between FB_1 and fructose. When heated to 80°C for 48 h, fructose reacts with the amino terminus of FB_1, resulting in more than 95% FB_1 conjugation to fructose.

Ellagic acid is a phenolic compound that occurs naturally in some foods, such as strawberries, raspberries, and grapes. It has both antimutagenic and anticarcinogenic activity as demonstrated in a wide range of in vitro and in vivo assays. Loarca Piña et al. *(74, 75)* in in vitro tests on *Salmonella* cells showed that ellagic acid inhibited AFB_1 direct-acting mutagenicity, particularly when incubated with metabolic enzymes. The result of sequential incubation indicated that the formation of an AFB_1–ellagic acid chemical complex should be the involved mechanism of inhibition.

Another study emphasized the role of phenolic compounds in the activation and detoxification processes and hence in modulating the carcinogenicity of AFB_1 *(2)*. In tests performed on rats fed a synthetic diet containing various food-associated phenolic compounds each at the 0.5% level, the authors observed a marked decrease in the ability of liver microsomes to catalyze reactions of AFB_1, leading to its activation and DNA adduct formation. The phenolic compounds tested were several flavonoids (fisetin, kaempferol, morin, naringin, and catechin), phenolic acids (caffeic acid and chlorogenic acid), and other phenolic (eugenol, vanillin) and synthetic phenolics (butylated hydroxyanisole and butylated hydroxytoluene) antioxidants. Some phenolic compounds (naringin, catechin, eugenol, vanillin, and butylated hydroxyanisole) were also found to induce cytosolic glutathione S-transferase activity that stimulated the formation of specific AFB_1-glutathione conjugate. Rompelberg et al. *(95)* found that eugenol does not modify the unscheduled DNA synthesis in hepatocytes exposed to AFB_1.

Williams and Iatropoulos *(110)* and Manson et al. *(78)* confirmed that butylated hydroxyanisole and butylated hydroxytoluene inhibited the initiation of hepatocarcinogenesis by AFB_1 in rats. However, Allameh *(5)* demonstrated that the permitted dose of butylated hydroxytoluene, added to processed food as preservative, plays no role in the biotransformation of AFB_1. Skrinjar et al. *(102)* confirmed that ellagic acid and butylated hydroxyanisole ameliorate aflatoxin-induced mutagenicity and carcinogenicity. The same authors also found that certain food additives and/or active ingredients with general antioxidant properties, such as turmeric (*Curcuma longa*), curcumin (diferlolyl methane), and garlic, have the same properties. Firozi et al. *(33)* demonstrated that curcumin reduces the formation of AFB_1-DNA adducts by modulating cytochrome P-450 function.

Takahashi et al. *(103, 104)* and Im et al. *(55)* demonstrated that two flavonoids, alpha- and beta-naphtoflavone,

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strongly inhibit microsome-catalyzed AFB_1-DNA binding in trout.

In an in vivo study on rats, Hendrich et al. *(50)* reported that soy isoflavone extract has a marked protective action against FB_1 hepatotoxicity by the suppression of FB_1-stimulated prostaglandin production.

Natural phenolics such as quercetin and kaempferol, ellagic acid, and curcumin generally reduced the in vitro enzyme activity consequent to AFB_1 treatment *(82)*. According to the authors, the suppression of protein kinase C activity by phenolic compounds could be a way to control AFB_1 carcinogenicity.

A range of natural dietary constituents, including garlic oil, ethoxyquine, indole-3-carbinol, and phenethyl isothiocynate, have in vitro chemoprotective actions toward AFB_1 *(78)*.

Some natural polyphenolic compounds, i.e., polyhydroxylated flavonoids and phenolic acids, were found to be effective in reducing AFB_1-DNA adducts *(34)*.

S-methyl methanethiosulfonate, a compound present in the juice of cabbage and onion, has been demonstrated to have a suppressive effect on AFB_1- or methyl methanesulfonate–induced chromosome aberrations in rat bone marrow cells *(58)*. Also, the precursor of S-methyl methanethiosulfonate S-methyl-L-cysteinesulfoxide significantly suppressed AFB_1- or methyl methanesulfonate–induced chromosome aberrations. According to the authors, although other mechanisms are not excluded, the protective properties of S-methyl methanethiosulfonate may result from its ability to modify -SH groups in proteins.

Cavin et al. *(19)* identified two diterpenes, cafestol and kahweol, present in green and roasted coffee beans as potentially chemoprotective agents against the covalent binding of AFB_1 metabolites to DNA of rats. It has been postulated that these compounds may act as blocking agents by producing a coordinated modulation of multiple enzymes involved in carcinogen detoxification. Manson et al. *(78)* found that caffeic acid also has in vitro chemoprotective actions toward AFB_1. On the contrary, caffeine has been demonstrated to potentiate the in vitro genotoxicity of AFB_1 *(63)*.

Theafulvins, compounds isolated from black tea aqueous infusions, despite their overall ability in reducing the mutagenicity of other food carcinogens, enhanced the mutagenicity of AFB_1 *(18)*.

Jesval *(59)* attributed protective abilities against OTA-induced hepatoma and renal carcinoma in mice to leaf juice of the common grape (*Vitis vinifera*).

L-Methionine supplementation was found to inhibit the developmental toxicity induced by OTA in pregnant female rats and provided partial protection for renal and liver tissue *(1)*.

Propionic acid and potassium sorbate used as preservatives in bread making in France destroyed AFB_1 from approximately 52 to 71% *(6)*.

Piperine. According to Reen et al. *(93)*, piperine (1-piperoylpiperidine), the major alkaloid constituent of pepper (*Piper nigrum*), is potentially a protective agent against carcinogenic effects of AFB_1. It is well known that the AFB_1 toxicity is bioactivated by the cytochrome P-450 monooxygenases (CYP450). In rat cultured cells, piperine reduced dramatically CYP4502B1 activity and counteracted CYP4502B1-mediated toxicity of AFB_1, thus offering a chemopreventive effect against procarginogens activated by CYP4502B1.

Coumarins. More than 300 coumarins with general pharmacological and biochemical properties have been identified from green plants *(53)*. Coumarin (1,2-benzopyrone), a natural food constituent especially present in *Fava tonka,* has a chemoprotective action against AFB_1. As demonstrated by Goeger et al. *(42)* in in vitro studies on hamster ovary cells, liver cells from rats, and chick embryos, coumarin decreased cytotoxicity and mutagenicity of AFB_1, although with marked species differences in chemoprotection. However, it must be considered that coumarin also has toxic properties and, because of their structural similarity, counteracts vitamin K absorption. Raj et al. *(90)* showed that different oxygenated substituents on 4-methylcoumarins (i.e., acetoxy>hydroxy>methoxy, in order of inhibition ability) also have chemopreventive properties on AFB_1-DNA binding in vitro.

Chlorophyll and its derivatives. Dashwood et al. *(27)* demonstrated that chlorophylline (a food grade, water-soluble derivative of the green plant pigment chlorophyll) has chemopreventive properties against wide classes of mutagens, including AFB_1. Breinholt et al. *(16)* showed that chlorophylline acts as an interceptor molecule by forming a strong noncovalent complex with AFB_1, reducing hepatic AFB_1-DNA adducts and liver tumors. In particular, it has been demonstrated that the complex formation occurs between the porphyrinlike structure of chlorophyllin and the planar molecular surface of AFB_1. Arimoto Kobayashi et al. *(7)* demonstrated that chlorophyllin mixed with chitosan, a polyglucosamine, can form an insoluble saltlike material able to trap AFB_1. The chemoprotective effect of chlorophyllin was confirmed in an in vivo study of Breinholt et al. *(17)*, who showed that 2,000 to 4,000 ppm of chlorophylline reduced AFB_1-DNA adduction up to 77% in rainbow trout.

Aspartame. Four studies *(9, 10, 23, 24)* reported that aspartame (L-aspartyl-L-phenylanine methyl ester) has a wide protective action against OTA-induced subchronic effects. Studies on monkey kidney cells showed that aspartame prevents or partially protects against some typical cytotoxic effects of OTA, such as inhibition of protein synthesis, lipid peroxidation, and leakage of certain enzymes, such as lactate dehydrogenase, gamma-glutamyl transferase, and alkaline phosphatase. In vitro, aspartame prevented OTA bindings to plasma proteins. When given to rats, aspartame prevented OTA genotoxicity and nephrotoxicity. When given after intoxication of animals with OTA, aspartame eliminated the toxin efficiently from the body. The protective action should be due to its structural similarity to OTA and phenylalanine. According to the detailed account by Creppy et al. *(25)*, the molecular mechanism me-

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diating the preventive effect of aspartame is not only the delivery of phenylalanine by cleavage of the peptide but also the direct effect of the peptide on the bending capacity and transport of the toxin in vivo and in vitro. To the authors, aspartame is the best candidate for preventing OTA-induced subchronic effects, also considering the absence of adverse effects in humans and animals.

Cyproheptadine. Cyproheptadine, a serotonine antagonist with appetite-stimulant properties, has been tested to reduce feed refusal due to the presence of DON *(87)*. The authors observed that dosing levels, including various combinations of cyproheptadine and DON, offset the reduction of feed intake. It was concluded that, although serotoninergic mechanism is involved in reducing DON-induced feed refusal, further investigations are needed to better understand the reasons of anorectic effect.

Allyl sulfides. Le Bon et al. *(70)* showed that the dietary administration of diallyl sulfide, diallyl disulfide, and allyl mercaptan to rats strongly reduced hepatic DNA breaks induced by AFB_1 and, to a lesser extent, its mutagenicity.

MEDICINAL HERBS AND PLANT EXTRACTS

Some studies highlighted the capability of several extracts from medicinal herbs and plants to counteract the AFB_1 toxicity.

Al Dakan et al. *(4)* reported that an ethanol extract from a concentrate of *Cassia senna* (a medicinal herb commonly used as vegetable laxative) inhibits the in vitro mutagenic effect of AFB_1 at low concentrations but not at higher ones. Anthraquinone aglycones and naphtopyrone glycosides, two compounds detected in methanol extract of *Cassia tora,* showed a marked in vitro antimutagenic activity against AFB_1 *(21)*. *Semecarpus anacardium* nut extract was demonstrated to be effective in reducing AFB_1-induced hepatocarcinoma *(88)*. The administration of a methanol extract of the leaves of *Piper argyrophyllum* normalized the genotoxic effect of AFB1 on rat cells *(89)*.

A study on rats showed that an extract from *Thonninga sanguinea* is able to protect against acute hepatotoxicity caused by AFB_1 *(47)*. Carnosol and carnosic acid, two natural polyphenols found in *Rosmarinus officinalis* L., are potent inhibitors of in vitro AFB_1-induced DNA adduct formation *(84)*.

A reduction of in vitro metabolic activation of AFB_1 by *Azadirachta indica* var. *siamensis* and *Momordica charantia* L. was observed by Kusamran et al. *(68)*. In a study on rats, a lignin-enriched extract of the fruit of *Schisandra chinensis* was found to provide hepatoprotective action against AFB_1 by enhancing the hepatic antioxidant and detoxification system *(57)*.

Majonoside-R2, a saponin extracted from rhizomes and roots of *Panax vietnamensis* (also referred to as Vietnamese ginseng), showed a potent anti–tumor-promoting activity against FB_1 applied to mouse skin *(65)*.

MYCOTOXIN-BINDING AGENTS

Addition of nutritionally inert sorbents is one of the most recent approaches to reduce mycotoxin toxicity in animals and carryover of mycotoxin from contaminated feeds to animal products (milk, meat, and eggs). After a generalized initial skepticism, the interest of researchers about sorbents has increased in the last years. Sorbents act by reducing the bioavailability of mycotoxins by adsorption on their surface. Indeed, if a stable sorbent-mycotoxin complex is formed, the absorption of mycotoxins in the gastrointestinal tract can be reduced, decreasing both toxic effects for the animal and carryover in animal products for human consumption.

With this aim, numerous sorbents from different sources have been tested, such as hydrated sodium calcium aluminosilicate (HSCAS), zeolites, bentonites, clays, and activated carbons *(86, 91)*.

HSCAS. HSCAS, a phyllosilicate derived from natural zeolite, is perhaps the most extensively investigated sorbent. Evidence of a high in vitro and in vivo affinity of HSCAS for AFB_1 arises from numerous studies reviewed by Piva et al. *(86)* and Ramos et al. *(91)*. However, to these latter authors, enthusiasm for the efficacy of HSCAS must be tempered by the fact that other studies demonstrated its ineffectiveness in binding dangerous mycotoxins other than AFB_1. Indeed, its protective properties are very low toward OTA and ZEN and nil toward trichothecenes.

Zeolites. Zeolites are hydrated aluminosilicates of alkali and alkaline-hearth cations characterized by infinite three-dimensional structure *(91)*. Although contrasting results are present in the literature, an overall efficacy of zeolite in binding AFB_1 and ZEN has been reported *(86, 91)*. As evidenced by Piva et al. *(86)*, the origin of zeolite can widely affect results of adsorption tests. In fact, the pore size distribution of synthetic zeolites, as opposed to natural ones, varies very little, being generally concentrated within a narrow diameter range. If the size of the pores is compatible with those of the mycotoxin molecules, adsorption can occur. On the contrary, adsorption can be low or nil because of the absence of intermediate-sized pores. The use of a zeolite, the clinoptilolite, was shown to reduce liver accumulation also when administered to laying hens exposed to AFB_1, although it had no effect on liver mixed-function oxygenase activities *(113)*. In contrast, Mayura et al. *(79)* observed dangerous synergetic toxic effects between AFB_1 and clinoptilolite, resulting in severe liver lesions in female rats.

Bentonites. Bentonites are sorbents with layered (lamellar) crystalline microstructure and variable composition. Their adsorption properties mainly depend on the interchangeable cations (NA^+, K^+, Ca^{++}, and Mg^{++}) present in the layers *(91)*.

Bentonite has been demonstrated to be able to bind efficaciously AFB_1 in vitro and reduce its toxic effects in trout and swine and also to reduce toxic effects of T2 toxin in rats, as referenced by Ramos et al. *(91)*. Sodium benton-

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ite and a synthetic zeolite mixture (80:20 ratio) was reported not to depress feed intake or apparent nutrient digestibility *(94)* but to prevent AFB_1 accumulation in the liver of growing lambs and decrease by several folds AFB_1 recovery in urine *(112)*. To the authors, the detoxifying properties of bentonite could be enhanced by its ability to reduce the transit time of digestion through the gastrointestinal tract, thus increasing the fecal losses of the toxins. However, this ability was not observed concerning ZEN and nivalenol *(91)*.

Clays. Other clays, such as kaolin, sepiolite, and montmorillonite, have a variable ability to reduce toxic effects of AFB_1 as reviewed by Ramos et al. *(91)*. However, their efficacy is limited to AFB_1 and is lower than that of HSCAS and bentonite.

Activated carbons. Activated carbons (AC) are an important group of sorbents. They are a family of carbonaceous substances obtained by pyrolysis of several organic compounds and manufactured by activation processes aimed at developing a highly porous structure *(38)*. Generally, the adsorption properties of AC are strictly dependent on the source materials and physicochemical parameters, such as surface area and pore size distribution. Preparation methods and chemical treatments can strongly modify the surface characteristics of AC. Because of the numerous possible combinations between typology of carbonaceous substances and activation processes, many AC with different adsorbing properties exist. This fact could explain some contrasting results reported in a recent review concerning the ability of AC in binding mycotoxins *(91)*.

Since there is no reliable, universal test of adsorptive properties of AC, they must be selected under application conditions. However, to characterize AC well, several physicochemical parameters can be considered *(38)*. Indeed, it is known that the adsorption properties are roughly correlated with the total surface area measured by adsorption of a very small molecule (nitrogen). The pore size distribution of AC is another important characteristic that affects the accessibility of the internal carbon surface. Since diffusion effects inside the pores can slow the adsorption process, the effective pore size distribution of AC can influence it as a function of the molecular size of the adsorbate. Information about mesopores and macropores can be obtained by mercury porosimetry, which allows determination of the size distribution of pores of inside diameter greater than 75 Å. Iodine number is a relative indicator of the microporosity of AC, and it is often used as an approximation of surface area, whereas methylene blue index is a test that established the medium-sized pore (mesopores) range and is an important indicator in practice of the ability of AC to adsorb organic molecules of medium-to-large size from a solution *(38)*.

In a series of preliminary in vitro tests (Table 1), we investigated the adsorption ability of 19 experimental AC from different source materials (i.e., exhausted olive residues, peach stones, and almond shells) obtained with laboratory equipment by several experimental activation processes appropriately selected to obtain the desired physicochemical parameters and four commercial AC produced in industrial processing equipment.

TABLE 1. *In vitro adsorption of mycotoxins from standard solution: saturation limit of activated carbons and HSCAS for aflatoxin B_1, fumonisin B, aflatoxin B_1 plus fumonisin B_1 (simultaneously), Ochratoxin A, and Deoxynivalenol*

	Saturation limit[b]				
Sorbents	Aflatoxin B_1	Fumonisin B_1	Aflatoxin B_1 + fumonisin B_1	Ochratoxin A	Deoxynivalenol
CAC1	123.0	3.8	25.0 + 2.1	115.0	2.0
CAC2	123.0	9.9	25.0 + 5.0	119.0	1.8
CAC4	95.0	9.6	25.0 + 4.7	91.0	1.9
AF32	112.0	3.4	25.0 + 1.8	80.0	2.0
AF48	>125.0	9.8	25.0 + 5.0	121.0	>2.0
HSCAS	79.0	0.3	NA	1.3	0.8

[a] Data are from Galvano et al. (36). HSCAS, hydrated sodium calcium aluminosilicate; CAC, commercial activated carbon; AF, olive residue CAC; NA, not assayed.
[b] Values indicate μg of mycotoxin per mg of sorbent.

Overall evidence of the high ability of AC in binding mycotoxins in vitro arises from our studies *(35, 36, 38)*. The highest abilities have been observed in the adsorption of AFB_1 and OTA, whereas the lowest in the adsorption of DON (Table 1). AC have been demonstrated to adsorb efficiently FB_1 simultaneously with AFB_1. When compared with HSCAS, AC showed much higher adsorption abilities toward all the tested mycotoxins. Thus, AC are capable of binding in vitro several mycotoxins, and it is reasonable to consider their potential use as multimycotoxin-sequestering agents differently from other extensively studied sorbents, such as HSCAS and bentonite, which are not capable of adsorbing efficiently mycotoxins other than AFB_1.

The molecular size and physicochemical properties of the mycotoxins clearly affect the efficiency of the binding action. For this reason, further studies on the mechanism of the binding action (i.e., performing studies on chemisorption indices) are needed to clarify the mechanism of the binding process and improve the adsorption performance. We performed two studies with the aim of verifying the in vivo ability of one of the AC that showed the highest in vitro adsorption abilities. In one experiment on dairy cows, we compared the abilities of AC and HSCAS in reducing carryover of AFB_1 from dairy cows' feed to milk. AC reduced carryover up to 50%, whereas HSCAS reduction of carryover was 36% *(37)*. In a study performed on rats fed an FB_1-contaminated diet, the bioavailability of the toxin was indirectly monitored by measuring the sphinganine concentration and the sphinganine/sphingosine ratio in urine, liver, and kidney. The addition of 2% AC was effective in avoiding the increase of liver weight. AC also reduced the sphinganine concentration in liver and both sphinganine concentration and sphinganine/sphingosine ratio in kidney *(101)*. Even though the results of the in vivo study were lower than those expected based on the in vitro

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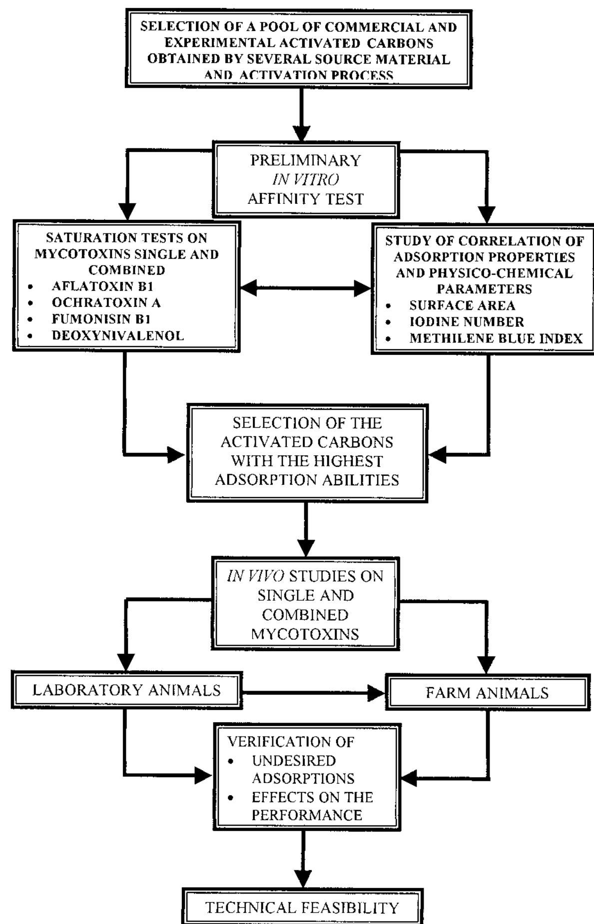

FIGURE 1. *Methodological approach to the study of activated carbons as mycotoxin-sequestering agents.*

results, data are promising, and further studies are in progress in our laboratory. In any case, that is the first report, to our knowledge, of an in vivo protective action of a sorbent toward FB_1.

Although the referenced tests are strongly encouraging, we are extremely conscious that additional in vivo studies are required to confirm the efficacy of AC in preventing or reducing the toxic effects of mycotoxins. We are sure that the in vivo efficacy of AC will be lower, since the practical conditions are widely different from the experimental ones, e.g., the binding sites of AC can be occupied by many other compounds present in the feed.

Assuming that in vivo studies would confirm the effectiveness of AC in detoxifying mycotoxins, three questions on whether AC could be added to feeds remain open.

The first is the possible long-term undesired adsorption of essential nutrients (i.e., vitamins and minerals). If long-term in vivo studies should confirm it, two strategies could be adopted: (i) addition of supplemental essential nutrients demonstrated to be excessively adsorbed onto AC or (ii) increase of the selectivity of AC toward mycotoxins by modulating the activation process and the physicochemical properties. The second question, as indicated by Ramos et al. *(91)*, is related to the property of AC to blacken the environment, the animals, and the feed. Some manufacturers have overcome this problem by producing AC that contain up to 65% water and have the consistency of brown sugar, thus eliminating the problems associated with the use of the powder form. We suggested that some of these problems could be also eliminated by pelleting the feed. Furthermore, we observed that, at least for AFB_1, the pelleting of the feed could increase the efficacy of AC *(37)*. The third is the economic evaluation of the addition of AC to feed. Today the price of AC is perhaps prohibitive to the feed industry. However, the possibility to include AC in feed should largely increase the demand and, consequently, decrease the price. In any case, evaluation of the cost-benefit balance is needed.

Cholestyramine. In a study on rats, cholestyramine, a bile acid–binding resin, was tested as protective agent against OTA-induced nephrotoxicity *(62)*. Cholestyramine decreased the concentration of OTA in plasma and the excretion of OTA and its metabolites in urine and bile and increased OTA excretion in feces. These results agree with those of Madhyastha et al. *(77)*. The authors attributed the decreasing of OTA nephrotoxicity to the reduction of its bioavailability and/or enterohepatic circulation. Cholestyramine was also capable of binding efficiently ZEN *(106)*. However, to the authors, its high cost would make its commercial use economically prohibitive.

Polyvinylpolypyrrolidone. Polyvinylpolypyrrolidone, a synthetic resin, can bind AFB_1 from feed *(105)*. A total of 0.4 g/kg of polyvinylpolypyrrolidone can bind up to 50 μg/kg of AFB_1 contained in feed. Polyvinylpolypyrrolidone plus bentonite partially ameliorated some hematological parameters altered by AFB_1 administration to broiler chickens *(61)*.

Bovine serum albumin. Hirano et al. *(51)* demonstrated that bovine serum albumin provides protection from AFB_1 toxic effects. In studies on 1-day-old chicks, the authors observed a marked reduction of histological and biochemical symptoms of exposure to AFB_1 and of AFB_1 levels in the plasma and liver. The authors noted that bovine serum albumin is able to bind AFB_1 in the intestinal tract and is excreted with it. The binding mechanism occurring between bovine serum albumin and AFB_1 was highlighted by Vyjaynthi et al. *(107)*.

Microbiological-binding agents. Increasing interest has also been generated by the possibility of using microorganisms to reduce the bioavailability of mycotoxins to farm animals, trying to overcome the inherent drawbacks associated with the use of described sorbents.

Saccharomyces cerevisiae 1026, initially used as a performance promoter in the early 1990s, was found to have beneficial effects on weight gain and immune response in broilers exposed to AFB_1 *(29)*. In vitro studies showed an AFB_1 dose-dependent binding capacity of *S. cerevisiae* up to 77% *(28)*. Interestingly, modified mannanoligosaccharide derived from the cell wall of *S. cerevisiae* was reported to have even higher binding capacity (95% AFB_1, 80% ZEN, FB_1 up to 59%, and DON up to 12%) *(28)*. These indica-

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tions were further confirmed by the addition of 0.11% of modified mannanoligosaccharide to the diet of layers receiving 2.5 ppm of AFB_1. AFB_1 did not contaminate the egg, but a 46% decrease of AFB_1 level in the liver was observed (4.13 versus 2.21 ppb) *(113)*. The transformation of mycotoxins on fermentation has been repeatedly reported. DON and ZEN were degraded in vitro by the normal bacterial gut flora from the distal sections (cecum, colon, and rectum) of the gastrointestinal tract of pigs *(64)*, whereas microorganisms from the cranial segments showed no transformation activity. DON was deepoxidated and ZEN was hydrolyzed to alpha-zearalenol and an unknown metabolite. These observations appear to be confined to the type of mycotoxin considered. In fact, various gram-positive and gram-negative bacteria, incubated with up to 1,000 mM of FB_1, were shown not to be affected in the growth rate or to be associated with any reduction of FB_1 concentration during the incubation time *(11)*.

Flavobacterium aurantiacum was the only microorganism that was shown to significantly remove AFB_1 from liquid medium and food products without the production of toxic by-products *(71, 73)*, although another study conducted by Dsouza and Brackett *(30)* observed that the presence of trace divalent metal ions, such as Cu, Mn, Zn, and Co, strongly inhibits the binding properties of *F. aurantiacum.*

More recently, some dairy strains of lactic acid bacteria were found capable of removing AFB_1 from contaminated liquid media via a rapid process involving the removal of approximately 80% of AFB_1 immediately on contact without further incubation *(31)*. Heat-treated bacteria had the same ability to remove AFB_1 as viable bacteria; consequently, metabolic degradation by viable bacteria has been ruled out as a possible mode of action under the experimental conditions tested. All the gram-positive strains tested were more efficient than *Escherichia coli*, suggesting that the bacterial ability to remove AFB_1 is dependent on bacterial cell wall structure. The process is temperature and bacterial concentration dependent, whereas no difference was observed due to pH variation across the range of 4 to 6. Furthermore, treatment with hydrochloric acid, autoclaving, or boiling enhanced the binding activity of the bacterial pellets, confirming that the type and structure of the cell wall is crucial for an effective binding of mycotoxins *(32)*.

Megharaj et al. *(81)* demonstrated the ability of a mixed culture of bacteria to remove ZEN from culture media. Bolognani et al. *(13)*, in a test aimed to determine whether viable cultures of lactic acid–producing organism can bind dietary carcinogens, observed that *Bifidobacterium longum* and *Lactobacillus acidophilus* poorly bound AFB_1, whereas Lankaputhra and Shah *(69)* reported that live cells of several strains of the same bacteria bound or inhibited AFB_1.

Pseudomonas aeruginosa was able to inhibit the growth of *Pennicilium citrininum* and the production of citrinin *(41)*.

Some strains of yogurt bacteria and bifidobacteria (*Streptococcus salivarius* subsp. *thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium bifidum*) were found to reduce the content of OTA in milk by fermentation *(99)*.

CONCLUSIONS

Encouraging results have been obtained in studies on the protective action of a large number of nutrients, food components, additives, microorganism, and sorbents. The increasing interest of researchers in this field is demonstrated by a constantly growing literature. Nevertheless, most studies were conducted in vitro and confined to AFB_1. Much less information is available from studies on other mycotoxins, such as OTA, FB_1, T2 toxin, ZEN, and citrinin. No studies have been performed on recently discovered toxins such as beauvericin, fusaproliferin, moniliformin, and fusaric acid. In addition, most of the studies have been conducted on laboratory animals, whereas only a few were conducted on farm animals. Furthermore, there is a limited knowledge about the efficacy or drawbacks of any of the investigated strategies to prevent extended exposure to mycotoxins. This is comprehensibly due to the preliminary approach research stage in this field. Several natural (vitamins, provitamins, carotenoids, chlorophyll and its derivatives, phenolics, and selenium) and synthetic (butylated hydroxyanisole and butylated hydroxytoluene) compounds with antioxidant properties seem to be potentially very efficacious. The protective properties of antioxidants are probably due to their ability to act as superoxide anion scavengers, thereby protecting cell membrane by mycotoxin-induced damages. However, other more complex mechanisms involving modulation of metabolic detoxification pathways (i.e., microsome enzymatic activation of AFB_1), intercepting action and formation of stable nontoxic complexes (i.e., formation of noncovalent complex between chlorophylline and AFB_1, ellagic acid and AFB_1, and the reaction between fructose and FB_1), and structural similarity between mycotoxin and protective agent molecules (i.e., ZEN and vitamin K) have been purposed and are currently being investigated.

Interesting results have also been reported regarding other food components with or without antioxidant properties. Several compounds contained in foods such as coffee, strawberries, tea, pepper, grapes, turmeric, *F. tonka,* garlic, cabbage, onions, medicinal herbs, and plant extract seem to be potentially capable of protecting against mycotoxins. However, further investigations are needed to better understand the modes of action.

Aspartame, a sweetener without adverse effects for humans and animals, has been proposed as a protective agent against the toxic effects of OTA.

A curious fact is that in some cases the sources of potential protective agents are also a potential way to the assumption of mycotoxins, especially AFB_1 and OTA *(48)*.

These are the cases of coffee, pepper, tea, grapes, and medicinal herbs. Thus, caution should be used in promoting antimycotoxin action of discussed substances, since some may be carcinogenic (i.e., quercetin) or toxic (selenium and coumarin) properties.

Use of mycotoxin-binding agents seems to be a very

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promising approach to the detoxification of mycotoxins, as demonstrated by 23 studies and two extensive reviews. The positive outcome from the initial investigations of AFB_1 binding to HSCAS and bentonite fostered additional interest in this approach, and further studies were conducted on other sorbents and mycotoxins. Concomitantly, a number of products arrived on the food market claiming multimycotoxin-binding capabilities. It is unfortunate that only a limited number of sorbents were peer reviewed and found capable of binding effectively only AFB_1, making it difficult to objectively assess the capacity of other sorbents to bind different mycotoxins. None of the sorbents has been scientifically proven to bind simultaneously more than one mycotoxin in in vivo studies. Therefore, as emphasized by Dale *(26)*, it is time for real science where mycotoxin binders are concerned.

Figure 1 shows the scientific approach we are using to study AC as mycotoxin binders. Although obviously our approach could be modified, depending on the type of sorbent, we propose it as a general model for the study of sorbents as mycotoxin binders.

Interesting results have been obtained by using microorganisms (i.e., *F. aurantiacum* and some *Lactobacillus* spp.) to remove and/or destroy mycotoxins, even though procedures have not yet been applied.

We think that dietary approaches to reduce the toxic and economic impact of mycotoxins are the most promising approach to the problem, considering their potential ability to fulfill the efficacy, safety, safeguarding of nutritional elements, and cost requisites of a satisfactory detoxification-decontamination process.

ACKNOWLEDGMENTS

This work was financially supported by grants from the MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica), Ass. Ex-60%, 1997–98, Prof. Fabio Galvano, Università di Reggio Calabria, and Ass. Ex-60%, 1997–98, Prof. Giacomo Galvano, Università di Catania.

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