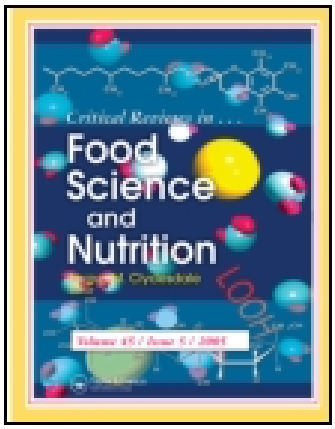
This article was downloaded by: [190.151.168.87]
On: 10 July 2014, At: 15:22
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Food Safety and Increasing Hazard of Mycotoxin Occurrence in Foods and Feeds

Stoycho D. Stoev [a]
[a] Department of General and Clinical Pathology , Faculty of Veterinary Medicine, Trakia University, Students Campus , 6000 , Stara Zagora , Bulgaria
Accepted author version posted online: 24 Feb 2012.Published online: 14 Jun 2013.

To cite this article: Stoycho D. Stoev (2013) Food Safety and Increasing Hazard of Mycotoxin Occurrence in Foods and Feeds, Critical Reviews in Food Science and Nutrition, 53:9, 887-901, DOI: 10.1080/10408398.2011.571800

To link to this article: http://dx.doi.org/10.1080/10408398.2011.571800

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Critical Reviews in Food Science and Nutrition, 53:887–901 (2013)
Copyright © Taylor and Francis Group, LLC
ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2011.571800

Taylor & Francis
Taylor & Francis Group

Food Safety and Increasing Hazard of Mycotoxin Occurrence in Foods and Feeds

STOYCHO D. STOEV

Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Students Campus, 6000 Stara Zagora, Bulgaria

Downloaded by [190.151.168.87] at 15:22 10 July 2014

The possible hazard of mycotoxin occurrence in foods and feeds and some food-borne mycotoxicoses is reviewed. Management of the risk of mycotoxin contamination using some useful preventive measures against mycotoxin contamination of foods/feeds during pre- and post-harvesting periods is considered. The physical and chemical methods of mycotoxin decontamination of foods/feeds are briefly described. The use of various feed additives as a method for prevention of the adverse effects of mycotoxins is reviewed. The processing of various foods and feeds is considered in a view to possible mycotoxin decontamination. The necessary hygiene control and risk assessment in regard to mycotoxin contamination of foods and feeds in addition to some useful prophylactic measures are briefly described. A short reference is made concerning the most successful methods of veterinary hygiene control in order to prevent a possible entering of some mycotoxins in commercial channels with a view to human health.

Keywords Food safety, mycotoxins, mycotoxicoses, hygiene control, risk assessment, feed additives

INTRODUCTION

Food safety and protecting the men or animals from various food-borne diseases become more and more important missions of many specialists in human and veterinary medicine from all over the world, especially with the challenge to ensure a safe and healthy feeds or foods for animals and humans in regards to their mycotoxin contamination. While developed countries have well-developed infrastructures for monitoring of food quality standards, people in developing countries are not protected by food quality monitoring and enforcement of safe standards within their countries. On the other hand, such standards would often encourage the exportation of the best-quality crops, because the foods being exported are expected to comply with the recognized, by most of the countries, quality standards and thereby possibly inadvertently resulting in higher risk of mycotoxins exposure of people in developing countries, because only the best-quality foods leave the country, whereas the poor-quality crops always remain for domestic consumption. In addition, the difficulty in meeting U.S. and European Union (EU) mycotoxin safety standards possibly means the loss of an export market. Or, alternatively, it means exportation of best-quality grains in order to keep the export market. By keeping the poorer quality grain in the domestic market, local consumers bear the health burdens (Stoev, 2007).

Safety and quality control activities along the food supply chain are very important to enhance the safety of various foods and feeds. However, all the activities at each level require integration into a coordinated system. The human resource capacity, various kinds of professionals and knowledge in field of biochemistry, agriculture, veterinary medicine, environmental health science, and food science and technology as well as the upgrading of knowledge and skills via professional courses are of significant importance in order to be able to realize these safety and quality control activities. Hazard Analysis and Critical Control Point (HACCP) system is also of a particular importance to improve the food safety management. Only integrated approach to food safety, which includes systematic identification and assessment of hazards in foods and various means to control them, could resolve various existing problems in this field. Effective enforcement of food safety laws and regulations in addition to surveillance control is also required to reduce the number of food-borne diseases and consequent social burdens of health care as well as to enhance the security of food. That is why a harmonization of various national standards with other

Address correspondence to Stoycho D. Stoev, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Students Campus, 6000 Stara Zagora, Bulgaria. E-mail: stoev@uni-sz.bg

standards and regulations in regards to various mycotoxins, heavy metals, pesticides, veterinary drugs, and pathogens is also necessary in order to protect the consumer and to ensure a global safety of various kinds of foods produced in various countries as well as to ensure fair international trade (Stoev, 2007). On the other hand, in the case of very strict regulations, an unjustified rejection of various kinds of commodities or raw food materials can have disastrous consequences for the producer and the distributor, respectively, which leads to unjustified trade barriers (Rosner, 1998).

HAZARD OF MYCOTOXIN OCCURRENCE AND SOME FOOD-BORNE MYCOTOXICOSES

The mycotoxins content in various foods and feeds presents a serious health problem for animal and humans in many developing as well as developed countries in all over the world. Most of mycotoxins are toxic secondary metabolites produced by certain fungi in agricultural products susceptible to mold infestation. Their production is unavoidable and depends on different environmental factors in the field and during the storage (Fig. 1). Due to its unavoidable and unpredictable nature, mycotoxin contamination presents a unique challenge to food safety. The discovery of new mycotoxins and co-contamination of known mycotoxins is occurring at a high rate; and although definitive evidence on the effect of mycotoxins on human diseases is limited, there is considerable evidence to support the association between mycotoxins and certain animal syndromes. The molds and mycotoxins have been associated with a variety of livestock diseases including ergotism, various kinds of fusariotoxicoses (Fig. 2), aflatoxicosis, mycotoxic porcine/chicken nephropathy (Fig. 3), stachybotryotoxicosis, equine leukoencephalomalacia, and many others (Stoev, 2007).

Ergotism, also known as St Anthony's fire, is one of the oldest food-borne diseases in man that caused hallucinatory symptoms and death of many people in France and other European countries during the Middle Ages (Betina, 1989). Some cases of ergotism have been found in a French village of Pont Saint Esprit in 1951 (Gabbai et al., 1951; Bennett and Bentley, 1999) and in Ethiopia in 1978 (Sibanda et al., 1997). *Clavisep purpurea* poisoning is known as ergotism and is still a disease of public health importance, especially in the developing world (IPCS, 1990; Schneider et al., 1996). It is predominantly characterized by dry gangrene of the extremities, loss of one or more limbs as well as the exhibition of gastrointestinal symptoms (IPCS, 1990; Bennett and Bentley, 1999).

Aflatoxins were discovered due to their devastating effect on turkey poults and some other chicks in 1960 (Allcroft and Carnaghan, 1962), while ochratoxin A was found as a major causal agent in nephropathy in pigs and probably Balkan Endemic Nephropathy in humans, widely encountered in Balkan countries (Krogh, 1972; Stoev, 1998, 2008).

Figure 1 An inappropriate store in rural area of Limpopo province of South Africa, which would not be able to preserve feed from rain. (Color figure available online.)

Downloaded by [190.151.168.87] at 15:22 10 July 2014

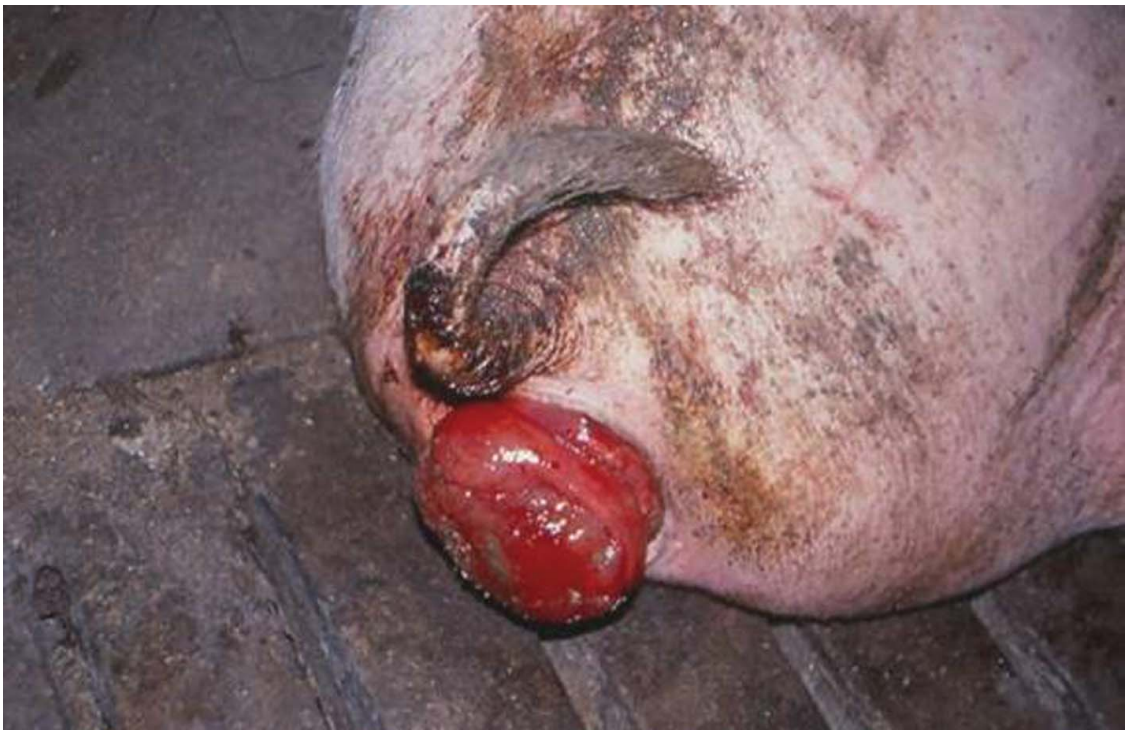


Figure 2 A rectal prolapse in spontaneous case of fusariotoxicosis in pig from Bulgaria due to oestrogenic effect of mycotoxin zearalenone. (Color figure available online.)

Downloaded by [190.151.168.87] at 15:22 10 July 2014

Mycotoxic nephropathy (MN), which is widely encountered disease in all over the world, can be considered as a renal disorder caused by alimentary ingestion of nephrotoxic mycotoxin ochratoxin A. This nephropathy is recently found to have much complicated pathology and etiology in some countries as Bulgaria and South Africa (Stoev et al., 2010a, 2010b). In these countries spontaneous nephropathy in pigs (Stoev et al., 1998a, 1998b, 1998c) or chicks (Stoev et al., 2002a) described previously, was recently found to be provoked by several mycotoxins as ochratoxin A, penicillic acid and fumonisin B_1, having synergistic interaction (Stoev et al., 2010a, 2010b). Some of the mycotoxins produced by storage fungi such as ochratoxin A and penicillic acid are secondary fungal metabolites encountered in feeds/foods/forages made mainly from cereals or fibrous plants, and kept in storehouse conditions and increased humidity, whereas the other target mycotoxin fumonisin B_1 produced by field fungi mainly contaminates maize before harvesting. The farms with nephropathy problems usually had a history of incorrect feed storage, but sometimes the problem seemed to come from certain feed plants whose grains, collected during moist and rainy days, had not been properly dried. All farms supplied by these plants subsequently produced some pigs with nephropathy and growth depression, but after changing the certain suspected feeds the problems with poor growth of pigs disappeared (Stoev et al., 1998a, 1998c). Because of the harmful effects, which can be observed in human kidneys after consuming the meat of animals with nephropathy the timely diagnosis of disease during the meat inspection at slaughterhouses is very important (Fig. 3). In such a way the exposure of humans to the most hazardous and relatively heat stable ochratoxin A from chicken/pigs meat can be prevented (Stoev, 1998, 2008). Similar growth depression and decrease of weight of the eggs can be observed in ochratoxin A-exposed chicks or laying hens (Stoev, 2010b; Stoev et al., 2002d).

Moldy maize was found to be a cause of horse or pig diseases, including death in many horses more than 150 years ago and this was subsequently associated with the presence of *Fusarium moniliforme* (*F. verticillioides*), but the mycotoxins responsible for these diseases, the fumonisins, were not well known until the 80s (Bezuidenhout et al., 1988; Gelderblom et al., 1988). The importance of fumonisin B_1 increased significantly after a number of outbreaks of equine leukoencephalomalacia (Conkova et al., 2003) and porcine pulmonary oedema killing many horses and pigs, fed on diets containing fumonisin-contaminated maize in the United States during 1989–1990 (Marasas et al. 1976; Marasas, 2001), which appeared to be due to the alteration of vascular function and endothelial cell permeability provoked by disruption of sphingolipid metabolism (Ramasamy et al., 1995). It is worth mentioning that the main concern for human health in regard to aflatoxins, ochratoxin A, and fumonisins in developed countries appeared to be their carcinogenic or genotoxic effects rather than their acute effects.

Although fumonisin B_1 was first discovered in connection with esophageal cancer in South Africa (Marasas et al., 1988) and was shown to be a liver carcinogen in rats (Gelderblom et al., 1992), it is also a nephrotoxin (Bucci et al., 1998; Howard

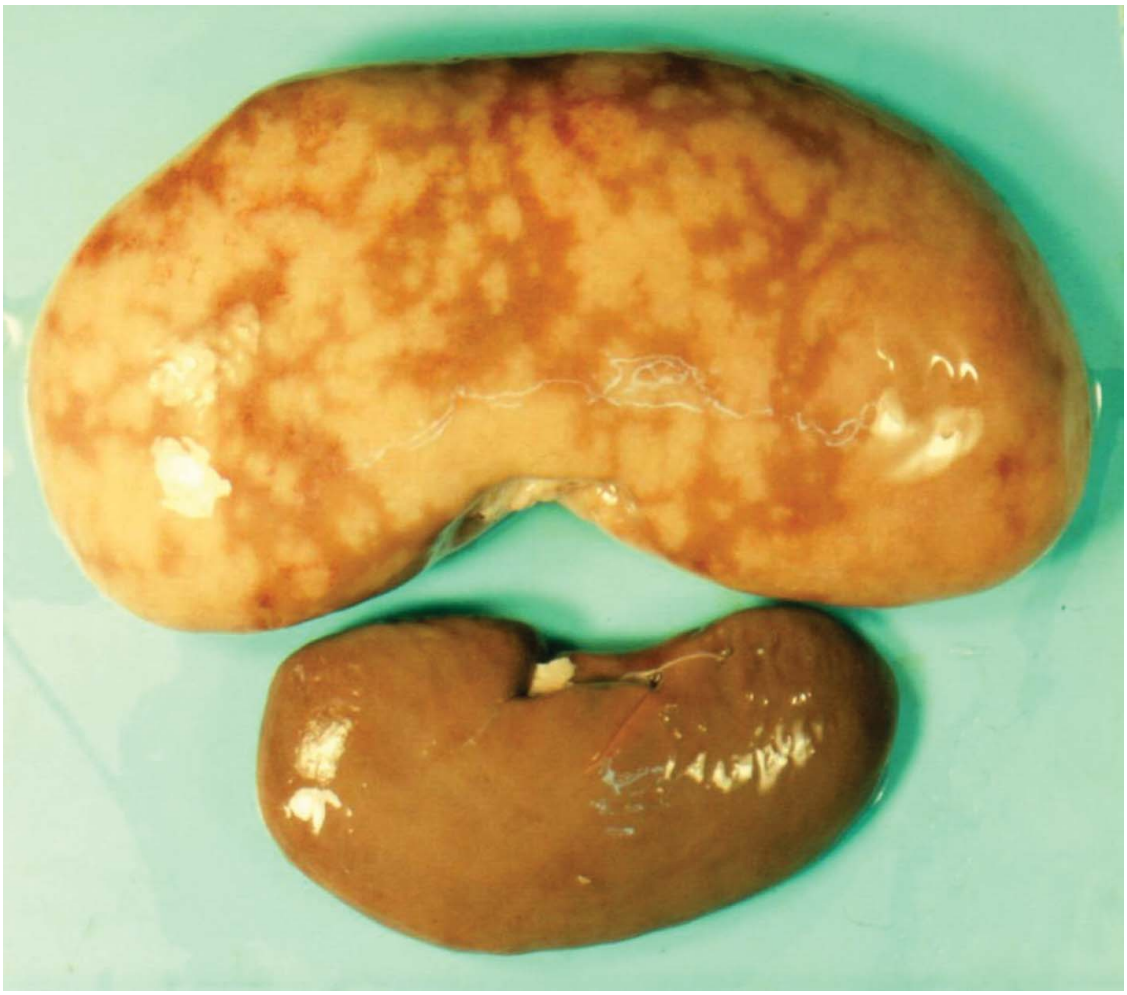


Figure 3 Macroscopic appearance of kidney with spontaneous mycotoxic nephropathy in Bulgaria. Enlarged and marbled appearance of kidney in pig of 6–8 month age (above) and normal kidney in pig of the same age (below). (Color figure available online.)

Downloaded by [190.151.168.87] at 15:22 10 July 2014

et al., 2001), and that is why it is suspected to be involved in human and animal nephropathies in Bulgaria and South Africa, as recently found (Stoev et al., 2010a, 2010b).

Zearalenone is a *Fusarium* mycotoxin, found mainly in the moldy maize, that has been recognized due to its estrogenic activity in animals as swelling of the vulva and mammary glands, infertility, vulvovaginitis, vaginal, and/or rectal prolapse in swine (Fig. 2; Shibamato and Bjeldanes, 1993; Shier, 1998; Friends et al., 1999).

However, the lack of sound evidences for the definitive relationship between mycotoxins and current human diseases in developing countries does not necessarily imply that dietary exposure does not represent a potential risk. In developing countries, many individuals are not only malnourished but are also chronically exposed to high levels of mycotoxins in their diet. *Fusarium* toxins, specifically fumonisins, are known carcinogens. With regard to human health, epidemiological studies established a correlation between the level of fumonisin in corn, the amount of corn consumed in the diet, and the rate of esophageal cancer (Marasas et al., 1988; Sydenham et al., 1990; Dutton, 1996). This mycotoxin has been reported to be associated with human esophageal cancer in China and South Africa. The greatest level of esophageal cancer in South Africa (Marasas et al., 1988; Sydenham et al., 1990; Rheeder et al., 1992) and China (Chu and Li, 1994) occurred among those populations consuming the largest amount of corn with the highest level of fumonisin B_1 contamination. When pregnant women are exposed to high levels of fumonisins in their diet, the risk of having a child with a birth defect of the brain or spinal cord increases significantly. Several studies suggested that maternal ingestion of high levels of fumonisin B_1 among human populations during early pregnancy may increase the risk of neural tube defects such as brain and spinal cord defects (van Waes et al., 2005; Missmer et al., 2006).

On the other hand, lots of mycotoxins are known to have many different adverse effects as cytotoxic, genotoxic, immunotoxic, carcinogenic, or teratogenic effects on animals. Trichothecenes, in addition to many other adverse effects are reported to be also immunotoxins (Sharma, 1993). Aflatoxins are proven hepatotoxins, carcinogens, genotoxins, immunotoxins suppressing both cellular and humoral response and cause growth retardation in animals (Dirheimer, 1998; Kubena et al.,

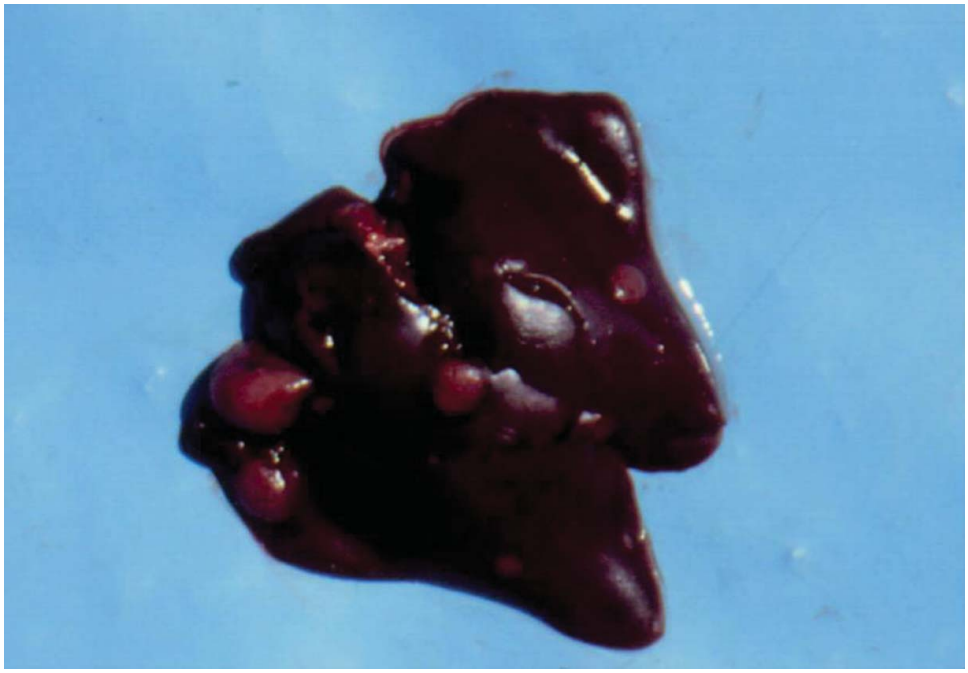


Figure 4 Adenocarcinoma in the liver of chick exposed to 5 ppm ochratoxin A for 10 months. Large grey-white neoplastic foci are seen in the liver and protruded significantly above its surface. (Color figure available online.)

Downloaded by [190.151.168.87] at 15:22 10 July 2014

1998; Coulombe et al., 2005) as well as human hepatosis (Hgindu et al., 1982). Ochratoxins (especially ochratoxin A) and fumonisins are proven nephrotoxins (Fig. 3; Krogh, 1972; Stoev et al., 1998a, 2002c), immunotoxins (Stoev et al., 2000a, 2000b, 2002b), genotoxins (Dirheimer, 1998), and carcinogens (Fig. 4; Stoev, 2010a).

Some recent experiments focused attention on the immunosuppression as the first expressed toxic effect of ochratoxin A, which may become evident clinically before nephropathy and its associated biochemical changes. For the first time, susceptibility to natural infectious disease has been demonstrated in pigs exposed to the immunotoxicity of ochratoxin A. Ochratoxin A suppression of humoral and cellular immunity, defined in principle (NNT, 1991), has been demonstrated in practice allowing development of secondary bacterial infections in pigs at only 1 ppm ochratoxin A in diet (Stoev et al., 2000b). Humoral immunity was affected to the extent of allowing development of clinical disease in pigs at only 1 ppm ochratoxin A in diet (Stoev et al., 2000b).

The repetitive exposures to the trichothecenes T-2 toxin increases susceptibility to *Mycobacterium bovis*, *Salmonella typhimurium*, *Lysteria monocytogenes*, and *Staphylococcus aureus* infections in animals or chickens (Boonchuvit et al., 1975; Ziprin et al., 1987; Tai and Pestka, 1988; Cooray and Jonsson, 1990; Oswald and Comera, 1998).

Ochratoxin A has also been described to increase the susceptibility of chickens to coccidiosis (Huff and Ruff, 1982; Stoev et al., 2002b; Koynarski et al., 2007), salmonellosis (Elissalde et al., 1994; Fukata et al., 1996; Gupta et al., 2008), and colibacillosis (Kumar et al., 2003). The high mortality among chicks/pigs fed on moldy diet containing ochratoxin A may be due to the increased susceptibility to secondary bacterial enteric disease (Stoev et al., 2000b) or to a heavy progression of some often encountered parasitic diseases (Stoev et al., 2002b; Koynarski et al., 2007) and microbial infections, because of the suppression in both humoral and cell-mediated immune response in such animals as an aspect of ochratoxicosis (Dwivedi and Burns, 1985; Oswald and Comera, 1998; Stoev et al., 2000a, 2000b).

MANAGEMENT OF THE RISK OF MYCOTOXIN CONTAMINATION OF FOODS/FEEDS

Usually, cereals are invaded by fungi both in the field and after harvest and have the potential for multitoxin contamination. The formation of each particular mycotoxin is depending on a number of factors but particularly on the climate, the type of cereal, drying at harvest, and storage conditions. Thus, cereals may be contaminated by any of the main mycotoxin groups that include aflatoxins, ochratoxin A, deoxynivalenol, and other related trichothecenes, zearalenone, fumonisins, or moniliformin. These mycotoxins can occur in wheat, barley, rye, oats, triticale, maize, sorghum, etc., although the fumonisins mainly occur in maize. Some of the mycotoxins are formed by the fungi encountered in the growing crop prior to harvesting and therefore development of the same fungi, mainly belonging to *Fusarium*, *Alternaria*, and *Aspergillus* genera, are often difficult to control. It is much easier to prevent the formation of those mycotoxins that arise during storage, mainly formed by species belonging to *Penicillium* or *Aspergillus* genera, which could be performed

by drying crops at harvest to safe moisture contents as soon as possible. However, sometimes this is not easily done or if it is done, the subsequent poor storage practice may result in mold growth and mycotoxin formation, which could have been prevented (Stoev, 2007).

One possible approach to the management of the risks associated with mycotoxin contamination is the use of the same integrated system of HACCP mentioned earlier. This proposed control program for processed foods/feeds should be based on the HACCP approach and should involve strategies for prevention, control, good manufacturing practices, and quality control used at all stages of production from the field to the final consumer. HACCP-like approaches are now being applied to mycotoxins and the Food and Agriculture Organization (FAO) have published a comprehensive manual on the application of the HACCP system in mycotoxin prevention and control (FAO, 2002). HACCP can be used to identify the steps at which mycotoxins might be prevented or removed and identifies the stages at which monitoring systems can be set up. Such approach is used in automated sorting and segregation of peanuts in order to remove those nuts highly contaminated with aflatoxins as well as in cleaning procedures for cereals prior to milling that removes dust, fungal spores, broken grains, and other debris that may contain high concentrations of mycotoxins (Dowell et al., 1990; FAO, 2002).

Downloaded by [190.151.168.87] at 15:22 10 July 2014

Preventive Measures of Mycotoxin Contamination of Foods/Feeds

The occurrence of molds and mycotoxins can be decreased by the application of a variety of preventative measures both before and after harvest including, for example, appropriate control measures, timely harvesting, cleanup, drying and storage practices, crop rotation, management of insect infestation, creating of plant cultures resistant to fungi infestation, and others. For example, Bt-corn (transgenic corn) products have less risk of fumonisin contamination depending on the types of insects attacking the corn. Genetic engineering has also been useful in the development of host resistance through the addition or enhancement of antifungal genes in order to create mold-resistant wheat cultivars or maize hybrids, etc. (Doko et al., 1995; Bata et al., 2001). Also, it is well known that mold contamination is more pronounced if wheat is sown after maize and vice versa. Applying multifield crop rotation in which, for example, rape, sugar beet, sunflower, or soya beans are present reduces degree of mold infestation. It should be taken into account that fertilization with nitrogen increase plants sensitivity to mold (Reid et al, 2001). On the other hand, fungicides, applied before blossoming, decrease *Fusarium* contamination and the respective mycotoxins production, whereas delayed harvesting favors *Fusarium* contamination (Peraica et al., 2002).

Another way to prevent the production of some mycotoxins such as ochratoxin A or aflatoxins is prevention of the growth of the storage fungi. Some studies indicate that fungi in cereal grains stored at moisture contents lower than 15% generally do not produce ochratoxin A, which suggests that the moisture content in the cereal grains have to be decreased below 15% via various drying procedures before storage (Frohlich et al., 1991). Storage at higher moisture levels requires that grain be maintained under anaerobic conditions, which prevent growth of fungi. Otherwise, a combination of mold inhibitors and sterilization techniques, possibly coupled with the inoculation of nontoxigenic competitive microflora, have to be applied to prevent the growth of ochratoxin A-producing fungi (Stoev, 2008).

On the other hand, Chelack et al. (1991a, 1991b) have used radiation to control the growth of ochratoxin A-producing fungi. They demonstrated that gamma or electron beam irradiation is a highly effective means of destroying spores from some ochratoxin A-producing fungi as *Aspergillus alutaceus* (Chelack et al., 1991a, 1991b).

Leitao et al. (1990) demonstrated that phosphine (PH_3) was effective at inhibiting both fungal growth and sterigmatocystin production by *Aspergillus versicolor*, and this could be considered as another specific way to control the growth of mycotoxin production by fungi.

For foods with pH values from 5 to 6, such as the cereals or sorghum, the antimicrobial agents (food additives) that are able to prevent the growth and ochratoxin A-production by *Aspergillus* and *Penicillium* species, are methyl paraben or potassium sorbate (Tong and Draughon, 1985). Small concentrations of these compounds are able completely to inhibit the growth by both genera of fungi and their ochratoxin A-production. At pH 4.5, as occurs in silage, fungal growth and ochratoxin A-production was completely inhibited by 0.02% potassium sorbate, 0.7% methyl paraben, and 0.2% sodium propionate (Tong and Draughon, 1985). The same could be also used to suppress the growth of various other fungi, producing different mycotoxins.

Prevention through preharvest management is the best method for controlling mycotoxin contamination. Ideally, risks associated with mycotoxin hazards should be minimized in each phase of the food processing. Control parameters in processing commodities susceptible to mycotoxin contamination should include time of harvesting, temperature, moisture during storage, and transportation as well as humidity of air in the storehouses (Scott, 1998). Adequate storage conditions with optimal temperature and humidity of grain and storehouses may decrease significantly the growth of toxigenic molds (Peraica et al., 2002).

However, once mycotoxin contamination occurs, the hazards associated with various mycotoxins must be managed through postharvest procedures, if the product is to be used as human food or animal feed. Such measures, which can decrease mycotoxin content in food or feed, include various physical or chemical decontamination methods such as cleaning, segregation, electronic sorting, steeping, etc., as well as various kinds of processing as milling, that usually used to manage mycotoxins in raw ingredients, etc. Further baking, cooking, extrusion, malting, and brewing can additionally decrease mycotoxin content in the final product.

Downloaded by [190.151.168.87] at 15:22 10 July 2014

Physical Methods of Mycotoxin Decontamination of Foods/Feeds

The physical methods of mycotoxin decontamination (Scott, 1998) includes various kind of procedures such as cleaning, mechanical sorting and separation, washing, segregation, thermal inactivation, irradiation, solvent extraction, etc.

Cleaning procedures are used for all cereals prior to food processing and this process includes removal of dust, broken grains, and other unwanted material and may involve the physical removal of some of the outer layers of grains by abrasion, e.g., "scouring." The data of the effectiveness of cleaning for the aflatoxins vary, but an average reduction of about 40% in concentration was usually reported (Brekke et al., 1975a). Generally, separation of corn screenings can significantly reduce fumonisin and aflatoxin concentration (Broggli et al., 2002). Cleaning of grain can remove ergot alkaloids from rye and wheat (Scott, 1998). Some reduction by cleaning and scouring procedures is also reported for ochratoxin-contaminated grain (Scudamore and Banks, 2004). The highest concentrations of deoxynivalenol can be usually found in the smaller broken and shrivelled grains and therefore mean toxin concentrations could be reduced by discarding the small grains (Chelkowski and Perkowski, 1992). Substantial reduction of zearalenone occurs in barley by polishing, but cleaning of maize grains appeared to have little effect on zearalenone concentration as reported by Scudamore and collaborators (Scudamore et al., 1998, 1999). Similarly, the exclusion of rotten or poor quality apples can significantly reduce concentrations of patulin in apple juice (Scudamore and Banks, 2004).

The automated sorting and segregation of peanuts can remove damaged or inadequately developed nuts highly contaminated with aflatoxins, which are usually used for oil production (Dowell et al., 1990). Electronic sorting is another method for decontamination of peanuts, which is based on the color of roasted, blanched peanuts (Pelletier and Reizner, 1992), whereas fluorescence sorting is used mainly for screening and decontamination of corn, cottonseed, and dried figs (Muller, 1983; Steiner et al., 1988).

Washing procedures by water or sodium carbonate solution is sometimes used for decreasing the concentration of some *Fusarium* mycotoxins as fumonisins, zearalenone, and deoxynivalenol in grains or corn cultures, but it might be a useful procedure only prior to wet milling, because of the eventual high price of the subsequent drying procedures (Scott, 1998).

Thermal inactivation is not a suitable method for decontamination, because most of the mycotoxins are heat stable. Partial decontamination can be only achieved by coffee roasting or by microwave treatment, which may destroy mainly aflatoxins in peanuts or trichothecenes in corn (Basappa and Shantha, 1996; Scott, 1998).

Different types of radiation as γ-irradiation, X-rays, ultraviolet light, etc., were explored for decontamination of some mycotoxins as T-2, deoxynivalenol, or aflatoxins as well as to control the growth of some fungi (Müller, 1983; Samarajeewa, 1991) but the same have some disadvantages, because the radiation is effective only when applied to a thin layer of grain (Peraica et al., 2002). Fortunately, solar radiation is an inexpensive way of partial detoxification of coconuts, peanuts, corn, sesame contaminated with aflatoxins, which is widespread in some tropical areas of the world (Basappa and Shantha, 1996).

Solvent extraction by ethanol, isoprapanol, or methoxymethane can be an effective way for decontamination of some mycotoxins as aflatoxins, but the high prices and possible solvents residues present barriers for commercial exploitation of such methods (Basappa and Shantha, 1996; Peraica et al., 2002).

Chemical Methods of Mycotoxin Decontamination of Foods/Feeds

There are also various chemicals as acids (e.g., formic and propionic acid), bases (e.g., ammonia, sodium hydroxide), oxidizing agents (e.g., hydrogen peroxide, ozone), reducing agent (e.g., sodium bisulphite), and some other chemicals as chlorine or formaldehyde, which have been studied for their effectiveness in mycotoxin decontamination (Peraica et al., 2002), but these usually leave some toxic metabolites or reduce the nutritional value of foods/feeds.

According to Laciakova et al. (1998), formic acid of 0.25% concentration degraded ochratoxin A after 3 hours exposure, propionic and sorbic acids in 1% concentration after 24 hours exposure, and benzoic acid in 0.5% concentration after 24 hours exposure (Laciakova et al., 1998).

Hydrogen peroxide has been used for detoxifying peanuts, whereas calcium hydroxide or monomethylamine were used for detoxifying oilseeds and corn, contaminated respectively with aflatoxins or fumonisins (Scott, 1998). Sodium bisulphite is another chemical used for detoxification of corn or dried figs contaminated with aflatoxins or deoxynivalenol (Scott, 1998).

Ammoniation and ozonation are other chemical methods that have received the most research attention as a practical solution to decontaminate aflatoxins- or fumonisins-contaminated feeds or peanuts, but such methods usually are not allowed within the European Community (EC) for human foods (Peraica et al., 2002). Chelkowski et al. (1981) reported that treatment of ochratoxin A-contaminated grain with ammonia reduced concentrations of this mycotoxin to undetectable levels. They concluded that ammoniation of grain not only detoxifies several mycotoxins, including fumonisins, but also inhibits mold growth (Chelkowski et al., 1981). Feeding studies have shown no toxic effects related to the ammoniation process, but there are some changes in the nutritional quality of the feed, such as a decrease in lysine and sulfur containing amino acids (Scott, 1998). In addition, adequate aeration after ammoniation is necessary for acceptance of the feed by animals.

Madsen et al. (1983) reported that treatment of ochratoxin A-contaminated barley with 5% NH_3 for 96 hours at 70°C or warming of grain to 105°C in the presence of 0.5% NaOH as well as autoclaving at 132°C for 0.5 hours can destroy the main

part of ochratoxin A in the barley, but such treatments are not practical and are not able to effectively reduce the mycotoxin contamination in grain.

Except ammoniation, many of the techniques proposed to remove mycotoxins are currently perceived as impractical, ineffective, and/or potentially unsafe for large-scale use (CAST, 1989).

Use of Feed Additives and Other Methods Preventing the Adverse Effects of Mycotoxins

There is also possibility of addition of various chemicals or feed additives with possibilities to fix and neutralize mycotoxins or to realize antidote effects against mycotoxin action (Stoev et al., 1999, 2000a, 2002d, 2004; Stoev, 2008, 2010b).

Some feed additives are designed to bind mycotoxins in gastrointestinal system and to reduce their bioavailability. The clay (hydrated sodium calcium aluminosilicate clay, HSCAS and others) as well as zeolitic minerals present a broad family of functionally diverse silicoaluminosilicates, which have shown promising effects on the binding of mycotoxins in gastrointestinal tract of animals, significantly decreasing their bioavailability and associated toxicities. The same (for example, HSCAS) are very useful for preventing aflatoxicosis in farm animals and for reducing aflatoxins concentrations in milk, but are not very effective for other mycotoxins as ochratoxin A, T-2 toxin, fumonisins, or deoxinivalenol (Ramos et al., 1996; Kubena et al., 1998; Stoev, 2008).

Several different approaches have been used to reduce ochratoxin A absorption, including the use of HSCAS, bentonite, charcoal, and cholestyramine. The addition of HSCAS (1%) and bentonite (1 and 10%) to a diet containing ochratoxin A had no effect on ochratoxin A concentration in swine blood, serum, tissues, and bile (Rotter et al., 1989). Benonite, however, can effectively absorb aflatoxins (Ramos et al., 1996).

The addition of 1% activated charcoal to the diet, in contrast, caused a slight decrease in the concentration of ochratoxin A in swine blood, whereas 10% charcoal decreased the concentration of ochratoxin A in blood, liver, kidney, spleen, and heart by 50–80% (Marquardt and Frolich, 1992). However, the supplementation of diet with activated charcoal was considered as an impractical method of reducing ochratoxin A toxicity in chicks or pigs that were continuously exposed to this mycotoxin, because of its high cost and the possibility to reduce minerals and vitamins in domestic animals (Rotter et al., 1989).

Cholestyramine, in contrast to the nonspecific absorbent discussed above, seems to be an effective absorbent of ochratoxin A, zearalenone, and fumonisins in the gastrointestinal tract of nonruminant animals. Cholestyramine is a commercial anion exchange resin that has been shown to reduce blood ochratoxin A concentrations by 50%, when it was included in 0.5% in a rat diet containing 1 ppm ochratoxin A (Marquardt and Frolich, 1992).

Another way to reduce toxicity of various mycotoxins is to elucidate a good understanding of the mechanisms of their toxicity. This approach includes an addition of specific antidotes or vitamins to the diet in order to prevent the specific toxic action of each mycotoxin (Stoev, 2008). For example, the ascorbic acid supplementation (300 mg/kg) of laying hen diet that contained 3 ppm ochratoxin A has been reported to have a good protective effect against ochratoxin action and can partially ameliorate its toxic effects (including the negative effect of ochratoxin (OTA) on the eggs production and on the weight of the eggs) (Haazele, 1992; Haazele et al., 1993).

The use of some other feed additives was also found to protect against the toxic effects of ochratoxin A, reducing significantly various farm losses from a decrease of weight gain in stock chicks (Stoev et al., 2000a, 2002d, 2004) and from a decrease of egg production in laying hens (Stoev, 2010b). For example, 5% total water extract of artichoke (*Cynara scolymus L*), prepared as a steam infusion from dried leaves of artichoke and given to chicks in concentration 5 mL/kg body weight (b.w.) via the feed or water, has been found to have a good protective effect against the toxic effect of ochratoxin A and to improve its fast elimination from animals (Stoev et al., 1999, 2000a, 2002d, 2004). Other similar antidotes against the toxic effects of ochratoxin A as Roxazyme-G (polyenzyme complement produced by the fungal genus "*Trichoderma*" given in concentration 0.2 g/kg feed) or Rosallsat (plant extract of bulbus *Allii Sativi* and seminum *Rosae caninae* given *per os* in 0.6 mL/kg body mass daily as a supplement to the feed) were also found to protect against various adverse toxic effects of ochratoxin A in chicks (Stoev et al., 1999, 2002d). Such antidotes, used as supplements to the feeds, could be used as a practical approach for safely utilizing of ochratoxin A-contaminated feed. In such a way, the rejection or condemnation of such feed will be avoided as well as there will be no need to eliminate this mycotoxin from the feed, if its contamination levels are similar to these encountered in the practice (normally up to 1–2 ppm; Stoev et al., 2002d).

In addition, the feeding of contaminated grain to animal species that are less susceptible to a particular mycotoxin is also a good measure to utilize mycotoxin-contaminated feeds. For example, feeding ochratoxin A-contaminated feeds to ruminants can safely utilize such feeds, because ruminants are less sensitive to this mycotoxin and are able to hydrolyze it in the rumen to its nontoxic form ochratoxin α (OTα) (Sreemannarayana et al., 1988).

Food/Feed Processing as a Method of Mycotoxin Decontamination

Processing can be defined as any chemical, biological, or physical treatment that is applied to a raw material to produce the final consumer product (Scudamore and Banks, 2004). That includes any procedure from dry and wet milling of grains, baking, extrusion, steaming, and brewing to feeding cereal-based complete feeds to animals to produce meat or milk. The stability of mycotoxins during processing may be affected by chemical or biological reactions and factors such as temperature, pH, moisture content, pressure, buffering conditions, and the presence of other constituents and enzymes. However, some important mycotoxins such as aflatoxins, ochratoxin A, deoxynivalenol,

Downloaded by [190.151.168.87] at 15:22 10 July 2014

Downloaded by [190.151.168.87] at 15:22 10 July 2014

zearalenone, and fumonisins can usually survive processing of raw cereals to some extent and thus occur in consumer foods but these may also undergo significant processing, so that the concentration of particular mycotoxin in a food item that reaches the consumer may be considerably lower than in the raw harvested crop. Therefore, appropriate management of the commodity during processing becomes a prime importance.

Wet and dry milling is an operation traditionally used for grain processing, but can be also used for mycotoxin decontamination of food in some cases (in zearalenone, fumonisins, aflatoxins, trichothecenes, and ochratoxin A contamination). This method separates the grain into different fractions and it is important to identify the fractions that remain toxic. In dry milling, mycotoxins are usually concentrated in the bran so that white flour from wheat (Osborne et al., 1996) or maize grits (e.g. Broggli et al., 2002) usually has significantly reduced mycotoxin levels. In wet milling of maize, significant amounts of most mycotoxins (especially fumonisins, zearalenone, and aflatoxins) are removed in the steep water and subsequent processing results in very low residues in maize starch (Bennett and Andersen, 1978) although high concentrations may remain in the gluten or germ fractions that are mainly used for livestock (Scudamore et al., 1998). In dry milling, aflatoxins concentrate in the bran and offal fractions of wheat and germs as well as in the outer layers of maize while being reduced in flour or corn grits (Brekke et al., 1975b). In wet milling, a large percentage of aflatoxins are similarly removed in the steep water (Bennett and Anderson, 1978). Similarly, ochratoxin A tends to be concentrated in the outer bran layers of cereals and this raises the possibility of redistribution during milling with the result that both reduction and increase in concentration can occur, depending on the milled fraction examined. Analysis of each milled fraction showed that high-ochratoxin A concentrations were present in the bran and offal fractions while those in the white flour fractions were much reduced unlike the wholemeal flour (Osborne et al., 1996). Therefore, ochratoxin A can be reduced during production of bread by elimination of the bran and offal components although the high temperature of baking had minimal effect on the final concentrations (Scudamore and Banks, 2004). After milling, higher concentrations of deoxynivalenol and zearalenone were found in bran and shorts, while lower concentrations were found in straight grade (white) flour (Scudamore and Banks, 2004). Dry milling results in a redistribution of fumonisins in the different fractions with reduced amounts in milling grits and flour and increased concentrations in bran and germ (Broggli et al., 2002). Industry information indicates that dry milling results in fumonisin-containing fractions in descending order of highest to lowest fumonisin levels as follow: bran, flour, meal, grits, and flaking grits (Broggli et al., 2002).

In some specific kinds of processings, as the treatment of corn with limewater in the manufacture of tortillas, levels of aflatoxins can be considerably decreased (Scott, 1998). Refining of oil successfully eliminates any extracted aflatoxins. Similarly, addition of sodium chloride (salt) during the cooking of unshelled peanuts under pressure can decrease significantly aflatoxins content, whereas vitamin C in apple juice can slowly decrease the content of patulin (Scott, 1998; Peraica et al., 2002).

Stability of aflatoxins to heat in processes such as baking (Stoloff and Trucksess, 1981), and extrusion (Martinez and Monsalve, 1989), depend on temperature and pH so that higher temperatures or alkaline processes such as the use of leavening agents or tortilla production can reduce content of aflatoxins (Price and Jorgensen, 1985; Abbas et al., 1988; De Arrola et al., 1988). The baking process itself is ineffective in destroying significant amounts of ochratoxin A. Deoxynivalenol is heat stable at 120°C, moderately stable at 180°C (deep frying), and decomposes within 30–40 minutes at 210°C (grilling; Kamimura, 1989). Zearalenone also survives baking (Scudamore and Banks, 2004). The fumonisins are unstable during roasting but comparatively stable during baking and canning and therefore can be considered as moderately stable compounds (Castelo et al., 1998). Ergot alkaloids are much less stable and are destroyed on baking bread up to 100% (Scott, 1998).

Breakdown by enzymes depends on the process but aflatoxins and most of other mycotoxins such as deoxynivalenol, zearalenone, and fumonisins can survive and occur in beer produced from maize or wheat (Scott and Lawrence, 1994; Scott, 1996; Scudamore and Banks, 2004). Malting of barley and brewing can partially destroy ochratoxin A but this does not happen for deoxynivalenol or zearalenone (Scott, 1996). Regardless of the circumstance that ochratoxin A is a relatively stable mycotoxin, under certain situations such as high temperatures and acid or alkaline conditions and in the presence of enzymes, some breakdown can occur. The fermentation by yeasts has been found to be effective in destroying patulin and rubratoxin B (Scott, 1998). Rotter et al. (1990) demonstrated that inoculation of barley with a *Lactobacillus* species followed by ensiling (decreasing of pH) can reduce the concentration of ochratoxin A by approximately 50%, but ensiling of corn does not usually destroy aflatoxins, zearalenone, or deoxynivalenol (Rotter et al., 1990).

While processing tends to reduce the concentration of mycotoxins, there are some circumstances that can increase their levels. Such circumstances are poor storage of intermediate materials or end products, which can lead to mold growth and mycotoxin formation. For example, wheat flour, if not stored properly could be contaminated by various storage mycotoxins such as ochratoxin A. On the other hand, the milling is a kind of processing, in which contamination levels of various mycotoxins often change in both directions, because mycotoxins are unequally distributed among the milled fractions reflecting how the same originally formed in each individual grain. In this context, the concentrations of mycotoxins are usually found to be higher in some fractions, commonly bran or germ, but reduced in flour (Scudamore and Banks, 2004).

Hygiene Control and Risk Assessment

The tracing of the fate of the mycotoxin at each stage of the process is required to optimize control and to reduce the

Downloaded by [190.151.168.87] at 15:22 10 July 2014

quantity of various mycotoxins reaching the consumer. With a view of eliminating or minimizing mycotoxins contents in the food supply, the primary objective should be to introduce effective agricultural practices and storage protocols that prevent their formation. However, complete removal of mycotoxins is not always possible. If the risk from a particular mycotoxin is considered as significant, legislation may be introduced together with some measures aimed to minimize consumer exposure. Obviously, it is very important to protect the consumer from the effects of a particular mycotoxin in the food supply when a risk assessment of the contaminant indicates that its level of exposure is likely to be unacceptable. As a rule, there are two main compounds taken into account in risk assessment: the toxicologic effect of particular mycotoxin and an estimate of the mycotoxin exposure of the consumer. If the risk assessment of a particular mycotoxin suggests a significant risk to the consumer, setting maximum permissible limits presents a powerful tool for its effective control in the food chain (Rosner, 1998). However, all limits must be supported by reliable and extensive research in order to avoid unnecessary restrictions and economic loss.

Maximum permissible limits have been set for some mycotoxins by international and national organizations. Therefore, acquisition of more information about the content of various mycotoxins during processing may allow more lenient limits for raw ingredients to be set in the cases when it can be clearly shown that there is a significant decrease through the food-processing chain. Moreover, a sound knowledge of processing techniques and how mycotoxins are destroyed can indicate those food products that are more susceptible to mycotoxins, which may suggest that the same should be monitored more frequently than the other products (FAO, 1988, 1997). This is very important for mycotoxins such as deoxynivalenol, zearalenone, and fumonisins in maize that can occur in very high concentrations in raw cereals, but the same are much reduced during processing. Any failure to take this into account could result in unnecessary penalizing of the cereal producers without increasing consumer safety. The balance between economic considerations and consumer risk should be also taken into account in such cases. Sometimes, a kind of processing such as the removal of bran from the food supply might lower human exposure to mycotoxins but such processing must be considered against the nutritional and health benefits provided by the final product. Another important question is, whether the consumer should consume wholemeal bread with its known health benefits or white bread in order to ensure less intake of particular mycotoxin as ochratoxin A (Scudamore and Banks, 2004).

Regulatory control of nephrotoxic mycotoxins in animal feeds in European countries has been summarized by van Egmond (van Egmond, 1989) and by Boutrif and Canet (Boutrif and Canet, 1998). On the other hand, EU decided on an official limit for OTA in cereals designed for direct consuming of about 5 μg OTA/kg, whereas for end consumer products and foods is 3 μg/kg (Rosner, 1998; Scudamore and Banks, 2004). The official limits for OTA in cereals in some countries can be seen in various FAO reports and commonly ranged between 2 μg/kg (Switzerland) and 20 μg/kg (Czech Republic), and rarely reached up to 50 μg/kg (Uruguay; Boutrif and Canet, 1998). Some limits were also introduced for the maximum allowed amount of aflatoxin B_1 in animal feeding stuffs (MAFF, 1982). Many countries in the world have limits or legal regulations for the effective control of some mycotoxins in foods or feeds (Rosner, 1998). How the limits for various mycotoxins are chosen in each country, and for which commodities, depend on several factors such as: the availability of toxicological data; the availability of data on the occurrence of mycotoxins in various commodities; the availability of methods of sampling and analysis; the inter-country trade implications; as well as the existence of sufficient food supply (Boutrif and Canet, 1998). The European Community has introduced maximum permissible limits for aflatoxins, ochratoxin A, and patulin in specific products and is actively involved in considering which other mycotoxins need regulation (WHO/FAO, 1995; FAO, 1997; Boutrif and Canet, 1998; Scudamore and Banks, 2004). Such statutory limits may have important implications in international trade especially if the export of a commodity may represent a significant percentage of trade earnings of particular country.

Any development of internationally harmonized regulatory control measures for mycotoxins in order to protect public health and promote fair trade at international level is of particular importance these days (Boutrif and Canet, 1998). On the other hand, it usually takes a lot of time to agree and implement internationally recognized limits so it may be useful to introduce guideline limits as temporary measure when a risk to health is considered to be significant. In this regard, JECFA (Joint FAO/WHO Expert Committee on Food Additives) has recently evaluated the most important mycotoxins (JECFA, 2001). The JECFA provides a mechanism for assessing the toxicity of additives, veterinary drugs, and contaminants such as mycotoxins. The safety evaluation for contaminants incorporates various steps of a formal health risk assessment approach (Boutrif and Canet, 1998). The toxicological evaluation carried out by JECFA normally results in the estimation of a provisional tolerable weekly intake (PTWI). In principle, the evaluation is based on the determination of no-observed-effect-level in toxicological studies, and the application of a safety factor. This approach for establishing maximum tolerated levels of mycotoxins in foods does not apply for toxins where carcinogenicity is the basis for concern as is the case with aflatoxins. In such cases, the necessary approach must be "as low as reasonably achievable" (ALARA principle) or as low as possible but technologically feasible and analytically detectable in the food ready for consumption (Rosner, 1998). In this regard, International Agency for Research on Cancer (IARC) has a program for the evaluation of carcinogenic effects of some mycotoxins on humans and according to it, mycotoxins can be distributed in group 1 (carcinogenic to humans as many aflatoxins), group 2A (probably carcinogenic to humans), group 2B (possibly carcinogenic to humans as fumonisins or ochratoxin A), group 3 (not classifiable as to its carcinogenicity to humans; Castegnaro and McGregor, 1998). Some revisions of the criteria for this classification and

Downloaded by [190.151.168.87] at 15:22 10 July 2014

reclassification of some mycotoxins are made periodically by IARC.

The quantitative evaluation of the degree of exposure and risk likely to occur in the population are incorporated in the JECFA work (Boutrif and Canet, 1998). In the last years, various meetings of the JECFA evaluated the hazards of specific mycotoxins, and the respective reports issued after such meetings are widely distributed (JECFA, 2001; Scudamore and Banks, 2004). On the other hand, the question of establishing maximum levels of mycotoxins and other chemical contaminants in foods and various commodities is considered by the Codex Committee on Food Additives and Contaminants in consultation with the Codex Commodity Committee, but the final decision is usually taken by the Codex Alimentarius Commission (CAC). The conclusions are based on the scientific evaluation made by JECFA and other relevant expert meetings (Boutrif and Canet, 1998).

Because of the increase of scientific reports on ochratoxin A contamination in beverages and many kinds of food, the JECFA assessed the available information and proposed 112 ng/kg b.w. as a PTWI for ochratoxin A (WHO, 1991). That corresponds to about 16 ng/kg b.w. per day. Having in mind the strong carcinogenic effect of this mycotoxin, its PTWI was subsequently decreased to 100 ng/kg b.w., which corresponds to about 14 ng/kg b.w. per day (JECFA, 1997). With a big worry we have to mention, that the calculated average daily intakes of humans in the endemic for Balkan nephropathy areas in Bulgaria from 26.8 ng/kg b.w. for 1988, 36.4 ng/kg b.w. for 1989, and 34.2 ng/kg b.w. for 1990, respectively (Stoev, 1998, 2008), exceeds strongly the PTWI (100 ng/kg b.w. or 14 ng/kg b.w. per day), proposed by the JECFA (JECFA, 1997). JECFA bases its calculation of the tolerable intake mainly on the nephrotoxicity of ochratoxin A and does not address the question of the toxin's carcinogenic effect. Kuiper-Goodman and Scott, on the other hand, regard the carcinogenic effect as the most important effect and base their analysis on this. Tolerable daily intakes (TDIs), depending on the method used and calculated on the base of carcinogenic effect of ochratoxin A, range from 0,2 to 4,2 ng/kg b.w. If the maximum TDI of ochratoxin A is about 5 ng/kg b.w. (Kuiper-Goodman and Scott, 1989), it can be seen that the average daily intake in humans from endemic areas in Bulgaria exceeds strongly the TDI calculated on the base of cancerogenic effect of ochratoxin A (Stoev, 1998, 2008).

In regard to various animal products, there are regulations in Denmark, according to which all "enlarged and mottled kidneys" are investigated for residues of ochratoxin A at slaughter time and all carcasses, whose kidneys contained ochratoxin A levels above 10 μg/kg are condemned (Boutrif and Canet, 1998). These regulations are not very safe and satisfactory, because macroscopic changes in kidneys can be found only after 1–3 months of ochratoxin A exposure via the feeds (Krogh et al., 1973; Stoev et al., 2001). In spite of the toxicological investigations of such kidneys, ochratoxin A-contaminated pork may enter the human food chain and thus represents a potential public health hazard (Stoev et al., 1998c; Stoev, 2008).

Because of the assumption that mycotoxins and especially ochratoxin A are involved in etiology of Balkan endemic nephropathy (Krogh, 1972; Stoev, 1998, 2008), the exposure of humans to this very hazardous toxin from pork or chicken meat (by the way "feed—pork/chicken—food") need to be prevented. A much better procedure for preventing the exposure of humans to this mycotoxin from meat would be a toxicological analysis of a few blood samples of pigs/chicken from risky farms suspected of MN several weeks (in pigs) or several days (in chicken) before slaughter and a change in the feed source for a week (pigs) or for 2–3 days (chicken), if it is necessary. Also, the period of feed deprivation of pigs/chicken before slaughter could be prolonged (Stoev et al., 1998c, 2002a; Stoev, 2008). Because of the short half-life of ochratoxin A in pigs (72–120 hours) and especially in chickens (4 hours; NNT, 1991), its concentration in blood and various tissues quickly decreases after changing the feed source or after prolonging the period of feed deprivation before slaughtering. Thus, the loss of condemnation of pig/chicken production would be prevented and a better procedure (than toxicological investigations of "enlarged mottled kidneys" accepted in Denmark) would be realized for preventing the exposure of humans to ochratoxin A from meat. The preventive measures in already slaughtered chicks could include condemnation and removing of the kidneys and liver, where ochratoxin A is accumulated (Stoev, 2008; Stoev et al., 2002a).

It is also expected that mixtures of mycotoxins would have at least an additive, if not synergistic toxic effect (Stoev, 2008). A potent synergistic effect was found between ochratoxin A and penicillic acid, mycotoxins produced by the same ochratoxinogenic fungi, when the same mycotoxins were given simultaneously to pigs and chickens (Micco et al., 1991; Stoev et al., 1999, 2000a, 2001, 2004). The presence of multiple toxins in various foods presents new concerns since toxicological information on the effects of simultaneous exposure is still very limited (Stoev, 2008; Stoev et al., 2010a, 2010b). However, in a diverse human diet, exposure will be to multiple toxins at a low concentration on an intermittent rate over long periods of time (Stoev, 2008). The ultimate effect of this constant exposure is still unknown, although there is some evidence of strong synergistic or additive effect between some mycotoxins as ochratoxin A, penicillic acid, fumonisin B_1, and citrinin (Micco et al., 1991; Stoev et al., 2001, 2004) or between ochratoxin A and fumonisin B_1 (Klaric et al., 2007; Stoev et al., 2010a, 2010b, 2012). The simultaneous exposure to those mycotoxins might be an important factor for development of chronic renal diseases in animals and humans, especially after long-term exposure as the same mycotoxins were recently found in high-contamination levels (especially fumonisin B_1 and penicillic acid) in most of the feeds originated from farms with mycotoxic porcine or avian nephropathy in Bulgaria and South Africa (Stoev et al., 2010a, 2010b).

Mycotoxins are natural contaminants, and therefore, human exposure cannot be completely prevented. Some mycotoxins as aflatoxin B_1, zeqralenone, and ochratoxin A would represent safety hazard twice, due to the possible transmission in milk of lactating cows of either parent toxin of toxic metabolites as

aflatoxin M_1 or α zearalenol (Galtier, 1998) as well as due to the possible transmission of many mycotoxins in eggs or meat (Dailey et al., 1980; Galtier, 1998). Obviously, the development of national programs for the monitoring, prevention, and control of mycotoxin contamination based on the assessment of the situation in each individual country is not sufficient these days. The factors which are compromising the quality of the products of the commodity system, and leading to the production of molds and mycotoxins, could be evaluated by the implementation of: carefully designed surveillance studies, and modern internationally recognized biomonitoring methods measuring the exposure to mycotoxins of individuals. The need for networking for both dissemination of information and staff training at regional and international basis are often identified as important activities to be sustained in the future.

Whenever efforts are made to improve the quality of foods and feeds, it should be clearly established that there is a definite need for a better quality product, and that the community is prepared to bear any associated increase in the cost of the improved commodity.

Downloaded by [190.151.168.87] at 15:22 10 July 2014

ACKNOWLEDGMENTS

This research has been financially supported in part through European Community under Marie Curie Outgoing International Fellowship under 6th framework, Department of Science and Technology in South Africa, UK Royal Society Joint Project with Central and Eastern Europe, NATO grant, and Foundation of Ministry of science and education of Bulgaria via 5 Research projects.

REFERENCES

Abbas, H. K., Mirocha, C. J., Rosiles, R. and Carvajal, M. (1988). Effect of Tortilla-preparation process on aflatoxin B_1 and B_2 in corn. *Mycotox. Res.* **4**:33–36

Allcroft, R. and Carnaghan, R. B. A. (1962). Groundnut toxicity. Aspergillus flavus toxin (aflatoxin) in animal products: Preliminary communication. *Vet. rec*. **74**:863–864

Basappa, S. C. and Shantha, T. (1996). Methods for detoxification of aflatoxins in foods and feeds—A critical appraisal. *J. Food Sci. Technol.* **33**:95–107.

Bata, A., Rafai, P. and Kovacs, S. (2001). Investigation and a new evaluation method of the resistance of maize hybrids grown in Hungary to *Fusarium* moulds. *Phytopathology.* **149**:107–111.

Bennett, G. A. and Anderson, R. A. (1978). Distribution of aflatoxin and/or zearalenone in wet-milled corn products: A review. *J. Agric. Food Chem.* **26**:1055–1060

Bennett, J. W. and Bentley, R. (1999). Pride and prejudice: The story of ergot. *Perspect. Biol. Med.* **42**:333–355.

Betina, V. (1989). Biological effects of mycotoxins. **In**: Mycotoxins: Chemical, Biological and Environmental Aspects, vol **9**, pp. 42–58. Betina, V., Ed., Elsevier Science Publishers, Amsterdam.

Bezuidenhout, S. C., Gelderblom, W. C. A, Gorst-Allman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G. and Vleggaar, R. (1988). Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc. Chem. Comm.* **11**:743–745

Boonchuvit, B., Hamilton, P. B. and Burmeister, H. R. (1975). Interaction of T-2 toxin with *Salmonella* infection in chickens. *Poultry. Sci.* **54**:1693–1696.

Boutrif, E. and Canet, C. (1998). Mycotoxin prevention and control: FAO programes. *Revue. Med. Vet.* **149**:681–694

Brekke, O. L., Peplinski, A. J. and Griffin, E. L. Jr. (1975a). Cleaning trials for corn containing aflatoxin. *Cereal Chem.* **52**:198–204.

Brekke, O. L., Peplinski, A. J., Nelson, G. E. N. and Griffin, E. L. Jr. (1975b). Pilot-scale dry milling of corn containing aflatoxin. *Cereal. Chem.* **52**:205–211

Broggli, L. E., Resnik, S. L., Pacin, A. M., Gonzalez, H. H. L., Cano, G. and Taglieri, D. (2002). Distribution of fumonisins in dry-milled corn fractions in Argentina. *Food Addit. Contam.* **19**:465–469.

Bucci, T., Howard, P., Tolleson, W., Laborde, J. and Hansen, D. (1998). Renal effects of fumonisin mycotoxins in animals. *Toxicol. Pathol.* **26**:190–194.

CAST (Council for Agricultural Science and Technology). (1989). Mycotoxins: Economic and Health Risk. Task Force Report 116, pp 1–91, Ames, Iowa.

Castegnaro, M. and McGregor, D. (1998). Carcinogenic risk assessment of mycotoxins. *Revue. Med. Vet.* **149**:671–678.

Castelo, M. M., Sumner, S. S. and Bullerman, L. B. (1998). Stability of fumonisins in thermally processed corn products. *J. Food Protect.* **61**:1030–1033.

Chelack, W. S., Borsa, J., Marquardt, R. R. and Frohlich, A. A. (1991a). Role of competitive microbial flora in the radiation induced enhancement of ochratoxin production by *Aspergillus alutaceus* var alutaceus NRRL-3174. *Appl. Environ. Microbiol.* **57**:2492–2496.

Chelack, W. S., Borsa, J., Szekely, J. G., Marquardt, R. R. and Frohlich, A. A. (1991b). Variants of *Aspergillus alutaceus* var alutaceus formely *Aspergillus ochraceus*, with altered ochratoxin A production. *Appl. Environ. Microbiol.* **57**:2487–2491.

Chelkowski, J., Golinski, P., Godlewska, B., Radomyska, W., Szebiotko, K. and Wiewiorowska, M. (1981). Mycotoxins in cereal grains. Part IV. Inactivation of ochratoxin A and other mycotoxins during ammoniation. *Nahrung.* **25**:631–637.

Chelkowski, J. and Perkowski, J. (1992). Mycotoxins in cereal grain (part 15). Distribution of deoxynivalenol in naturally contaminated wheat kernels. *Mycotox. Res.* **8**:27–30.

Chu, F. S. and Li, G. Y. (1994). Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions of high incidences of oesophageal cancer. *Appl. Environ. Microb.* **60**:847–852.

Conkova, E., Laciakova, A., Kovac, G. and Seidel, H. (2003). *Fusarial* toxins and their role in animal diseases. *Vet. J.* **165**:214–220.

Cooray, R. and Jonsson, P. (1990). Modulation of resistance to mastitis pathogens by pre-treatment of mice with T-2 toxin. *Food Chem. Toxicol.* **28**:687–692.

Coulombe, R. A., Guarisco, J. A., Klein, P. J. and Hall, J. O. (2005). Chemoprevention of aflatoxicosis in poultry by dietary butylated hyodroxytoluene. *Anim. Feed Sci. Tech.* **121**:217–225.

Dailey, R. E., Reese, R. E. and Brouwer, E. A. (1980). Metabolism of 14^C-zearalenone in laying hens. *J. Agric. Food Chem.* **28**:286–291.

De Arrola, M., Del, C., de Porres, E., de Cabrera, S., de Zepeda, M. and Rolz, C. (1988). Aflatoxin fate during alkaline cooking of corn for tortilla preparation. *J. Agric. Food Chem.* **36**:530–533.

Dirheimer, G. (1998). Recent advances in the genotoxicity of mycotoxins. *Revue. Med. Vet.* **149**:605–616.

Doko, M. B., Rapior, S., Visconti, A. and Schjoth, J. E. (1995). Incidence of levels of fumonisin contamination in maize by genotypes grown in Europe and Africa. *J. Agric. Food Chem.* **43**:429–434.

Dowell, F. E., Dorner, J. W., Cole, R. J. and Davidson, J. I. (1990). Aflatoxin reduction by screening farmers stock peanuts. *Peanut. Sci.* **17**:6–8.

Dutton, M. F. (1996). Fumonisins, mycotoxins of increasing importance: Their nature and their effects. *Pharm. Ther.* **70**:137–161.

Dwivedi, P. and Burns, R. B. (1985). Immunosuppressive effects of ochratoxin A in young turkeys. *Avian. Pathol.* **14**:213–225.

Elissalde, M. H., Ziprin, R. L., Huff, W. E., Kubena, L. F. and Harvey, R. B. (1994). Effect of ochratoxin A on *Salmonella*-challenged broiler chicks. *Poul. Sci.* **73**:1241–1248.

FAO. (1997). Food and Nutrition Paper No. 64. Worlwide Regulations for Mycotoxins, Rome, Italy.

Downloaded by [190.151.168.87] at 15:22 10 July 2014

FAO. (1988). Distribution of mycotoxins: an analysis of worldwide commodities data. The second Joint FAO/WHO/UNEP International Conference on Mycotoxins. Bangkok, Thailand, 28 September–2 October 1987, Rome, Italy, p. 28.

FAO. (2002). Manual on the Application of the HACCP System in Mycotoxin Prevention and Control. Joint FAO/WHO Food Standards Programme FAO, Rome, Italy.

Friends, D. W., Trenholm, H. L., Thompson, B. K., Hartin, K. E., Fiser, P. S., Asem, E. K. and Tsang, B. K. (1999). The reproductive efficiency of gilts fed very low levels of Zea. *Can. J. Anim. Sci.* **70**:635–645.

Frohlich, A. A., Marquardt, R. R. and Ominski, K. R. (1991). Ochratoxin A as a contaminant in the human food chain: A Canadian perspective. In: Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours, M. Castegnaro, R. Pleština, G. Dirheimer, I. N. Chernozemsky, and H. Bartsch (eds)., publication 115, pp. 139–144, IARC, Lyon, France.

Fukata, T., Sasai, K., Baba, E. and Arakawa, A. (1996). Effect of ochratoxin A on *Salmonella typhimurium*-challenged layer chickens. *Avian. Dis.* **40**(4):924–926.

Gabbai, A., Lisbonne, M. and Pourquier. P. (1951). Ergot poisoning at Pont St. Esprit. *Brit Med. J.* **Sept 15**:650–651.

Galtier, P. (1998). Biological fate of mycotoxins in animals. *Revue Med. Vet.* **149**:549–554.

Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Theil, P. G., Horak, R. M., Vleggaar, R. and Kriek, N. P. J. (1988). Fumonisins—Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microb.* **54**:1806–1811.

Gelderblom, W., Marasas, W. and Farber, E. (1992). The cancer initiating potential of the fumonisin B mycotoxins. *Carcinogenesis.* **13**:433–437.

Gupta, S., Jindal, N., Khokhar, R. S., Asrani, R. K., Ledoux, D. R. and Rottinghaus, G. E. (2008). Individual and combined effects of ochratoxin A and *Salmonella enterica* serovar *Gallinarum* infection on pathological changes in broiler chickens. *Avian. Pathol.* **37**:265–272.

Haazele, F. M. (1992). Response to Dietary Ascorbic Acid Supplementation in Laying Hens: Effects of Exposure to High Temperature and Ochratoxin Ingestion. Ph.D. Thesis, University of Manitoba, Winnipeg, MB, Canada.

Haazele, F. M., Guenter, W., Marquardt, R. R. and Frohlich, A. A. (1993). Benefical effects of dietary ascorbic acid supplement on hens subjected to ochratoxin A toxicosis under normal and high ambient temperatures. *Can. J. Anim. Sci.* **73**:149–157.

Hgindu, A., Johnson, B. A. and Kenya, P. R. (1982). An outbreak of acute hepatitis by aflatoxin poisoning in Kenya. *Lancet.* **319**:1346–1348.

Howard, P., Warbritton, A., Voss, K., Lorenzen, R., Thurman, J., Kovach, R. and Bucci, T. (2001). Compensatory regeneration as a mechanism for renal tubule carcinogenesis of fumonisin B_1 in F344/N/Nctr BR rat. *Environ. Health Persp.* **109**:309–314.

Huff, W. E. and Ruff, M. D. (1982). *Eimeria acervulina* and *Eimeria tenella* infections in ochratoxin A-compromised broiler chickens. *Poult. Sci.* **61**:685–692.

IPCS (International Programme on Chemical Safety). (1990). Selected mycotoxins: Ochratoxins, trichothecenes, ergot. International Program of Chemical Safety (IPCS). Environmental Health Criteria No. 105, WHO, Geneva.

JECFA. (1997). Toxicological evaluation of certain food additives, WHO Food Additives Series. 49th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Geneva.

JECFA. (2001). Evaluation of certain mycotoxins that may contaminate food. 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Geneva.

Kamimura, H. (1989). Removal of mycotoxins during food processing. **In**: Mycotoxins and Phytotoxins'88, pp. 169–176. Natori, S., Hashimoto, K. and Ueno, Y., Eds., Elsevier Science Publishers, B. V., Amsterdam.

Klaric, M. S., Rumora, L., Ljubanovic, D. and Pepeljnjak, S. (2007). Cytotoxicity and apoptosis induced by fumonisin B_1, beauvericin and ochratoxin A in porcine kidney PK15 cells: Effects of individual and combined treatment. *Arch. Toxicol.* **82**:247–255.

Koynarski, V., Stoev, S., Grozeva, N., Mirtcheva, T., Daskalov, H., Mitev, J. and Mantle, P. (2007). Experimental coccidiosis provoked by *Eimeria acervulina* in chicks simultaneously fed on ochratoxin A contaminated diet. *Res. Vet. Sci.* **82**:225–231.

Krogh, P. (1972) Mycotoxic porcine nephropathy: a possible model for Balkan endemic nephropathy. In: Proceedings of the Second International Symposium on Endemic Nephropathy; Puchlev, A. (ed); Publishing House of the Bulgarian Academy of Sciences: Sofia, Bulgaria, pp. 266–270.

Krogh, P., Hald, B. and Pederson, J. (1973). Occurrence of ochratoxin A and citrinin in cereals associated with mycotoxic porcine nephropathy. *Acta Path. Mcrobiol. Scand Sect. B.* **81**:689–695.

Kubena, L. F., Harvey, R. B., Bailey, R. H., Buckley, S. A. and Rottinghaus, G. E. (1998). Effects of a hydrated sodium calcium aluminosilicate (T-Bind^{TM}) on mycotoxicosis in young broiler chickens. *Poult. Sci.* **77**:1502–1509.

Kuiper-Goodman, T. and Scott, P. M. (1989). Risk assessment of the mycotoxin ochratoxin A. *Biomed. Environ. Sci.* **2**:179–248.

Kumar, A., Jindal, N., Shukla, C. L., Pal, Y., Ledoux, D. R. and Rottinghaus, G. E. (2003). Effect of ochratoxin A on *Escherichia coli*-challenged broiler chicks. *Avian. dis.* **47**:415–424.

Laciakova, A., Cabadaj, R., Conkova, E. and Pastorova, B. (1998), Degradation of ochratoxin A by feed additives. *Revue Med. Vet.* **149**:567.

Leitao, J., Bailly, J. R. and de Saint Blanquant, G. (1990). Action of phosphine (PH_3) on production of sterigmatocystin by various fungal strains isolated from foodstuffs. *Food Addit. Contam.* **7**:26–28.

Madsen, A., Hald, B. and Mortensen, H. P. (1983). Feeding experiments with ochratoxin A contaminated barley for bacon pigs-3. Detoxification by ammoniation heating + NaOH, or autoclaving. *Acta Agric. Scand.* **33**:171–175.

Marasas, W. F. O. (2001). Discovery and occurrence of the fumonisins: A historical perspective. *Environ. Health Persp.* **109**:239–243.

Marasas, W. F. O., Kellerman, J. S., Pienaar, J. G. and Naude, T. W. (1976). Leukoencephalomalacia: A mycotoxicosis of Equidae caused by *Fusarium moniliforme* Sheldon. *Onderstepoort J. Vet. Res.* **43**:113–122.

Marasas, W. F. O., Jaskiewics, K., Venter, F. S. and van Schalkwyk, D. J. (1988). *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei. *S. Afr. Med. J.* **74**:110–114.

Marquardt, R. R. and Frolich, A. A. (1992). A review of recent advances in understanding ochratoxicosis. *J. Anim. Sci.* **70**:3968–3988.

Martinez, A. J. and Monsalve, C. (1989). Aflatoxin occurrence in 1985–86 corn from Venezuela and its destruction by the extrusion process. **In**: Biodeterioration Research 2, pp. 251–259. O'Rear, C. E. and Llewellyn, G. C., Eds., Plenum Press, New York.

Micco, C., Miraglia, M., Onori, R., Libanori, A., Brera, C., Mantovani, A. and Macri, C. (1991). Effect of combined exposure to ochratoxin A and penicillic acid on residues and toxicity in broilers. *La Ravista della Societa Italiana di Scienza dell'Allimentazione.* **20**:101–108.

Ministry of Agriculture, Fisheries and Food (MAFF). (1982). The Fertilisers and Feedingstuffs (Amendment) Regulations 1982. SI No 386.

Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H. Jr., Rothman, K. J. and Hendricks, K. A. (2006). Exposure to fumonisins and the occurrence of neural tube defects along the Texas-Mexico border. *Environ. Health Persp.* **114**:237–241.

Muller, H. M. (1983). A survey of methods of decontaminating mycotoxins. I. Physical methods. *Anim. Res. Develop.* **18**:70–96.

Nordic Working Group on Food Toxicology and Risk Evaluation (NNT). (1991). Health Evaluation of Ochratoxin A in Food Products. Nordiske Seminar- og Arbeidsrapporter 1991:545; Nordic Council of Ministerrs, Copenhagen, Denmark, pp. 1–29.

Osborne, B. G., Ibe, F. I., Brown, G. L., Patagine, F., Scudamore, K. A., Banks, J. N. and Hetmanski, M. T. (1996). The effects of milling and processing on wheat contaminated with Ochratoxin A. *Food Addit. Contam.* **13**:141–153.

Oswald, I. P. and Comera, C. (1998). Immunotoxicity of mycotoxins. *Revue, Med. Vet.* **149**:585–590.

Pelletier, M. J. and Reizner, J. R. (1992). Comparison of fluorescence sorting and colour sorting for the removal of aflatoxin from large group of peanuts. *Peanut. Sci.* **19**:15–20.

Peraica, M., Domijan, A.-M., Jurjevic, Z. and Cvjetkovic, B. (2002). Prevention of exposure to mycotoxins from food and feed. *Arch. Ind. Hyg. Toxicol.* **53**:229–237.

Price, R. L. and Jorgensen, K. V. (1985). Effects of processing on aflatoxin levels and on mutagenic potential of tortillas made from naturally contaminated corn. *J. Food Sci.* **50**:347–349.

Ramasamy, S., Wang, E., Hennig, B. and Merrill, A. H. (1995). Fumonisin B_1 alters shingolipid metabolism and disrupts the barrier function of endothelial cells in culture. *Toxicol. Appl. Pharmacol.* **133**:343–348.

Ramos, A. J., Fink-Gremmels, J. and Hernandes, E. (1996). Prevention of toxic effects of mycotoxins by means of non-nutritive absorbent compounds. *J. Food Protect.* **59**:631–641.

Reid, L. M., Zhu, X. and Ma, B. L. (2001). Crop rotation and nitrogen effects on maize susceptibility to *Giberella* (*Fusarium graminearum*) ear rot. *Plant Soil.* **237**:1–14.

Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Shephard, G. S. and van Schalkwyk, D. J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human oesophageal cancer in Transkei. *Phytopathology.* **82**:353–357.

Rosner, H. (1998). Mycotoxin regulations: An update. *Revue. Med. Vet.* **149**:679–680.

Rotter, R. G., Frohlich, A. A. and Marquardt, R. R. (1989). Influence of dietary charcoal on ochratoxin A toxicity in Leghorn chicks. *Can J. Vet. Res.* **53**:449–453.

Rotter, R. G., Marquardt, R. R., Frohlich, A. A. and Abramson, D. (1990). Ensiling as a means of reducing ochratoxin A concentrations in contaminated barley. *J. Sci. Food Agric.* **50**:155–156.

Samarajeewa, U. (1991). In situ degradation of mycotoxins by physical methods. **In**: Mycotoxins and Animal Foods, pp. 785–796. Smith, J. E. and Henderson, R. S., Eds., CRC press, Boca Raton, Florida.

Schneider, D. J., Miles, C., Garthwaite, I., van Halderen, A., Wessels, J. C. and Lategan, H. J. (1996). First report of ergot-alkaloid toxicity in South Africa. *Onderstepoort J. Vet. Res.* **63**:97–108.

Scott, P. M. (1996). Mycotoxins transmitted into beer from contaminated grain during brewing. *J. AOAC Int.* **79**:875–882.

Scott, P. M. (1998). Industrial and farm detoxification processes for mycotoxins. *Revue Med. Vet.* **149**:543–548.

Scott, P. M. and Lawrence, G. A. (1994). Analysis of beer for fumonisins. *J. Food Protect.* **58**:1379–1382.

Scudamore, K. A. and Banks, J. N. (2004). The fate of mycotoxins during cereal processing, In: Meeting the mycotoxin menace, Barug, D., van Egmond, H., López-García, R., van Osenbruggen, T. and Visconti, A. (eds), Proceedings of the 2nd World Mycotoxin Forum, Nordwijk, Netherlands, 17–18 February 2003, Wageningen Academic Publishers, pp. 165–181.

Scudamore, K. A., Nawaz, S. and Hetmanski, M. T. (1998). Mycotoxins in ingredients of animal feeding stuffs: II. Determination of mycotoxins in maize and maize products. *Food Addit. Contam.* **15**:30–55.

Scudamore, K. A., Patel, S. and Breeze, V. (1999). Surveillance of stored grain from the 1997 harvest in the United Kingdom for ochratoxin A. *Food Add. Contam.* **16**:281–290.

Sharma, R. P. (1993). Immunotoxicity of mycotoxins. *J. Dairy Sci.* **76**:892–897.

Shibamoto, T. and Bjeldanes, L. F. (1993). Fungal toxins occurring in foods. In: Introduction to Food Toxicology. Chapter 6, Academic Press, Elsevier Inc., San Diego, pp. 97–116.

Shier, W. T. (1998). Estrogenic mycotoxins. *Revue Med. Vet.* **149**:599–604.

Sibanda, L., Marovatsanga, L. T. and Pestka, J. J. (1997). Review of mycotoxin work in sub-Saharan Africa. *Food Control.* **8**:21–29.

Sreemannarayana, O., Frohlich, A. A., Vitti, T. G., Marquardt, R. R. and Abramson, D. (1988). Studies of the tolerance and disposition of ochratoxin A in young calves. *J. Anim. Sci.* **66**:1703–1711.

Steiner, W. E., Rieker, R. H. and Battaglia, R. (1988). Aflatoxin contamination in dried figs: Distribution and association with fluorescence. *J. Agric. Food Chem.* **36**:88–91.

Stoev, S. D. (1998). The role of Ochratoxin A as a possible cause of Balkan endemic nephropathy and its risk evaluation, *Vet. Hum. Toxicol.* **40**: 352–360.

Stoev, S. (2007). Food Safety and Some Foodborne Mycotoxicoses, Vet Africa 2007 Congress, 27–28 July, 2007, Johannesburg, South Africa.

Stoev, S. D. (2008). Complex etiology, prophylaxis and hygiene control in mycotoxic nephropathies in farm animals and humans, special issue "Mycotoxins: Mechanisms of toxicological activity—treatment and prevention", section "Molecular Pathology." *Int. J. Mol. Sci.* **9**:578–605.

Stoev, S. D. (2010a). Studies on carcinogenic and toxic effects of ochratoxin A in chicks. *Special Issue "Ochratoxins". Toxins*, **2**:649–664.

Stoev, S. D. (2010b). Studies on some feed additives and materials giving partial protection against the suppressive effect of ochratoxin A on egg production of laying hens. *Res. Vet. Sci.* **88**:486–491.

Stoev, S. D., Anguelov, G., Ivanov, I. and Pavlov, D. (2000a). Influence of ochratoxin A and an extract of artichoke on the vaccinal immunity and health in broiler chicks. *Exp. Toxicol. Pathol.* **52**:43–55.

Stoev, S. D., Angelov, G., Pavlov, D. and Pirovski, L. (1999). Some antidotes and paraclinical iInvestigations in experimental intoxication with ochratoxin A and penicillic acid in chicks. *Vet. Arhiv.* **69**:179–189.

Stoev, S. D., Daskalov, H., Radic, B., Domijan, A. and Peraica, M. (2002a). Spontaneous mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxin aetiology. *Vet. Res.* **33**:83–94.

Stoev, S. D., Denev, S., Dutton, M. F., Njobeh, P. B., Mosonik, J. S., Steenkamp, P. A. and Petkov, I. (2010b). Complex etiology and pathology of mycotoxic nephropathy in South African pigs. *Mycotox. Res.* **26**:31–46.

Stoev, S. D., Djuvinov, D., Mirtcheva, T., Pavlov, D. and Mantle, P. (2002d). Studies on some feed additives giving partial protection against ochratoxin A toxicity in chicks. *Toxicol. Lett.* **135**:33–50.

Stoev, S. D., Dutton, M., Njobeh, P., Mosonik, J. and Steenkamp, P. (2010a). Mycotoxic nephropathy in Bulgarian pigs and chickens: Complex aetiology and similarity to Balkan enedemic nephropathy. *Food Addit. Contam. A.* **27**:72–88.

Stoev, S. D., Goundasheva, D., Mirtcheva, T. and Mantle, P. G. (2000b). Susceptibility to secondary bacterial infections in growing pigs as an early response in ochratoxicosis. *Exp. Toxicol. Pathol.* **52**:287–296.

Stoev, S. D., Grozeva, N. and Hald, B. (1998b). Ultrastructural and toxicological investigations in spontaneous cases of porcine nephropathy in Bulgaria. *Vet. Arhiv.* **68**:39–49.

Stoev, S.D., Gundasheva, D., Zarkov, I., Mircheva, T., Zapryanova, D., Denev, S., Mitev, Y., Daskalov, H., Dutton, M., Mwanza, M. and Schneider, Y-J. (2012). Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and fumonisin B1. *Exp. Toxicol. Pathol*. **64:** 733–741

Stoev, S. D., Hald, B. and Mantle, P. (1998a). Porcine nephropathy in Bulgaria: A progressive syndrome of complex of uncertain (mycotoxin) etiology. *Vet. Rec.* **142**:190–194.

Stoev, S. D., Koynarsky, V. and Mantle, P. G. (2002b). Clinicomorphological studies in chicks fed ochratoxin A while simultaneously developing coccidiosis. *Vet. Res. Commun.* **26**:189–204.

Stoev, S. D., Paskalev, M., MacDonald, S. and Mantle, P. G. (2002c). Experimental one-year ochratoxin A toxicosis in pigs. *Exp. Toxicol. Pathol.* **53**:481–487.

Stoev, S. D., Stefanov, M., Denev, S., Radic, B., Domijan, A.-M. and Peraica, M. (2004). Experimental mycotoxicosis in chickens induced by ochratoxin A and penicillic acid and intervention by natural plant extracts. *Vet. Res. Commun.* **28**:727–746.

Stoev, S. D., Stoeva, J., Anguelov, G., Hald, B., Creppy, E. E. and Radic, B. (1998c). Haematological, biochemical and toxicological investigations in spontaneous cases with different frequency of porcine nephropathy in Bulgaria. *J. Vet. Med. Series A.* **45**:229–236.

Stoev, S. D., Vitanov, S., Anguelov, G., Petkova-Bocharova, T. and Creppy, E. E. (2001). Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and penicillic acid. *Vet. Res. Commun.* **25**:205–223.

Stoloff, L. and Trucksess, M. W. (1981). Effect of boiling, frying, and baking on recovrery of aflatoxin from naturally contaminated corn grits or cornmeal. *J. Assocf. Off. Anall. Chem.* **64**:678–680

Sydenham, E. W., Thiel, P. G., Marasas, W. F. O., Shepard, G. S., van Schalkwyk, D. J. and Koch, K. R. (1990). Natural occurrence of some *Fusarium*

Downloaded by [190.151.168.87] at 15:22 10 July 2014

mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei, southern Africa. *J. Agric. Food Chem.* **38**:1900–1903.

Tai, J. H. and Pestka, J. J. (1988). Impaired murine resistance to *Salmonella typhimurium* following oral exposure to the trichothecene T-2 toxin. *Food Chem. Toxicol.* **26**:691–698.

Tong, C. and Draughon, H. (1985). Inhibition by an antimicrobial food additives of ochratoxin A production by *Aspergillus sulfureus* and *Penicillium viridicatum*. *Appl. Environ. Microbiol.* **49**:1407–1411.

Van Egmond, H. P. (1989). Current situation on regulations for mycotoxins: Overview of tolerances and status of standard methods of sampling and analysis. *Food Addit. Contam.* **6**:139–188.

van Waes, J. G., Starr, L., Maddox, J., Aleman, F., Voss, K. A., Wilberding, J. and Riley, R. T. (2005). Maternal fumonisin exposure and risk for neural tube defects: Mechanisms in an in vivo mouse model. *Birth Defects Res. A. Clin. Mol. Teratol.* **73**:487–497.

WHO. (1991). Evaluation of certain food additives and contaminants. 37th Report of the Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series 806), World Health Organization, Geneva, pp. 29–31.

WHO/FAO. (1995). Report of the Joint FAO/WHO Expert Consultation on Application of Risk Analysis to Food Standards Issues. WHO/FNU/FOS 95.3, Geneva.

Ziprin, R. I., Holt, P. S. and Mortensen, R. (1987). T-2 toxin effects on the serum amyloid P-component (SAP) response of *Listeria monocytogenes* and *Salmonella typhimurium* infected mice. *Toxicol. Lett.* **39**: 177–184.

Downloaded by [190.151.168.87] at 15:22 10 July 2014