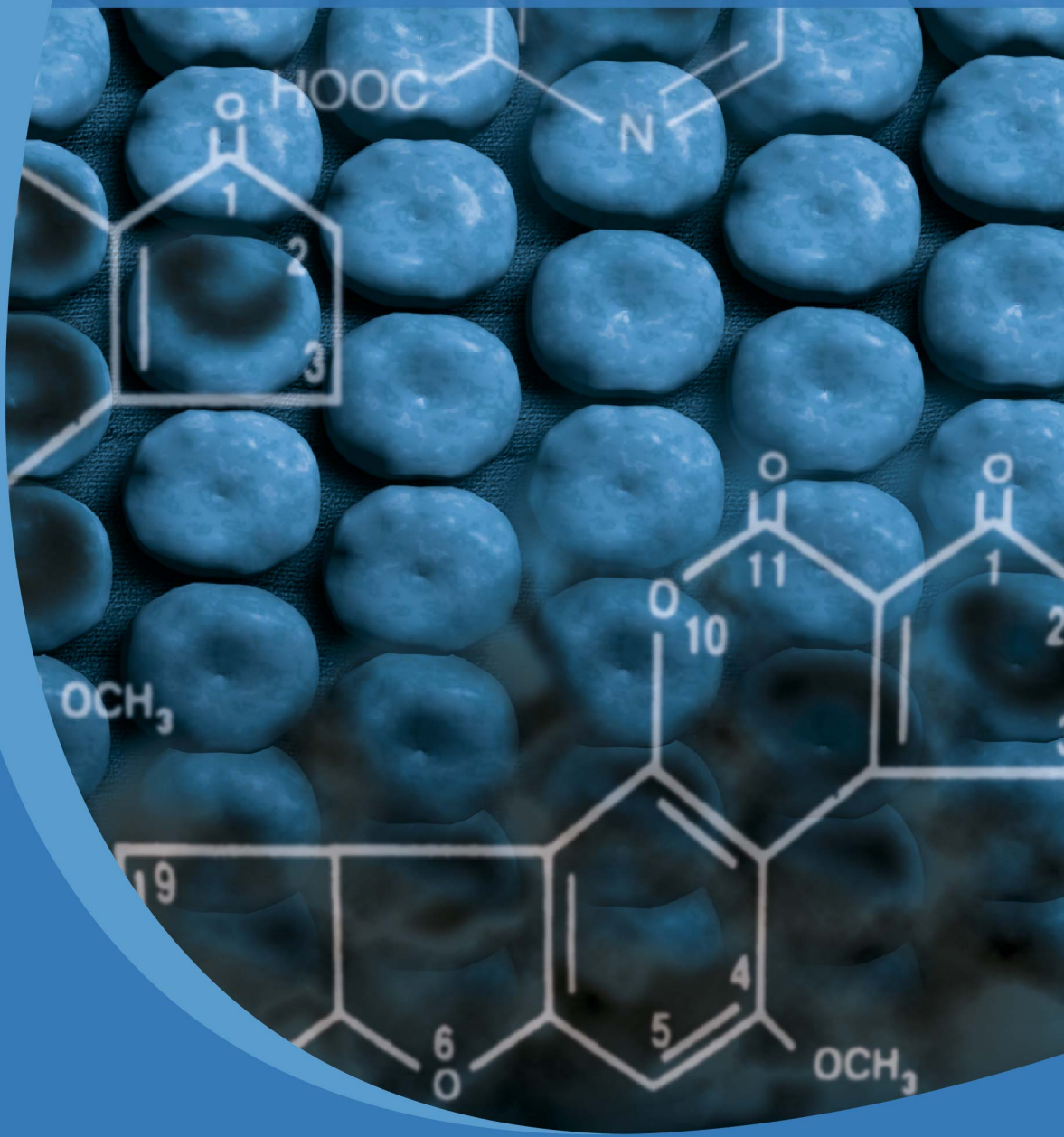
The Mycotoxin Blue Book



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PREFACE

The Food and Agriculture Organization of the United Nations stated, in a recent report, that at least 99 countries around the world, representing 87% of the world's inhabitants, had some type of mycotoxin guidelines for foodstuffs and/or feedstuffs. Such worldwide interest is also reflected in the great volume of research published since the discovery of aflatoxin B_1 as the causative agent in Turkey X disease in the early 1960s. Due to the ubiquitous nature of mycotoxin-producing moulds and our general inability to prevent many of the conditions favorable for their growth and mycotoxin production, mycotoxin contamination of foods and feeds is an inevitable part of modern agriculture. The increasing irregularity of meteorological events related to the ever-changing global climate will further challenge us in the years to come. Although the true impact of moulds and mycotoxins is impossible to measure, the risks associated with their presence is well established.

The main aim of this book is to highlight the practical aspects of mycotoxin contamination. Careful attention is given to the understanding of toxicity and its impact on performance of animals exposed to mycotoxin contaminated feedstuffs. Chapters within this volume will also discuss the basis of proper feedstuff sampling and the principles of mycotoxin analysis. Additionally, this book will attempt to review concepts of mould growth and mycotoxin production, mycotoxin contamination of forages, and the contamination of human foods. Other chapters will focus on the mechanisms of toxicity, and on the specific effects of mycotoxins on antioxidant status. Therefore information provided in this book will be useful to professionals associated with the fields of veterinary and medical sciences, biotechnology, physiology, immunology, food technology, toxicology and nutrition, as well as students interested in this rapidly-developing field.

The Mycotoxin Blue Book owes its existence to the labour and commitment of many contributors to whom I am indeed grateful. I want to particularly acknowledge Professor Lon Whitlow for his support and encouragement.

Duarte E Diaz

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SAMPLING FEEDS FOR MYCOTOXIN ANALYSIS

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Introduction

Mycotoxins are toxic and/or carcinogenic compounds produced by various fungal species that grow on various agricultural commodities (Cullen and Newberne, 1994). There are many different mycotoxins (CAST, 2003) and a partial list is shown in Table 1.

Table 1. Partial listing of several mycotoxins produced by various fungi.

- Aflatoxins
- Fumonisins
- Ochratoxin A
- Deoxynivalenol
- Patulin
- Zearalenone

Commodities can be contaminated either in the field or in storage. Pre- and post-harvest strategies to prevent crop contamination include yearly crop rotations, irrigation in hot and dry weather, use of pesticides to reduce the insect population, drying crops to a safe moisture level, and providing protective storage (Phillips *et al*., 1994). Because mycotoxins are toxic and carcinogenic in animals, many countries regulate the maximum level that can occur in foods and feeds. Most regulations are concerned with controlling aflatoxin because it is considered the most toxic and carcinogenic of the naturally occurring mycotoxins. A recent FAO/WHO survey indicated that almost 100 countries regulate aflatoxin in foods and feeds (FAO, 1995). However, maximum levels differ widely from country to country because of a lack of agreement on what constitutes a safe maximum level for humans. Some of the maximum levels found in the FAO/WHO survey for aflatoxin are shown below in Table 2 (FAO, 1995).

Table 2. Examples of aflatoxin legal limits found in various countries.

Country	*Aflatoxin B_1 (ppb)*	*Total aflatoxin (ppb)*
US		20
EU	8	15
Australia		15
Canada		15
Egypt		
Maize		20
Peanuts		10
Nigeria	20	
Philippines		20
South Africa		10

It is important to be able to detect and quantify the mycotoxin concentration in foods and feeds destined for human and animal consumption. In research, quality assurance, and regulatory activities, correct decisions concerning the fate of commercial lots can only be made if the mycotoxin concentration in the lot can be determined with a high degree of accuracy and precision. The mycotoxin concentration of a bulk lot is usually estimated by measuring the mycotoxin

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MYCOTOXINS: THEIR EFFECTS IN POULTRY AND SOME PRACTICAL SOLUTIONS

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Introduction

During the past few decades there has been a steady increase in global production of poultry meat and eggs. Although the high nutritive value of eggs and poultry meat has resulted in increasing demand, food quality and safety factors are becoming increasingly significant in determining market value of poultry products. As mycotoxins are one of the major factors suppressing poultry productivity and also product quality, control of their impact is critical.

According to the United Nation's Food and Agriculture Organization (FAO), approximately 25% of the world's grain supply is contaminated with mycotoxins. The greatest economic impact of mycotoxin contamination is felt by crop and poultry producers, as well as food and feed producers (CAST, 2003). Worldwide, the economic losses due to mycotoxins in poultry feeds can be as much as several hundred million USD annually. The adverse effects of mycotoxin-contaminated diets on performance range from undetectable to devastating in terms of reduced egg production in layers and breeders, and growth depression in broilers, turkeys, ducks etc.

The threat of mycotoxins to humans was described during World War II when Russian soldiers suffered severe dermal necrosis, haemorrhage and destruction of bone marrow after eating mouldy grains (*Fusarium*-contaminated). However, it was not until 1960, when the entire turkey population of Britain was decimated by a fatal liver disease called **Turkey X Disease**, that the scientific community recognized the negative effects associated with mycotoxins. British agriculture officials later traced the source of the outbreak to aflatoxin in a shipment of peanut (groundnut) meal that originated in Brazil.

Mycotoxins of importance in poultry are mainly produced by fungi of the genera *Aspergillus, Fusarium* and *Penicillium* either pre-harvest, during harvest, or in storage or during feed processing whenever conditions are favorable. No region of the world escapes these silent killers; and their negative impact on poultry productivity and human health is enormous.

Geographic distribution of mycotoxins

At the First Food and Agricultural Organization (FAO) - World Health Organization (WHO) - United Nations Environment Programme (UNEP) Conference on mycotoxins in 1977, a review was presented on the occurrence of mycotoxins in various commodities throughout the world. Only seven mycotoxins were reported to occur significantly in naturally contaminated foods and feeds: aflatoxins, ochratoxin A, patulin, zearalenone, trichothecenes, citrinin and penicillic acid

Poor general resistance to disease and increased mortality are also observed in birds fed OTA-contaminated diets. Young chicks in the initial few weeks of life are highly sensitive. Though older birds are less sensitive, laying hens show reduced egg production in a dose-related fashion with a characteristic yellow staining of egg shells. Higher levels of OTA result in poor egg shell quality and higher incidence of eggs with blood spots (Shirley and Tohala, 1983).

Decreased embryo viability and poor hatchability are observed in eggs from hens fed OTA-contaminated feed. Chicks from such eggs continue to perform below average. Delayed sexual maturity is commonly seen in chicks from hens exposed to OTA.

Since the main consumer of poultry products are humans, it becomes relevant to also view the problem of mycotoxin residues in poultry products from a human health standpoint (Table 12).

Table 12. Occurrence of mycotoxin residues in poultry products.

Mycotoxin	*Poultry production*	*Carry over in meat and eggs*
Aflatoxin B_1	+	Liver
Ochratoxin A	+	Hatching eggs
Cyclopiazonic acid	+	Meat and eggs
Deoxynivalenol	+	Hatching eggs
Zearalenone	+	Eggs
T-2 toxin	-	-
Diacetoxyscripenol	-	-
Fusarochromanone	+	Hatching eggs
Aurofusarin	+	Eggs

TURKEYS

High levels of OTA can have devastating effects on turkeys. Table 13 shows the impact of ochratoxicosis on a commercial turkey farm (Hamilton *et al.*, 1982). The farm lost over 50% of the flock in just nine days. The level of ochratoxin in the feed was very high; and it was reported that even wild birds foraging on spilled feed were dying.

Table 13. Turkey mortality during an outbreak of ochratoxicosis.

Age (Days)	*Mortality (Number of poults)*
6	19
7	12
8	57
9	586
10	246
11	983
12	863
13	370
14	547
15	1033
16	826

Hamilton *et al.*, 1982

IMMUNE STATUS

Ochratoxin is known to cause regression and cellular depletion (lymphocytes) of major lymphoid organs, significantly affecting cellular immunity in poultry. A lesser secondary suppression of humoral immunity with lower circulating immunoglobulins is observed in ochratoxicosis as a result of depletion of immune system effector cells, especially the macrophages.

SIGNIFICANCE OF THE PROBLEM

Ochratoxin has been detected in a number of foods and feeds in various countries (Van Egmond and Spijers, 1994). A recent survey, where 1431 samples of wheat, barley, rye, oats and bran were analysed for OTA in Denmark (Hohler, 1999), showed that 40% of feed samples were contaminated with OTA. Among the commodities analysed, bran products had higher OTA levels than cereal kernels.

RESIDUES

Ochratoxin A, CPA and citrinin bind to plasma proteins once absorbed, resulting in accumulation and a prolonged residence

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EFFECTS OF MYCOTOXINS IN HORSES

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Until recently, studies investigating the effects of mycotoxins on horses were limited to trials with few animals or depended on extrapolations based on data from other species (the pig or ruminant being the most obvious choices). However, a unique challenge is presented when attempting to use non-equine data effectively since the horse is comparable to the ruminant in that it is a forage-grazing animal but has a gastrointestinal tract more closely related to the pig with the addition of a hindgut fermentation process. The nature of the horse farm also makes the equine quite different from other livestock species. These other species are bred for growth and meat yield and have a relatively short lifespan while in most cases the horse is bred for athletic performance, conformation, temperament, beauty and/or durability.

Limiting values or safe concentrations of specific mycotoxins are, for the most part, unknown for the horse. Historically, mycotoxins were identified by their ability to produce severe, overt disease syndromes in animals as a result of relatively high intakes of mycotoxins, however low levels of exposure over long periods can elicit chronic or subchronic toxicological manifestations. A concern is that for horses, especially elite athletes or breeding stock, exposure to low levels of mycotoxins may affect performance or breeding ability without the appearance of overt signs of disease. The outcome from exposure to chronic low levels of toxin may include general unthriftiness, suppression of the immune system and increased risk of secondary infections. Unlike other livestock species, horses can have a long lifespan and are expected to be reproductively sound in their later years. For these reasons, probably more so than for other species, the safe amount of specific mycotoxins is unknown for the horse. In addition to the degree of exposure, incidence of disease can also be influenced by the presence of multiple mycotoxins. Factors influencing susceptibility to mycotoxins include disease, heat stress, marginal nutritional profile, drug interactions, presence of multiple toxins, crowding, age and reproductive status. The intent of this chapter is to inform the reader of available data on mycotoxins and horses or the possible implications of toxins for the horse in the absence of data.

Moulds and production of mycotoxins

The occurrence of mould and mycotoxins in food and animal feed is a problem of major concern internationally (Wood, 1992). Mycotoxin production can occur when favourable conditions allow fungi to grow on crops in the field, at harvest, in storage or during the processing of feed (Palmgren and Lee, 1986). Mycotoxins are the products of secondary fungal metabolism although not all fungi produce mycotoxins. Environmental

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EFFECTS OF MYCOTOXINS ON DOMESTIC PET SPECIES

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Introduction

Domestic pets include all animals commonly cared for in households, such as cats, dogs, pet birds, rabbits, guinea pigs, and mink. Over the last decade, the importance of domestic pets in developed countries has grown dramatically. As an example from the EU, more than 28% of households in Austria keep cats, followed by dogs in 16%, caged birds in 5%, aquarium fish in 4%, rabbits in 2.5%, guinea pigs in 2%, and hamsters and turtles are kept in 1% of households. The adverse effects of fungal toxins have been well documented in many species, particularly farm animals. However, only a few papers address mycotoxicoses in pets. Puschner (2002) has reviewed the clinical and pathological effects of some mycotoxins on pet animals. In addition, Devegowda (2000) and Bird (2000) have reported on the relevance of mycotoxins in pet foods. This general lack of information makes it difficult to assess the relevance of mycotoxins to pet health.

In the cases of aflatoxins, penitrem A, roquefortine, and ochratoxins in dogs, descriptions of feed-induced natural mycotoxicoses have been reported. Experimentally, the effects of trichothecenes, patulin, penicillic acid, moniliformin, and cyclopiazonic acid have been studied in dogs, cats, rabbits, and mink. For the most toxic compound within the aflatoxin family, aflatoxin B_1, maximum regulatory limits in the EU have been defined as 10 μg/kg in complete feedstuffs (Council Directive 1999/29/EC). The US Food and Drug Administration has established only an action level (FDA Regulatory Guidance for Toxins and Contaminants) of 20 ppb for corn, peanut products, cottonseed meal, and other animal feeds and feed ingredients.

This chapter provides an overview of the toxicological effects of mycotoxins on domestic pets, as well as on the occurrence of mycotoxins in pet foods. Different mycotoxins and their relevance, as well as clinical signs of their mycotoxicosis, are described.

Aflatoxins

Aflatoxins are toxic secondary metabolites of *Aspergillus* spp., such as *A. parasiticus* and *A. flavus*. Aflatoxins have been shown to be the cause of toxicities in livestock, domestic animals, and humans throughout the world. Due to their high toxicity and carcinogenicity, aflatoxins have received greater attention than other mycotoxins. In the early 1960s, when more than 100,000 young turkeys in England died as the result of an apparently new disease, later named **Turkey X disease**, aflatoxicosis was soon found to be the cause of death.

In the 1950s, before Turkey X disease was reported, epizootics of fatal hepatitis in dogs

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EFFECTS OF MYCOTOXINS ON ANTIOXIDANT STATUS AND IMMUNITY

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Effects of mycotoxins on antioxidant systems

A delicate balance between antioxidants and pro-oxidants in the body in general and specifically in the cell is responsible for regulation of various metabolic pathways leading to maintenance of immuno-competence, growth and development and protection against stress conditions associated with commercial poultry production (Surai and Dvorska, 2001). This balance can be regulated by dietary antioxidants, including vitamin E (Surai *et al.,* 1999), carotenoids (Surai and Speake, 1998; Surai *et al.,* 2001) and selenium (Se) (Surai, 2000). On the other hand, nutritional stress factors have a negative impact on this antioxidant/pro-oxidant balance. In this respect mycotoxins are considered to be among the most important feed-borne stress factors.

It is not clear at present whether mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or if the increased tissue susceptibility to lipid peroxidation is a result of a compromised antioxidant system. It seems likely that both processes are at work. In most cases lipid peroxidation in tissues caused by mycotoxins was associated with decreased concentrations of natural antioxidants. For example, in an experiment with quail, levels of the primary liver antioxidants (α–tocopherol, γ–tocopherol, carotenoids and ascorbic acid) were significantly decreased as a result of T-2 toxin consumption (Dvorska and Surai, 2001; Figure 1).

Similarly, the presence of T-2 toxin in the diet decreased the concentration of α–tocopherol in the chicken liver (Hoehler and Marquardt, 1996). T-2 toxin consistently depressed concentrations of vitamin E in chicken plasma (Coffin and Combs, 1981). Addition of micelle-promoting compounds (taurocholic, monoolein, and oleic acids) alleviated depression in plasma vitamin E, indicating interference of T-2 toxin with micelle formation during vitamin E absorption. Similarly, aflatoxin B_1 (AFB_1) in the feed interfered with the accumulation of carotenoids in chicken tissues (Schaeffer *et al.,* 1988) inducing pale bird syndrome in birds. In fact, AFB_1 caused a significant depression of lutein in the toe web, liver, serum and mucosa (Schaeffer *et al.,* 1988a). Pigment restoration was accomplished by feeding the same diet supplemented with lutein (70 mg/kg). In young chickens AFB_1 reduced the lutein content of jejunal mucosa up to 35% while serum lutein was reduced up to 70% (Tyczkowski and Hamilton, 1987), suggesting that AFB_1 interfered with the absorbtion, transport and deposition of carotenoids. More precisely, AFB_1 impaired lutein absorption in chickens (Tyczkowski and Hamilton, 1987a). In similar fashion, ochratoxin A (OTA) was shown to affect

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MYCOTOXINS IN AQUACULTURE

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Introduction

An understanding of how mycotoxins affect aquaculture becomes more important as fish nutritionists rely more heavily on plant ingredients to formulate fish diets. Commercial diets for warmwater fish, such as channel catfish, *Ictalurus punctatus*, have evolved over the past decade to include little or no animal protein. It has been common practice during the past 10 to15 years to include in the diets of channel catfish up to 10% fish meal or other sources of animal protein. More recently, nutritional research with salmonids is striving to develop practical diets that contain reduced levels of animal protein that are replaced with higher levels of plant protein and energy sources from oilseed meals and cereal grains. Therefore, with increasing use of plant sources of protein or energy, the likelihood of exposing cultured fish to mycotoxin-contaminated diets becomes eminent.

Concerns about mycotoxins in aquaculture began over 40 years ago when hepatocellular carcinomas of rainbow trout, *Onchorhyncus mykiss,* being cultured in commercial and governmental fish hatcheries in the US were determined to be caused by aflatoxin-contaminated cottonseed meal. Since that time, many of the recognised mycotoxins have been evaluated with coldwater fish like salmonids and warmwater species such as channel catfish and tilapia. Other mycotoxins that have been investigated with rainbow trout and more recently with warmwater aquaculture fish include the *Aspergillus* or *Penicillium* mycotoxins, cyclopiazonic acid and ochratoxin A (OTA), and the *Fusarium* trichothecene mycotoxins T-2 toxin and deoxynivalenol (DON). Other *Fusarium* mycotoxins like fumonisin B_1, (FB_1) and moniliformin have been studied recently.

Background

The occurrence of aflatoxicosis at fish hatcheries in the US producing rainbow trout and in turkey farms in the UK caused great concern in the early 1960s about the safety of the food supply for both domesticated animals and humans. Initially, the condition affecting turkeys was referred to as **turkey X disease** (Blount, 1961), but was subsequently identified as being caused by mouldy peanut meal imported from Brazil that was infected with *Aspergillus flavus*.

In the US, rainbow trout reared in government and commercial hatcheries were found to have developed liver nodules or hepatomas (Post, 1987). Much laboratory and research work went into understanding the cause of the hepatomas that appeared in rainbow trout. These efforts have been thoroughly described by Dr. J.E. Halver (1969). After numerous studies it was found that trout fed pelleted dry diets containing

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PRINCIPLES AND APPLICATIONS OF MYCOTOXIN ANALYSIS

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Introduction

Analysis forms an integral part of almost all studies involving mycotoxins. There are many reasons for mycotoxin analysis that range from the need to enforce legislative limits, to on-line monitoring for quality control of materials in the food and feed industries and surveillance to monitor occurrence to estimate consumer exposure. In addition, research studies require, for example, measurements to determine the fate of the toxins during food processing, means for their prevention and control, research into the relationship between fungal species and the toxin produced, toxicological studies and veterinary investigations.

Analysis is often regarded as something best left to the specialist chemist to provide a service whose results are accepted without question by the customer. Analysts are often regarded as cautious about their results and liable to wrap these up in all sorts of provisos and warnings. This chapter will show why this caution is justified and will illustrate that the determination of mycotoxins, in common with many other types of natural contaminants, is a complex and difficult field. It is thus essential that the analyst has full confidence in his methods and takes all necessary precautions to ensure the reliability of his results and the suitability of the method for the task in hand. At the same time the customer should demand evidence that appropriate quality checks are incorporated as routine. This is incumbent on all parties because important decisions that sometimes affect national and international trade relations and involve large amounts of money may need to be made on the basis of a few analytical results.

This chapter does not merely provide a list of selected methods and references but aims to discuss many of the factors that influence how a method is selected, developed and operated effectively, together with the controls necessary to ensure that it continues to work reliably and accurately. It is aimed to help those new to the field to understand and avoid some of the pitfalls in analytical development and method application and to assist in general improvement in the standard of mycotoxin analysis. In addition, it is hoped that managers and food quality technicians may gain an insight into the complexities of mycotoxin analysis and assist in communication with the analytical fraternity.

Aims and preparation for mycotoxin analysis

OBJECTIVE OF THE ANALYSIS

In this section it is assumed that the need for the determination of mycotoxins has arisen and that the organisation requiring this has no

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MYCOTOXINS IN THE HUMAN FOOD CHAIN

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Introduction

The impact of mycotoxins in the human food chain is an important issue worldwide. In general, consumers perceive less risk from mycotoxins than from other food-related threats such as pesticides, additives, heavy metals and microbial agents. This is due to the fact that, at least in developed countries, mycotoxins very rarely cause acute intoxication outbreaks or health emergencies, the impact of which is often amplified by the media. Nevertheless, the real danger of mycotoxins is potentially very high, which is why they have been called 'hidden killers'. Mycotoxins are highly undesirable substances that should not be present in food and for which a zero tolerance would be ideal. However, even good agricultural, storage and processing practices cannot completely prevent contamination; and it is impossible to achieve a truly mycotoxin-free food chain. Consequently, small quantities of mycotoxins believed not to be dangerous are legally tolerated. Despite incomplete toxicological, epidemiological and exposure data needed to fully assess human exposure and health risks, and establish causal relationships between mycotoxins in foods and human disease, responsible risk management makes it necessary to take action (Kuiper-Goodman, 1995). For these reasons and in order to achieve realistic goals for mycotoxin management, public authorities of many countries have fixed legal limits for mycotoxin presence in foods. Worldwide legal limits have been recently reviewed by van der Westhuizen *et al.* (2002), who recorded that 77 countries have specific regulations for mycotoxins, 13 countries have no specific regulations, and no data are available for about 50 countries, many of them in Africa. However, the matter of the legal limits is questionable. In the recent past the regulatory limits were highly variable depending on the degree of development and economic involvement of the countries. In recent years serious efforts at harmonization have been made worldwide, but further work is still needed, as regulatory limits are often a practical compromise between the need for safe commodities and the economic consequences of the regulatory level chosen.

Suspected human mycotoxicosis

Mycotoxins can enter the human food chain directly by cereals, seeds, spices, fruits, beverages and other plant materials, and indirectly by food products obtained from animals given contaminated feeds through residues in milk, meat, eggs and their derivates. Consumers in developed countries are surely less exposed to mycotoxins than those in developing countries. This is due to several factors, e.g. abundance of food resources, modern food handling and preservation technology, and effective

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MOULD GROWTH AND MYCOTOXIN PRODUCTION

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Introduction

The significant advance in intensive animal production systems is at once the cause and the consequence of the increase in grain production. Production of poultry and swine, in particular, requires a large amount of grain to fulfill their dietary needs. For this reason grain production increases every year; and following harvest, most of the grain is stored until utilized. Storage conditions are determined by the complex interaction among the grain, the macro- and microenvironments, and a variety of organisms including microorganisms, insects, mites, rodents, and birds. Grain provides an abundant source of nutrients, but the natural consequences of the ecosystem during storage often will be spoilage of the grain.

Mould growth in grain is a normal occurrence in both the field and in storage. Mould growth can spoil the nutritional aspects of the grain and also result in secondary metabolites that are highly toxic to animals, humans, and plants. These so-called mycotoxins have been extensively studied since 1961, when groups of highly toxic *Aspergillus flavus* metabolites, the aflatoxins, were isolated from groundnut meals that had been imported into the UK.

In general, pre-harvest control of mould growth is somewhat compromised by the inability of man to control the climate, since both insufficient and excessive rainfall during critical phases of crop development can lead to mould contamination, spoilage of grain, and mycotoxin production. However, the post-harvest handling of grain presents many more opportunities for controlling mould growth and its consequences. Careful drying of grains and good storage management should minimize post-harvest fungal growth and therefore, mycotoxin production.

This review introduces certain aspects of mould growth in grains, the production of mycotoxins by these moulds, and their relationship with animal production.

Grain mould pathogens

Moulds are fungi that grow by producing long filaments called **hyphae**. They are plants that contain no chlorophyl and can grow in the absence of natural light. Moulds grow from single cells to a body of branched hyphae. In general, hyphae are important to the survival and dispersal of fungi. A network of hyphae is referred to as **mycelium**. This hyphal network is responsible for 'cementing' kernels together, which results in columns of grain that cannot be separated. Grain mould fungi also produce **spores** (conidia) capable of aerial dispersal in the field as well as within a grain storage bin. It is usually masses of these spores that give the mould a characteristic colour. Spores are dispersed passively by wind and rain. Insects can serve as vectors of these fungi, usually by transporting the spores on their bodies. Insects also increase the surface area available for

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CURRENT CONCEPTS IN MYCOTOXICOSES IN SWINE

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Introduction

Mycotoxins are fungal metabolites that can reduce performance and alter metabolism of livestock and poultry (Wannemacher *et al.*, 1991). The pathological states arising from the consumption of feeds contaminated with mycotoxins are referred to as **mycotoxicoses**. Mycotoxins can be formed in the field pre-harvest and may continue to be formed under suboptimal storage conditions post-harvest. High moisture content often predisposes feedstuffs to fungal growth and mycotoxin formation. Temperature is another key factor. Some fungi, such as *Aspergillus flavus*, are usually found in tropical and semi-tropical climates. This mold produces the carcinogenic hepatotoxin aflatoxin. *Fusarium* fungi, however, are more common in temperate climates and *Fusarium* mycotoxins are likely the most common mycotoxins on a global basis (Wood, 1992).

Aflatoxicosis in swine

Aflatoxins are produced mainly by *Aspergillus flavus* and *A. parasiticus*. Aflatoxicosis has been investigated in many different animal species and in more depth than other mycotoxins (Smith and Ross, 1991). This is because aflatoxins are among the most acutely toxic of mycotoxins causing extensive liver pathology. There is also concern about residues of aflatoxin and metabolites in foods because of the well-documented carcinogenicity of these compounds.

EFFECTS OF DIETARY AFLATOXIN ON GROWING AND FINISHING PIGS

Chronic aflatoxin B_1 toxicity in growing and finishing pigs (40 to 140 kg live weight) was described by Bonomi *et al.* (1992). The feeding of 500, 650 and 800 μg/kg aflatoxin B_1 reduced weight gain, feed utilisation, lipid digestion and renal function. In an experiment of similar design, it was shown that chronic feeding of aflatoxin G_1 resulted in more severe liver pathology than was observed with aflatoxin B_1 (Bonomi *et al.,* 1993). The feeding of up to 400 μg/kg aflatoxin B_1, however, had little effect on performance and mycotoxin tissue residues in pigs grown from 65 to 95 kg live weight (Wu *et al.,* 1989). Studies of feeding aflatoxin contaminated corn to weanling and growing pigs described reduced growth rates and feed consumption as well as elevated serum γ-glutamyltransferase (GGT) activity at a dietary aflatoxin concentration of 922 μg/kg B_1 (Schell *et al.,* 1993). Feeding 800 μg/kg aflatoxin for four weeks to starter pigs resulted in changes in numerous serum parameters including albumin and total protein concentrations as well as activities of GGT and alkaline phosphatase indicating liver damage (Schell *et al.,* 1993). A similar

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MYCOTOXINS IN FORAGES

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Introduction

Each year, many million tonnes of forage consumed by animals is contaminated with fungal species invading forage plants prior to harvest or during storage as hay, straw, or silage. The interactions between fungal species and plants are diverse, and comprise a true symbiosis, as observed for many endophytes, an aggressive invasion of living plants resulting in visible plant disease and deterioration as being characteristic for many *Fusarium* species as well as *Rhizoctonia* spp., and finally the utilisation of organic biomass in the decay of plant material.

Fungi produce a wide diversity of secondary metabolites to increase their competitiveness in nature, and many of these metabolites exert antifungal and antibacterial activity. These specific properties have long been recognized and initiated the synthesis of medicinal products, the most prominent one being penicillin. In the definition of the term **mycotoxins**, these effects are often neglected. As most mycotoxin problems in animal husbandry involve contaminated feed grains and oilseeds that are used worldwide in the production of formulated feeds, research focused also on investigation of toxins, which are regularly observed in feeds. Ruminant species are less sensitive to many mycotoxins, as the forestomach flora effectively degrades large parts of the ingested toxins. The mycotoxins that exert antimicrobial effects, however, are of great significance in ruminants as they impair ruminal function, resulting not only in malnutrition, but also facilitate the unexpected absorption of other mycotoxins, which under normal circumstances do not pass the rumen. As forage is still the most economic way to feed ruminant animals, the economic losses encountered with mycotoxin contamination can be significant.

Mycotoxins in growing forage grasses

Natural grassland covers large areas in moderate climates across the world. Whilst grass and hay have been monitored for many decades for environmental pollutants, either naturally occurring substances or industrial products, natural toxins such as secondary plant metabolites or fungal metabolites were considered to be only of local interest. The two major diseases related to either tall fescue (fescue toxicosis) or perennial ryegrass (staggers) and the considerable local economic losses related to these disease complexes in grazing animals, like ruminants and horses, stimulated research activities in this area (Table 1).

TOXINOGENIC ENDOPHYTES IN PASTURE GRASS

Various endophytic fungi may infect growing pasture grass. In particular, species of the

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MYCOTOXIN INTERACTIONS

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Introduction

Mycotoxicology can be defined as the study of highly toxic metabolic by-products, resulting from the growth of moulds on a feedstuff, such that when an organism is exposed to these metabolic by-products in relatively minute amounts, an alteration of the structure or disruption of the function of cells, tissues, organs, or organ systems results. When animals are exposed to a mycotoxin, the net result of exposure to the toxin is defined as a response. This mycotoxin-related response may be subclinical with no visible changes in the animal. On the other hand, this response may result in clinical changes of the animal with these changes being easily detectable and of such a unique nature that they can be used to accurately diagnose the toxicity. It can be inferred that a response, whether subclinical or clinical, is associated with substandard productivity or poor health of the affected animal.

The manifestation and magnitude of a mycotoxin-related response is dependent upon three situations. First, the severity of the response will be dictated by the dose of the mycotoxin. Since mycotoxins are usually introduced into an animal via the feed, the dose is directly related to 1) the concentration of the mycotoxin in the feed source, 2) the daily intake of feed, and 3) the body size of the target animal. When these variables are known, a daily dose or intake can be accurately determined and is usually expressed as units of toxin intake/unit of body weight/unit of time (i.e. mg toxin/kg of BW/day).

Although the dose may be accurately determined, another important variable is the duration or time frame that the determined dose is exposed to the animal. For example, an animal receiving a particular dose for one day will obviously exhibit a less severe response compared to an animal receiving the exact same dose for 30 days. The longer the administration of a particular dose the more severe will be the response.

The third determinant of a response to a feed-borne mycotoxin is typically referred to as interactions. Interactions constitute a very broad set of circumstances that can enhance, diminish or have no impact on the response of an animal to a defined dose for a defined length of time. For example, a group of animals receiving a feed with a defined mycotoxin concentration for a defined length of time may respond quite differently than a group of the same animal species receiving the same dietary concentration of the mycotoxin for the same length of time, but managed in a different manner, exposed to a different set of environmental conditions, or influenced by some other factor.

Specific interactions known to affect response to mycotoxins include; age and gender of the target animal, nutritional status at the time of mycotoxin exposure, genetic make-up, environmental extremes, presence of multiple mycotoxins in the ration, and

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MYCOTOXINS: METABOLISM, MECHANISMS AND BIOCHEMICAL MARKERS

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Introduction

There are probably tens of thousands of fungal metabolites, however, the food-borne fungal metabolites that are suspected or known to cause disease in humans or animals number in the hundreds (Riley, 1998). For this review, mycotoxins are defined as those metabolites that when consumed or absorbed by animals cause sickness or behavioural changes. This definition distinguishes between 'fungal metabolites', 'toxic fungal metabolites', and 'mycotoxins'.

The number of known mycotoxins that pose a measurable health risk to farm animals is quite limited because, while many fungal metabolites have the potential for toxicity, only a relative few are documented and confirmed to cause toxic effects in field situations. Nonetheless, the knowledge derived from studies with laboratory animals and *in vitro* studies serves as a warning for the possible contribution of mycotoxins in altering immune function (Bondy and Pestka, 2000), contributing to unexplained animal diseases, and in performance problems in farm animals (Osweiler, 2000). Several excellent reviews document the toxicology of mycotoxins in farm animals with extensive descriptions of the clinical manifestations (National Academy of Sciences, 1979; Richard and Thurston, 1986; Raisbeck *et al.*, 1991; JECFA 56th, 2001; CAST, 2003; Haschek *et al.*, 2002; Cousin *et al.*, in press). This review describes the main clinical signs in farm animals exposed to levels of mycotoxins encountered in field outbreaks, metabolism, mechanisms of action and biochemical markers of exposure. The purpose of the review is to highlight those observations that could help to identify the responsible mycotoxin when it is known that the outbreak is a result of consumption of mouldy feed. Some clinical signs are unique and some are highly suggestive of a particular mycotoxin. While it would be most useful to have simple mechanism-based biochemical markers that provide definitive identification of a specific mycotoxicosis, this is only possible for a very few mycotoxins. Thus, from a forensic perspective, the investigator must use all the tools available to identify the cause of a feed-borne disease outbreak.

The mycotoxins that present the greatest risk to farm animals are those that occur in commodities that are consumed in large amounts, and include aflatoxin B_1, ochratoxin A, fumonisins B_1 and B_2, deoxynivalenol, T-2 toxin, zearalenone, ergot alkaloids, ergot-like alkaloids, and macrocyclic trichothecenes. This review will cover only those mycotoxins for which exposure is known to be high and those that are known to cause disease in animals or are suspected to be modifying factors in disease processes. Mycotoxins with biochemical mechanisms of action that strongly suggest that the action could modulate disease processes will also be reviewed. Because there are literally thousands of research articles on this subject,

implicated as a causative agent in several cases of field intoxication, involving pigs, cattle, quail, and man (Bryden, 1991).

Acute doses of CPA administered to rats cause toxic lesions in liver, spleen, gastrointestinal tract and skeletal muscle. CPA is a potent inhibitor of Ca^{2+} uptake and Ca^{2+}-dependent ATP-ase activity in both sarcoplasmic and endoplasmic reticulum (Riley *et al.*, 1995) (Figure 2). Other than disrupted calcium metabolism, there is no useful biochemical marker for exposure to CPA, however, because it binds directly to the sarcoplasmic/endoplasmic reticulum calcium ATP-ase (SERCA), it is possible that the CPA-SERCA adduct could be detected in membrane vesicle preparations using electrophoresis.

Deoxynivalenol and the trichothecenes

Trichothecenes are a group of over 180 sesquiterpenoid mycotoxins produced by *Fusarium*, *Stachybotrys* and other fungi.

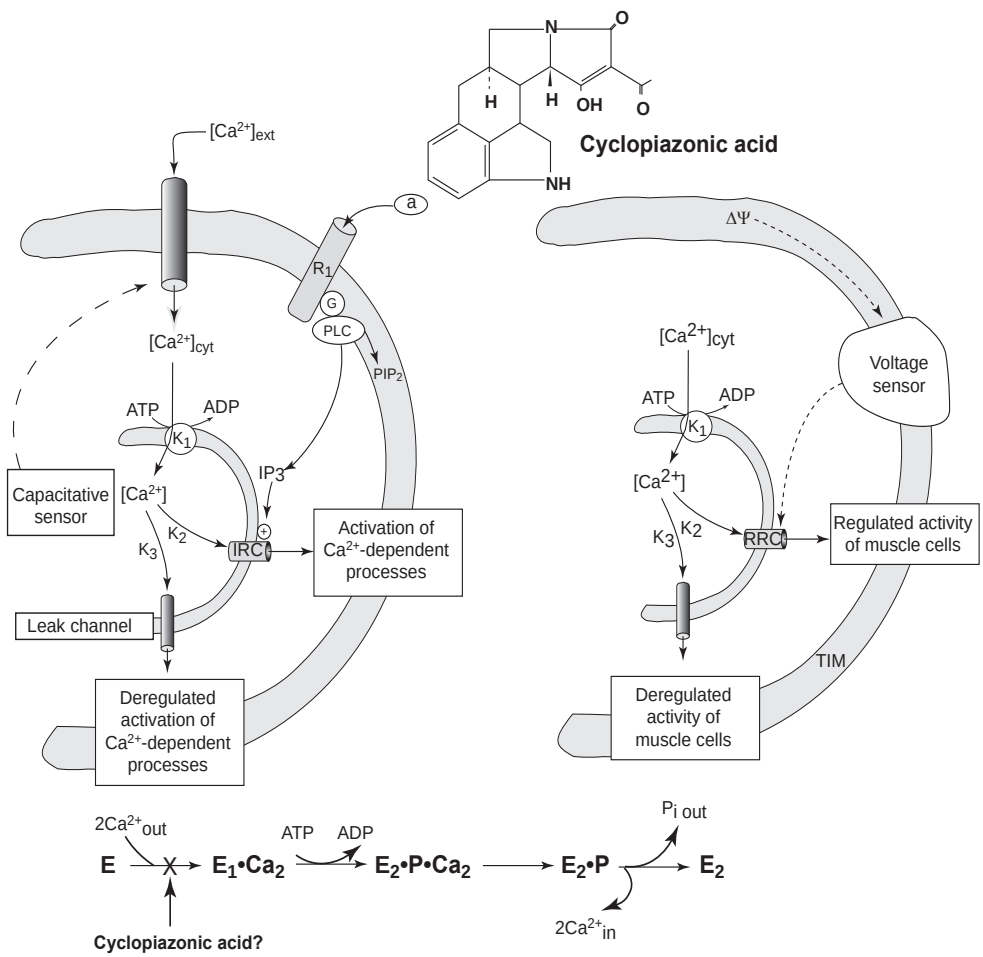

Figure 2. Biochemical mechanism of action of cyclopiazonic acid. The chemical structures of cyclopiazonic acid and a schematic showing inhibition of sarcoplasmic or endoplasmic reticulum calcium-dependent ATPases (Modified from Riley, 1998).

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EFFECTS OF MYCOTOXINS IN RUMINANTS

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Introduction

Mycotoxins are toxic, biologically diverse, secondary fungal metabolites produced by several fungi, particularly by species of *Aspergillus*, *Fusarium* and *Penicillium*. Apart from the threat to public health, mycotoxins are associated with significant economic losses for both crops and animals, including ruminants. Although several hundred mycotoxins have been described in the scientific literature, less than 10 have been extensively studied since the discovery of aflatoxin in the early 1960s. Mycotoxin-producing moulds are ubiquitous in nature and are commonly in contact with forages and cereals in the field, during harvest, drying and transport as well as during storage. Depending on the specific crop, mycotoxin, and environment, contamination with mycotoxins may be more likely at certain phases of production and handling, but contamination is rarely associated with just one mycotoxin. Because the same fungus is able to produce several mycotoxins and several fungi can produce the same mycotoxin, it is difficult to correlate mycotoxins to the presence of moulds in feeds. The presence of moulds does not directly indicate presence of mycotoxins, but if toxigenic moulds are present and the proper conditions exist (stress), there is a potential for mycotoxin production. Conversely, the absence of moulds does not guarantee the absence of mycotoxin, because the toxin may be present long after the death of the toxin-producing moulds.

Ruminant diets generally include both forages and concentrate, which may increase the risk of mycotoxins in comparison with animals that do not consume forages. The multiplicity of ingredients in complex diets may increase the probability of multiple mycotoxin contamination but decrease the risk of high mycotoxin concentrations because any one feed ingredient is diluted in the final diet. New techniques for preservation of wet forages, such as silages and wrapped bales, have been developed over the past 20-30 years. Preserved forages are more likely to harbour moulds and associated toxins than dry forages when anaerobic conditions are not strictly controlled. Many agricultural and food industry by-products such as fruit pulp, beet pulp and brewery wastes commonly used in ruminant diets are often handled in wet form, which means that moulds can grow and mycotoxins be produced during the storage and transport phases. Furthermore, grazing systems cannot be considered completely safe from mycotoxin contamination. Fresh grasses can be contaminated with mycotoxins (Erasmuson *et al.*, 1994; Sporsen *et al.*, 1995) including fungal endophytes that produce toxins such as ergovaline, lolitrem B and peramine (Lewis and Clements, 1986). Prohibition of antifungal agents in

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MYCOTOXIN SEQUESTERING AGENTS: PRACTICAL TOOLS FOR THE NEUTRALISATION OF MYCOTOXINS

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Introduction

Ever since the first report of a mycotoxicosis in the early 1960s researchers all over the world have been meticulously researching ways to eliminate or minimise the effects of these inevitable contaminants. Due to the wide range of mycotoxins that can contaminate animal feeds and their variable chemical compositions, protection against mycotoxicosis is a relatively difficult task.

Among all the available approaches to control mycotoxin contamination the simplest strategy is based on the prevention of the formation of mycotoxins in feeds (Lopez-Garcia and Park, 1998). Even with current technologies it is very difficult to predict or to prevent their occurrence either preharvest or during storage and feed processing (Wood, 1992). Once ingredients become contaminated with mycotoxins, elimination of the contaminated product is the most effective method of avoiding the problems related to their ingestion (CAST, 2003). Unfortunately, due to the difficulties in obtaining a representative sample it is quite difficult to accurately determine the level of contamination of a specific feedstuff. The impracticality and cost associated with the complete substitution of these ingredients means that this practice is not performed as frequently as recommended. Therefore, mycotoxins are often present in animal feedstuffs and pose a substantial economic loss to agricultural animal industries. Moreover, mycotoxins represent a public health concern as animals consuming mycotoxin-contaminated diets may leave residues of these toxins in animal-derived food products.

Mycotoxin **decontamination** refers to methods by which the mycotoxins are removed or neutralised from the contaminated feed while mycotoxin **detoxification** refers to methods by which the toxic properties of the mycotoxins are removed. These strategies include physical, chemical and biological methods. Chemical procedures like treatment with acid/base solutions or other chemicals, ammoniation, ozonation, and reaction with food grade additives such as sodium bisulfite have proven effective in degrading and detoxifying aflatoxin contaminated feedstuffs (CAST, 2003). Biological methods primarily involving the degradation of the toxin by microorganisms are receiving increasing interest among researchers and have shown positive results (Volkl *et al.,* 2004). Physical procedures like sorting, thermal inactivation, irradiating or extracting contaminated products have been attempted with variable success (CAST, 2003).

Any detoxification or decontamination method for mycotoxin-contaminated feedstuffs should fulfil the following prerequisites (Sinha, 1998).

1. Be effective in removing, destroying and inactivating the mycotoxin,
2. Do not produce toxic or carcinogenic/ mutagenic residues in the treated

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The **Mycotoxin** Blue Book

Edited by Duarte Diaz

Once thought to be only a problem in tropical regions, moulds and the mycotoxins they produce have wide-ranging economic impact on animal agriculture on every continent of the globe. Mould growth robs feed nutritive value and reduces intake, which lowers efficiency. Mycotoxins, even when present at levels previously considered 'trace', have negative effects on performance and health, particularly in the context of today's more highly productive modern livestock genetics. Food-borne toxins also threaten human health through contaminated cereal and protein sources and transfer of toxins in food animal products.

The Mycotoxin Blue Book focuses on the physiological effects and field occurrence of mycotoxins. Detailed information on types of moulds and mycotoxins and the conditions under which moulds flourish is included. Implications of mycotoxin contamination of feedstuffs for all major food animal species are presented in addition to aquaculture and companion animals. Sampling and analytical issues are covered in depth; as is the topic of mycotoxins in human foods. Finally, practical means of ameliorating mycotoxin effects are addressed. It is the hope of the editor and authors that the material herein will lead to clearer recognition of mycotoxin problems and ultimately to ways of reducing their impact on food animal production.

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CONTEXT