Mycotoxicological challenges to european animal production: A review

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1.0 Multifactorial challenge

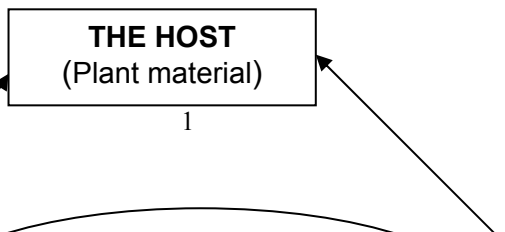
The spotlight in animal production systems across Europe is increasingly on the husbandry detail associated with improving efficiency and thereby business competitiveness within the livestock sector. For these reasons and others, farmers and those involved throughout the modern food chain are continually challenged and encouraged to adopt best practice. The whole of the production process is being subjected to closer external scrutiny and accountability reinforced by increasing regulation and legislation (Knock, 2003; Jeuring, 2004). One of the major determinants of livestock profitability is correct nutrition together with the requirement that such feedstuffs offered are characterised by high quality, hygiene standards. In turn, the European consumer demands quality assured food (edible animal products) which are free from chemical and microbiological contaminants. Included as such are the moulds which have the potential to produce secondary metabolite products (mycotoxins) capable of producing a toxic response (mycotoxicosis) when ingested and inhaled by higher animals.

Toxins of agricultural significance globally but also within Europe, include those produced by the fungi genera of *Aspergillus, Fusarium* and *Penicillia* (Veldman, 2004; Bhatnagar *et al., 2004;* Deregowda *et al* 1998). The chemical properties and biological activities of individual mycotoxins vary widely and can be responsible for causing a wide range of pathological and physiological effects in animals and potentially, humans. In addition to representing a threat to consumer health, the adverse economic effects due to reduced crop yields through premature senescence and impaired animal performance and reproductive capability, are only too apparent (Tucker, 2004). Indeed economic loss can be sustained across the whole food chain (Trenholm *et al*., 1996). There is a cost also incurred to countries seeking to export commodities to the EU. Globalisation of trade has complicated the way we deal with mycotoxins in that regulatory standards often become bargaining chips in world trade negotiations. Countries vary in their sophistication of infrastructures for the monitoring of internal food standards. Food security combined with quality are goals which are difficult to achieve simultaneously.

This review, with a focus on Europe, highlights the complexity of interaction between host, toxin properties and consumer (animal and human), Figure 1, and seeks to reflect some of the significant progress made by researchers into this increasingly topical issue.

2.0 The dynamics of mycotoxin contamination

Mycotoxity and its consequences occur within an agricultural system's context which possesses many variable elements (Figure 1). Although three separate systems can be identified and implicated, research endeavour in this field necessarily has to evaluate scientifically the dynamics and relationships of a total system which embraces all three aspects and modified by environmental impact factors. Mycotoxin contamination of forages and cereals (important crops within the European livestock sector) frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Further significant spoilage may also occur during processing and storage of harvested crops and feed whenever environmental conditions are conducive to active toxin formation. Moisture content and ambient temperature are considered to be key determinants of fungal colonisation and mycotoxin production although a range of other predisposing factors such as presence of oxygen, nature of the substrate and pH should not be overlooked (Tucker, 2004). In addition, Yiannikouris and Jouany (2002) emphasised the possible physical damage to plants caused by rodents, birds and insects which can provide a route of entry into the plant for fungal species.



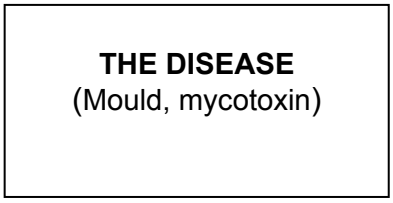


Figure 1. The multifactorial challenge of mycotoxin contamination

It is traditional in terms of classification to subdivide toxigenic fungi into 'field' (or plant-pathogenic) and 'storage' (or saprophytic/spoilage) organisms. *Claviceps*, *Fusarium* and *Alternaria* are classic representatives of field fungi, while *Aspergillus* and *Penicillium* exemplify storage organisms. Mycotoxigenic species may be further distinguished on the basis of geographical prevalence reflecting specific environmental requirements for growth and secondary metabolism, thereby yielding of toxins. Geographical dispersion of toxic substances linked to prevailing climate in various regions has been considered by Deregowda *et al* (1998). Characteristically found in the cooler more temperate countries of Europe are the economically important mycotoxins such as vomitoxin or deoxynivalenol. (DON) zearolenone (ZEN), ochratoxin A (OTA), diacetoxyscirpenol (DAS), T-2 and HT-2 toxins whilst aflatoxins are not considered such a major problem. However, it should be noted that even within Europe prevalence of fungal species is influenced by climatic differences between north and south. In the north OTA is seen with increased frequency compared with southern regions where mainly fusariotoxins are found with greater regularity. Ergot contamination has been noted in the more temperate regions of Europe. *Fusarium* fungi are more ubiquitous, but even this genus contains toxigenic species that are almost exclusively associated with cereals cultivated in warm countries. The change in climate patterns witnessed at present including perhaps some evidence of global warming and irregular rainfall distribution, may indicate that contamination levels and type may be less predictable in the future (Tucker, 2004). The current trend albeit rather slow, to convert land to organic (non interventionist) methods of production, may be an additional factor in compounding the uncertainty over the extent of mycotoxin occurrence in Europe.

3.0 Mycotoxin identity and characterisation

The challenge remains for research to extend our knowledge of the toxicological properties of mycotoxins. Many of those deemed agriculturally significant have been characterised and they show significant diversity in their chemical structures and biological activity (CAST, 2003) and more recently, Bhatnager *et al.* (2004) have provided updated information on the genomics of toxigenic fungi. Even the *Fusarium* mycotoxins are very diverse in chemical structure and in the characteristics of the mycotoxicoses they produce. These would include the trichothecenes, the fumonisins, moniliformin and fusaric acid, in addition to ZEN, already stated. Some toxic metabolites are simple aromatic compounds while others are complex organic molecules that have stability over a significant range of environmental conditions (Trenholm *et al.*, 1996). To add to the complexity the chemistry of toxins may resemble that of certain micronutrients present in some feed and food commodities. Traditional fungal toxonomy has been reliant on using morphological criteria as a basis for identification and

more recently, biochemical or physiological markers (Perez-Sierra and Henricot, 2002). The authors suggested, however, that complementary DNA information could provide a more complete picture and assist in the more rapid identification of pathogens.

The negative impact on livestock health and productivity can be more serious on occasions when mixtures and combinations of mycotoxins are found to be infecting a feedstuff. It is a reminder that seldom will specific mycotoxin production occur in an isolated way. When combined, a mycotoxin 'cocktail' could act in an additive or antagonistic way (Magan, 2004). Much research has focused, however, on the fact that co-occurrence of mycotoxins may result in producing a toxicological synergistic effect between different categories of toxic substances (Flanagan, 1991; Trenholm *et al.* 1994; Smith and Seddon,1998). For example Tucker (2004) supports the concept of a toxicological synergism between fusaric acid and the trichothecenes. The former, when acting alone, has relatively low toxicity status.

Crop and feedstuff association

There is a potential vulnerability of plant tissue to succumb to varying levels of spoilage moulds which may or may not yield eventual toxic secondary metabolites. Mycotoxin contamination can represent a challenge and threat to the normal growth and development expectancy of a specific crop and occur at seed germination, crop establishment, vegetative, reproductive phases and/or at harvest. In addition, there is the critical period between immediately post-harvest and the occasion when crop products are offered in various raw and further processed forms as livestock feed. The opportunity and likelihood of mycotoxin development will largely depend firstly, on the presence of those climatic and environment variables which are conducive to and encourage specific primary mould growth organisms and secondly, inadequacy of on-farm prevention and control measures (section 5). Although mycotoxin infection has a global impact on crop and livestock performance, a significant number of toxin - producing fungal agents can be identified within the confines of the regions of Europe as indicated in Table 1. The range of toxins stated can be found in close association with the typical feedstuffs commonly available to the ruminant and monogastoric livestock sectors. Feeds of plant origin are of increasing importance in the industry one reason being the demise of proteins of animal origin. In addition to animals accessing feeds containing toxic properties, mould contamination indicates inferior nutritional value of the product and its palatability compromised. The Table 1 information does not distinguish between 'field' and 'storage' specific spoilage organisms.

Fresh and conserved forages are not immune to fungal contamination (Smith *et al.,* 2001; Fink-Gremmels, 2004; Tucker, 2004 and Veldman, 2004). Furthermore, Tucker (2004) links apparent changes in global weather patterns to the increased production of mycotoxins and higher incidence of mycotoxicosis in Europe since 1998. Changes in agricultural husbandry practice may have also exacerbated the situation. Clearly the fundamental principles of preservation technique must be adhered to at the farm level in order to minimise risk of mould formation, whether it be conservation by acidic ensiling or through dehydrating the crop product as with hay production. According to Fink-Gremmels (2004) *Penicillium roqueforti* and *Aspergillus fumigatus* are the first and second most frequently found mould species in silage. Minimising the possibility of aerobic instability is of paramount importance and toxins formed at the pre-harvest stage can remain intact during the ensiling process. Scudamore and Livesey (1998) in their review of mycotoxin presence in forage crops, suggest that OTA and citrinin may be produced in contaminated maize silage and dry forages.

Table 1. A range of European feedstuffs and their possible mycotoxin associations.

Commodity	Genera	Species	Toxin
Pasture Grass	*Claviceps*	*purpurea*	Ergot alkaloids
Grass Silage	*Penicillium* *Aspergillus* ‘’ *Fusarium*	*roqueforti* *fumigatus* *flavus* *moniliforme*	Roquefortine, Patulin Aflatoxin ‘’ Fusaric acid
Hay	*Fusarium* *Aspergillus*	*sporotrichioides* *fumigatus*	T-2 toxin Aflatoxin, Patulin
Straw	*Fusarium* *Aspergillus* ‘’	*sporotrichioides* *fumigatus* ‘’	T-2 toxin Aflatoxin Patulin
Cereal Grains	*Penicillium* *Penicillium* *Penicillium* *Fusarium* ‘’ *Fusarium* *Fusarium* ‘’ *Alternaria*	*verrucosum* *verrucosum* *viridicatum* *graminearum* ‘’ *sporotrichioides* *culmorum* ‘’ *alternata*	Ochratoxin A Citrinin Citrinin Deoxynivalenol Diacetoxyscirpenol T-2 toxin Nivalenol Zearalenone Alternariol
Maize Silage	*Fusarium* *Fusarium* *Fusarium* *Fusarium* *Penicillium*	*graminearum* *roseum* *verticillioides* *moniliforme* *verrucosum*	Zearalenone Fumonisins B1, B2 Fumonisins B1, B2 Ochratoxin A ‘’
Oilseed products	*Penicillium*	*viridicatum*	Ochratoxin A
Imported Raw Materials	*Aspergillus* *Aspergillus*	*fumigatus* *flavus*	Aflatoxins Aflatoxins
Compound feeds	*Penicillium*	*viridicatum*	Ochratoxin A

Regarding other more nutrient dense animal feedstuffs, mycotoxin contamination is a continuous challenge and threat for the producers of small grain cereals and maize crops which are among Europe's most valuable for food and feed. The mycotoxins DON, nivalenol (NIV) and ZEN are considered important and caused by *Fusarium* head blight of small grains and *Giberella* eat rot of maize in the humid temperate regions. DON is the most frequently reported Fusarium – produced mycotoxin (Diaz, 2003) and together with the others have been the subject of much survey reporting of cereal crops across many European countries (Robb, 1990; Liebetseder, 1995; Prickett *et al*., 2000). Fumonisins occur commonly in corn and corn by-products and have been the subject of review by Marasas (1996). Although there is minimal evidence that such toxins occur in (north) European commodities imported feedstuff products, however, many contain such (Veldman, 2004).

In terms of threat to animal and human health, OTA is the most important of the *Penicillium* mycotoxins. OTA contaminates a variety of plant and animal products. Although presenting a worldwide problem, its impact is greatest in temperate climates. Unlike the field fungal contaminants of cereal crops represented mainly by the trichothecene group, OTA is produced by *Penicillium verrucosum*, a mould that occurs widely in poorly stored cereals in northern European countries and its occurrence is associated with storage of damp grain. Studies conducted in the UK by Banks *et al.,* (2002) concluded that, in addition to a fairly well defined combination of moisture and temperature responsible for promoting contamination, there is a strong likelihood that grain may become infected during or shortly after harvest,

perhaps due to the presence of this fungus within the storage environment or in handling facilities. Appropriate management of the OTA problem is of paramount importance to the UK grain trade. In addition, the food and drink industries require assurance of freedom from significant amounts of OTA (it having much greater toxicity relative to DON). To this end the European Community have introduced maximum permitted limits for OTA of 5mg/kg and 3mg/kg for whole cereal grains and processed products respectively (Banks *et al.,* 2002).

The feed compound manufacturing industry represents a very significant business within the agricultural economies of most European countries. For this reason alone it is imperative that feed products with a high hygiene standard are produced and quality assurance and control procedures adhered to in individual feed mills. Most countries, in addition to the utilisation of indigenous home-produced raw materials, will necessarily need to supplement these in the manufacture of compound mixtures by acquiring imported feedstuff commodities. The latter are likely to carry the risk of being contaminated with fungal poisons associated with hotter climates where, for example, aflatoxin may be common place. Mycotoxins can be produced at any stage from the growing crop to the formulated compound end-product. Extensive laboratory evaluation of the microbiological profile of feed samples indicate that finished (compounded) feeds contain lesser amounts of mould contamination when compared with the original raw materials (Howell, 1983). It would seem that the pelleting process and the heat generated, reduces quite dramatically the quantity and viability of mould spores present in the original sample. Moulds appear much less heat resistant than some groups of bacteria (Wyatt, 1995). That said, recontamination of feed products during the post-pelleting phase is a real possibility perhaps through handling factilites or poor storage conditions and the profile of mycotoxin presence may change. Also, Scudamore and Banks (2004) point out the concern that residual effects of certain mycotoxins may occur in the foods destined for human consumption and gives examples such as aflatoxins, OTA, DON, ZEN and fumonisins as being able to survive the further processing of raw materials.

Appropriate sampling and analytical procedure

In order to implement prevention and control measures, to gather fungal spoilage intelligence information and thereby safeguard against animal and human health problems, effort is needed on-farm and in the laboratory to identify and quantify with accuracy the degree of mouldiness of crop and feedstuffs and determine any resultant mycotoxin concentration. Whittaker (2000) and others Armitage (2003) have emphasised that the most critical part of any analytical procedure needed to confirm mycotoxin presence and diagnosis of mycotoxicoses, is sampling the commodity in a truly representative manner. This is particularly crucial due to the potential uneven distribution of moulds and toxic substances in feed batches and the large variability associated with sampling, sub-sampling and condensing sample for final analysis (Diaz 2003). Smith and Seddon (1998) were right to point out that because visible moulding of a product is not obvious, this does not necessarily indicate the absence of mycotoxins. Conversely, the fact that moulding has occurred does not automatically mean there is an accompanying problem of mycotoxins or that moulds serve as a reliable guide or predictor of toxin content. Armitage (2003) in his extensive evaluation of the UK cereal grain problem, considers the origin of mycotoxin formation to influence the sampling procedure, based on whether contamination originates in the field or storage facility. Furthermore, in processed commodities such as groundnut cake and rice bran, were fungal infection took place in the whole kernel, it is difficult to distinguish contaminated from non-contaminated batches. After processing there is no causative sign of the fungus and the moisture level is normally acceptable. Mycotoxin contamination in this instance is not discovered until a chemical analysis is carried out.

Analytical procedures, the ‘tools’ of the trade are improving in sensitivity and accuracy whether it be the isolation and/or description of mould growth and harmful secondary metabolite substances. These include the use of high-performance liquid chromatography (Howell 1983), mould spore plate count and respirometry (Wyatt, 1995), and rapid, accurate ELISA (enzyme-linked immunosorbent assay) tests (Smith and Seddon, 1998). As it is the development of the mycelial mass and not sporulation which represents the actual dynamics of mould growth, then mould spore plates counts may not be an accurate reflection of such as it mainly indicates degree of sporulation. Respirometry is capable of simultaneous

measurement of CO_2 production and O_2 consumption which Wyatt (1995) felt offered a more detailed and accurate assessment of mould growth. Analytical costs are always a factor and the reduction of such is one of the claimed advantages of the more recently developed and sophisticated immunoassays. Tucker (2004) has considered the challenges for establishing guidelines which reflect acceptable (safe) levels of mycotoxins in feedstuffs and emphasised the problem of meaningful interpretation of laboratory studies when applied to field conditions where so many complex and interactive environmental variables need to be factored into the equation.

4.0 Compromised animal productivity

Since mycotoxin – producing fungi can contaminate commodities destined for use in animal feed and human food products, both populations can be affected by them (Figure 1). There is always the potential for the carry - over of mycotoxins into animal products such as meat, milk and eggs and this representing a challenge to the food industry from a food safety viewpoint (Charmley *et al***.** (1995). As mould growth can modify the nutrient status of a feedstuff and in addition, supply a toxic secondary metabolite, it is not surprising that animal performance is compromised in various ways faced with these twin challenges to optimal productivity. The range of clinical toxicological syndromes caused by ingestion of moderate to high amounts of mycotoxins have been often characterised and reviewed in the literature either under the specific mycotoxin or animal species categories (Smith and Seddon, 1998; Charmley *et al.* 1995; Lawlor and Lynch, 2001; Danicke, 2002). The severity of mycotoxicosis is dependent on a range of factors including, the duration (intensity) of exposure to the toxin, its degree of toxicity and animal parameters such as age, nutritional status and health. Acute toxicity is likely to be manifest in an animal which has ingested an elevated level of the toxic compound or alternatively, the condition may be described as chronic, if resulting from longer-term exposure. Bhatnager *et al* (2004) have produced a classification of mycotoxins based on their ability to promote certain responses in higher vertebrates and other animals, namely as possessing, acute toxic or chronic carcinogenic, mutagenic, teratogenic or oestrogenic properties. However, across Europe overt toxicosis, morbidity and death occur relatively infrequently with most economic losses being due to subtle non-specific and perhaps sub-clinical effects associated with impaired animal performance and increased disease incidence.

Immunosuppression effects

Much of the literature on the subject of animal interaction with mycotoxin load, gives consideration to the threat that such toxins pose to immunocompetence. The ensuing consequence to compromised livestock welfare and performance is all too obvious and demonstrates the potential significance of mycotoxin infection at the subclinical level. Robb (1990) for example noted a failure in immunogenesis in pigs and poultry. Vaccination during a period of aflatoxin intake, did not generate an adequate immunity against subsequent challenge inoculations. A major concern at the commercial level of malfunction of the immune system is the fact that animals with sub-optional health status will show greater susceptibility to other ‘opportunisitic’ diseases. Devegowda *et al* (1997) in their study of the immunosuppressive effect of aflatoxins in poultry, suggested a cascade effect (Figure 2) in terms of demonstrating the sequence of events, the consequence of which mean that normal body immune status can be breached.

Depressed protein synthesis

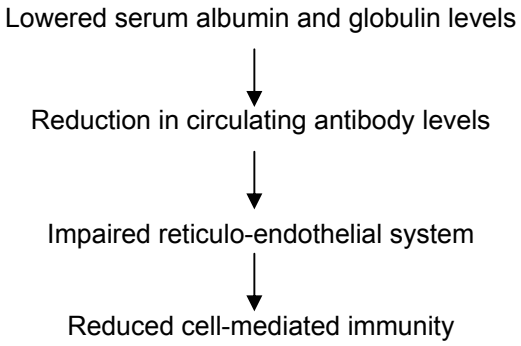


Figure 2. A cascade of immunosuppression effects in aflatoxin-challenged poultry (adapted from Deregowda *et al.*,1997)

Failure of immune response in mycotoxin-challenged livestock is often closely associated with altered neuroendocrine function together with disrupted metabolism of feed nutrients (Diaz, 2003). Research interest in this area has been documented in relation to laboratory animals (Bondy and Pestka, 2000), starter pig immunological measurements (Swamy *et al.*, 2003) and by way of pig immune system review (Oswald *et al.,* 2003).

Ruminants vs monogastrics

There are clearly differences in the degree of negative impact that various levels of ingested mycotoxins have on the health and welfare of livestock. Table 2 provides a crude analysis of the degree of sensitivity to specific toxins that the range of animal categories display when confronted with such a challenge. Levels of resistance are variable even when pigs and poultry are compared. Smith and Seddon (1998) ranked the tolerance capabilities demonstrated by species with pigs being rated the most sensitive, ruminants the least and poultry somewhat intermediate. The apparent advantage of ruminants over other species is linked to the ability of rumen microorganisms to degrade potentially harmful poisons, although the extent of and nature of such modification benefit is questioned by some (Diaz, 2003). Protozoa are considered to be more proactive in the detoxification role within the rumen compared with bacteria. The author goes on to outline the other factors which can compromise the effectiveness of such a process. These include a range of production stresses and genetic vulnerability. Tucker (2004) mentioned incidences of ochratoxicoses in ruminant animals being rarely observed due to the microbes hydrolysing OTA to a much more benign form. However, it was noted that in cases of severe poisoning, the detoxification capacity of the rumen may be exceeded.

Table 2. Rated sensitivities of livestock to selective mycotoxin ingestion.
(X = Low, XX= Moderate XXX High Level)

Mycotoxin	Ruminants	Pigs	Poultry	Horses
Deoxynivalenol	X	XXX	X	
Diacetoxyscirpenol T-2 toxin	X	XX	XXX	
Zearalenone	XX	XXX		
Fumonisin	X	X	X	XXX
Ochratoxin A	X	XXX	XX	
Aflatoxin	X	XX	XXX	
Roquefortine	XX			

The biological manifestation in livestock experiencing mycotoxicosis is dependent upon the specific mycotoxin implicated in the toxicological occurrence (Table 3). It is acknowledged that these are broad descriptions of associations when in practice, the deleterious physiological effects on affected animals and the consequences for impaired performance, are more common place. For example, a compromised immune system is likely to be a characteristic outcome of a range of mycotoxin type challenges. The degree to which clinical symptoms are observable compared with the more subtle subclinical effects of influence on animal performance criteria, is very much dependent on the level and duration of exposure to toxic substances and the animal's natural tolerance level. Research work is actively engaged in more accurately quantifying the latter. Despite the fact that aflatoxins have been the most studied group of mycotoxins over time, in the context of European animal production then their likely occurrence is fairly remote. However, others such as the most commonly reported trichothecene, DON and the nephrotoxic effects of OTA have been proven to have a significant impact on the European pig and poultry industries.

Table 3. **Productivity consequence of livestock consuming selective mycotoxins**.

Mycotoxin	Impact
Deoxynivalenol + (other trichothecenes)	Feed refusal (impaired nutrition)
Zearalenone	Reproductive dysfunction (hyperestrogenic)
Fumonisin/ Roquefortine/Patulin	Neural dysfunction
Ochratoxin A	Impaired kidney function (nephrotoxicity)
Aflatoxin/trichothecenes	Immunosuppression
Penicillic Acid	Antimicrobial
Trichothecenes	Inhibition of protein synthesis
Fusaric acid	Toxicological synergies (symptom enhancement)

Diagnostic challenges

Accurate diagnosis of both offending feedstuff mycotoxin and/or an instance of mycotoxicosis in farm livestock is essential in order to determine as rapidly as possible the most appropriate preventative and control measures. Unfortuantely the situation observed in the field or in the laboratory locations can be confounded by a number of other complex interacting factors. For example, even though relatively low levels of mycotoxins are detected in feed, classic symptoms of mycotoxicosis may be evidenced in the animal (Tucker 2004). Furthermore, it is not necessarily sound to interpret sub-optimal performance of livestock, as judged by reduced feed consumption, inferior than expected growth and symptoms in common with immunosuppression, as definite evidence of say symptoms of trichocethene toxicosis, although characteristic of such. Rather the spotlight may need to be on dubious management practices or the closer examination of secondary symptoms perhaps resulting from the presence of other opportunistic disease. Care needs to be also taken in experimental situations particularly when extrapolating or making inference from data accumulated under 'controlled' circumstances and the application of such findings to the commercial setting. This caution needs to be exercised too when comparing *in-vitro* with *in-vivo* studies.

Unexpected toxicity scenarios may be due to additive and interactive effects between different mycotoxins which may exaggerate the intensity of toxicity effect, post-ingestion. Co-contamination of feedstuffs may produce an enhanced negative impact on health and productivity of farm livestock when compared to their presence as the sole mycotoxin. For example, a survey conducted in France, amongst other countries, demonstrated the co-occurrence of DON and NIV in the range of cereal grains (Devegowda *et al.,* 1998). The *Fusarium* phytotoxin fusaric acid has been implicated and indeed has been demonstrated to have a toxicological synergistic effect when fed experimentally and supplementary to DON in young pig diets (Smith *et al.,* 1997; Smith and Seddon, 1998). Its phytotoxicity has been manifest in the pathology of soybean. Although its chemical characterisation has been established for some time, fusaric acid has not been considered an integral factor in *Fusarium* mycotoxicoses because of its relatively low toxicity. However, this pharmacologically active substance which is thought to alter brain chemistry in a number of animal species, appears to enhance the toxicological effects of the trichothecene DON as manifested by growth depression and lethargy in starter pigs (Smith *et al.,* 1998). This example and many other idiosynchracies characteristic of mycotoxin behaviour often lead to considerable difficulty in the correct diagnosis of mycotoxicoses.

5.0 Managing the risk of mycotoxity

This review highlights the far-reaching consequences that mould formation and mycotoxin occurrence can have in economic terms across the crop and livestock production sectors and the ultimate health-associated risks of contamination within the higher food chain (Figure 1). With a significant number of industries and organisations involved in feed and food production and technologies employed to improve efficiency, it is imperative that in the future more integrated approaches to prevention and control are considered and adopted. It is acknowledged by many within the agricultural industry including those who engage in research support that, due particularly to the vagaries of climate and other husbandry variables it is virtually impossible to completely eliminate mould and mycotoxin contamination of crops and feed commodities. That said, effective management and response to the risks associated with contamination must continue to be priority. In practice this means the adoption of appropriate intervention and prevention strategies ideally, coupled with meaningful control measures throughout the cropping phases and food chain. Assuming that the crop is being cultivated for the purpose of being offered as a feed to farm livestock in a fresh, conserved or further processed state, then sound (best) agricultural practice needs to be applied at the strategic pre- and post- harvest times. The final opportunity, essentially, when contaminatory impact can be minimised, is at the point of feed ingestion by livestock.

Pre-harvest agronomic practice

This relates to the time between crop establishment and full development when each growth stage has a particular vulnerability to a range and intensity of mould growth and contamination. Crop husbandry practice worthy of careful scrutiny includes cropping history, soil management and effective use of rotation although a study conducted by Schaafsma *et al.* (2001) indicated that the effect of environment and host susceptibility were much greater than that of rotation in respect of occurrence of *F.graminearum* and *Fusarium* head blight condition. In a number of studies linked with cereal mycotoxin evaluation in the field, it would seem that ploughing rather than minimal tillage is beneficial to subsequent cropping (Miedaner, 2004; Nicholson *et al.,* 2004). It is imperative that good quality fungus-free viable seed is used. Crop nutrition and in particular the application of nitrogen, has an important role to play in the maintenance of a healthy mould-free crop, although the level of influence is still debated (Schaafsma *et al.*. 2001). Although fungicide products are currently the key method of controlling cereal diseases across Europe, the efficiency of using this approach in isolation may be limited due to fungal species interaction and the potential for resistance to develop among the pathogen population. Limited success has been documented in respect of breeding plants for fungal resistance, for example, resistance in cereal crops to infection by *Fusarium* species (WHO, 2002). The breeding of mycotoxin-prone varieties with a high level of resistance is problematical due to the fact that such disease is quantitatively inherited (Nicolson *et al.,* 2004). Miedaner (2004) optimistically considers plant breeding to be an efficient method to combat *Fusarium* and *Aspergillus* species contamination in the field and supports the further pursuit of realistic breeding research and selection programmes. Such challenges for the geneticists and breeders could be assisted by the increased use of gene technologies, optimised selection procedures and molecular marker techniques. Competitive exclusion, the introduction of non-toxinogenic strains in the field as means of biological control has been considered but with variable results (WHO, 2002). Evidence suggests a greater success achieved using this methodology with a reduction in aflatoxin B, formation in a number of crops compared with mycotoxin producing *Fusarium* species.

Post-harvest storage and handling issues

Of particular interest and concern in the UK and across European countries is the presence and contamination of stored cereal grains by the mycotoxin OTA produced mainly but not exclusively by *Penicillium verrucosum* (Table 1). Indeed maximum permissible levels of 5ppb in raw cereals and 3 ppb in processed products imposed within the EU has been the focus of a European project research which has recently been reported on by Banks *et al.*, (2004). One purpose of this investigation was the identification of critical control points in order to achieve compliance with such limits. It is worth noting that in general, the EU has the lowest permitted mycotoxin ceilings globally in respect of food products (Gadd, 2003) The current evidence presented by Banks *et al.* (2004) and indeed previous work conducted by Banks *et al.* (2002), demonstrates the need for prompt and effective drying of cereal grains immediately after harvest in order to achieve safe moisture storage levels. The wettest grain is at greatest risk from toxin production. It follows that high hygiene standards in store must be adhered to and this also applies to grain handling facilities as this apparatus has the potential to harbour harmful sources of inoculum.

Mould inhibition can also be effected in moist storage grains by the application of an appropriate chemical preservative and has been an established practice for some time. The methodology is not just confined to cereal protection but also, for example, in hay preservation. Individual or combinations of organic acids (propionic, sorbic, benzoic, acetic) represent the main classification of mould inhibiting substances although salts of organic acids (i.e. calcium propionate) and copper sulphate have been used. Thorough distribution of liquid forms in the crop is essential and the acid form of inhibitors tend to be more active than its corresponding salt. A word of caution has been expressed by some regarding the administration of sub-lethal concentrations of propionic acid. Howell (1983) provided the example of *Fusarium culmorum*, associated with the mycotoxin ZEN exhibiting strong growth in the presence of an inadequate dose level of propionic acid when applied to stored grain.

Dietary modification

Following the storage phase the priority becomes prevention of mycotoxin-contaminated feedstuffs from causing performance impairment of the animal. Researchers have, therefore, exploited decontamination options at the point of ingestion. Various methods used to modify the diet have included the removal of toxins by solvent treatment, also physical and chemical detoxification procedures (Flannigan, 1991). It has been claimed, for example, that ammoniation is effective in rendering harmless, aflatoxin-contaminated oilseeds and cereals. Furthermore, dilution of contaminated with normal grain is considered an effective method for counteracting the adverse impact on animal productivity of toxin-containing feed (Charmley *et al.,* 1995). Success, however, with some of these methodologies is variable and often costly and impractical in commercial farming situations. Another possibility is to deliberately enhance the nutrient status of the diet by elevating energy, protein and micronutrient levels, particularly the amino acid methionine and trace element, selenium (Tucker, 2004).

A unique nutritional strategy to overcome mycotoxicoses is being explored and developed by the research team at the University of Guelph to investigate the potential for specific feed ingredients and nutrients to manipulate brain neurochemistry. Protein feed supplements have been identified such as corn gluten meal and blood protein components, that are rich in large neutral amino acids. Serotonin is a brain neurotransmitter that, when produced at elevated levels, is associated with lethargy and impaired appetite. Smith and Seddon (1998) have described the mechanism of action by which the synergistic effects of fusaric acid and DON raise blood and brain tryptophan and subsequently, brain serotonin. The dietary inclusion of large neutral amino acids could result in competition with tryptophan for transport sites through the blood-brain barrier. The consequence of this would be a reduction in the quantity of tryptophan entering the brain and decrease the production of serotonin. The papers produced by Cavan *et al.,* (1988) and Smith *et al.,* (2000) expand and elaborate on the important relationship between amino acids and neurochemical activity, an improved knowledge of which could assist in the dietary treatment of various mycotoxicoses.

In addition to such methods which are at times associated with unpredictable results, there has been an increased impetus in recent years to discover more routine but effective dietary treatments which will withstand rigorous scientific evaluation and scrutiny. A focus of attention by researchers has been the mycotoxin-neutralising capabilities of a range of so-called binding agents. The required mode of action and essential characteristics of such in order for them to be effective in minimising mycotoxin exposure to animals, have been comprehensively dealt with in the literature (Smith *et al.,* 2001; Diaz, 2003). Mycotoxins are essentially adsorbed to an inert compound (the binding product) and in so doing the toxins and their deleterious effects on animal health are prevented from being absorbed from the intestines in the conventional way. It is vital that the properties of a binding agent permit and facilitate a tight association with the offending toxin(s) in contaminated feed and to reduce the possibility of disassociating in the gastrointestinal tract. The range of binding additives which have been examined include bentonites, bleaching clays, activated carbons and alfalfa. A concern over the efficacy of some binders is their undesirable tendency to bind other dietary components. Most are non-nutritive therefore acting as dietary diluents.

More recently, biotechnologists have discovered the role carbohydrates can play, extra to their energy-yielding properties, in other biological functions such as immune response enhancement and neutralising various pathogens (Devegowda *et al.,* 1997). Of the oligosaccharides, mannanoligosaccharides derived from the yeast cell wall, have shown most promise in animal production due to their high degree of antigenicity facilitated by the mannon and glucan components. Such organic polymers like esterified glucomannans have demonstrated a strong affinity for certain mycotoxins. Although a significant number of binding agents have been marketed commercially in a number of European countries, not all have published reports which demonstrate the efficacy of the product due to sound experimental evaluation. An esterified glucomannan enzymatically extracted from the cell wall of *Saccharomyces cereviciae*, has received much attention by researchers, 'Mycosorb' produced by Alltech Inc. This has shown much promise as demonstrated in *in vitro* and *in vivo* studies. Mainly beneficial binding of mycotoxin effects have been reported in the literature particularly in poultry and pig experiments (Smith *et al.,* 2001; Tucker, 2004). However, in a recently published study by Swamy *et al.,* (2003) in which in addition to immunological response measurements, the efficacy of a similar adsorbent was evaluated,

prevention of reduced feed intake which is a consequence of *Fusarium* exposure, was not fully demonstrated. *In vitro* evaluation of Mycosorb has revealed considerable binding effectiveness with a range of mycotoxins with the highest being aflatoxin (85%) and lowest, NIV (8%) (Devegowda *et al.,* 1998). The importance of conducting *in vivo* studies to complement any *in vitro* findings has been emphasised in the literature (Ledoux and Rottinghaus, 1999) in order for a more complete and accurate assessment to emerge.

6.0 Concluding comments

Our knowledge of the defined area of mycotoxity has increased significantly in recent years. The advent of biotechnology in the field of animal health in addressing the hidden dangers inherent in mycotoxins continues to hold a promise for the future. However, complacency is not appropriate. Despite technological progress, as a rapidly increasing world population demands to be fed, natural environmental factors which favour mould infestation and mycotoxin contamination of high value crops and livestock, will continue to dictate the nature and speed of innovative responses to ensure a safe, high quality food supply. From the perspective of the production end of the food chain, Bhatnager *et al.,* (2004) has identified a twin approach to the priorities for research. Firstly, emphasis on devising more rapid procedures for detecting the presence of fungal and toxin contamination of crops which embrace sensitivity, accuracy and sustainability and secondly, the creation and adoption of effective strategies for controlling contamination prior to harvest and the decontamination of commodities post-harvest.

Maximising protection of the whole food chain is of paramount importance, which includes the consumer of animal products (Figure 1). In addition, there is the challenge within the industry of minimising the possibility of workers and processors being exposed to excessive and potentially harmful levels of fungal spores and contaminants in the building environment. Magan (2003) suggests that this aspect has received insufficient attention. Veldman (2004) in his paper on mycotoxins in the animal production chain, considered the transmission risk of toxin contaminatory effects from animal tissue to humans to be fairly minimal and concluded that direct consumption of plant-derived products carries a greater risk. The main drivers in enforcing strict appliance of quality assurance and HACCP principles in the food chain across Europe appear to be the regulatory framework and EU directives which have been established to maintain standards. At the farm level, however, many challenges remain in respect of the ongoing effort required to maintain the crop and animal hygiene standards required. Best agricultural practice remains an essential and integral aspect of managing the mycotoxin risk.

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