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Mycotoxin contamination: assessment of risk in livestock systems

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Glossary

AC	Activated Carbon
AFB1	Aflatoxin B_1
AFLs	Aflatoxins
AFM1	Aflatoxin M_1
AME	Alternariol Monomethyl Ether
AOH	Alternariol
CEN	European Committee for Standardization (Comité Européen de Normalisation)
CIT	Citrinin
DAS	Diacetoxyscirpenol
DOM	Deepoxy-deoxynivalenol
DON	Deoxynivalenol
EAs	Ergot Alkaloids
EFSA	European Food Safety Authority
EGM	Esterified Glucomannan
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organisation
FB1	Fumonisin B_1
FDG	Fusarium Damaged Grain
FHB	Fusarium Head Blight
FUM	Fumonisins
GI	Gastro Intestinal
GMA	Glucomannan Mycotoxin Adsorbent
HACCP	Hazard Analysis Critical Control Point
HSCAS	Hydrated Sodium Calcium Aluminosilicate
HT-2	HT-2 toxin
LAB	Lactic Acid Bacteria
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LFD	Lateral Flow Device
LOD	Limit of Detection
LOEL	Lowest Observed Effect Level
LOQ	Limit of Quantification
MDP	Mycotoxin Deactivation Products

MPL	Maximum Permitted Limit
MU	Measurement Uncertainty
NIV	Nivalenol
NOEL	No Observed Effect Level
PGR	Plant Growth Regulators
OTA	Ochratoxin A
m/z	Mass to Charge Ratio
QUECHERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RSD	Relative Standard Deviation
RSD_r	Repeatability
S/N	Signal to Noise Ratio
T-2	T-2 Toxin
TeA	Tenuazonic Acid
TMR	Total Mixed Ration
TTX	Tentoxin
UPLC-MS/MS	Ultra Performance Liquid Chromatography Tandem Mass Spectrometry
VS	Veterinary Surgeons
WHO	World Health Organisation
YCW	Yeast Cell Wall
α-ZEL	α-Zearalenol
β-ZEL	β-Zearalenol
ZEN	Zearalenone

1. Abstract

A desk review to assess the risk to livestock systems (pigs and ruminants) from mycotoxins was conducted by a group of researchers and veterinarians with experience of mycotoxins, cereal crops/agronomy and livestock. Information was collected from many sources – published scientific literature and grey literature/trade journals. Direct information of mycotoxin issues and other anecdotal evidence was collected from farmers and industrial sources by surveys.

The first part of this review summarises the various mycotoxins, legislation applicable to feed materials and the occurrence of mycotoxins in feed materials, as well as straw. Agronomic practices that can impact on mycotoxin levels in feed, silage, pasture and straw were reviewed and prevention strategies to minimise mycotoxin formation in the field and store were recommended.

Next, the potential effects of mycotoxins for pigs and ruminants, including dairy cattle and sheep, were considered by summarising published information on acute and chronic effects of mycotoxins on each species. Analytical testing methods, both screening and confirmatory, for mycotoxins in animal products, feed, forage straw and live animals were reviewed.

The scientific literature on the different classes of mycotoxin binders, their activity and effectiveness, was reviewed and included in this report. A substantial number of commercial mycotoxin binders/deactivators are available with suppliers offering a range of solutions from single component products to more complex, multicomponent sequestering formulations. Many producers try to provide a holistic approach towards mycotoxicosis and compensate for adverse effects. However, findings suggest that there is no sequestering product versatile enough to effectively remove low level mycotoxin mixtures from feed. Only bentonite is approved for use as a mycotoxin binder, and is approved for ruminants, pigs and poultry. Two other products approved by EFSA are biotransforming products. They each have specific activity to one type of mycotoxin, so the presence of these should be confirmed before these products are used.

Surveys of vets and farmers showed the use of binder products appears to be considerably different within the different livestock agricultural sectors. The cattle industry appears to be using binder products as part of treatment-based protocols when suspicious of mycotoxin exposure. In contrast, the use in the pig industry has much more of a prophylactic stance, often being used all year round, and primarily focussed on the adult breeding herd.

A series of key recommendation factsheets for farmers was produced. These include the need to carry out surveys of UK feed materials to determine mycotoxin levels, the need to establish more accurately animal intake of straw bedding, and research on levels of mycotoxins in pasture. Toxicity studies on lower levels of mycotoxins as well as the effects of mixtures are needed and coupled with this, there is a need to develop diagnostic analytical methods that measure biomarkers or markers of effect rather than parent mycotoxin. Finally, controlled studies on binders in real situations would be of benefit to produce conclusive evidence of their efficacy.

2. Introduction

A desk review to assess the risk to livestock systems from mycotoxins was conducted by a group of researchers and veterinarians with experience of mycotoxins, cereal crops/agronomy and livestock (veterinary and husbandry). Information was collected from many sources including published scientific literature, and grey literature/trade journals. Direct information of mycotoxin issues and other anecdotal evidence was collected from farmers and industrial sources by survey to ensure all possible information sources are captured.

The review covers analytical testing methods for mycotoxins in animal products, feed, forage straw and live animals, and considers issues such as availability of tests (screening and confirmatory); their cost and reliability and availability of diagnostic tests for animals. Potential effects of mycotoxins for different species, indicators for chronic and acute exposure and the possible effects of interactions of multiple mycotoxins are summarised. Prevention strategies, including good agronomic practice, the use of risk management practices to cover all possible exposure routes, and a review of the availability and use of commercial “mycotoxin binders” are also included.

The review sought to determine the severity of the mycotoxin problem in the UK, highlight research gaps and collect evidence that can be used for regulatory purposes. A key aim was to develop recommendations and advice for farmers. To achieve this, a series of key recommendation fact sheets for farmers were produced.

3. Literature searching methodology

3.1. Peer review literature

Partners completed searches using their own available facilities. The partners had access to numerous secondary sources indexing an immense body of the relevant scientific literature. For example, on the OVID host access includes:

- CAB Abstracts – 1973 to present (includes full-text access to 117 CABI book titles)
- BIOSIS Previews – 1985 to present
- Medline – 1946 to present
- Zoological Record – 1993 to present
- Food Science and Technology Abstracts – 1969 to present

For the above, simultaneous searching with duplicate removal is the standard approach. Via the Web of Knowledge host there was access to:

- Web of Science – 1981 to present

In addition, via the Proquest Dialog host pay-as-you go access to circa 250 science databases with the follow being of obvious potential relevance to this project was available:

- *CAB Abstracts – 1972 to present
- *Biosis Previews(R) – 1926 to present
- *Zoological Record Online(R)
- Current Contents Search(R) – 1990 to present
- AGRICOLA – 1970 to present
- CSA Life Sciences Abstracts – 1966 to present
- *SciSearch(R) Cited Ref Sci – 1990 to present
- *Medline – 1950 to present
- EMBASE – 1974 to present
- AGRIS – 1974 to present
- Pascal – 1973 to present
- ELSEVIER BIOBASE – 1994 to present
- Environmental Sciences – 1966 to present
- GEOBASE(TM) – 1980 to present
- SciSearch(R) Cited Ref Sci – 1974 to 1989
- Periodical Abs Plustext – 1986to present
- Inside Conferences – 1993 to present
- FEDRIP
- Dissertation Abs Online
- Enviroline(R) – 1975 to 2008

3.1.1. Recording results

Results were stored in EndNote software record storage and retrieval system.

3.2. Grey literature search

3.2.1. Search criteria

A grey literature review was carried out to complement the peer reviewed literature search carried out by other project partners. The search included online databases, including PubMed, Web of Science, CAB Abstracts and Google Scholar. The objective of the grey

literature review was to collate non-peer reviewed reports and data on the potential effects of mycotoxin exposure in livestock (cattle/pigs/sheep), the availability and use of binders and the availability of testing services. The search criteria were set to include results that originated from the UK, and that were reported between January 2004 and January 2015. It included all material relative to the search terms below that had not been published in academic journals. For example; conference proceedings, industry reports, theses/dissertations, newsletters, research reports, technical specifications and informal communications. The exact search terms and sources of information are highlighted below (Table 1). The search was carried out between the 14th December 2014 and the 5th January 2015.

Table 1. Search terms and locations searched for grey literature.

Search Term	**Locations searched**
Mycotoxin awareness	Opengrey
Veterinary mycotoxin awareness	PubMed
Veterinary mycotoxin understanding	Medline
Experience with mycotoxins in cattle	Scopus
Experience with mycotoxins in sheep	Web of Science
Experience with mycotoxins in pigs	CAB direct
Mycotoxin perceptions in cattle	Agricola
Mycotoxin perceptions in sheep	Biosis Citation Index
Mycotoxin perceptions in pigs	ISRCTN directory
Appreciation of mycotoxicosis in cattle	Global Health Database
Appreciation of mycotoxicosis in sheep	Europe PubMed Central
Appreciation of mycotoxicosis in pigs	Google Scholar

When searching within Google Scholar, irrespective of the total number of search hits received, only the initial 1000 were visible to read.

Nine key industry individuals were contacted via email to acquire any additional unpublished data or personal communications (See appendix 5.).

3.2.2. Recording results

Results were recorded in Microsoft Excel and included search terms, total number of references returned, number of published references returned, number of grey literature references returned that fit the search criteria and the URL for the documents.

4. Mycotoxins in feed

4.1. Introduction

Mycotoxins are a group of 300 to 400 secondary metabolites produced by microscopic, filamentous fungi which differ both in size and structure while sharing a common trait of toxicity to vertebrates and other animals, at low levels (Bennett and Klich, 2003). They are mostly produced by fungi of *Claviceps, Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* genus (da Rocha *et al.*, 2014) with the first three being plant pathogens and the next two spoilage organisms (D'Mello, 2003). Thus, the contamination may take place during crop production but also during their preservation (in case of hay and silage) or storage if good manufacturing practice is not adhered to (Zachariasova *et al.*, 2014). The main groups of mycotoxins which have been recognised as those of the highest significance regarding public health and agro-economics include aflatoxins (AFL), fumonisins (FUM), ochratoxin A (OTA), trichothecenes (TRIC) and zearalenone (ZEN) (Zain, 2011). The abbreviation ZEN has been chosen to follow convention suggested by Metzler (2011).

Nevertheless, EFSA has recently issued scientific opinions on the risk to human and animal health related to the reported presence of ergot alkaloids (EAs), *Alternaria* toxins, as well as citrinin (CIT) in food and feed, indicating that the spectrum of compounds of concern is broadening (EFSA, 2011a; 2011b; 2012a; 2012b).

4.2. Current legislation

After carrying out risk assessments for various mycotoxins in feed, the European Food Safety Authority (EFSA) has listed a number of compounds that pose a potential risk to human or animal health including AFB_1, DON, ZEN, OTA, fumonisins, T-2 and HT-2 toxins (EFSA, 2004a; 2004b; 2004c; 2004d; 2005; 2011b). Due to its carcinogenicity, aflatoxin B_1 (AFB_1) is the only mycotoxin with established maximum permitted level (MPLs) in feedstuffs under the Directive 2003/100/EC (amending Directive 2002/32/EC), while the remainder assessed by EFSA have had guidance values established (Table 2) under Commission Recommendation 2006/576/EC (EC, 2003; 2006; 2013b). In 2002, the European Commission (EC) also established an MPL for the fungal sclerotia of rye ergot (*Claviceps purpurea*) in feedstuffs containing unground cereals. Ten years later, a follow-up recommendation was issued to monitor ergot alkaloids in cereals and cereal products intended for animal feed (EC, 2012). Apart from the EU, harmonization of mycotoxin-related regulations also takes place in some free trade zones such as Mercado Común del Sur (MERCOSUR), Association of Southeast Asian Nations (ASEAN), Gulf Co-operation Council

(GCC), Australia/New Zealand, while the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) supported *Codex Alimentarius* Commission develops food and feed standards on the international level. According to the FAO, Europe has the most extensive regulations for mycotoxins in feed. Canadian regulations are among the most detailed as they additionally include mycotoxins not regulated in EU feedstuffs such as ergot alkaloids and diacetoxyscirpenol (a trichothecene), with China and the Islamic Republic of Iran also having demanding limits in place. Nevertheless, regulations in the rest of the world undoubtedly focus majorly on AFLs, with only 15 countries in Africa having specific, feed-oriented mycotoxin regulations in place (FAO, 2004).

Table 2. Maximum permitted levels and guidance levels of mycotoxin in products intended for animal feed in the EU (EC, 2003; 2006; 2012; 2013b). Where: a - mycotoxin content is relative to a feedingstuff with a moisture content of 12%.

Toxin	Products intended for animal feed	Content mg/kg [a]
AFB_1	Feed materials	0.02
	Complementary and complete feed with the exception of:	0.01
	-compound feed for dairy cattle and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry animals	0.005
	-compound feed for cattle (except dairy cattle and calves), sheep (except dairy sheep and lambs), goats (except dairy goats and kids), pigs (except piglets) and poultry (except young animals).	0.02
DON	Feed materials	
	-cereals and cereal products with the exception of maize by-products	8.0
	-maize by-products	12.0
	Complementary and complete feedingstuffs with the exception of:	5.0
	-complementary and complete feeding stuffs for pigs	0.90
	-complementary and complete feeding stuffs for calves (<4 months), lambs and kids	2.0
ZEN	Feed materials	
	-cereals and cereal products with the exception of maize by-products	2.0
	-maize by-products	3.0
	Complementary and complete feedingstuffs	
	-complementary and complete feeding stuffs for piglets and gilts	0.10
	-complementary and complete feeding stuffs for sows and fattening pigs	0.25
	-complementary and complete feedingstuffs for calves, dairy cattle, sheep (including lambs) and goats (including kids)	0.50
OTA	Feed materials	
	-cereals and cereal products	0.25
	Complementary and complete feedingstuffs	
	-complementary and complete feeding stuffs for pigs	0.05
	-complementary and complete feeding stuffs for poultry	0.10
FB_1 and FB_2	Feed materials	
	-maize and maize by-products	60
	Complementary and complete feedingstuffs for:	
	-pigs, horses (Equidae), rabbits and pet animals	5
	-fish	10
	-poultry, calves (< 4 months), lambs and kids	50
	-adult ruminants (> 4 months) and mink	20
T-2 and HT-2	Cereal products for feed and compound feed	
	Oat milling products	2
	Other cereal products	0.5
	Compound feed, with the exception of feed for cats	0.25
Rye ergot *(Claviceps purpurea)*	All feedingstuffs containing unground cereals	1,000

4.3. Reported levels of mycotoxins in feed

There is a very large number of studies available in the literature investigating mycotoxins occurrence in feed, and their levels as well as factors influencing their production (Table 3). Results of a worldwide animal feed survey point to the problem of high levels of feed co-contamination with a number of different mycotoxins. Out of 72% of samples containing detectable levels of mycotoxins, 38% were co-contaminated with more than one toxin (Streit *et al.*, 2013), while only 1 – 18% samples (depending on the toxin) presented levels above the EU guidelines or regulations. Also, an EU feeding materials study of Monbaliu *et al*., reports that out of 82% contaminated samples, 75% were co-contaminated with more than one mycotoxin (some with up to 10 toxins), with only two samples exceeding the recommended EU levels (Monbaliu *et al.*, 2010). This high percentage of contaminated samples reported in the literature contradicts FAO's claims stating that only 25% of the world's crops are contaminated with mycotoxins (Park *et al.*, 1999). What is more, the low percentage of samples containing mycotoxins exceeding recommended values, accompanied by high co-contamination rates, was judged to pose a possible toxicological hazard which should not be underestimated and there have been calls for in-depth investigations (Grajewski *et al.*, 2012; Zachariasova *et al.*, 2014).

Mycotoxin production by pathogenic fungi is greatly influenced by a number of physical, chemical and biological factors such as pre- and post-harvest temperature, CO_2 and moisture levels but also nutrient availability, pesticide usage, physical damage or pest attacks. The climate is thought to be a key driving force influencing all of the factors mentioned, thus having a profound impact on host-pathogen dynamics (Tirado *et al.*, 2010). Some authors, taking the UK's predicted future weather patterns as an example, suggest that fungal species, the range of mycotoxins produced, as well as their levels may be affected by the ongoing climate change, most probably increasing both the severity and the area of the outbreaks (Paterson and Lima, 2010).

A summary of mycotoxin surveys conducted in years 2004 – 2011 (Streit *et al.*, 2013) encompassing more than 17,000 feed samples from all over the world revealed that globally, the percentage of samples with detectable levels of AFLs, ZEN, DON, FB_1 or OTA over the time of the study was fairly stable. However, an increasing trend in positive samples from south-east Asia was noted, with climate change quoted as a possible explanation. The authors also point out that, indeed stress growth conditions, resulting from the climatic variations, may be the reason of the migration of mycotoxin contamination from 'traditional' regions to novel locations an example also being AFLs migration from (sub-) tropical regions

to southern Europe. Some more detailed studies have also connected changes in the climate with large year to year variations in mycotoxins concentrations as well as variation in contamination patterns (Goertz *et al.*, 2010; Grajewski *et al.*, 2012). Also, a growing concern related to the high prevalence of the 'emerging' toxins such as *Alternaria* toxins and enniatins in a broad range of feedstuffs has been reported, underlining the need for the complex assessment of their toxicological properties (Zachariasova *et al.*, 2014).

Straw is commonly used as animal bedding as well as food, but compared to feed, there is relatively little information on animal exposure to straw. Two small-scale studies in the UK identified higher mycotoxin contamination in straw compared to grains and a higher concentration in wheat compared to barley straw (White *et al*., 2007; Edwards & Stewart, 2010). In the delayed harvest of 2008, nearly 50% of straw samples exceeded the feed guidance limits for DON and ZEN in feed (Edwards & Stewart, 2010). A recent study by Nordkvist *et al.* (2014) found that 86 and 58 % of surveyed straw samples (n=79) collected from Swedish pig farms were contaminated with DON (average 884 µg/kg) and ZEN (average 134 µg/kg), respectively. The most recent study showed up to 100% incidence of DON in wheat, barley and oat straw with an average DON concentration in oat straw bales from one field of over 16,000 µg/kg. Contamination levels of DON and ZEN were higher in oat straw and the contamination observed was more uniform. The mycotoxin levels varied greatly between bales, and there was no obvious correlation between DON and ZEN levels, which may make sampling difficult (Häggblom & Nordkvist, 2015).

Predominant fusarium mycotoxins of concern in wheat are deoxynivalenol (DON) and zearalenone (ZEN). Both of these mycotoxins are produced predominantly by *F. graminearum* and *F. culmorum* as a result of fusarium head blight (FHB) infections. Wheat is most susceptible to FHB during flowering (Obst *et al.*, 1997; Lacey *et al.*, 1999) with symptoms developing two to four weeks later. Flowering in the UK occurs from late May in the south of England to mid-July in the north of Scotland. Flowering time varies with drilling date, weather and variety. Flowering duration varies with weather and variety. FHB is assessed in the field after flowering, usually one to four weeks post-anthesis and is based on the number of heads with blight symptoms (incidence) or the number of spikelets with blight symptoms (severity). The two measurements are closely correlated (Xu *et al.*, 2004). At harvest, grains can be visually assessed for *Fusarium* damaged grain (FDG) or infection can be measured by culturing the *Fusarium* from grain on blotting paper or microbiological media to determine *Fusarium* infected grain. Many studies have been directed at the control of FHB and have not assessed mycotoxin concentration. In most countries where these studies have been performed, *F. graminearum* is the predominant FHB pathogen, and as this is the

most potent DON producing species, there is a reasonable relationship between FHB severity, %FDG and DON concentration. It is, however, important to note that in the UK, *Microdochium* species can be the predominant FHB pathogen and these species do not result in FDG or fusarium infected grain or any known mycotoxin. For UK data, it is therefore advisable not to assume that a measurement of FHB is closely related to DON concentration at harvest (Edwards *et al.*, 2001). A similar situation has been reported in France (Champeil *et al.*, 2004).

DON is the predominant *Fusarium* mycotoxin found in cereals worldwide. As a result, DON is the most common *Fusarium* mycotoxin quantified in research studies. In the UK there is a reasonable correlation between DON and ZEN concentrations in UK wheat at harvest, although the relationship varies between seasons. DON and ZEN are produced at different stages of crop development, with DON being produced during infection (flowering and milky ripe stages) and ZEN during ripening (dough development onwards). Once *Fusarium* infection has occurred, levels of ZEN are strongly dependent on rainfall during ripening and delayed harvests (Kharbikar, 2013). DON and ZEN are produced by the same species of *Fusarium* and as such methods which reduce DON should have a corresponding benefit in the reduction in ZEN as well.

The distribution of *Fusarium* mycotoxins is highly skewed with the majority of UK wheat samples containing levels well below guideline limits for feed, but a few samples contain levels above these limits. The overall risk of exceeding DON and ZEN limits in unprocessed cereals in the UK is low for wheat and very low for barley and oats. This risk varies with year and region. Lowest levels are in the north of the UK. High levels of DON have been found after all forms of agronomy. Modifying agronomy can reduce the risk of exceeding guideline limits for feed, but it cannot remove this risk.

5. Agronomic factors impacting on mycotoxin contamination of feed

5.1. Crop rotation

Numerous studies have shown that FHB and/or DON in wheat are affected by the previous crop. Maize is a major host of *F. graminearum*, which is the most potent producer of DON and ZEN. An early observational study of wheat fields (n=28) in Illinois identified that a higher incidence of FHB occurred in wheat after maize and in particular, wheat after a succession of two maize crops, and in wheat following grain maize compared to silage

maize (Holbert *et al.*, 1919). There is also anecdotal evidence from the epidemic years of FHB in the USA from 1991–1996 when high levels were contributed to by a high proportion of cultivated land under min-till and planted to susceptible host crops and short rotation intervals between susceptible crops (McMullen *et al.*, 1997). In Ontario, Canada in 1983, fields where maize was the previous crop (n=5, FHB incidence = 0.036%) had a significantly higher incidence of FHB than fields following small grain cereal (n=4, FHB incidence = 0.007%) or soybean (n=13, FHB incidence = 0.005%). In this case the severity of the infection was not recorded, only the incidence, and although the values are low the difference observed was statistically significant (Teich & Nelson, 1984). In a repeated study the following year, fields where maize was the previous crop (n=7, DON = 657 µg/kg) had significantly higher DON than fields following a crop other than corn (n=14, DON = 54 µg/kg) (Teich & Hamilton, 1985). Sturz & Johnston (1985) found higher levels of FHB in wheat following wheat rather than wheat following fallow. In replicated field experiments in Minnesota, previous crop and tillage were compared in a three-year factorial experiment (Dill-Macky & Jones, 2000). On average, the DON concentration was 25% lower in wheat following soybean compared to wheat following wheat, and 50% lower in wheat following soybean compared to wheat following maize. *Fusarium* species were isolated from all crop residues. *F. graminearum* was the predominant species present on maize and wheat residues whereas other *Fusarium* species, in particular *F. sporotrichioides* predominated on soybean residues.

In the 1990s, a large observational study of FHB and DON was conducted in Bavaria, Germany (n=1600). On average, wheat following grain maize had the highest DON concentrations (mean ca. 500 µg/kg), followed by wheat following silage maize (mean ca. 300 µg/kg). It is proposed that this difference was due to the higher quantity of crop residue present after harvest of grain maize (Obst *et al.*, 1997). However, some of this difference may be due to differences in maize variety susceptibility or harvest dates. This study also showed that DON concentration was lower in wheat following wheat, barley or oilseed rape (means ca. 100 µg/kg) compared to wheat following potatoes or sugar beet (means ca. 200 µg/kg). The authors postulated that this may be due to the later sowing of wheat following potatoes or sugar beet. A large replicated field experiment in Germany identified that wheat following wheat had a higher FHB incidence and DON concentration compared to wheat following sugar beet. There was also a significant interaction with cultivation technique (Koch *et al.*, 2006). The difference between the two German studies above may be due to differences in agronomy, such as sowing date (as postulated in the observational study), which were standardised in the field experiment. An observational study performed using commercial fields (n=233) in Canada from 1996 to 1999 (Schaafsma *et al.*, 2001) identified

significantly lower DON in wheat following soybeans or wheat compared to wheat following maize. Three percent of the variance (P=0.05) was accounted for by the crop two years previous. UK studies have shown that maize as a previous crop greatly increases the risk of high DON and ZEN in subsequent wheat crops (Edwards, 2007). This first UK study did not show increased risk with other previous crops, however, more recent data has shown a higher risk after sugar beet (unpublished AHDB Cereals & Oilseeds data) although this risk is still much lower than that of maize as a previous crop. This risk maybe confounded with regional differences as sugar beet production is now restricted to the eastern counties of England.

5.2. Cultivation

In the 1990s, a large observational study of FHB and DON was conducted in Bavaria, Germany (n=1600). On average, DON concentration of wheat crops after maize was ten-times higher if the field was min-tilled compared to ploughed (Obst *et al.*, 1997). An observational study performed using commercial fields (n=233) in Canada from 1996 to 1999 (Schaafsma *et al.*, 2001) determined that tillage was only a significant factor (P=0.004) in one year, 1997, when it accounted for 16% of the variation observed. In 1997, wheat DON concentration after min-till was 1300 µg/kg, after no-till was 700 µg/kg and after ploughing was 500 µg/kg. In replicated field experiments in Minnesota, previous crop and tillage were compared in a three-year factorial experiment (Dill-Macky & Jones, 2000). DON concentration was significantly reduced by ploughing compared to min-till and no-till in wheat plots following maize, but not in plots following wheat or soybean. A four-year field experiment failed to identify differences in FHB incidence using different tillage systems (Miller *et al.*, 1998). The lack of differences may have been due to spread of inoculum between plots. Large replicated field trials in Germany identified that there was a significant interaction between previous crop and cultivation technique (Koch *et al.*, 2006). Following sugar beet, there was no significant difference in DON concentration between wheat plots receiving different methods of cultivation, however, following a wheat crop without straw removal, direct drilled wheat had a significantly higher DON compared to wheat from plots which were either ploughed or min-tilled (Koch *et al.*, 2006). Studies in France have determined that crop debris management can have a large impact on DON concentration at harvest, particularly after maize. Highest DON concentration was found after no-till, followed by min-till and then lowest levels after ploughing. The reduction in DON has been linked to the reduction in crop residue on the soil surface (Blandino, 2010). However, the reduction in DON with min-till, compared to no-till is usually greater than the reduction of crop residue on the soil surface (Labreuche *et al.*, 2005; Maumene, 2005). This is probably due to the fact

that min-till increases the colonisation of crop debris with soil saprophytic microorganisms, which compete with *Fusarium* species. Chopping of maize debris before minimum tillage or zero tillage also caused a marked decrease in DON concentration in the following wheat crop (Maumene, 2005), again this is likely to reduce the size of crop debris particles and increase the mixing of crop debris with soil.

5.3. Crop nutrition

No significant differences were reported by Teich and Hamilton in 1984, either in the rate of application or the form of nutrient application (Teich & Hamilton, 1985). In a previous study, they had identified that soils high in phosphorus and nitrogen had a lower incidence of FHB (Teich & Nelson, 1984). In both studies, urea was associated with lower FHB incidence compared to ammonium nitrate, but this was not significant in either year. Results from split field experiments performed in 1985 and 1986 identified significantly lower FHB incidence in wheat receiving urea rather than ammonium nitrate as a source of nitrogen. The incidence of FHB was about 30% lower with urea treatment (Teich, 1987). In a large survey conducted in Saskatchewan, Canada from 1999 to 2002 (n=659), nitrogen fertiliser had no impact on FHB infection. A field study in Germany compared a number of agronomic factors within replicated field trials. One factor was the form of nitrogen applied, with nitrolime (a mixture of ammonium nitrate and limestone) treated plots having significantly less FHB incidence that calcium ammonium nitrate treated plots (Yi *et al.*, 2001). Glasshouse studies indicated that nitrolime is fungistatic to *F. graminearum* (Yi *et al.*, 2002). A replicated factorial experiment of nitrogen source and rate identified higher FHB with natural infection occurred with increasing rate of nitrogen applied from 0 to 160 kg ha^{-1} for all forms of nitrogen used. The form of nitrogen, both inorganic (ammonium nitrate urea and ammonium nitrate) and organic had no significant effect (Lemmens *et al.*, 2004). A repeated study with artificial inoculation provided similar results with a ca. two-fold increase in DON after an application of 160 kg ha^{-1} ammonium nitrate urea (Lemmens *et al.*, 2004). The fact that an artificially inoculated trial gave similar results indicates that nitrogen rate does not affect inoculum production or dispersal to the ear. The authors postulated that nitrogen can modify crop canopy, and thus alter the microclimate or can lead to extension of the flowering period, during which the crop is most susceptible to infection. The fact that high nitrogen is required to produce economically viable yields and quality (i.e. protein content) means modification of nitrogen inputs is not a valid method of reducing DON. It should be also noted that nitrogen inputs above the optimum increases the risk of lodging, which will result in an increased risk of high DON in harvested grain (see Section on PGR and Lodging).

5.4. Fungicides

5.4.1. Fungicide seed treatment

Only a few experiments have shown the ability of a fungicide seed treatment to reduce FHB or *Fusarium* mycotoxins at harvest. This is probably because most experiments are performed on small plots and spread of inoculum between plots over the growing season results in no significant differences later in the season (e.g. (Sturz & Johnston, 1985; Schaafsma & Tamburic-Ilincic, 2005). One observational study by Teich and Hamilton in Ontario, Canada in 1984 (Teich & Hamilton, 1985) showed a significant reduction in head blight incidence (only incidence was recorded, not severity) after seed treatment (n=10, mean %FHB incidence = 0.091) compared to fields with no seed treatment (n=3; mean %FHB incidence = 0.144) in fields of wheat following maize. As seed treatments reduce the amount of *Fusarium* present on the stem base of cereals during early growth stages, this could reduce the amount of inoculum present. However, there is much evidence to suggest that crop debris is the main source of inoculum and therefore, fungicide seed treatment is likely to be of only occasional benefit; i.e. where seed-borne infection is the main source of infection within a field. In a series of experiments over five years, it was determined that severe FHB only occurred at a UK site when local inoculum was present (Bateman, 2005). Infected seed did not result in increased FHB incidence when tested under conditions that resulted in increased FHB after application of infected crop debris (two of two years). In a study of organic and conventional production at three sites over three years, there was no correlation between the incidence of *Fusarium* species on seed and in the resultant grain at harvest (Birzele *et al.*, 2002). A large-scale long-term observational study of commercial wheat crops in France (n=2958) identified that wheat crops from seed treated with fludioxonil had, on average a 26% lower DON content compared to crops treated with other seed treatments (Syngenta, 2009).

5.4.2. Foliar fungicides

One study has identified that the application of prothioconazole at the traditional foliar pathogen spray timings of T1 (GS31) and T2 (GS39) can significantly reduce FHB and DON. The reduction achieved at T1 and T2 was much less than at the traditional flowering T3 (GS59-63) timing but the reductions were additive with a cumulative beneficial effect from all three timings (Edwards & Godley, 2010).

5.4.3. Head fungicides (T3 application)

Numerous studies have been conducted to identify the extent to which fungicides applied during flowering can reduce FHB and subsequent DON in harvested grains. The factors determined to be important are the fungicide used, the rate and the timing of application. Most experiments are conducted with inoculation of the crop with *Fusarium* spores and mist irrigation to ensure severe FHB occurs. The most recent, independent studies performed in the UK were performed by Nicholson *et al.* (2003) over three sites and three years. Results from this study identified that the azoles, tebuconazole, metconazole and prothioconazole significantly reduced FHB symptoms and fusarium mycotoxin concentrations. At full rate, the greatest reduction in DON concentration occurred with prothioconazole (10-fold). Efficacy was reduced as dose was reduced. Azoxystrobin had little impact on mycotoxin concentration in harvested grain when *Fusarium* species dominated the site but could result in an increase in mycotoxin concentration in grain when *M. nivale* was the predominant species present. The ability of azoxystrobin to result in an increase in FHB and DON concentration in harvested grain has been reported on a number of occasions (Mesterhazy *et al.*, 2003; Ioos *et al.*, 2005). Fungicide mixtures of azoxystrobin and an azole resulted in a lower reduction of DON compared to an azole alone (Edwards *et al.*, 2001; Nicholson *et al.*, 2003). Reductions in DON observed in field experiments using fungicides against natural infections of *Fusarium* are lower and inconsistent (Ioos *et al.,* 2005; Simpson *et al.*, 2001). On average, a two-fold reduction was observed in large-scale field experiments in Germany from a full rate of tebuconazole (Koch *et al.*, 2006). This is probably because during natural infection, infection occurs over greater a period of time. In trials with spray inoculation, the application of pathogen and fungicide are synchronised. Some studies have shown that fungicide application must be close to inoculation time (± 2 days) for optimum control (Nicholson *et al.*, 2003) whilst others have shown a wider window and variable optimum timing dependent on season (D'Angelo *et al.*, 2014). Later application of fungicides after anthesis can reduce mycotoxin content but not FHB (Yoshida *et al., 2012*).

5.5. Insecticide use and insect transmission

A few studies have identified a role of insect in the transmission or infection of fusarium species. *Fusarium* species were found on a wide range of insects, indicating that they can act as a vector (Miller *et al.*, 1998). Mongrain *et al.* (2000) determined that *F. graminearum* could be found at low incidence on wheat blossom midge, and that under laboratory conditions, could transmit *F. graminearum* to wheat plants resulting in FHB infection. The low incidence of *F. graminearum* on midges would suggest this is not a major route of infection.

5.6. Herbicide use and weed density

There are conflicting results as to the impact of herbicide use and weed density on FHB and DON concentration in harvested grain. *Fusarium* species were isolated from 14 of 15 broad leaf weeds surveyed on three fields in fallow in the UK. *F. culmorum* was the second most common species whereas *F. graminearum* was the least common of the species isolated (Jenkinson & Parry, 1994a). *F. graminearum* was the predominant *Fusarium* species isolated from 34 species of wild grasses in Canada (Inch & Gilbert, 2003). In 1983, Teich and Nelson did not identify any difference in FHB incidence in fields with and without an herbicide treatment. However, they did identify a higher incidence of FHB in fields with a high weed density (n=13, 0.064%) compared to fields with a low weed density (n=4, 0.029%) (Teich & Nelson, 1984). The authors later reported that the predominant weed was quack grass (*Agropyron repens*) (Teich & Hamilton, 1985). In the following year, Teich and Hamilton did not find any difference in FHB incidence with herbicide use or with weed density. They reported that weeds were mainly dicotyledons in fields studied that year (Teich & Hamilton, 1985). In a large survey conducted in Saskatchewan, Canada from 1999 to 2002 (n=659), the application of glyphosate within 18 months previous to sowing significantly increased FHB in min-tilled fields (Fernandez *et al.*, 2005). As this was an observational study, then a “cause and effect” relationship is not proven, however there is experimental data to show that glyphosate treatment of weed and crop species can result in increased colonisation of the roots by *Fusarium* species and increased numbers of *Fusarium* propagules in soil (Levesque *et al.*, 1987; Levesque *et al.*, 1993).

5.7. Plant Growth Regulators (PGR) and lodging

Few reports have detailed any effect of PGR on FHB parameters. One study found an increase in *Fusarium*-infected grain when a PGR (ectophon) was used (Martin *et al.*, 1991). A second study found the use of PGR with foliar fungicides resulted in increased FHB and DON concentration in harvested wheat (Oerke *et al*., 2002 as reported in Oldenburg, 2004). This may be due to a direct effect of the altered crop physiology due to the application of the PGR or due to the reduction in height resulting in greater numbers of *Fusarium* spores splash dispersed from the soil surface (Jenkinson & Parry, 1994b). Such an effect has to be balanced against the risk of lodging, as PGR are primarily used in cereal production to reduce lodging risk. An early observational study of wheat fields (n=28) in Illinois identified that a higher incidence of FHB occurred in lodged areas of fields (Holbert *et al.*, 1919). Similar results of high levels of DON in lodged plots were reported during fungicide efficacy

experiments (Nicholson *et al.*, 2003). Nakajima (2008) showed that DON content of lodged wheat and barley increased by 30–50% after 5 days.

5.8. Host resistance

Plant breeding can be considered as the best solution for *Fusarium* disease control and subsequent mycotoxin contamination in crops. However, current UK wheat varieties have a narrow range of FHB resistance and in comparison to material available worldwide, are considered susceptible. Many studies have been conducted on host resistance to FHB and resultant reduction in *Fusarium* mycotoxin in harvested grain (Miedaner, 1997). There are a number of wheat varieties worldwide which have good resistance to FHB and quantitative trait loci (QTLs) for *Fusarium* resistance have been identified in these varieties (Liu *et al.*, 2009). The main dwarfing gene in UK varieties, *Rht-D1b* (Rht2) is associated with a significant increase in susceptibility to initial *Fusarium* infection (Srinivasachary *et al.*, 2008). Studies are currently being conducted to break this linkage (AHDB, unpublished).

5.9. Adjacent crops

No evidence that the adjacent crop has an effect on DON concentration of wheat (Schaafsma *et al.*, 2005). If any effect does occur, it would be expected to be limited to the field margin and, therefore, unlikely to be detectable in observational studies of samples from whole fields.

5.10. Drilling date and seed rate

In a large survey conducted in Saskatchewan, Canada from 1999 to 2002 (n=659), drilling date or seed rate had no impact on FHB infection (Fernandez *et al.*, 2005). Field trials have also failed to identify an effect of seed rate on FHB incidence or DON concentration (Schaafsma & Tamburic-Ilincic, 2005). In Croatia, field trials of cultivar and drilling date identified that over a three year period, later drilling date resulted in significantly higher *Fusarium* infected grain (5th Nov compared to 25th Sept and 15th Oct) (Jurkovic *et al.*, 2006). Differences between countries may be due to differences in prevailing weather conditions when early and late drilled crops are in flower.

5.11. Harvest

There are conflicting results as to the effect of delayed harvest. In a single site study in Germany (Matthaus *et al.*, 2004), the concentration of DON in grain increased after flowering until ripeness was reached and then declined slightly one week later when harvested. In a

replicated study in the US (Farrer *et al.*, 2006), a delayed harvest of 8–19 days resulted in a significant increase in DON at three out of four sites. The largest increase was five-fold from 590 to 2900 µg/kg after a harvest delay of 16 days. Another study identified no difference in DON after a one or two week delay in harvest of infected barley (Pageau *et al.*, 2009). Differences in the effect of experimental delays in harvest can be expected due to the weather experienced during the delay. In commercial practice, delays usually occur due to wet weather. A current AHDB-funded project has identified that in commercial crops, a high level of DON and ZEN occurs in crops harvested more than two weeks after the long-term average harvest date for that region (AHDB, unpublished). Results would suggest that a short delay in harvest has little effect but a longer delay with an extended ripening phase due to wet weather can result in dramatic increases in *Fusarium* mycotoxins as experienced in the UK in 2008 (Edwards, 2011).

Fusarium mycotoxins occur at higher concentration in FDG and the components of wheat ears other than grain (Bechtel *et al.*, 1985; Savard *et al.*, 2000; Brinkmeyer *et al.*, 2006). Comparison of hand-harvested and combine samples have shown lower levels of FDG and DON in combine samples, due to the loss of smaller and lighter grains (Schaafsma *et al.*, 2001). It is, therefore beneficial to ensure correct aeration settings of combine harvesters and driers to maximise the removal of FDG and chaff (Salgado *et al.*, 2011).

5.12. Storage

Fusarium species can produce mycotoxins post-harvest if grain is held at high moisture content for any period of time. The rate of mycotoxin production is dependent on moisture content and temperature (Hope *et al*, 2005). It is therefore important that grain is dried and cooled quickly after harvest. Once below 15% moisture content, no further *Fusarium* mycotoxin production will occur. However, grain held at high moisture is more at risk from ochratoxin A contamination.

Ochratoxin A is a storage mycotoxin that can be produced on various feed ingredients and compound feeds if stored under adverse conditions, primarily high moisture content. Consequently, the risk of ochratoxin A can be eradicated if consignments of feedstuffs and feed are stored appropriately. The moisture available for fungal species to use within stored goods is dictated by the product moisture content (%MC), the temperature and the product itself. As the temperature decreases the biological availability of water decreases. This biological availability of moisture is measured as water activity or availability (Aw). Ochratoxin A is produced by *Penicillium verrucosum* in temperate climates and several

Aspergillus species in warmer climates. For cereals the method of control is by two strategies. Firstly, store hygiene to minimise the *P. verrucosum* within stores and secondly, grain must be stored dry and cool to avoid conditions that promote ochratoxin A production. *P. verrucosum* is not found on grain before harvest and survives between harvest on old grain and dust present on machinery and in stores. Thorough cleaning of machinery and stores before each new crop is harvested greatly reduces the levels of *P. verrucosum* on stored grain. At harvest, grain should be dried to, and stored below 17%MC to avoid a direct risk of ochratoxin A contamination. However, conditions conducive to ochratoxin A production can develop as an indirect result of insect or mite activity as they result in increased temperature and moisture levels. Consequently, wheat should be stored below 15%MC to avoid an indirect risk of ochratoxin A contamination. Cooling grain reduces fungal growth and ochratoxin A production, as well as reducing the biological availability of water. Grain should be cooled to below 5°C as night time temperatures drop after harvest.

During delayed, wet harvests the temporary storage of grain at high moisture content must be avoided. The higher the moisture content and the higher the temperature of grain, then the shorter the period required for ochratoxin A contamination to exceed guideline limits for feed. Detailed guidelines on grain drying and storage are provided by AHDB (AHDB, 2011).

5.13. Interactions

As DON concentration is largely dependent on suitable weather conditions for FHB infection, then there is a significant temporal (year) and spatial (location) interaction. If suitable weather conditions do not occur at a specific location in a particular season, then DON contamination of grain will not occur, irrespective of agronomic practices employed. All evidence available indicates that particular crop residues are an important source of *Fusarium* inoculum. As a result of this, there is a significant interaction between previous crop and crop residue management. If the previous crop is a host of *Fusarium,* then it is important to reduce the amount of crop debris on the soil surface. The greater the reduction in crop residue on the soil surface, and the greater the mixing of this crop debris with soil, then the greater the reduction in DON concentration in harvested grain.

Studies have shown that control measures in combination can have an additive effect on the reduction of mycotoxins in grain and, as no individual control measure can result in a large reduction in mycotoxin risk then an integrated approach using several control measures in combination is necessary to cause a large scale reduction in mycotoxin risk (Blandino, 2010).

5.14. Silage

To minimise mycotoxin content of silage, it is important that the crop to be ensiled is at the optimum moisture for ensiling, the harvested crop is adequately compacted and the cover adequately restricts the access of oxygen. Any ingress of oxygen will result in increased fungal growth and mycotoxin production. For baled silage, it is important to correctly handle bales to avoid damage to the plastic wrapping and to avoid pest damage. For clamped silage, it is important that the silo front is systematically removed during feed out so no one area remains exposed for long periods of time (Cheli *et al.*, 2013).

5.15. Straw

Higher concentrations of *Fusarium* mycotoxins exist on straw compared to harvested grain. As a consequence, it is important that the consumption of straw by animals housed on straw bedding is taken into consideration within any investigation of presumed mycotoxicosis. However, there is little data as to the degree that straw forms part of an animal's diet and how this varies for different stock (e.g. growers compared to dry sows) (van Barneveld *et al.*, 2005). One estimate was that 13–14% of the diet of 20–30 kg pigs was straw from wheat and barley (van Barneveld, 2003), whilst Staals *et al.* (2007) identified grower pigs consumed between 96 and 234 g/day dependent on diet composition when feed was limited. More recent research identified that sows on a restricted diet consumed up to 600 g of bedding straw per day, whilst growers on an *ad-lib* diet consumed negligible quantities of straw bedding (Mansbridge & Stewart, 2012).

Results also indicate that mycotoxin concentrations can be highly variable between bales harvested from the same field (Haggblom & Nordkvist, 2014). This could, therefore, result in intermittent mycotoxin issues on farm. The authors recommended the use of careful sampling, including the use of a sampling probe, where available, to identify bales of straw safe for use for bedding (Häggblom & Nordkvist, 2015). With regards to straw management, straw should be harvested and stored undercover as soon as possible to minimise exposure to rain. For straw stored outdoors and exposed to rain the concentration of DON decreased over time whereas the concentration of ZEN increased over time (Rohweder *et al.,* 2011).

5.16. Pasture

Occurrence of *Fusarium* spp. and some of their mycotoxins have been reported in grass. Detection of zeranol in cattle in Northern Ireland was shown to be caused by ingestion of

ZEN from pasture (Kennedy et al 1998). The majority of the research in this area has been conducted in New Zealand and Australia. A survey published in 2009 showed an approximate 50% incidence of ZEN in pasture samples analysed, many at levels above 1 mg/kg dry matter. DON was detected in 6 of 13 pastures and ergovaline and lolitrem-B were detected above tolerable levels in 8 ryegrass dominant samples tested (Reed and Moore, 2009). The same authors also reported 80% incidence of ZEN in 87 samples of pasture, and found occurrence was independent of annual rainfall, date of sampling and height and age of pasture (Reed *et al.*, 2004). Smith and Morris (2006) concluded that control of ZEN production or of *Fusarium* growth on pasture on a large scale was currently not feasible. The other prevalent issue is the occurrence of ergot alkaloid type toxins, ergovaline being the most frequently reported. Most reports in the literature originate from North America, Australia and New Zealand. However, a recent paper has reported incidences of ergot alkaloid in Irish perennial rye grass and that this was associated with poor performance in animals (Canty *et al.*, 2014).

5.17. Conclusions – Agronomic factors

Mycotoxins are frequent contaminants of cereals and their by-products (Table 3). Fungal infection and subsequent mycotoxin production primarily occurs pre-harvest. Mycotoxin concentration is highly variable, ranging from µg/kg to mg/kg quantities. Variation in mycotoxin concentration depends on climate, microclimate, soil type, varietal resistance, rotation, cultivation, pesticide applications and harvest date. Mycotoxins rarely occur alone and are usually found in combination with related derivatives, other mycotoxins produced by the same species or mycotoxins produced by different species. There are several claims of potential “cocktail effects” with some evidence to support this. However, it should not be assumed that there is a synergistic effect (i.e. the combined effect of two or more mycotoxins is greater than the sum of the individual mycotoxins) and it should not be assumed that a synergistic effect, if present, would occur across the range of concentrations routinely detected in feed.

It is important to consider straw as a source of mycotoxins when animals are housed on straw bedding. To minimise contamination straw should be baled and removed from fields as quickly as possible.

Table 3. Levels of mycotoxins detected worldwide in feed. Where: nd – not detected,; a – total number of samples is supplied with information about the percentage of samples contaminated with at least one mycotoxin in the whole study (% cont.) as well as the percentage of contamination with more than one toxin (%co-cont.); b – feed sample types are supplied with the number of samples in category or a range if not all samples were tested for the presence of each toxin in a study, where: M – maize, W - wheat, S – soy meal, B – barley, R – rye, O – oats, Sil – silage, FF – finished feed, FM – feed mixtures, CF – complex feed; c – Maximum concentrations of the toxin reported in a study is quoted (unless the range was provided) with the percentage of positive samples for each toxin tested expressed in the bracket (%cont.). Where data was unavailable, cells were left blank.

Region	Total number of samples (% cont / % co - cont) [a]	Feed type (number of samples) [b]	AFLs	FB_1	FB_2	FB_3	OTA	DON	NIV	T-2	HT-2	ZEA	AOH	AME	BEA	ENNA	ENNB	References
								Concentration [µg/kg] (% pos) [c]										
Czech		W (n = 3)		78	28	nd		nd - 8,841	nd	nd	nd	nd - 155						
Republic	82	M (n = 7)		nd - 853	nd - 329	nd - 84		81 - 9,528	2,547	nd - 18	nd - 33	387						
Hungary	(75 / 61)	W (n = 4)		nd	nd	nd		nd - 2,113	nd	nd	nd	nd						Monbaliu et al., 2010
Spain		M (n = 14)		nd - 5,114	nd - 1,527	nd - 192		nd - 1,920	94	10	nd	nd - 356						
Portugal		M (n = 11)		127 - 3,761	98 - 611	nd - 246		nd - 3,039	nd - 435	nd	nd	nd - 281						
North America		M (126 < n < 466)	920 (26)		22,900 (39)		18 (10)	24,900 (79)				4,787 (29)						
		S (18 < n < 74)	2 (1)		nd		6 (17)	5,500 (45)				144 (10)						
		W (2 < n < 25)	9 (20)		nd		1 (50)	7,000 (76)				513 (13)						
		DDGS (24 < n < 80)	14 (29)		6,400 (84)		4 (24)	10,100 (96)				849 (80)						
		FF (21 < n < 55)	56 (24)		11,400 (47)		nd	6,100 (65)				1,710 (52)						
Central Europe		M (16 < n < 535)	3 (31)		7,680 (60)		3 (10)	26,121 (72)				849 (39)						
		W (9 < n < 436)	2 (31)		450 (33)		331 (23)	49,000 (55)				336 (12)						
	7,049	FF (45 < n < 579)	1 (2)		2,282 (40)		30 (37)	25,759 (67)				1,045 (48)						
Southern Europe	(81 / 48)	M (31 < n < 59)	44 (36)		11,050 (47)		46 (90)	3,851 (47)				1,546 (21)						Rodrigues et al., 2012
		S (21 < n < 25)	3 (22)		5,088 (29)		1 (18)	908 (24)				nd						
		W (10 < n < 24)	6 (43)		925 (30)		1 (8)	3,505 (38)				nd						
		FF (48 < n< 104)	103 (47)		7,008 (37)		17 (53)	1,252 (37)				165 (18)						
North Asia		M (420 < n < 477)	4,687 (12)		23,499 (75)		19 (10)	15,073 (92)				7,446 (67)						
		S (33 < n < 37)	3 (6)		321 (6)		19 (24)	314 (38)				398 (35)						
		W (67 < n < 76)	20 (7)		874 (11)		7 (22)	5,331 (87)				465 (42)						
		FF (575 < n < 671)	225 (20)		77,502 (67)		60 (32)	19,141 (89)				5,791 (79)						
Spain	122 (97 / 67)	B						1,111 (95)	143 (20)	332 (10)	200 (254)							Ibanez - Vea et al., 2012a
	122 (100 / 80)		0.75 (100)				4 (58)					19 (39)						Ibanez - Vea et al., 2012b
Slovakia	50 (100 / 86)	FM		nd - 798 (98)	nd - 362 (86)				nd									Labuda et al., 2005a
	50 (100 / 84)							nd - 1,230 (44)	nd	nd - 130 (90)	nd - 173 (88)	nd - 77 (12)						Labuda et al., 2005b
Poland		W+R+B+O (2 < n < 143)	0.90 (3)		nd		657 (47)	7,356 (78)	449 (69)	27.1 (38)	124 (47)	108 (57)						
	1,255	M (1 < n < 68)	0.61 (3)		9,409 (82)		13.6 (36)	6,817 (98)	77 (72)	289 (84)	106 (85)	603 (91)						Grajewski et al., 2012
		FM (1 < n < 89)	0.23 (12)		39.2 (92)		135 (81)	2,739 (93)	88 (75)	29.3 (83)	57 (93)	229 (89)						
		Sil	nd		6,821 (83)		29.0 (32)	14,470 (92)	85 (87)	8 (7)	21.2 (83)	1,150 (79)						
Germany	88	M		20,690 (17)	6,710 (12)			19,570 (83)	4,410 (36)	340 (12)	500 (13)	14,580 (60)					2,960 (36)	Goertz et al., 2010
Switzerland	12	M			300 - 265,000 (17)			160 - 8,570 (92)	440 - 1,530 (25)			60 - 2,240 (92)						Dorn et al., 2011
UK	27	M			30,500 (96)		nd		2,300 (89)	170 (41)	210 (48)	1,800 (100)						Scudamore et al., 1998
UK		W (n = 21)		nd	nd		56	1,038	nd	10	nd	131	29	133	34	1,326	2,287	
Czech		M (n = 8)		189	64		nd	1,523	580	nd	nd	159	7	34	685	734	35	
Republic		B (n = 16)		nd	nd		nd	1,582	nd	nd	nd	17	nd	nd	nd	669	1,676	
	343	O (n = 3)		nd	nd		nd	296	nd	48	64	21	523	444	48	225	nd	
	(>80 / 0 < n < 100)	DDGS_M (n = 71)	nd	3,719	542	241	20	5,981	nd	36	112	258	17	35	237	17	1,839	Zachariasova et al., 2014
		DDGS_W (n = 16)		262	48	29	20	588	nd	7	54	50	30	33	nd	40	4,614	
		Sil_M		190	36	nd	nd	2,950	823	nd	111	120	12	30	128	845	101	
		CF (n = 26)		10	nd		65	1,735	nd	15	nd	104	31	24	36	2,816	799	
Middle East		M (n = 63)	310 (35)		4,398 (84)		nd		3,035 (38)			310 (16)						
Africa	324	W (n = 32)	7 (19)		404 (9)				11,022 (53)		nd	392 (25)						Rodrigues et al., 2011
		FF (n = 119)	213 (42)		2,588 (83)				2,786 (62)			135 (28)						
Asia	1,291	M (36 < n < 312)	457 (17)		14,714 (69)		143 (25)	10,626 (70)		nd		6,468 (41)						Binder et al., 2007
Oceania		W (8 < n < 98)	nd		646 (4)		23 (75)	18,991 (81)		266 (1)		1,489 (27)						

6. Mycotoxin impact on livestock

6.1. Mycotoxin impact on ruminants (cattle and sheep)

Ruminants such as cattle and sheep are thought to be protected from a selection of mycotoxins (found in contaminated feed) due to the function of the ruminal microflora (Swanson *et al*., 1987). This was based on a number of reports showing that the rumen can degrade a range of mycotoxins to less toxic components (e.g. ochratoxin A to ochratoxin α, Duarte *et al*., 2011). However, recent research has shown that a number of mycotoxins can resist rumen degradation (e.g. fumonisins; Caloni *et al.*, 2000). Furthermore, prolonged exposure to complex mixtures of mycotoxins, some of which have antimicrobial effects (e.g. patulin; Margavi *et al.*, 2003) can impair the detoxifying effect of the rumen microbiota (Fink-Gremmels *et al.*, 2008 and 2014). A recent review has considered the effects of mycotoxins in dairy ruminants. The impacts of mycotoxins were classed as falling under the following headings: rumen function and rumen health; reproduction; feed intake and performance; carry-over into milk; liver toxicity; foot health, lameness and other metabolic disorders; and sub-clinical, immunosuppressive and pro-inflammatory effects. The overall conclusion was that mycotoxins can exert effects through four main mechanisms, these being intake reduction and feed refusal, reduced nutrient absorption and impaired metabolism, endocrine system alterations and suppression of the immune system (Rodrigues, 2014).

The main mycotoxins (or groups of mycotoxins) and their effects on ruminants are summarised below.

6.1.1. Aflatoxins (AFLs)

Aflatoxins are difuranocoumarin derivatives produced mostly by two species of the *Aspergillus* genus i.e. *A.flavus* and *A.parasiticus* (EFSA, 2004a). The four major aflatoxins are AFB_1, AFB_2, AFG_1, AFG_2. While natural mixtures of these toxins are recognised human carcinogens (IARC, 1993a), AFB_1 is the main toxin produced and the most potent natural carcinogen known (Bennett and Klich, 2003). Aflatoxin is a potent hepatotoxin and hepatocarcinogen as the liver is the primary target organ for this group of mycotoxins (Upadhaya *et al.*, 2010). Clinical signs resulting from animals' exposure to aflatoxins are collectively referred to as aflatoxicosis and include jaundice, weight loss, depression, haemorrhages, immunosuppression or pulmonary oedema as well as decreased milk production in dairy cattle and hepatic carcinoma in pigs under chronic exposure (Eaton *et al.*, 2010; EFSA, 2004a). Also, after consumption of AFB1 by lactating animals, the toxin is

metabolized to AFM1 (considered as possible human carcinogen (IARC, 1993a)) which is later on excreted into the milk (Kourousekos, 2011).

6.1.2. Fumonisins

Fumonisins are produced predominantly by *Fusarium* species, mainly by *F.verticillioides* and *F.proliferatum* (EFSA, 2005). The most important of the 28 known fumonisin analogues are those belonging to the B group i.e. FB_1, FB_2 and FB_3, with FB_1 being the most prominent (Marin *et al.*, 2013). The International Agency for Research on Cancer (IARC) classified FB_1 as a possible carcinogen to humans while also recognising its toxicity to animals (IARC, 2002). Fumonisins are incompletely degraded by the rumen and have been reported to be excreted mainly unmetabolised. Only at very high levels of contamination in feed were biochemical changes reported, indicating liver damage. A decrease in milk production from dairy cattle was observed although there was very limited carry-over of the toxin to milk (EFSA, 2005; Yazar and Omurtag, 2008). Decreased feed intake and milk production were observed in Jersey cows after a daily intake of 3mg fumonisin B_1 per kg of bodyweight (Richard *et al.,* 1996).

6.1.3. Ochratoxin A

OTA is the most frequently occurring member of the ochratoxin family. Discovered in 1965 this mycotoxin is produced by several species of *Aspergillus* and *Penicillium* (Bennett and Klich, 2003). IARC has classified OTA as a potential human carcinogen (IARC, 1993b). Ruminants, however, are thought to be less sensitive to OTA's toxicity due to extensive rumen metabolism and remained clinically normal when exposed (EFSA, 2004d). Apart from apparent influence on animals' health, OTA residues have been reported in meat, milk and dairy products which indicates possible indirect consumers' exposure through animal derived food commodities (Duarte *et al.*, 2011). Boudra et al have recently shown that both ochratoxin A and ochratoxin α were found in ewes' milk in a dose dependant concentration after ingestion of ochratoxin A. However, the rate of carryover was very low (<0.02%), and chronic administration of toxin did not increase the concentration in the milk. The majority of the ochratoxin was excreted in the faeces (Boudra *et al.,* 2013).

6.1.4. Trichothecenes

Trichothecenes consist of approximately 170 structurally related compounds, divided into 4 types (A-D) with deoxynivalenol (DON) and nivalenol (NIV) representing type B, while T-2 toxin (T-2) and its major metabolite HT-2 toxin (HT-2) being type A toxins (Marin *et al.*, 2013). DON is majorly produced by *F.graminearum* and *F.culmorum* and is frequently

referred to as vomitoxin. Both T-2 and HT-2 can be found in feed contaminated with *Fusarium* species. However, since T-2 is rapidly metabolised to HT-2 after ingestion it is difficult to distinguish between the toxic effects of the two compounds *in vivo*, thus the risk assessment is usually performed for both toxins as feeding studies for HT-2 alone are not available (Eriksen and Pettersson, 2004).

Cattle and sheep are resistant to the emetic effect of deoxynivalenol but effects reported in ruminants include immunosuppression and decreased feed intake although sheep and cattle did not show any signs of decreased performance or sickness (EFSA, 2004b). DON levels in feed have also been associated with reduced milk production in cattle (Whitlow and Hagler, 2005). However, a recent study found no effects on performance parameters in dairy cows fed at a DON level of 5mg/kg. Danicke and Brezina (2013) recently thoroughly reviewed the kinetics and metabolism of deoxynivalenol in farm animals. They noted the low bioavailability of DON to sheep (6–10%) and the formation of de-epoxydeoxynivaleol and glucuronide metabolites were the main reason DON was less toxic to ruminants than pigs.

EFSA has associated immunosuppression, delayed ovulation and decreased semen quality with exposure to T-2 in cattle. In sheep dermatitis, diarrhoea and ovarian malfunction were reported (EFSA, 2011b). A recent study of T-2 mycotoxicoses in sheep observed acute symptoms including anorexia, ruminal atony and soft faeces. In animals that died rumenitis, myocarditis and oedema of the skin and brain were observed (Ferreras *et al.,* 2013). However, it should be noted no tissue samples were tested to confirm the presence of T-2.

6.1.5. Zearalenone

ZEN belongs to the group of *Fusarium* toxins and was previously referred to as F-2 toxin. Nevertheless, its classification as a mycotoxin has been controversial as ZEN, even though biologically active, is rarely toxic. Thus, some believe it is better classified as a mycoestrogen due to is molecular resemblance to 17β-estradiol and resulting agonistic activity towards estrogenic receptors (Bennett and Klich, 2003). Disorders connected to ZEN in exposed livestock include hyperestrogenism resulting in disturbances in the oestrous cycle, uterus weight and poor pregnancy outcomes, however, the results vary between studies depending on the feeding conditions (EFSA, 2004c). ZEN has been implicated in reproduction functions in sheep in Australia and New Zealand as a result of consumption of contaminated pasture (Morris *et al.,* 2005, Reed *et al.,* 2004, 2009). It was also reported cows from herds with low fertility had higher levels of ZEN metabolites in their blood (Towers

et al., 1995). Takagi *et al.* (2011) suggested that measuring bovine urinary ZEN and its metabolites could be a useful approach to assess the exposure of dairy cows to ZEN.

6.1.6. Ergot alkaloids

EAs are a group of toxins which are produced in sclerotia of common fungal grass pathogens – *Claviceps* and *Neothyphodium* species that infect tall fescue grass. The most prominent of EAs include compounds such as ergotamine, ergometrine, ergocristine and ergosine which usually co-appear in contaminated feed. Perennial rye grass contains a different profile of alkaloids from tall fescue when infected with *Neothyphodium lolii,* as in addition to ergovaline, infected rye grass also contains lolitrems and other indoles. Lolitrem B is the causal alkaloid of rye grass staggers. Human ergotism was first recognised in the Middle Ages and even though modern grain cleaning methods have now largely eliminated human exposure to EAs, it still remains an issue from an agricultural point of view (Bennett and Klich, 2003). Fungal alkaloids are not inactivated by rumen microflora, and can have significant effects on rumen function. There have been several cases of cattle ergotism outbreaks where over ingestion of feed contaminated with a cocktail of EAs resulted in symptoms such as diarrhoea, lameness and gangrene. (EFSA, 2012b). More recently ergot toxicoses was shown to be the cause of death of eight calves fed a pelleted creep feed in the USA (Leuschen, *et al.,* 2014). Ergovaline and other ergot alkaloids such as ergotamine have been reported to cause cardio vascular, pulmonary and body temperature effects on sheep (McLeay, *et al.,* 2002). The ingestion of alkaloids in dairy cattle has been correlated with decreased feed intake, reduced milk production and loss of body weight. There is evidence that ergot alkaloids can have a negative effect on dairy reproduction by decreasing the secretion of luteinising hormone, plasma prolactin and follicle-stimulating hormone. This combined with the fact that ergot alkaloids act as dopamine receptor agonists and vaso-constrictors explain why they interfere with ovulation, luteal function and pregnancy maintenance leading to reduced pregnancy rates and increased embryo mortality.

6.1.7. ‘Emerging’ mycotoxins

Enniatins A, A_1, B, B_1 (ENNs) and beauvericin (BEA) are considered to be further emerging mycotoxins produced by *F.oxysporum* and *Beauveria bassiana,* respectively. The four ENNs are ionophores and can form anion (multi-)complexes which are thought to contribute to their *in vitro* cytotoxic effects; however, there are no studies to show *in vivo* toxicity (Jestoi, 2008). They were recently evaluated by EFSA (2014). Based on current information and feeding practices it was concluded there are not likely to be any acute adverse health effects for

livestock species under current feeding practices. It was, however, noted that adequate toxicity data was limited.

Toxins produced by *Alternaria* species include alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TTX) and tenuazonic acid (TeA). *Alternaria* isolates have been shown to be toxic to chickens while TeA was associated with decreased feed efficiency and weight gain, nevertheless there is no other available evidence of their toxicity in other farm animal species (EFSA, 2011a).

Citrinin (CIT) was first isolated from *Penicillium citrinum* and later on from other *Penicillium* and *Aspergillus* species, which also produce other mycotoxins such as OTA and patulin. While there are no studies available on CIT's toxicity in ruminants, the few studies available in pigs report that the toxin may cause renal failure, feed refusal and depression and in poultry diarrhoea, decreased body weight and nephrotoxicity have been reported (EFSA, 2012a).

6.1.8. Mycotoxin mixtures

Since raw feed materials are frequently infected with different fungal pathogens and these are known to produce more than one mycotoxin, adverse effects of mycotoxin mixtures on animals' performance have been studied (Streit *et al.*, 2012). Numerous reports of synergistic or additive effects mainly for aflatoxins in combination with fumonisins, trichothecenes, OTA and/or CIT, or mixtures of various *Fusarium* toxins have been reviewed (Grenier and Oswald, 2011). The studies show that co-contaminated samples may express adverse health effects even with concentrations of toxins being within regulatory limits, underlining the need for combined toxicological assessment of the range of co-existing mycotoxins.

6.1.9. Mycotoxin deactivation products

The use of mycotoxin deactivation products (MDP) in contaminated feed is dealt with in more depth elsewhere in this report. They are reported to have a positive impact on the rumen microorganism population in the presence of mycotoxins. Kiyothong *et al.* (2012) fed contaminated feed (DON, FB1, ZEN and OTA, T-2 and AFB1 at concentrations of 720, 701, 541, 501, 270 and 38 µg/kg, respectively) to dairy cows (Holstein-Friesian x Red Sindhi crossbred) with and without MDP as a supplement. Supplemented cows show higher bacterial and fungal zoospore levels in the rumen compared to non-supplemented animals. It was also noted that ruminal pH of non-supplemented cows (6.1) was lower compared to

supplemented cows (6.7). Many of the recent studies on the effects of mycotoxins in feed to farm animals were carried out with reference to the use of MDP (e.g. Glucomannan Mycotoxin Adsorbent (GMA), aminosilicates). It is therefore difficult to differentiate if the effects observed in these studies were due to the absence of mycotoxin effects, the beneficial effects of the MDP or a combination of both. A number of other mycotoxins, e.g. fungal alkaloids, and fusaric acid, have also been reported to alter rumen function (Schummann *et al.,* 2008; May *et al.,* 2000). A summary of some of the more recent studies involving ruminants is given in Table 4. The reporting of metabolomics markers is also reported. These could have potential for use to detect intoxications in the future.

Table 4. Summary of some of the clinical signs and metabolomics markers associated with mycotoxin exposure in ruminants from more recent studies.

Toxins (conc µg/kg)	Country	Animal	Clinical observation	Metabolomic Effects	Reference
DON (761-205) ZEN (240-91) PAT (311-105) GLI (1870-<10) MYA (7565-588)	Netherlands	Dairy cows	Loss of body condition, lose faeces, lameness, low milk production	+ chlolesterol values +bilirubin +rumen volatile fatty acid - oxidative stress markers (G6PD, GSH-Px, TEAC)	Santos *et al*., 2014
DON (2500)	Uruguay	Dairy cows	Reduced milk fat and yield No effect on body weight	+milk somatic cell count	Mendoza *et al.,* 2014
ZEN (7500)	Japan	Japanese black female cattle	Not reported	+ no. animals with detectable Anti-mullerian hormone	Fushimi *et al.,* 2014
Ergocornine (4000) Ergosine (4000) Total ergot alkaloids (14000)	Switzerland	Dairy cows	Hyperthermia Heavy dyspnoe Increased water consumption Reduced milk production	Not reported	Rosch *et al.,* 2013
T-2 (56-16)	Spain	Ewes	Death Reduced weight gain Wool loss High % abortion Acute rumenitis	+aspartate aminotransferase +creatine kinase +lactate dehydrogenase	Ferreras *et al.,* 2012
Aflatoxin B1 (3-1) Fumonisin B1 (250-50)	Canada	Calves	Promotes STEC associated haemorrhagic enteritis		Baines *et al.,* 2013
Sterigmatocystin (7775)		cows	Death Bloody diarrhoea Loss of milk production		EFSA, 2013
Sterigmatocystin (2000-3000)		Sheep	No difference	No difference : e.g. Erythrocytes, leucocytes, haematocrit, haemoglobin. Aspartate aminotransferase, creatinine, etc.	EFSA, 2013

Key to table: + increased levels, - decreased levels – significant difference compared to controls

Table 5. Summary of some of the clinical signs and metabolomics markers associated with mycotoxin exposure in pigs

Toxins	**Feed conc µg/kg (if known)**	**Type of pigs**	**Clinical observation**	**Metabolomic or Other effects**	**Reference**
Aflatoxins	2000	Gilts and sows	Acute hepatosis. Death in 3-10 days		Osweiler 2006
	500-750	Gilts	Reduced growth due to aflatoxins in milk		Osweiler 2006
	500-800	-	Reduction in average daily gain due to reduced feed intake		Schell *et al.,* 1993
	0-280	-	Reduction in weight gain Alteration of immune response (changes to cytokines expression)	Lower cellular immunity	Van Heugten *et al.,* 1994, Martin *et al.,* 2002
Ochratoxin A	2300	Swine	Reduction in feed consumption. Reduction in weight gain. Increase water consumption		Madsen *et al*., 1982
Ochratoxin A	400, 800	Weaned piglets	Growth performance reduced. Degenerative changes in epithelial cells in proximal tubules and hepatic cells. High serum and kidney OTA concentrations	Increased AST, creatinine and urea in serum Decreased glucose, total protein, albumin and globulin	Zhang *et al.,* 2015
Fumonisin B1	5000-8000	Young pigs	Reduction in weight gain	Promotes *E. Coli* colonisation in small and large intestines	Oswald *et al.,* 2003
	>23000		Pulmonary edena Hepatic lesions Hepatocyte proliferation		Haschek *et al*., 2001
ZEN	1000-10000	Gilts	Vulvovaginitis Retained corpora lutea Pseudopregnancy		Osweiler, 2006
	>30000	Pregnant sows	Early embryonic death when fed 1-3 weeks post mating		Osweiler, 2006
T-2	0.5 mg/kg bw	Piglets	Altered nucleotide and energy metabolism, protein synthesis, oxidative stress,	Increased amino acids. Reduction of glucose and lipid in the plasma	Wan *et al.,* 2015
T-2/DAS	3000 10000 20000		Decreased feed consumption Oral/derma irritation Complete feed refusal		Osweiler, 2006
Toxins	Feed conc µg/kg (if known)	Type of pigs	Clinical observation	Metabolomic or Other effects	Reference
DON	3400-19100		Reduction in feed consumption Reduction in weight gain		Trenhom *et al.,* 1994
DON	4000	Piglets	Disturbances in amino acid, lipid, and energy metabolism	Increased low-density lipoprotein, glycoprotein, urea, trimethylamine-N-oxide (TMAO), lactate and most essential Amino Acids Decreased high-density lipoprotein (HDL), unsaturated lipids, citrate, choline, and fumarate	Xiao *et al.,* 2015

6.2. Mycotoxin impact on pigs

Pig feed ingredients in the UK, in descending order, are primarily wheat, barley, wheatfeed and soybean meal. The proportions vary with age, sex and breeding cycle for sows, as well as the price of competing sources of carbohydrate and protein. Young stock (weaners) have a high proportion of flaked cereals (wheat, maize and oats) as they are easier to digest. Based on the standard components within pig compound feeds then the main mycotoxins of concern are deoxynivalenol, zearalenone, ochratoxin A and aflatoxin. Other toxins are commonly detected but are presently only detected at low levels. If compound feeds were altered to contain a higher proportion of maize or oats then issues could arise due to the higher potential risk of contamination from fumonisins and aflatoxins in maize and HT-2 and T-2 in oats.

6.2.1. Deoxynivalenol

Deoxynivalenol (DON) is a fusarium mycotoxin produced in field during *Fusarium* head blight infections of small grain cereals or ear rot of maize. As such it is a common contaminant of small grain cereals and maize. *Fusarium* infection severity is highly seasonal due to the importance of rainfall during short periods of time when crops are flowering. Maize and wheat are the most susceptible host crops, followed by barley then oats. High concentrations of DON rarely occur in barley or oats. DON is also regularly detected on straw with highest concentrations again detected most frequently on wheat straw in the UK (Edwards & Stewart, 2010).

Pigs are regarded as the most susceptible animal species to DON with reduced feed intake and reduced weight gain the most sensitive indicators in feed studies. There are discrepancies between experiments. One consistent discrepancy is that naturally contaminated feed is more toxic than feed spiked with pure DON. This is maybe due to other co-contaminants present within naturally infected feed. DON is rarely found alone and is usually detected in the presence of related mycotoxins including acetylated DON (3-ACDON and 15-ACDON) as well as a glucosylated DON (DON-3-glucoside). The lowest observable effect limit (LOEL) of reduced feed intake varies from 0.35–2 mg/kg feed (EFSA, 2004b). The effect measured was feed intake, age and weight varied or were not stated for some studies – therefore a concentration range has been given to give guidance as to concentrations that may cause reduced feed intake/reduced weight gain.

At higher concentrations (3 mg/kg) delays in immune responses have been reported (Rotter *et al.*, 1994) and vomiting occurred at 20 mg/kg (Young *et al.*, 1983).

6.2.2. Zearalenone

Zearalenone (ZEN) is another *Fusarium* mycotoxin produced in field during *Fusarium* head blight infections of small grain cereals or ear rot of maize. However, ZEN is produced at the end of the growing season as the crop ripens. During dry ripening periods then no ZEN may be produced even if severe head blight has occurred. However, if the ripening period is prolonged and harvests delayed due to wet weather then the ZEN levels can increase rapidly (Edwards, 2011).

ZEN is similar in chemical structure to oestrogen and as a consequence causes hyperestrogenism. Pigs are particularly sensitive to ZEN, and within pigs, pre-pubescent females are the most sensitive. A NOEL of 0.2 mg/kg feed was derived from a study of female growers (prepubertal piglets) fed naturally contaminated maize (Döll *et al.*, 2003). Visual symptoms include swollen and reddened cervix and vulva and at high concentrations rectal and vaginal prolapses can occur. In sows, higher concentrations (2–3 mg/kg feed) are required to observe symptoms including a range of fertility issues including prolonged anoestrus, reduced litter size, reduced piglet weight and survival (EFSA, 2004d). Gilts are more susceptible than sows and this in part can be attributed to the greater feed intake per kg of body weight. A diagnostic indication that sows have been exposed to ZEN during pregnancy or lactation is the presence of swollen red vulva on suckling piglets. Very high concentrations (>40 mg/kg feed) can reduce boar libido but such concentrations should not occur in naturally contaminated feed (Berger *et al.*, 1981).

6.2.3. Ochratoxin A

Pigs are the most sensitive farm animal to Ochratoxin A. The mycotoxin is nephrotoxic, in that it reduces kidney function. A range of concentrations have been shown to result in decreased kidney function as measured by enzyme activity, although the lowest concentrations determined in some studies may have been influenced by the presence of other mycotoxins. Based on various studies a LOEL of 0.2 mg/kg feed for reduced kidney function in female growers was accepted by EFSA (2004c). A lower concentration of ochratoxin A (0.025 mg/kg feed) was reported to cause a significant reduction in the daily weight gain and feed efficiency for growing pigs (Malagutti *et al.*, 2005), and an even lower concentration (estimated as 0.003 mg/kg feed) was reported to reduce spermatological parameters (volume, viability and motility) in boars (Biró *et al.*, 2003).

6.2.4. Aflatoxin B_1

Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* on a wide range of agricultural products both pre- and post-harvest. There are four commonly detected aflatoxins, B_1, B_2, G_1 and G_2. Aflatoxin B_1 occurs at the highest concentration and is the most toxic of the group. Legislative limits for food apply to the combined concentration of these four aflatoxins and for aflatoxin B_1 alone (European Commission, 2006), animal feed legislation exists for aflatoxin B_1 alone (European Commission, 2011). The legal limit for compound feed for pigs is 0.02 mg/kg and for piglets is 0.01 mg/kg aflatoxin B_1.

Aflatoxins are primarily an issue in tropical and sub-tropical climates on maize and nuts although they can occur on a wide range of products including protein and oil crop by-products (e.g. soybean meal) from these climates at low concentrations (Scudamore *et al.*, 1997). Aflatoxins are rarely detected on European agricultural products although recently high levels have occurred in maize produced in central and southern Europe during severe droughts (Piva *et al.*, 2006, Kos *et al.*, 2013).

Effects of aflatoxins on pigs include reduced feed intake and reduced weight gain as well as reduced liver function at 0.25 mg/kg and 0.5 mg/kg feed (Rustemeyer *et al.,* 2010). Reduced immunity and liver lesions have been reported above 0.4 mg/kg (Osweiler & Ensley, 2012). The Norwegian Safety Committee for Food Safety proposed a NOEL of 0.2 mg/kg feed for all pigs (VKM, 2013). Hepatic carcinomas (liver cancers) can be induced with high concentrations of aflatoxins, but these have not been reported under European farming conditions (EFSA, 2004a).

6.3. Economic impact

The economic impact exerted by mycotoxins can be classified using multiple criteria such as reduced yield and value of contaminated crops translating to profound trade losses due to product rejection; reduction in animal productivity followed by increased medical cost of toxicosis treatment. The potential for animal based products recalls due to carry-over of mycotoxins and their biotransformation products resulting in possible human health management costs can also be considerable. This was exemplified by the incident in 2013 when several European countries, including Romania, Serbia, and Croatia reported nationwide contamination of milk for human consumption with aflatoxin M_1. It was reported that feed originating from Serbia and imported to the Netherlands and Germany was contaminated with aflatoxins, and tests revealed contamination in milk produced by two Dutch farms. Romanian farmers dumped milk in protest after the government banned milk

from five farms and an international dairy company withdrew some 75 tons of milk products from the shelves in Romania (All About Feed, 2013).

The additional costs related to regulatory monitoring and research strategies to relieve the impact and severity of mycotoxin exposure must also be included (Zain, 2011). These costs are borne by crop and animal producers, raw products distributors, processors and marketers, and finally consumers and society as a whole (Rodrigues *et al.*, 2011). In the US alone, the potential mean annual cost of mycotoxin contamination of crops was estimated somewhere between $0.42 -– $1.66 billion, with another $466 million and $6 million added for mitigation and livestock losses, respectively (Rodrigues *et al.*, 2011) while India's exports have suffered by millions of dollars of losses due to groundnut contamination alone (Zain, 2011).

6.4. Conclusions – Mycotoxin exposure in animals

As previously discussed in Section 4.3 animal feed can be contaminated with mycotoxins from various different sources, and due to the varied and complex nature of animal feeds, it is common to find several mycotoxins in a batch of contaminated feed. This can often make it difficult to pinpoint the exact effect a particular mycotoxin will have on animals since they are more than likely being exposed to several mycotoxins at the same time. Pure mycotoxin introduced artificially to the animal feed can often display less toxic effects compared to naturally contaminated animal feed containing a mixture of mycotoxins.

An overview of field disease outbreaks known or suspected to be caused by *Fusarium* toxins reported many cases, but highlighted that for the majority the presence of the causal toxin was not confirmed by analysis of animal samples (Morgavi and Riley, 2007). They highlighted other, as yet unknown mycotoxins could be the cause of other inexplicable animal production problems.

The European Commission has laid out a set of guidelines on the presence of DON, ZEN, OTA, T-2 and HT-2 and FUM in products intended for animal feeding (European Commission 576/2006 2006b). However, Stinshoff *et al.* (2013) have reported that animal feed containing ZEN at a concentration within the EC recommended guidelines (0.5 mg/kg) can still show an endocrine disruptive effect to dairy cows. This suggests a revision of the EC guidelines is required to reflect the current state of research.

7. Binders and feed additives

7.1. Mycotoxin management

The unavoidable problem of mycotoxin presence in animal feed is a great health, agricultural and economic issue. The changing world climate, gaps in knowledge regarding mycotoxins interactions and emergence or reclassification of mycotoxin compounds of significant concern mean that strict control of mycotoxin occurrence and exposure reduction is a complicated task (Zain, 2011). Thus, various strategies have been developed to manage mycotoxins in feed such as the FAO Hazard Analysis and Critical Control Point (HACCP) management system establishing controls such as good agricultural practice, breeding for resistance, (post-) harvesting control and decontamination (Lopez-Garcia *et al.*, 1999). Good agricultural and manufacturing practice are the best methods of controlling mycotoxin contamination, however, in the case of such contamination occurrence the hazard has to be managed if the feed is to be utilised (Park *et al.*, 1999). The EC has recognised the possibility of usage of mycotoxin-detoxifying agents defined as *'…substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action'* as feed additives (EC, 2009). In 2009 EFSA commissioned a review of mycotoxin-detoxifying agents used as feed additives under an Article 36 Grant. The document produced covered mode of action, efficacy and feed/food safety. It highlighted several areas for concern in the use of detoxifying agents, including possible anti-nutritional effects, reversibility of the risk-reduction, possible excretion of toxic components, particularly into edible products (milk, eggs) and contamination of the agent with other agents such as pathogenic bacteria. It was recognised that each situation is different and recommendation was made that recommendations or proposals to revise established guidelines could be useful for assessing risk in the agreement on how the products should be used (Boudergue et al 2009). Subsequently the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDA) published a guidance document on the preparation of dossiers for technological additives (EFSA, 2012).

A wide range of materials that can be classified as 'mycotoxin-detoxifying agents' are currently available on the market and a review of these has been undertaken.

7.2. Adsorbing agents

Adsorbing agents are large molecular weight compounds which adsorb the toxins present in the feed and thus limit their bioavailability after ingestion, limiting animal's exposure. Since the complex has to be stable throughout the entire digestive track its stability in varying pH is

one of the crucial parameters to be evaluated and is influenced by the agent's physical structure and targeted toxins' physicochemical properties (Huwig *et al.*, 2001). These agents can be divided into two sub-groups – mineral and organic adsorbents, and biological adsorbents (Jard *et al.*, 2011).

7.2.1. Mineral and organic adsorbents

The most studied group of mineral adsorbents are the aluminosilicates. This group can be divided into two subclasses, according to their spatial structure – phyllosilicates and tectosilicates. Phyllosilicates are characterised by crystalline, sheet-like structure with a common chemical formula $Si_2O_5^{2-}$ (or a ratio 2:5) such as montmorillonite; its impure form is created from the weathering of volcanic ash – bentonite; its heat-processed form – hydrated sodium calcium aluminosilicate (HSCAS); or one of the natural nanomaterials – halloysite. Phyllosilicates can adsorb substances on their surface or within their interlaminar space, however, to provide more targeted adsorption modified mineral and organic clays have been developed (Boudergue *et al.*, 2009). Tectosilicates include hydrated aluminosilicates with a three dimensional structure, consisting of tetrahedra of SiO_4 and AlO_4^- such as zeolites e.g. clinoptiolite or hectorite. They provide large and specific binding surface but also size, shape and charge selectivity due to which they have been compared to molecular sieves (Huwig *et al.*, 2001). Also, other mineral materials like diatomite and talc have been employed for the purpose of mycotoxin sequestering (Sprynskyy *et al.*, 2012).

Activated carbon (AC) is a highly porous powder, produced through pyrolysis of several organic compounds, followed by its chemical or physical activation. Even though AC has been shown to be an effective mycotoxin adsorbent, its lack of specificity was discussed with regards to diminished nutrients content of the treated feed (Van Alfen, 2014).

Two polymeric resins have also been employed as mycotoxin adsorbents i.e. cholestyramine – an insoluble, quaternary ammonium anion exchange resin, and polyvinylpyrrolidone – a highly polar, water soluble polymer with amphoteric properties (Jard *et al.*, 2011).

7.2.2. Biological adsorbents

Bi-layered cell wall of yeast *Saccharomyces cerevisiae* mainly consists of mannoproteins and carbohydrates fractions with glucans and mannans being the two main constituents of the latter fraction. As a result, yeast cell wall (YCW) exhibits a great variety of accessible adsorption loci. Nevertheless, it was the cell wall polysaccharides that were later directly connected to specific toxin binding properties rather than other constituents (Shetty and

Jespersen, 2006). Thus, apart from YCW, an extracted, esterified glucomannan (EGM) has also been employed in mycotoxin detoxification (Avantaggiato *et al.*, 2005). It has to be noted that adsorption efficiency of native and extracted cell wall will differ due to varying processing (Fruhauf *et al.*, 2012) and was also shown to be strain specific (Shetty and Jespersen, 2006).

Some strains of bacteria such as *Lactobacillus* as well as *Bifidobacterium* were shown to be able to bind certain compounds in the small intestine with cell wall peptidoglycans, polysaccharides and teichoic acid proposed as crucial elements in that process. Cell wall and envelope structures were shown to have influence on the binding efficiency with gram-positive bacteria being more efficient towards non-polar toxins (e.g. ZEN) due to higher hydrophobicity of the cell surface (Kabak *et al.*, 2006). As with yeast, binding efficiency was shown to be dependant both on the strain employed but also on the cells pre-treatment (El-Nezami *et al.*, 1998a; El-Nezami *et al.*, 2002a).

Also, alfalfa fibre, as well as micronized wheat fibres, consisting mainly of cellulose, hemicellulose and lignin, has been utilised as mycotoxin adsorbents due to favourable gut adsorption and enhanced faecal excretion (Aoudia *et al.*, 2009). What is more, a possible application of a problematic industrial waste – grape pomace as mycotoxin binding agent has been recently investigated (Avantaggiato *et al.*, 2014).

7.2.3. Biotransforming agents

Microbial degradation has become an important part in mycotoxin decontamination strategies in food and feed. A variety of microbial species belonging to genres of bacteria, fungi and yeast have been recognised for their ability to biotransform mycotoxins into less toxic metabolites through routes such as (de)acetylation, oxygenation, ring/side chain cleavage, deepoxidation, isomerisation or glucosylation. They may be encountered in environments likely to contain mycotoxins such as infested cereals or soil (McCormick, 2013). Also, some of the enzymes responsible for biotransforming characteristics recognised in these microbial species, have been isolated and applied directly as detoxifying agents.

Bacteria, both gram positive (Corynebacterium rubrum, Nocardia corynebacterioides, Rhodococcus erythropolis, Bacillus sp., Rhodococcus sp., Eubacterium BBSH 97) as well as negative (Stenotrophomonas sp., Phenylobacterium immobile, Sphingopyxis sp., family of Enterobacteriaceae, Mycobacterium smegmatis (acid-fast)) have been described in the literature as possible mycotoxin detoxifying agents along with some yeast (Exophiala

spinifera, Trichosporon mycotoxinivorans, Trichomonascus sp., Saccharomyces sp., Rhodotorula sp.) and fungal species (Hyphomycetes rostellum, Rhizopus and Aspergillus sp., Clonostachys Rosea) (Boudergue et al., 2009; McCormick, 2013).

The strategy of promoting selected microbes growth in the gut to facilitate the detoxification process has been proposed, nevertheless better characterisation of the microbes themselves, their influence on the overall intestinal microbiome as well as optimising growth and mycotoxin-degradation activity is required. What is more, detailed characterisation of the resulting metabolites is pivotal as some of the toxins' metabolites exhibit higher bioactivity than their parent compounds such as ZEN's highly estrogenic metabolite α-zearalenol (Frizzell *et al.*, 2011). Additionally, microbial action resulting in the production of mycotoxins' glycosides has to be ruled out as these metabolites are regarded as masked forms of the parent compounds which are deconjugated in the gut after ingestion (Dall'Erta *et al.*, 2013).

Extracelullar enzymes of two mushrooms i.e. *Armillariella tabescens* and *Pleurotus ostreatus* were shown to have detoxyfying properties which were associated with laccase (Alberts *et al.*, 2009). Other enzymes employed in the field include carboxylestrase, amino-/acetytransferase (McCormick, 2013), protease A, pancreatin, epoxidase and lactonohydrolase (Boudergue *et al.*, 2009).

7.3. Efficacy of mycotoxin sequestering agents

A substantial number of both *in vitro* and *in vivo* studies have been performed to assess the efficacies of proposed mycotoxin managing agents. *In vitro* studies usually include single concentration experiments but also more informative sorption isotherms in buffer/matrix or gastro-intestinal models, while *in vivo* studies employ animal models to assess agents' performance in realistic conditions. *In vitro* assays are an important pre-screening tool for initial assessment of the mode of action and performance of the sequestering materials before moving on to costly and demanding *in vivo* testing. Nevertheless, numerous discrepancies between results have been reported which may be attributed to the design of *in vitro* studies (Kolosova and Stroka, 2012) which frequently do not account for differences between animals and their metabolism but also a number of variables encountered in the gastrointestinal (GI) track such as individual physiology and intestinal microbiome as well as other factors for instance variability in animal feed composition. Thus, EFSA concluded that '*…efficacy can only be fully demonstrated by in vivo studies*' and that '*the dietary concentration of mycotoxin(s) used in such studies should not exceed official or advisory*

limits' as the end-product can only be used on feed materials containing mycotoxins below the guidance levels (EFSA, 2010).

7.3.1. *In vitro* studies

The comparison of numerous *in vitro* studies available in the literature regarding mycotoxin binders/deactivators has proven highly challenging due to the different designs and approaches (Table 6). Binding efficacy of a tested product was shown to be mainly dependant on the detailed composition of the binder, physicochemical properties of both the binder and the toxins and the pH. However, it may also be influenced by the levels of mycotoxins themselves, their co-occurrence or possible cooperative effects and in some cases was shown to be hard to predict (Faucet-Marquis *et al.*, 2014; Santos *et al.*, 2011). Also, the binding efficiency of a product is highly affected by the presence of the matrix or even gastric juice which can decrease its performance even up to 100 times when compared to the results obtained in buffer (Jaynes *et al.*, 2007; Vekiru *et al.*, 2007). Additionally, products of the same company sold in different countries were shown to differ in the type of the clay, its content and composition, which was proven to affect binding efficiencies (Fruhauf *et al.*, 2012). All of which should be kept in mind during an *in vitro* studies review.

In the case of mycotoxin adsorbents, hydrophobic clays with a planar structure such as bentonite, HSCAS or montmorillonite favour binding of planar toxins, being able to form bipolar bond with metal ions, such as AFLs, which have far lower binding to zeolite due to their 3D structure (Phillips *et al.*, 2002). Also lactic acid bacteria (LAB) were shown to efficiently bind AFB_1 (El-Nezami *et al.*, 1998b).

Modified clays, such as cation modified montmorillonite, were shown to be more efficient in the case of hydrophobic mycotoxins with higher pK_a such as ZEN and OTA, in which case the cationic modification introduced prevents mycotoxin repulsion and so desorption at higher pH (Dakovic *et al.*, 2003; Feng *et al.*, 2008). For these toxins cholestyramine, polyvinylpyrrolidone or humic acid polymers were also shown to be efficient (Avantaggiato *et al.*, 2005; Ramos *et al.*, 1996; Santos *et al.*, 2011) as well as YCW derived products (Fruhauf *et al.*, 2012; Ringot *et al.*, 2007) and LAB (El-Nezami *et al.*, 2002b). In the case of mycotoxins being zwitterions such as FB_1 or FB_2, organic modified clays such as octadecyldimethylbenzyl ammonium modified clinoptiolite (zeolite) or methyl dihydrogenated tallow quaternary ammonium montmorillonite were shown to be more effective than natural clays (Baglieri *et al.*, 2013; Dakovic *et al.*, 2010).

Table 6. In vitro binding efficiencies of various mycotoxin binding agents. Where: a – products concentration (percentage w/w, w/v or number of cells in case of microbial strains) employed is quoted in brackets; b – In the case of feed matrix letters in brackets denote (a) artificially or (n) naturally contaminated feed. In the case of buffers, pH values assessed, if more than one, are separated by a slash and percentage adsorption achieved is presented accordingly. Where data was unavailable, cells were left blank.

Product (%) [a]	Matrix [b]	AFB_1	FB_1	FB_2	OTA	DON	NIV	T-2	HT-2	ZEA	References
						% adsorption					
		193 µg kg^{-1}	19.9 mg kg^{-1}	5.9 mg kg^{-1}	187µg kg^{-1}	5.6 mg kg^{-1}				1.3 mg kg^{-1}	Avantaggiato et al.,
Standard Q/FIS (2)	Corn (a)	88	29	27	29	20				44	2007
		1 µg mL^{-1} *	2 µg mL^{-1}		2 µg mL^{-1} *	2 µg mL^{-1}	2 µg mL^{-1}			2 µg mL^{-1}	
Standard Q/FIS (0.1)		97	100/100		97	50/53	20/25			100/100	
Myco AD A - Z		99	91/89		89	11/1	10/4			93/90	
Cholestyramine			89/91		94	4/10	5/12			100/100	
AC			100/100		94	84/95	62/63			100/100	
Mycosorb			25/5		42	18/3	6/10			38/42	
Bentonite		92	100/18		17	2/3	4/3			42/35	
Activated bentonite		98			9						
Clinoptiolite		61			20						
CAB 70		26			19						
Zeovet	Buffer pH 3.0/8.0				30						Avantaggiato et al.,
Celite	Buffer pH 7.0 *		36/9		25	4/1	3/2			25/19	2005, 2007
Dowex Marathon MSA			51/42			2/10	3/14				
Amberlite XAD - 2			67/20			2/2	4/5			88/66	
Amberlite IRA-900			48/47			6/2	9/4			35/59	
Dowex 1 - X8			55/86			2/9	4/11			85/100	
Dowex MR - 3			44/27			5/8	7/9			33/39	
Zeolite			59/9			5/2	3/2			33/17	
Florsil			100/25			5/9	7/10			61/100	
Glucomannan			49/10			1/1	2/3			21/11	
Flo Bond			100/22			9/0	11/0			37/27	
Microton			91/10			3/0	4/4			39/11	
						2.8 mg kg^{-1}	3.8 mg kg^{-1}			4.1 mg kg^{-1}	
Cholestyramine (2)	Wheat (a)					45	41			52	Avantaggiato et al.,
AC (0.5/2)										84	2003, 2004
			6.9 mg kg^{-1}	1.8 mg kg^{-1}							
Montmorillonite (1):											
Dellite LVF			18	52							
Modified montmorillonites (1):											Baglieri et al., 2013
Dellite 43B	Corn (n)		80	68							
Nanofil 2			70	69							
Cloisite 93A			83	85							
Nanofil 5			80	79							
		10 ng mL^{-1}				250 ng mL^{-1}					
Bentonite (0.5)		99				7					
Cellulose		4				17					
Yeast cell wall	Buffer pH 6.8	0.93				23					Kong et al., 2014
AC		1				14					
Mixture of all above		97				4					
	Synthetic gastric									8.3 ng mL^{-1}	
Talk	and bile fluid pH									70/50	Sprynskyy et al., 2012
Diatomite	1.0/7.4									50/40	
										50 mg mL^{-1}	
Halloysite nanotubes (0.1)	Synthetic gastric									22/38	Zhang et al., 2014
Modified Halloysite nanotubes	and intestinal									27/55	
Montmorillonite	fluid pH 1.2/7.5									27/55	
		50 ng mL^{-1}									
A.vasica Nees	Aqueous plant	98									Vijayanandraj et al.,
A.paniculata Wall. ex Nees	extract	86									2014
					0.1 µg mL^{-1}					0.5 µg mL^{-1}	
Organic activated bentonite (0.5)	Buffer pH				99/99/98					98/98/99	Santos et al., 2011
Humic acid polymer (0.5)	7.4/3.0/8.4				58/99/67					97/96/94	
	Buffer pH		2 mg kg^{-1}								Dakovic et al., 2010
Organic clinoptiolite (OZ - 5) (1)	3.0/7.0/9.0		98/95/97								
								10 mg L^{-1}			
Montmorillonites (1)								8			
Clinoptiolite	Buffer pH 3.0							13			Dakovic et al., 2009
Hectorite								95			
	Buffer pH 3.0/9.0	1 µg mL^{-1}	1 µg mL^{-1}		1 µg mL^{-1}					1 µg mL^{-1}	Avantaggiato et al.,
Grape pomace (0.5)		83/83	27/34		68/61					68/65	2014
						3.34 µg mL^{-1}				0.33 µg mL^{-1}	
AC (0.08)						67				100	
Cholestyramine						10				94	
Modified aluminosilicate						17				81	
Toxisorb						1				55	
Mycosorb extra	Buffer pH 7.0					24				24	Doll et al., 2004
Klinosan						0				20	
Mycofix Plus						1				17	
Bentonite						1				13	
Fix a Tox						21				5	
Likratox						2				5	
		0.5 µg mL^{-1}			0.5 µg mL^{-1}					20 µg mL^{-1}	
AC	Buffer pH 3.0	100			99					97	Faucet-Marquis et al.,
Inactivated yeast /YCW (0.5)		45			70					60	2014
		5 µg mL^{-1}				20 µg mL^{-1}	20 µg mL^{-1}	20 µg mL^{-1}	20 µg mL^{-1}	2 µg mL^{-1}	
L. rhamnosus GG (10^{10})	Buffer pH 7.3	78				52	44	49	33	70	El-Nezami et al.,
L. rhamnosus LC705		79				1	43	13	7	76	1998b, 2002a,b

In the case of hydrophilic, non-ionisable toxins such as the majority of the trichothecenes, no binder was effective to date due to the lack of structural complementation (Doll *et al.*, 2004; Sabater-Vilar *et al.*, 2007). Only commercial Standard Q/FIS, cholestyramine and LAB showed moderate toxin binding, up to 53% and 41% for DON and NIV, respectively (Avantaggiato *et al.*, 2004; Avantaggiato *et al.*, 2005). Nevertheless, T-2 toxin, was shown to be effectively bound by hectorite (Dakovic *et al.*, 2009).

In the case of mycotoxin biotransformation (Table 7), successful AFB_1 degradation was reported for a couple of microorganisms such as *N.corynebacterioides* NRRL 24037 and *M.fluoranthenivorans* or *M. fulvus* ANSM068 which were shown to decrease the toxin's concentration by more than 75% (Guan *et al.*, 2010; Line *et al.*, 1994; Teniola *et al.*, 2005). Also, extracellular extracts of AFB_1 degrading *M. fulvus* ANSM068 and *R. erythropolis* were shown to decrease the toxin concentration present by almost 70% (Alberts *et al.*, 2006; Zhao *et al.*, 2011).

Brevibacterium sp., which can be commonly found in cheese isolates, were shown to be a very efficient OTA biotransforming agents attributed to the species ability of hydrolytically degrading a number of aromatic compounds (Rodriguez *et al.*, 2011). *A.niger* strain CBS 120 49 was also shown to be an efficient OTA deactivator (Varga *et al.*, 2000) while yeast *T.mycotoxinivorans* were reported to entirely degrade OTA. *T.mycotoxinivorans* were also capable of neutralising ZEN without creating estrogenic metabolites (Molnar *et al.*, 2004) which was also reported for bacterial strain of *B.natto* CICC 24640 (Tinyiro *et al.*, 2011). A variety of fungal *Rhizopus* sp. isolates were shown to be able to degrade OTA and ZEN, with *R.oryzae* NRRL 1526 being able to metabolise patulin as well, while *R.stolonifer* isolate was proven to remove almost all OTA directly from contaminated wheat (Varga *et al.*, 2005). Also, *A.niger* isolates were capable of complete detoxification of OTA with higher efficiency than other commercial enzymes such as protease A or pancreatin (Abrunhosa *et al.*, 2006).

In the case of trichothecenes, *Eubacterium* BBSH 797 strain isolated from bovine rumen fluids was one of the most studied and was shown to efficiently degrade DON, T-2 and HT-2 toxins (Binder *et al.*, 1998; Fuchs *et al.*, 2002) which after *in vivo* testing (Awad *et al.*, 2006) was later introduced to the market by Biomin® as a commercial biotransforming product – Mycofix® Plus. Additionally, chicken intestine isolates have also shown very promising DON, NIV, T-2 and HT-2 degrading properties (Young *et al.*, 2007).

Fumonisins biotransformation has not been studied extensively, however, a bacterial soil isolate as well as two carboxylesterases were shown to be capable of completely removing FB_1 (Benedetti *et al.*, 2006; Duvick *et al.*, 2003; Heinl *et al.*, 2010). FUMzyme®, fumonisin esterase, an enzyme isolated from a genetically modified strain of *Komagataella pastoris* has been reviewed by EFSA and authorised for use as a technological feed additive for pigs (EC, 2014, EFSA, 2014)

Table 7. In vitro biotransforming efficiencies of various mycotoxin detoxifying agents.

Toxin	Biotransforming agent	Type	Origin	% degradation	References
AFB_1	*B.licheniformis*	Bacteria	Soybean	74%	Petchkongkaew et al., 2008
	N.corynebacterioides NRRL 24037	Bacteria		76%	Line et al., 1994
	M.fluoranthenivorans	Bacteria	Soil of a gas plant	97%	Teniola et al., 2005
	R. erythropolis			83%	
	M. fulvus ANSM068	Bacteria	Deer faeces	77%	Guan et al., 2010
	MADE enzyme	Enzyme	*M. fulvus* ANSM068 isolate	67%	Zhao et al.,2011
	Extracellular extract		*R. erythropolis*	67%	Alberts et al., 2006
	P.putida	Bacteria	Sugarcane isolate	90%	Samuel et al., 2014
FB_1	*Delftia/Comamonas* group	Bacteria	Soil isolate	100%	Benedetti et al., 2006
	Recombinant carboxylesterase	Enzyme	*Sphingopyxis* sp.	100%	Heinl et al., 2010
	Fumonisin-degrading esterase	Enzyme		100%	Duvick et al., 2003
OTA	*T.mycotoxinivorans*	Yeast	Hindgut of the lower termite	100%	Molnar et al., 2004
	A. niger strain CBS 120.49	Fungus		80%	Varga et al., 2000
	Protein isolate	Fungus	*A.niger* MUM 03 55 isolate	100%	Abrunhosa et al., 2006
	Commercial *A.niger* enzyme		Protease A	87%	
	Commercial porcine pancreas isolate		Pancreatin	43%	
	P.parvulus UTAD 473	Bacteria	Douro wines isolate	100%	Abrunhosa et al., 2014
	B.licheniformis	Bacteria	Soybean	93%	Petchkongkaew et al., 2008
	B.casei DSM 20657T *B.casei* DSM 9657 *B.casei* DSM 20658 *B.casei* RM101 *B.linens* DSM 20425T *B.iodinum* DSM20626T	Probiotic bacteria		100%	Rodriguez et al., 2011
	Ochratoxinase	Enzyme		50%	Dobritzsch et al., 2014
DON	Mainly *Serratia*	Bacteria	Agricultural soil isolates	62%	Islam et al., 2012
	Large intestine chicken isolates			100%	Young et al., 2007
	Soil isolates			>87%	Zhou et al., 2008
	Nocardioides WSN05-2	Bacteria		90%	Ikunaga et al., 2011
ZEA	*B.natto* CICC 24640	Bacteria		100%	Tinyiro et al., 2011
	B. subtilis 168			81%	
	T. mycotoxinivorans	Yeast		95%	Vekiru et al., 2010

There are a number of factors which have to be taken into consideration when choosing a mycotoxin adsorbent for further *in vivo* testing such as nonspecific binding or raw material contamination. Activated carbon performs very well with the majority of mycotoxins under various conditions, however it was shown to be a highly unspecific binder, which strongly adsorbs not only toxins themselves but also vitamins and minerals essential for growth and development which are also crucial for fighting toxicoses, thus it is not to be employed in a real setting (Vekiru *et al.*, 2007). In the case of clays, accumulation in manure as well as possible contamination with toxic metals or dioxins have to be taken into consideration

(Yiannikouris *et al.*, 2004) while yeast cell walls, even though claimed to have high nutritional value resulting in growth promotion, express low binding affinity towards AFLs (Fruhauf *et al.*, 2012). In the case of biotransforming agents' application for animal feed detoxification, obtaining complete information on their safety for target species, consumers and environment is pivotal. Also, assessing their stability in the varying conditions of the gastrointestinal tract (GI), studying degradation products and their toxicity is crucial. Similarly, to the adsorbents, their influence on nutritive and organoleptic properties of the feed needs to be investigated (Awad *et al.*, 2010). Since microbes' adaptation and survival in the target animal's digestive tract is of most importance, anaerobic microbes, isolated form GI tracts are most suited for usage as feed additives while strains isolates from environmental samples are thought to have more potential as fermentation detoxifiers. Due to all these factors, enzymes are thought to be the most convenient and ultimately the optimal detoxifying strategy (Zhou *et al.*, 2008). Based on the available peer reviewed scientific literature, there is still no sequestering product versatile enough to effectively remove low level mixtures of various mycotoxins from feed.

7.3.2. *In vivo* studies

The efficacy assessment of feed additives used for the reduction of feed contamination with mycotoxins can only be fully demonstrated by *in vivo* studies (EFSA, 2010) as the *in vitro* activity of binders was shown to be not completely related to the *in vivo* efficacy (Trailovic *et al.*, 2013). Reports available in the literature aim at determining the ability of the binder/deactivator to detoxify the contaminated feed during the passage through animal's digestive system under its pH, temperature and moisture conditions which is assessed mainly by monitoring animal's zootechnical parameters such as feed efficiency or weight gain but also other variables such as mycotoxin residues concentration in plasma or tissues, target organs weight or blood composition (Boudergue *et al.*, 2009). In 2010 EFSA released a scientific opinion, setting specific guidelines regarding design of such studies for the purpose of EFSA binder assessment with the ultimate goal of obtaining EU accreditation. A minimum of three *in vivo* studies conducted in at least two different locations are required, employing preferably naturally contaminated feed with mycotoxin levels under the EC guidelines. What is more, product safety as well as significant improvement of the most relevant end-points such as toxins/metabolite excretion, their levels in blood, tissues or products destined for human consumption (eggs, milk) have to be presented as a result of sequestering agents employment. EFSA also concluded that zootechnical parameters should be reported but cannot be used as a measure of the detoxifiers efficacy (EFSA, 2010).

As in the case of *in vitro* studies*, in vivo* studies also suffer from a level of variability as clinical signs of toxicosis are influenced not only by the levels of mycotoxins tested but also the animal's age, nutritional and health status (Neeff *et al.*, 2013). What is more, some authors suggest that additional variations in the responses may arise due to differences in the sensitivity of the animal populations assessed (Magnoli *et al.*, 2011b) which has to be kept in mind while comparing different experiments (Table 8).

Contaminated AFB_1 feed supplemented with HSCAS was shown to alleviate most of the negative effects exerted after ingestion of the toxin in pigs while additional supplementation with an antioxidant was proven to improve their nutrient status (Harper *et al.*, 2010). The effect of ZEN on pig growth performance was shown to be species-, sex-, and dose-dependent at higher toxin concentrations, while at a concentration of 1mg/kg no symptoms of toxicosis were presented. However, hyperestrogenism, lower weight of testis and altered levels of other hormones were noted even after ingestion of feed contaminated with low ZEN levels which symptoms were alleviated by clays (Jiang *et al.*, 2012; Wang *et al.*, 2012). Faster recovery was also noted for pigs fed diets contaminated with ZEN, additionally supplemented with montmorillonite by preventing toxin (re-)absorption (Wang *et al.*, 2012). The decrease in pig feed intake is one of the most obvious effects on pigs exposed to DON, visible at as low as 1mg/kg levels in feed (Boudergue *et al.*, 2009). To date, no effective DON binder was proposed (Danicke and Doll, 2010; Doll *et al.*, 2005) with clays having been shown to have a limited effect or even increased levels of toxin in tissues (Boudergue *et al.*, 2009; Osselaere *et al.*, 2012).

Since ruminants are far more resistant to mycotoxins, available *in vivo* studies focus more on the possible transfer of AFM_1 to milk in case of AFB_1 feed contamination. Study design, feed form (pellet/meal) as well as its pre-treatment were all shown to influence AFB_1 bioavailability (Masoero *et al.*, 2009; Rojo *et al.*, 2014). Nevertheless, in the case of dairy cows AFB_1 was also shown to be effectively bound by aluminosilicates, significantly lowering AFM_1 concentrations in milk (Masoero *et al.*, 2009; Rojo *et al.*, 2014).

Clay binders are employed in the majority of formulations tested *in vivo* as they were shown not to affect animal performance (Neeff *et al*., 2013) and effectively bind some of the regulated mycotoxins such as AFLs. Nevertheless, altered pharmacokinetics of drugs typically applied in farming should be taken into consideration. Some studies suggest that binders' inclusion (both organic as well as inorganic) in feeds already contaminated with mycotoxins may significantly elevate pharmaceuticals bioavailability e.g. oxytetracycline by

complex interaction of mycotoxins, binders and tested drugs (Goossens *et al.*, 2012; Osselaere *et al.*, 2012). It may be a matter of concern with regards to consumer safety due to possibly elevated drugs residues in animal tissues.

Table 8. In vivo efficacies of various mycotoxin sequestering products. Where: N/A – not assessed; a – letters in brackets describe natural (n) or artificial (a) contamination usage in the study; b – detoxifiers' concentration is denoted in brackets as a % of feed weight; c – effects of the sequestering agents include changes in BW – body weight, BWG – body weight gain, FI – feed intake, F:G – feed conversion ratio, $C_{bi, p, t}$ – mycotoxin concentration in bile, plasma and tissues respectively, BC - blood composition (serum chemistry, haematology measurements), OW – organ weight, asterisk signifies the variables which were not affected by the mycotoxin contaminated feed ingestion in the study.

Species	Feed [a]	AFB_1	FB_1	OTA	DON	T-2	ZEA	Detoxifier (% content in feed) [b]	Effect of the addition of a sequestering agent to the contaminated feed	References
		mg/kg								
Broilers	Corn - soybean meal (n)	0.01		0.11			1.0	Bentonite (1)	No effect on BW/Ct/BC/OW (no positive control)	Pappas et al., 2014
	Corn (n)	0.45		0.07		0.32		EGM (0.05) HSCAS (0.2) CMA - mix of the above (0.1)	No effect on BW/FI/F:G/NR Partially recovered NR; no effect on BW/F:G/FI Recovered FI/NR; no effect on BW/F:G	Liu et al., 2011
	Corn - soybean meal (a)	0.1						Sodium bentonite (0.3)	No effect on BW*/FI*/F:G*/BC*/OW* Altered pharmacokinetics	Magnoli et al., 2011a
	Corn meal (n)	0.2 0.4 0.8						Probiotics + AFB_1 degrading enzyme (0.15)	Recovered BW/BWG/FI/F:G/BC Recovered BC; partially recovered BW/BWG/FI/F:G Partially recovered BC; no effect on BW/BWG/FI/F:G	Zuo et al., 2013
	Corn - soybean meal (n)	2.5						HSCAS (0.5)	Recovered Ct; no effect on BWG/FI/F:G*/BC/OW	Neff et al., 2013
	Corn - soybean meal (n)	0.20						MOS (0.2) HSCAS LAB	Recovered F:G; partially recovered BW/FI/OW, Recovered F:G; partially recovered BW/FI/OW Recovered BW/F:G; partially recovered OW; increased FI	Attia et al., 2013
	Corn - soybean (a)	0.05						Sodium bentonite (0.3)	Partially recovered Ct, liver damage; no effect on BWG*/FI*/F:G*; altered pharmacokinetics	Magnoli et al., 2011b
	Maize (a)				2.4/7.6			Illite - smbrosite (0.15) Bentonite - montmorillonite + yeast (0.15)	No effect on BW*/BWG*/FI*/Cbi/Ct; increased Cp; altered pharmacokinetics No effect on BW*/BWG*/FI*/Cbi/Ct; increased Cp; altered pharmacokinetics	Osselaere et al., 2012
	Corn (n)			2.0				Mycosorb® (EGM) (0.2) Mycofix Plus ® (0.2)	Partially recovered Ct Partially recovered Ct	Trailovic et al., 2013
	Commercial broiler cheek feed (n)		59					Bentonite (0.5, 1) Zeolite (0.5, 1)	Recovered BW/BC/liver damage Recovered BW/liver damage; partially recovered BC	Vizcarra - Olvera et al., 2012
Ducks	Corn - soybean meal (n)	0.10	2.25		0.84		0.19	Ca - montmorillonite (0.1)	Recovered OW/mortality rate; no effect on BWG*/FI*/F:G*	Wan et al., 2013
Pigs	Commercial feed mixture						3.84	Minazel Plus® (clinoptilolite) (0.2) Mycosorb® (EGM) (0.1)	Partially recovered BWG/F:G/vulva size Partially recovered BWG/F:G/vulva size	Nesic et al., 2008
	Conventional feed (n/a)				0.80	0.99	0.44	Glucomannan (0.2)	Altered pharmacokinetics	Goossens et al., 2012
	Corn (n)						0.2 0.4 0.8	Montmorillonite (0.05) (0.1) (0.2)	Recovered BW; partially recovered BWG/FI/F:G; no effect on vulva size Partially recovered BW/BWG/FI/F:G/vulva size Recovered BWG/FI/F:G; partially recovered vulva size; no effect on BW	Wang et al., 2012
	Corn - soybean meal (n)	0.50						HSCAS (0.5)	Recovered BW/BWG/FI/BC, no effect on F:G*, partially recovered NR	Harper et al., 2010
	Corn - soybean meal (a)						1.0	Calibrin - Z (montmorillonite) (0.4)	Recovered BC/genital OW; partially recovered vulva size; no effect on BWG*/FI*/F:G*/NR*	Jiang et al., 2012
	Wheat, barley (n)				2.8			BioPlus 2B (*Bacillus* sp.) ($2.3E6$ CFUs g^{-1})	No effect on BWG/FI/F:G/Cp	Danicke et al., 2010
Cattle	Corn (n)	0.03						Mg - smectite clay (2)	Reduction of AFM_1 in milk	Masoero et al., 2009
	Corn silage/grain concentrate (n)	0.04						Aluminosilicate (0.2)	Reduction of AFM_1 in milk	Rojo et al., 2014
	Corn (n)				10.0	0.20	1.81	YCW (1)	No effect on BW*/FI*/F:G/BC	Martin et al., 2010

7.3.3. Commercial mycotoxin sequestering products

In order to supply the agricultural sector with a solution to mycotoxin feed contamination, a substantial number of commercial mycotoxin binders/deactivators are available on the market (Table 9) with suppliers offering an array of products from unicomponent, usually clay based, products to more complex, multicomponent sequestering formulations. They usually consist of a mixture of available binders to combine their effects and include clays and microbial additives such as yeast and their cell walls but also fungi, bacteria or enzymes. Many suppliers try to provide a holistic approach towards mycotoxicosis and address not only mycotoxin removal but also compensate for adverse effects connected with mycotoxin exposure such as acidosis. Also, decreased zootechnical parameters such as production yield or feed efficiency are tackled by supplementation with yeast, which are considered to be a high protein source, or by the employment of bacteria with catalytic properties while mineral and vitamin deficiencies are addressed by including nutritional additives. Thus, commercial mycotoxin binders are not only advertised as toxin sequestering agents but also as complex products which aim at stimulation of the immune system, optimisation of GI metabolism and promotion of beneficial microbiota activity, with the ultimate goal of improving overall animal health status. Nevertheless, for the product to be authorised by the EU, a complex assessment must be performed by EFSA (Galobart, 2014). Due to these strict EFSA requirements, only one product has been approved for use as mycotoxin binder to be employed in animal feed – bentonite, (absorbent aluminium phyllosilicate, impure clay consisting mostly of montmorillonite). It has been used as an anticaking agent for a considerable period of time and was shown to act as a gut protector with a capacity to bind heavy-metals and non-polar substances such as polycyclic aromatic hydrocarbons and dioxins (WHO, 2005). Its AFB_1 binding capacity has been recognised by the EC, thus bentonite was authorised as the first adsorbent for usage as an AFB_1 sequestering agent in ruminants, poultry and pigs (EC, 2013a). Another clay product, Friedland clay, a montmorillonite-illite mixed layer clay was evaluated by EFSA, who concluded it was safe for use for animals and for the environment but insufficient evidence was provided about its binding and anti-caking properties. It was not evaluated for mycotoxin reducing properties as the proposed classification as a substance for reduction of the contamination of feed by mycotoxins was withdrawn during the application process (EFSA, 2014a). What is more, Biomin® BBSH is the first biotransforming product to be used in pigs which received a positive opinion from EFSA (EFSA, 2013) and has been authorised by the EC for reducing trichothecenes toxicity (EFSA, 2013), (EC, 2013c). In addition, FUMzyme®, a fumonisin esterase, was also authorised by the EC in 2014 (EC, 2014a).

Table 9. Commercially available mycotoxin sequestering products, with information provided on the producers' websites where: N/A – information not available on the producer's website

Producer	Product	Targeted toxins	Declared toxins binding [%]	Composition
Biotal UK	Biotal SC Micorbind Biotal SC toxisorb	Aflatoxin, ZEA	75, 10	Mannan oligosaccharides, beta glucans Live yeast: *S.cerevisiae* , mannan oligosaccharides, beta glucans
Micron Bio-systems Ltd UK	UltraSorb 20 Mycotex	AFB_1, FB1, OTA, DON, ZEA	95, 70, 20, 90, 90	Sodium and calcium aluminosilicate, dried yeast culture extract, dried molasses, dried yeast culture, vitamin A, vitamin E, manganese sulphate, vegetable oil Mineral binders, yeast, microbial fermentation products and extracts, plus enzymes, vitamins and microbial stimulants
Alltech USA	Mycosorb Mycosorb A+			Glucomannan, HSCAS, $CaCO_3$
Special nutrients Inc. USA	Myco AD MYCOAD-AZ	AFB_1, FB1, OTA, DON, T-2, ZEA AFB_1, FB1, OTA, DON, T-2, ZEA	95, 70, 72, 84, 94, 43	HSCAS Activated bentonite
Biomin® AT	Mycofix® 5.0 line: Mycofix® Secure FUMzyme® Biomin® BBSH Biomin® MTV	 AFLs Fumonisins Trichothecenes OTA, ZEA		 Bentonite Carboxylesterase enzyme *Eubacterium* BBSH 797 *T. mycotoxinivorans*
Agri-tech USA	Flo-Bond Flo-Bond Plus	AFB_1, AFM_1, FB_1, OTA, DON, T-2, ZEA, CIT	90, 100, 70, 80, 80, 90, 60, 95	HSCAS HSCAS, buffered propionic acid
Kiotechagil UK	Sorbatox Neutox	AFLs, DON, T-2, ZEA AFLs, DON, T-2, ZEA, ergot alkaloids		Hydrated aluminium silicate Mixed silicates, yeast cell wall, kieselguhr, propionic acid
BASF SE DE	Novasil™ Plus	AFM_1, FB_1	50, 30	Ca - montmorillonite
Select Sires Inc. USA	Select BioCYCLE™ Select BIOCYCLE Plus™			Yeast culture, *S.cerevisiae* (active dry yeast), potassium iodide, dried egg product, dried *A.oryzae* and *A.niger* fermentation extract, dried *B. subtilis* fermentation product, lactic acid, calcium lactate, malic acid, aspartic acid, tartaric acid, and $CaCO_3$ Montmorillonite clay, dried yeast culture, dried egg product, calcium carbonate, aspartic acid, lactic acid, calcium lactate, calcium pantothenate, papain, *S.cerevisiae* (active dry yeast), dried *A.oryzae* and *A.niger* fermentation extract, dried *B. subtilis* fermentation product, sodium potassium tartrate, potassium iodide, and silicon dioxide
Dox-al AU	Captex® Captex T2® Captex FUSA®	All purpouse toxin binder AFLs, fusarium toxins, DON Fusarium toxins		 Absorptive and enzymatic - degrading activities Modified hydrated calcium sodium alumino silicates and oligosaccharides
Olmix FR	MTX+®and MMIS	AFLs, fumonisin, DON, trichotecenes, ZEA, ergot alkaloids		Montmorillonite, algae *Ulva Lactuca*
EW nutrition GmbH DE	Mastersorb® Mastersorb® FM Mastersorb® Gold	AFLs AFLs and fumonisins AFLs, fumonisins, DON, ZEA		Bentonite Natural plant extracts, yeast cell walls and minerals Natural plant extracts, yeast cell walls and minerals
Selco NL	TOXO®-XL TOXO®-XXL	Broad spectrum of mycotoxins Broad spectrum of mycotoxins		Mixture of smectite clays Smectite clays, yeast cell wall fractions, activated beta glucans, vitamins, anti-oxidants
Cenzone Europe Ltd. USA	Microbond	Aflatoxin, fumonisin, OTA, DON, T-2, ZEA		Activated clinoptilolite, modified mannan-oligosaccharides, beneficial microorganisms, digestive enzymes and chelated organic minerals
Biorigin BR	Protemyc	Broad spectrum of adsorption		
ALINAT S.N.R AR	DETOXA PLUS® SINTOX PLUS® SINTOX	FB_1, OTA, T-2, ZEA Aflatoxin, OTA, DON, T-2, ZEA Aflatoxin, OTA, DON, T-2, ZEA		Enzymes from *Saccharomyces telluris* sp. Natural minerals, mannan-oligosaccharides, beta glucans Enhanced minerals
Leiber GmbH DE	BIOLEX® MB40			Cell walls of *Saccharomyces cerevisiae*
ICC BR	StarFix Zenifix	Wide range of mycotoxins AFLs		Beta glucans and aluminiosilicates Aluminiosilicate
Kemin Industries, Inc USA	TOXFIN™	Broad-spectrum mycotoxins		Several activated clays
OptiVite UK	Mycobond Ultrabond	Broad-spectrum mycotoxins AFB_1, FB_1, DON, T-2, ZEA		Natural mineral materials
Alman International USA	Calibrin-Z Calibrin-A	AFL, fumonisin, OTA, T-2, ZEA, ergotmaine, cyclopiazonic acid AFB_1, ergotamine, ergovaline, cyclopiazonic acid	100, 90, 95, 75, 95, 100, 100 100, 100, 100,	Montmorillonite Montmorillonite
Devenish™ UK	DaviSafe	AFB_1, FB_1, OTA, DON, ZEA	95, 100, 80, 70, 100	

7.4. Conclusions from the peer review on 'binders'

EFSA guidelines regarding mycotoxin levels in feed destined for animal consumption were established to protect animal performance and health as well as EU consumers from mycotoxin exposure through foods of animal origin. However, farm animals have been shown to express symptoms of chronic toxicoses when exposed to feed contaminated with toxins below the guideline levels. In this case, employment of mycotoxin binders and deactivators seems a viable solution to this problem. Indeed, the majority of the revised *in vivo* studies report that detrimental effects on animal health can be alleviated to some extent with the use of mycotoxin managing agents. The majority of recent studies report improvement of blood parameters such as serum chemistry or haematology as well as reduced tissue concentrations of the toxins and thus improved final product safety. The most significant economic effect of toxicosis in broilers and pigs is reduced feed conversion ratios (Neeff *et al.*, 2013) and their improvement due to sequestering agents is reported very infrequently. Thus, the issue of effective removal of low level mixtures of mycotoxin present in feed remains to be addressed as reduced feed conversion ratios may significantly reduce the agricultural production efficiency and so reduce net incomes of those involved in farming. What is more, climate change is predicted to influence global distribution and increase mycotoxins (co-)occurrence in feeds. At the same time, among the large number of mycotoxin sequestering or binding products available on the market, only one is EC accredited and intended for feed detoxification from AFB_1 only. Two biotransformation products have recently been approved for trichothecenes and fumonisins. In the light of high mycotoxins co-occurrence with new groups being identified as potential hazard, the challenging task of developing a more versatile solution for multi-toxin feed decontamination is an important one and requires attention.

8. Review of rapid tests for mycotoxin analysis

8.1. Introduction

An extensive literature survey on rapid methods of analysis for mycotoxin testing was conducted. The results of this survey including quantification range, detection format, time for analysis and costs are shown in the tables in Section 9.2.

For mycotoxins, the companies that provide rapid tests include Unisensor, Neogen, R-Biopharm, Vicam, Charm Sciences, Europroxima, Envirologix, Romer and Toximet. The assays included Lateral Flow Devices (LFDs), ELISAs and fluorescence based analyser. A selection of the methods available are capable of multi-analyte detection which make them

attractive as potential for rapid screening of samples for many of the commonly found mycotoxins.

8.2. Rapid tests and readers available for mycotoxin analysis

Table 10 and Table 11 summarise the available mycotoxin tests such as lateral flow devices (LFDs), ELISAs etc. and the readers needed for analysis, respectively. The tables also include the recommended sample types, approximate timings for analysis, number of test per kit and costs. Additional information, such as whether the test kit has any official status or approval is also given.

It should be noted that no approval of this type is given within the UK by any official body (such as British Standards Institute). Individual laboratories may choose to obtain ISO 17025 accreditation in the use of tests of this type after following the necessary in-house validation and UKAS assessment procedure. There are no standard or official methods for mycotoxins that employ methods of this type. It is only within the last year that increased use of screening methods for control of mycotoxins was acknowledged and criteria with which screening methods have to comply with for use for regulatory purposes were included in EU legislation (EC2014a). The European Reference Laboratory (EURL) for Mycotoxins is currently organising an interlaboratory evaluation of rapid tests for DON. The Regulation requires that a false positive and false negative rate be determined before using screening method for control. This is critical to understanding the performance of the method, this information is not always provided by the manufacturers and should be determined by any laboratory that uses these tests.

The relative performance of test kits for DON and T-2 and HT-2 toxins were recently evaluated (Aamot *et al.,* 2012, Aamot *et al.,* 2013). Many of the rapid tests are antibody based and there have been reports of over estimation of results from these tests, caused by cross reaction of the antibody with structurally similar molecules. In many cases cross reactivity is not known or not well characterised. For example, in the case of DON cross reactivity with 3 and 15 acetyl-DON and DON-3- glucoside has been reported. This is one reason why these methods are considered screening methods and further confirmatory analysis would normally be required, in particular in the event of a result exceeding a maximum limit or in the case of control or enforcement.

Some of the kits are relatively simple to operate and can be used without readers, or have portable battery operated readers. Indeed, many products now use similar readers, with the

key difference being in the software each has to allow use of a particular consumable test strip or kit (e.g. Vertu). However despite being marketed as rapid skilled personnel and more sophisticated equipment are often needed to perform the test accurately.

It should be noted that all the tests listed have been validated or approved or are marketed for food or feed applications, with a strong emphasis on cereals. None have been validated or have established performance characteristics for hay, straw or silage, and there are no tests of this type available for use to test samples from animals such as urine, blood or animal tissue.

Table 10. Survey on rapid methods of mycotoxin detection

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Neogen Reveal Q+										
Reveal Q+ for Aflatoxin / 8085	GIPSA approved for corn brewers rice, corn flaking grits, corn germ meal, corn gluten meal, corn meal, corn screenings, corn/soy blend, corn starch, cracked corn, distillers dried grains with solubles, popcorn, and sorghum			2-150 (5-100)	LFD/ Quantitative	6 mins	25	£150	£6.00	GIPSA approved
Reveal Q+ Aflatoxin Green (NEW) / 8086	Corn			2-150	LFD/ Quantitative	6 mins	25	£150	£6.00	
Reveal Q+ DON / 8385	GIPSA approved for wheat, corn, barley, corn gluten meal, DDGs with solubles, malted barley, oats, and rough rice			300-6000 (0.5-5mg/kg)*	LFD/ Quantitative	3 mins	25	£150	£6.00	GIPSA approved
Reveal Q+ Fumonisin / 8885	GIPSA approved for Corn Products			300-6000 (0.5-5mg/kg)*	LFD/ Quantitative	6 mins	25	£150	£6.00	GIPSA approved
Reveal Q+ Ochratoxin (NEW) / 8685	Grain and Grain products			2-20	LFD/ Quantitative	9 mins	25	£150	£6.00	
Reveal Q+ T-2/HT-2 Toxins (NEW) / 8285	Grain and Grain products			50-600	LFD/ Quantitative	6 mins	25	£150	£6.00	

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Neogen Reveal										
Aflatoxin / 8015	Corn, corn gluten meal, corn meal, corn/soy blend, cottonseed, cottonseed meal, hominy, milo, peanuts, popcorn, rice, soy meal and wheat		20		LFD/ Qualitative	3 mins	25	£135	£5.40	USDA-GIPSA approved
Aflatoxin SQ / 8020			<10, >20			5 mins	25	£135	£5.40	These kits are being replaced
DON SQ / 8315	*Wheat, corn, barley silages			<0.5 >2mg/kg	LFD/ Visual	5 mins	25	£135	£5.40	GIPSA approved These kits are being replaced

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
R Bio-pharm/Fannin RIDASCREEN										
RIDA Quick Aflatoxin RQS / R5205	Corn		4		LFD/ Quantitative-Reader	5 mins	20	£230	£11.50	
RIDA Quick Aflatoxin / R5204	Grain, Soy flour, nuts, pistachios, coconut flour, sunflower, seeds, figs, dates, cashew nuts		4, 10, 20		LFD/Semi-Quantitative	4-16 mins	20	£230	£11.50	
RIDA Quick Zearalenone RQS / R5504	Corn		75		LFD/ Quantitative-Reader	5 mins	20	£230	£11.50	
RIDA Quick DON / R5904	Wheat, triticale, corn		0.5, 1.25mg/kg		LFD/Semi-Quantitative	5 mins	20	£230	£11.50	
RIDA Quick Fumonisin / R5604	Corn		0.8, 4mg/kg		LFD/Semi-Quantitative	5 mins	20	£230	£11.50	
RIDA Quick Fumonisin RQS / R5606	Corn		0.8, 4 mg/kg		LFD/ Quantitative-Reader	5 mins	20	£230	£11.50	
Aflacard B1 / P27	Food and feed		varies depending on dilution used		Screening card/ Qualitative	10 mins	10 cards = 20 tests	£296	£14.80	
Aflacard Total / P38	Food and feed		varies depending on dilution used		Screening card/ Qualitative	10 mins	10 cards = 20 tests	£322	£16.10	
Ochracard / P48	Food and feed		varies depending on dilution used		Screening card/ Qualitative	30 mins	10 cards = 20 tests	£341	£17.05	

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
				Vicam						
Afla check/ 100000173	Food and grain		10, 20		LFD/ Qualitative	3 mins	25	£114	£4.56	USDA approved
Afla-V / 176002071	Peanuts, almonds, corn, wheat and rice samples can be used with this method			2-100	LFD/ Quantitative	5 mins	25	£156	£6.24	USDA approved
DON check / 100000198	Grain samples		1mg/kg		LFD/Qualitative	3 mins	25	£154	£6.16	USDA approved
DON V / 176002072	Grain & feed			0.2mg/kg-5mg/kg	LFD/ Quantitative	<5 mins	25	£178	£7.12	USDA approved
Fumo V / 176002810				0.2mg/kg-5mg/kg	LFD/ Quantitative	5 mins	25	£166	£6.64	USDA approved

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost US $	Cost per test US $	Additional Info
AC Diagnostics										
AFBI Screen (Methanol) / SS02LF1, LF2 or LF3	Grain, feed, grain derived products		5, 10, 20, 50		LFD/Visual Qualitative	5 mins	25 50 100	90 165 300	3.60 3.30 3.00	USDA approved
AFBI-Precise (Ethyl Acetate) / SS01LF1, LF2 or LF3	Grain, feed, grain derived products		5, 10, 20, 50		LFD/Visual Qualitative	5 mins	25 50 100	100 175 320	2.50 3.50 3.20	USDA approved
Fumonisin B1 / SS03 LF1, LF2 or LF3	Grain, feed		200ng/ml		LFD/Visual Qualitative	5-10 mins	25 50 100	120 195 360	4.80 3.90 3.60	USDA approved
Ochratoxin A / SS04 LF1, LF2 or LF3	Grain, feed		5		LFD/Visual Qualitative	10-15 mins	25 50 100	140 245 430	5.60 4.90 4.30	USDA approved
T-2 Toxin / SS05 LF1, LF2 or LF3	Grain, feed		100		LFD/Visual Qualitative	5-10 mins	25 50 100	140 245 430	5.60 4.90 4.30	USDA approved
Vomitoxin / SS06 LF1, LF2 or LF3	Grain, feed		10		LFD/Visual Qualitative	5-10 mins	25 50 100	130 215 390	5.20 4.30 3.90	USDA approved
Zearalenone / SS07 LF1, LF2 or LF3	Grain, feed		60		LFD/Visual Qualitative	5-10 mins	25 50 100	120 195 360	4.80 3.90 3.60	USDA approved

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Unisensor										
4 MycoSensor (Maize) / Kit 056	Maize, wheat and oat samples		280 (ZEN), 400 (T-2/HT-2), 1400 (DON), 3200 (FB1/FB2)		LFD/ Quantitative	<30 mins	24/96 tests	£450	£18.75 (multitest) £4.69 (analyte)	
4 MycoSensor (Wheat/Oat) / Kit 056	Maize, wheat and oat samples		80 (ZEN), 400 (T-2/HT-2), 1400 (DON)		LFD/ Quantitative	<30 mins	24/96 tests	£450	£18.75 (multitest) £4.69 (analyte)	
Don Sensor / Kit 065	Maize, wheat, barley, oat and cattle feed samples			200-15000	LFD/ Quantitative	5 mins	24			

Kit name	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences – Charm ROSA										
ROSA Aflatoxin Quantitative Test - Ethanol Extraction / LF-AFQ-ETOH-100ESK	Feed and Grain			0-150	LFD/ Quantitative	10 mins	100	£561.59	£5.62	5 positive controls
ROSA Aflatoxin Quantitative Test - Ethanol Extraction / LF-AFQ-ETOH-100K	Feed and Grain			0-150	LFD/ Quantitative	10 mins	100	£610.62	£6.10	1 positive control
ROSA Aflatoxin Quantitative Test - Ethanol Extraction / LF-AFQ-ETOH-20K	Feed and Grain			0-150	LFD/ Quantitative	10 mins	20	£133.71	£6.69	1 positive control
ROSA Aflatoxin Quantitative Test - Ethanol Extraction / LF-AFQ-ETOH-500K	Feed and Grain			0-150	LFD/ Quantitative	10 mins	500	£2,897.11	£5.79	5 positive controls
ROSA Aflatoxin Quantitative Test / LF-AFQ-100ESK	Feed and Grain			0-150	LFD/ Quantitative	10 mins	100	£708.68	£7.09	USDA GIPSA Approved -5 positive controls
ROSA Aflatoxin Quantitative Test / LF-AFQ-100K	Feed and Grain			0-150	LFD/ Quantitative	10 mins	100	£610.62	£6.11	USDA GIPSA Approved- 1 positive control
ROSA Aflatoxin Quantitative Test / LF-AFQ-20ESK	Feed and Grain			0-150	LFD/ Quantitative	10 mins	20	£157.78	£7.89	USDA GIPSA Approved- 2 positive controls
ROSA Aflatoxin Quantitative Test / LF-AFQ-20K	Feed and Grain			0-150	LFD/ Quantitative	10 mins	20	£133.71	£6.69	USDA GIPSA Approved- 1 positive control
ROSA Aflatoxin Quantitative Test / LF-AFQ-500ESK	Feed and Grain			0-150	LFD/ Quantitative	10 mins	500	£3,365.10	£6.73	USDA GIPSA Approved- 25 positive controls
ROSA Aflatoxin Quantitative Test / LF-AFQ-500K	Feed and Grain			0-150	LFD/ Quantitative	10 mins	500	£2,897.11	£5.79	USDA GIPSA Approved- 5 positive controls
Qualitative Test / LF-AFPN-20K					Qualitative					controls, 1 AFQ dilution buffer

Kit name / Product Code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences – Charm ROSA										
ROSA Fast Aflatoxin Quantitative Test / LF-FAST-100K	Feed and Grain		2, 10	0-150	LFD/ Quantitative	3 (corn)/5 (other)	100	£530.39	£5.30	GIPSA approved- 1 positive control
ROSA Fast Aflatoxin Quantitative Test / LF-FAST-20K	Feed and Grain		2, 10	0-150	LFD/ Quantitative	3 (corn)/5 (other)	20	£124.80	£6.24	GIPSA approved- 1 positive control
ROSA Fast Aflatoxin / LF-FAST-500K Quantitative Test /	Feed and Grain		2, 10	0-150	LFD/ Quantitative	3 (corn)/5 (other)	500	£2,224.09	£4.45	GIPSA approved-5 positive controls
ROSA WET™ Aflatoxin Quantitative Test / LF-AFQ-WET-100K	GIPSA approved for 16 commodities: barley, corn, corn flour, corn germ meal, corn gluten meal, corn meal, corn/soy blend, distiller's dried grain with solubles (DDGS), hominy, oats, popcorn, rice bran (defatted), rough rice, sorghum, soybeans, and wheat		2, 10	0-25 0-150	LFD/ Quantitative	5 mins	100	£530.39	£5.30	GIPSA approved-1 positive control
ROSA WET™ Aflatoxin Quantitative Test / LF-AFQ-WET-20K			2, 10	0-25 0-150	LFD/ Quantitative	5 mins	20	£124.80	£6.24	GIPSA approved-1 positive control
ROSA WET™ Aflatoxin Quantitative Test / LF-AFQ-WET-500K			2, 10	0-25, 0-150	LFD/ Quantitative	5 mins	500	£2,224	£4.45	GIPSA approved-5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA DON P/N Test / LF-DONPN-100ESK	USDA/GIPSA approved for barley, corn, wheat		0.5, 1(barley), 2, 5mg/kg	0-0.75, 0-1.5,0- 0.3, 0-7.5 reader range	LFD/Qualitative	3 mins	100	£668.56	£6.69	5 positive controls
ROSA DON P/N Test / LF-DONPN-100K	USDA/GIPSA approved for barley, corn, wheat		0.5, 1(barley), 2, 5mg/kg	0-0.75, 0-1.5,0- 0.3, 0-7.5 reader range	LFD/ Qualitative	3 mins	100	£579.42	£5.79	1 positive control
ROSA DON P/N Test / LF-DONPN-20ESK	USDA/GIPSA approved for barley, corn, wheat		0.5, 1(barley), 2, 5mg/kg	0-0.75, 0-1.5,0- 0.3, 0-7.5 reader range	LFD/ Qualitative	3 mins	20	£147.08	£8.17	2 positive controls
ROSA DON P/N Test / LF-DONPN-20K	USDA/GIPSA approved for barley, corn, wheat		0.5, 1(barley), 2, 5mg/kg	0-0.75, 0-1.5,0- 0.3, 0-7.5 reader range	LFD/ Qualitative	3 mins	20	£124.80	£6.24	1 positive control
ROSA DON P/N Test / LF-DONPN-500ESK	USDA/GIPSA approved for barley, corn, wheat		0.5, 1(barley), 2, 5mg/kg	0-0.75, 0-1.5,0- 0.3, 0-7.5 reader range	LFD/ Qualitative	3 mins	500	£3,204.65	£6.41	25 positive controls
ROSA DON P/N Test / LF-DONPN-500K	USDA/GIPSA approved for barley, corn, wheat		0.5, 1(barley), 2, 5mg/kg	0-0.75, 0-1.5,0- 0.3, 0-7.5 reader range	LFD /Qualitative	3 mins	500	£2,758.94	£5.52	5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA DON Quantitative Test / LF-DONQ-100ESK	Charm approved for barley, brewer's rice, buckwheat, corn, corn bran, corn germ meal, corn gluten meal, DDGS, hominy, malted barley Milled rice, oats, palm kernel meal, Rapeseed meal, rice bran, rough rice, rye sorghum, soybean meal triticale, wheat Wheat bran, wheat flour, wheat midds, wheat red dog			0-6, 6-12, 12-24mg/kg reader range	LFD/ Quantitative	10 mins	100	£757.70	£7.58	5 positive controls
ROSA DON Quantitative Test / LF-DONQ-100K				0-6, 6-12, 12-24mg/kg reader range	LFD/ Quantitative	10 mins	100	£668.56	£6.69	1 positive control
ROSA DON Quantitative Test / LF-DONQ-20ESK				0-6, 6-12, 12-24mg/kg reader range	LFD/ Quantitative	10 mins	20	£164.91	£8.24	2 positive controls
ROSA DON Quantitative Test / LF-DONQ-20K				0-6, 6-12, 12-24mg/kg reader range	LFD/ Quantitative	10 mins	20	£142.63	£7.13	1 positive control
ROSA DON Quantitative Test / LF-DONQ-500ESK				0-6, 6-12, 12-24mg/kg reader range	LFD/ Quantitative	10 mins	500	£3,650.36	£7.30	25 positive controls
ROSA DON Quantitative Test / LF-DONQ-500K				0-6, 6-12, 12-24mg/kg reader range	LFD/ Quantitative	10 mins	500	£2,476.16	£4.95	5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA Fumonisin Quantitative Test-Ethanol Extraction / LF-FUMQ-ETOH-100ESK	Charm approved for barley, corn, DDGS, hominy, oats, sorghum, and soybean meal			0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	100	£561.59	£5.62	5 positive controls
ROSA Fumonisin Quantitative Test-Ethanol Extraction / LF-FUMQ-ETOH-100K				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	100	£668.56	£6.69	1 positive control
ROSA Fumonisin Quantitative Test-Ethanol Extraction / LF-FUMQ-ETOH-20K				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	20	£142.63	£7.13	1 positive control
ROSA Fumonisin Quantitative Test-Ethanol Extraction / LF-FUMQ-ETOH-500K				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	500	£3,204.65	£6.41	5 positive controls
ROSA Fumonisin Quantitative Test / LF-FUMQ-100ESK	Charm validated for barley, corn, flaking corn grits, DDGS, millet, oats, rough rice, sorghum and wheat			0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	100	£757.70	£7.58	5 positive controls
ROSA Fumonisin Quantitative Test / LF-FUMQ-100K				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	100	£668.56	£6.69	1 positive control
ROSA Fumonisin Quantitative Test / LF-FUMQ-20ESK				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	20	£164.91	£8.25	2 positive controls
ROSA Fumonisin Quantitative Test / LF-FUMQ-20K				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	20	£142.63	£7.13	1 positive control
ROSA Fumonisin Quantitative Test / LF-FUMQ-500 ESK				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	500	£3,650.36	£7.31	25 positive controls
ROSA Fumonisin Quantitative Test / LF-FUMQ-500K				0-1, 0-6, 6-60mg/kg reader ranges	LFD/ Quantitative	10 mins	500	£3,204.65	£6.41	5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA Ochratoxin Quantitative Test / LF-Ochra-G-100ESK	Charm approved for 9 commodities (barley, corn, corn gluten meal, malted barley, oats, rye, sorghum, soybean meal and wheat)			0-12, 10-150 reader ranges	LFD/ Quantitative	10 mins	100	£846.85	£8.47	5 positive controls
ROSA Ochratoxin Quantitative Test / LF-Ochra-G-100K				0-12, 10-150 reader ranges	LFD/ Quantitative	10 mins	100	£757.70	£7.58	1 positive control
ROSA Ochratoxin Quantitative Test /				0-12, 10-150 reader ranges	LFD/ Quantitative	10 mins	20	£254.05	£12.75	2 positive controls
ROSA Ochratoxin Quantitative Test / LF-Ochra-G-20K				0-12, 10-150 reader ranges	LFD/ Quantitative	10 mins	20	£231.77	£11.59	1 positive control
ROSA Ochratoxin Quantitative Test / LF-Ochra-G-500ESK				0-12, 10-150 reader ranges	LFD/ Quantitative	10 mins	500	£4,096.06	£8.19	25 positive controls
ROSA Ochratoxin Quantitative Test / LF-Ochra-G-500K				0-12, 10-150 reader ranges	LFD/ Quantitative	10 mins	500	£2,971.39	£5.94	5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA T-2-HT-2 Quantitative Test / LF-T-2-HT-2-100ESK	Charm validated for barley, corn, corn gluten meal, oats, sorghum, soybean meal, wheat, wheat flour-feed and grain			0-250, 0-2500 reader ranges	LFD/ Quantitative	10 mins	100	£1,065.24	£10.65	5 T-2/HT-2 positive controls, 1 T-2/HT-2 dilution buffers
ROSA T-2-HT-2 Quantitative Test / LF-T-2-HT-2-100K				0-250, 0-2500 reader ranges	LFD/ Quantitative	10 mins	100	£976.10	£9.76	1 T-2/HT-2 positive controls, 1 T-2/HT-2 dilution buffers
ROSA T-2-HT-2 Quantitative Test/ LF-T-2-HT-2-20ESK				0-250, 0-2500 reader ranges	LFD/ Quantitative	10 mins	20	£240.68	£12.03	2 T-2/HT-2 positive controls, 1 T-2/HT-2 dilution buffers
ROSA T-2-HT-2 Quantitative Test / LF-T-2-HT-2-20K				0-250, 0-2500 reader ranges	LFD/ Quantitative	10 mins	20	£218.40	£10.92	1 T-2/HT-2 positive controls, 1 T-2/HT-2 dilution buffers
ROSA T-2-HT-2 Quantitative Test / LF-T-2-HT-2-500ESK				0-250, 0-2500 reader ranges	LFD/ Quantitative	10 mins	500	£5,005.31	£10.01	25 T-2/HT-2 positive controls, 5 T-2/HT-2 dilution buffers
ROSA T-2-HT-2 Quantitative Test / LF-T-2-HT-2-500K				0-250, 0-2500 reader ranges	LFD/ Quantitative	10 mins	500	£4,559.60	£9.11	5 T-2/HT-2 positive controls, 5 T-2/HT-2 dilution buffers

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA Zearalenone Quantitative Test / LF-ZEARQ-100ESK	USDA/GIPSA approved for barley, corn, DDGS, flaking corn grits, milled rice, oats, rough rice, sorghum, soybean meal, wheat, wheat flour-feed and grain			0-1400 reader range	LFD/ Quantitative	10 mins	100	£846.85	£8.47	5 Zearalenone positive controls, 1 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test / LF-ZEARQ-100K				0-1400 reader range	LFD/ Quantitative	10 mins	100	£757.70	£7.58	1 Zearalenone positive controls, 1 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test / LF-ZEARQ-20ESK				0-1400 reader range	LFD/ Quantitative	10 mins	20	£254.05	£12.70	2 Zearalenone positive controls, 1 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test / LF-ZEARQ-20K				0-1400 reader range	LFD/ Quantitative	10 mins	20	£231.77	£11.59	1 Zearalenone positive controls, 1 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test / LF-ZEARQ-500ESK				0-1400 reader range	LFD/ Quantitative	10 mins	500	£4,096.06	£8.19	5 Zearalenone positive controls, 5 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test / LF-ZEARQ-500K				0-1400 reader range	LFD/ Quantitative	10 mins	500	£3,650.36	£7.31	25 Zearalenone positive controls, 5 Zearalenone dilution buffers

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA Zearalenone Quantitative Test-Ethanol Extraction / LF-ZEARQ-ETOH-100ESK	Charm approved for corn, corn meal, DDGS, milled rice, palm kernel meal, rice bran, sorghum, soybean meal, wheat, wheat bran, wheat flour, wheat midds, wheat red dog			0-1400 reader range	LFD/ Quantitative	10 mins	100	£757.70	£7.58	5 Zearalenone positive controls, 5 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test-Ethanol Extraction / LF-ZEARQ-ETOH-100K				0-1400 reader range	LFD/ Quantitative	10 mins	100	£757.70	£7.58	1 Zearalenone positive controls, 1 Zearalenone dilution buffers
ROSA Zearalenone Quantitative Test-Ethanol Extraction / LF-ZEARQ-ETOH-20K				0-1400 reader range	LFD/ Quantitative	10 mins	20	£231.77	£11.59	1 positive control
ROSA Zearalenone Quantitative Test-Ethanol Extraction / LF-ZEARQ-ETOH-500K				0-1400 reader range	LFD/ Quantitative	10 mins	500	£3,650.36	£7.31	5 Zearalenone positive controls, 5 Zearalenone dilution buffers

Kit name / Product code	Testing material	Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences									
ROSA Fast 5 DON Quantitative Test / LF-DONQ-Fast5-100ESK	GIPSA approved for barley, corn, DDGS, malted barley, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour, wheat midds	0.1mg/kg	0-1.5, 1-6mg/kg reader range	LFD/ Quantitative	5 mins	100	£757.70	£7.58	5 positive controls
ROSA Fast 5 DON Quantitative Test / LF-DONQ-Fast5-100K		0.1mg/kg	0-1.5, 1-6mg/kg reader range	LFD/ Quantitative	5 mins	100	£668.56	£6.69	1 positive control
ROSA Fast 5 DON Quantitative Test / LF-DONQ-Fast5-20ESK		0.1mg/kg	0-1.5, 1-6mg/kg reader range	LFD/ Quantitative	5 mins	20	£164.91	£8.25	2 positive controls
ROSA Fast 5 DON Quantitative Test / LF-DONQ-Fast5-20K		0.1mg/kg	0-1.5, 1-6mg/kg reader range	LFD/ Quantitative	5 mins	20	£142.63	£7.13	1 positive control
ROSA Fast 5 DON Quantitative Test / LF-DONQ-Fast5-500ESK		0.1mg/kg	0-1.5, 1-6mg/kg reader range	LFD/ Quantitative	5 mins	500	£3,650.36	£7.31	25 positive controls
ROSA Fast 5 DON Quantitative Test / LF-DONQ-Fast5-500K		0.1mg/kg	0-1.5, 1-6mg/kg reader range	LFD/ Quantitative	5 mins	500	£3,204.65	£6.41	5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Charm Sciences										
ROSA Fast 5 Fumonisin Quantitative Test / LF-FumQ-Fast5-100ESK	GIPSA approved for barley, corn, flaking corn grits, millet, oats, rough rice, sorghum, wheat		0.25,1 mg/kg	0-1.5, 0-6mg/kg	LFD/ Quantitative	5 mins	100	£757.70	£7.58	5 positive controls
ROSA Fast 5 Fumonisin Quantitative Test / LF-FumQ-Fast5-100K			0.25,1 mg/kg	0-1.5, 0-6mg/kg	LFD/ Quantitative	5 mins	100	£668.56	£6.69	1 positive control
ROSA Fast 5 Fumonisin Quantitative Test / LF-FumQ-Fast5-20ESK			0.25,1 mg/kg	0-1.5, 0-6mg/kg	LFD/ Quantitative	5 mins	20	£164.91	£8.25	2 positive controls
ROSA Fast 5 Fumonisin Quantitative Test / LF-FumQ-Fast5-20K			0.25,1 mg/kg	0-1.5, 0-6mg/kg	LFD/ Quantitative	5 mins	20	£142.63	£7.13	1 positive control
ROSA Fast 5 Fumonisin Quantitative Test / LF-FumQ-Fast5-500ESK			0.25,1 mg/kg	0-1.5, 0-6mg/kg	LFD/ Quantitative	5 mins	500	£3,650.36	£7.31	25 positive controls
ROSA Fast 5 Fumonisin Quantitative Test / LF-FumQ-Fast5-500K			0.25,1 mg/kg	0-1.5, 0-6mg/kg	LFD/ Quantitative	5 mins	500	£3,204.65	£6.41	5 positive controls

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
Euro-Proxima										
Aflatoxin B1 FTR Test / 5127AFB	Food and feed- validated for cereals, soy beans, nuts and derived products		2		Rapid through test / Qualitative	10 mins	10	165 euro	16.5 euro	USDA-GIPSA approved
Aflatoxin Total FTR Test / 5127AFT	Food and feed- validated for cereals, soy beans, nuts and derived products		4		Rapid through test / Qualitative	10 mins	10	165 euro	16.5 euro	USDA-GIPSA approved
DON Gold FTR Test / 5127DONG	Cereals		1000		Rapid through test / Qualitative	approx. 20 mins		165 euro		USDA-GIPSA approved
Ochratoxin A FTR Test / 5127OCH	Food and feed-validated for cereals, wine, green coffee		4		Rapid through test / Qualitative	10 mins	10	165 euro	16.5 euro	USDA-GIPSA approved
Zearalenone Gold FTR Test / 5127ZEAG	Cereals		100		Rapid through test / Qualitative	approx. 20 mins		165 euro		USDA-GIPSA approved

	EnviroLogix									
	Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost US $	Cost per test US $	Additional Info
	QuickTox kit for Quickscan-Fumonisin / AQ111BG	Corn			0.2-25mg/kg	LFD/ Quantitative	5 mins	50	300	6.00	
	QuickTox kit for Quickscan-Ochratoxin / AQ113BG	Wheat			1.5-100	LFD/ Quantitative	10 mins	50	300	6.00	
	QuickTox kit for Quickscan-Zearalenone / AQ112BG	Corn			50-520	LFD/ Quantitative	5mins	50	300	6.00	
	QuickTox for Quickscan - Aflatoxin / AQ109BG	Corn and wheat			2.5-10 (5-100)*	LFD/ Quantitative	5 mins	50	300	6.00	USDA/GIPSA certified
	QuickTox for Quickscan - Aflatoxin / AQ109BG	DDGS			10-	LFD/ Quantitative	5 mins	50	300	6.00	USDA/GIPSA certified
	QuickTox for Quick Scan DON / AQ204BG	Corn, wheat, wheat derivatives, oats			0.2-5mg/kg/0.2-10mg/kg (0.5-5mg/kg)*	LFD/ Quantitative	10 mins	50	300	6.00	AOAC/USDA/ GIPSA
		Barley			0.2-5mg/kg (0.5-5mg/kg)*	LFD/ Quantitative	10 mins	50	300	6.00	AOAC/USDA/ GIPSA
	AQ204BG2 AQ204BG3	DDGS			0.2-10mg/kg (0.5-5mg/kg)*	LFD/ Quantitative	10 mins	50	300	6.00	AOAC/USDA/ GIPSA
	QuickTox kit for DON	Wheat, corn, barley		0.5, 1, 2mg/kg		LFD/ Qualitative	5 mins	50	250	5.00	USDA/GIPSA certified
	QuickTox kit for Aflatoxin	Corn		20		LFD/ Qualitative	2-5 mins	50	250	5.00	USDA/GIPSA certified

Kit name / Product code	Testing material		Limit of Detection µg/kg unless stated	Range of Quantitation µg/kg unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	Additional Info
				Romer						
AgraStrip Total Aflatoxin Test Kit / COKAS1200	Grains, grain products, peanuts, almonds		10		LFD /Qualitative	5 mins	24	£210	£8.75	USDA/GIPSA approved
AgraStrip Total Aflatoxin Test Kit / COKAS1000	Grains, grain products, peanuts, almonds		20		LFD/ Qualitative	5 mins	24	£209	£8.71	USDA/GIPSA approved
AgraStrip Total Aflatoxin Test Kit / COKAS1100	Grains, grain products, peanuts, almonds		4		LFD /Qualitative	5 mins	24	£209	£8.71	USDA/GIPSA approved
Agra Strip Aflatoxin / COKAS1600A	Corn and other commodities.		3.6	5-100	LFD/ Quantitative	3.5 mins	24	£210	£8.75	
Agra Strip DON Quantitative / COKAS4000A	Grains and grain products.		0.21 mg/kg (wheat) 0.19 mg/kg (corn)	0.25 – 5.0 mg/kg (wheat, corn)	LFD/ Quantitative	3.5 mins	24	£210	£8.75	
Agra Strip Fumonisin Quantitative / COKAS3000A	Corn and other commodities.		0.3 mg/kg	0.5-5mg/kg	LFD/ Quantitative	3.5 mins	24	£210	£8.75	

Kit name	Testing material		Limit of Detection µg/kg Unless stated	Range of Quantitation µg/kg Unless stated	Type of Test	Time of test/ incubation	Tests per kit	Cost	Cost per test	
Toximet										
ToxiQuant	Corn/Maize for Aflatoxin B1, B2, G1, G2, Ochratoxin A		sub µg/kg levels 0.3µg/kg		The extracted sample is immobilised on the ToxiTrace cartridge and using a spectrometer and chemometrics, the mycotoxins are detected/ Quantitative	5 mins /40 mins	20 Toxi-Sep, and 10 Toxi-Trace cart-ridges per package	Price depends on the number of tests carried out each year		

Table 11. Readers for Mycotoxin Analysis

		Readers			
Neogen		**Accuscan Reader £1080 Code:9565**			
RBio-pharm		**RIDA Quick Scan Optical reader £1825 Code: ZG5005, RIDA Quick Scan (including Printer and Scanning devices) £2858 Code: ZG5005-0**			
Vicam		**Vertu Reader £2,285.71 Code:725000574**			
Unisensor		**Readsensor APP038 (ESE Small case)/ APP039 (ESE Full case)-£1500**	**Heatsensor Duo(2)-Octo(8) Code:APP032-£150 Heatsensor Aerne Codes: APP004(12VDC), APP003(24VDC), APP004(230VAC), APP007(110VAC)**		
Charm Sciences		**Charm EZ-M Reader £3210.39 Code: LF-Rosa-EZ-M**			
EnviroLogix, Inc.		**Quickscan Reader US $3, 295 for ACC-131 scanner and ACC-134 PC**			
Romer Inc		**Agra Strip Reader and printer-£2071 Code: EQASR1000**	**AgraStrip Incubator (requires heat block) Code: EQOEV2060 £578, AgriStrip Heat Block Code: EQASR1005 £205**		

9. Review of Confirmatory Tests for Mycotoxin Analysis

9.1. Introduction

There is a huge amount of peer reviewed literature available on confirmatory tests for mycotoxins. The World Mycotoxin Journal publishes an article on developments in mycotoxin analysis each year which summarises the most recent developments in methods for individual or groups of mycotoxins. More recently it has become apparent that the use of LC-MS analysis has resulted in a large increase in the reports of methods capable of detecting several mycotoxins, (multi-mycotoxin methods) and therefore there is now also a section dedicated to these methods in the article (Shephard *et al.*, 2011, 2012, 2013, Berthiller, 2014).

9.2. Confirmatory analysis of mycotoxins in animal feeds and bedding

9.2.1. Methods for individual toxins

Immunoassays or immunoaffinity column clean-up along with HPLC-UV and/or FLD detection are traditionally used for mycotoxins analysis in foodstuff and animal feeds. These methodologies are often restricted to a limited range of mycotoxins due to the targeted and specific nature of the extraction and clean-up procedures used. Separate analyses would be required to cover the desired range of analytes. Therefore, reported incidence of mycotoxin contamination in the literature might not always provide the complete picture of contamination levels in the feed samples due to these limitations.

These methods still form the basis for all the standardised methods available within the UK and Europe for mycotoxins. Standardisation is carried out through the European Standardisation body (Comité Européen de Normalisation, CEN). Methods validated and standardised by this approach are automatically adopted by the member countries individual standardisation bodies, so in this case are also BSi methods. Currently there are a number of validated for mycotoxins in food and feed, all based on liquid chromatography (Table 12). Methods are validated for food or feed but there are no confirmatory or standard methods for mycotoxins in bedding, straw, hay or silage.

Table 12. Available international standardised confirmatory methods of analysis for mycotoxins in animal feed.

CEN/TC 327	**Animal feeding stuffs - Methods of sampling and analysis**
Standard Reference	**Title**
EN ISO 17375:2006	Animal feeding stuffs – Determination of aflatoxin B1 (ISO 17375:2006)
EN 15792:2009	Animal feeding stuffs – Determination of zearalenone in animal feed - High performance liquid chromatographic method with fluorescence detection and immunoaffinity column clean-up
EN 15791:2009	Foodstuffs – Determination of Deoxynivalenol in animal feed – HPLC method with immunoaffinity column clean-up
BS EN 16007:2011	Animal feeding stuffs. Determination of ochratoxin A in animal feed by immunoaffinity column clean-up and high performance liquid chromatography with fluorescence detection
BS EN 16006:2011	Animal feeding stuffs. Determination of the sum of fumonisin B1 & B2 in compound animal feed with immunoaffinity clean-up and RP-HPLC with fluorescence detection after pre- or post-column derivatisation
ISO Standards	
Standard Reference	**Title**
ISO6651: 2001 EDTN 3	ISO6651: 2001 Animal feeding stuffs- Semi-quantitative determination of aflatoxin B1 – Thin layer chromatographic methods
BS 5766-16:1999, ISO 14718:1998	Methods for analysis of animal feeding stuffs. Determination of aflatoxin B1 content of mixed feeding stuffs (high-performance liquid chromatographic method)
BS 5766-7:1988, ISO 6651-1987	Methods for analysis of animal feeding stuffs. Determination of aflatoxin B1 (Method for extraction and purification by thin-layer chromatography with visual or fluorodensitometric determination.)

9.2.2. Current developments and multi mycotoxin methods for feed

The advent of the LC-MS/MS detection allowed for a much wider range of mycotoxins to be analysed simultaneously, and in many cases at a greater sensitivity compared to traditional methods. There have been a number of reports of mycotoxin analysis using a generic extraction and clean-up technique followed by LC-MS/MS detection. In theory this would allow for the greatest number of analytes to be extracted (compared to immunoaffinity column clean-up). In practice however, a less specific cleanup will often result in dirtier extracts which can lead to matrix suppression (reduced sensitivity), contamination of the LC-MS instrument and less reproducible results. The use of isotopically labelled mycotoxin standard has been used to improve analytical precision, but this might not be possible due to the high expense and/or availability of the isotopically labelled standard. In order to reduce

the cost of analysis, Jackson *et al*., (2012) selected three isotopically labelled mycotoxin standards ($^{13}C_{18}$ zearalenone, $^{13}C_{15}$ deoxynivalenol and $^{13}C_{17}$ aflatoxin B_1) to validate an analysis method for more than twenty structurally different mycotoxins in the matrices corn, wheat, barley and distillers grains with solubles (see Table 13). In most cases the precision and recovery of the results improved with the use of one of the three isotopically labelled internal standards.

In the Swedish study on the occurrence of mycotoxins in cereals and bedding, an LC-MS/MS method previously published for analysis of several mycotoxins in vegetable animal feed was used. The method was developed and validated in-house. Performance data generated showed the method precision was within the performance predicted by the Horwitz equation for all analytes (Tevell Aberg *et al*., 2013). There have been a number of reports of methods for analysis of multi-mycotoxins in silage. Again all are based on LC-MS/MS. Rasmussen *et al*., (2010) developed a method for silage that was validated for quantitative analysis of 8 mycotoxins and qualitative analysis of 19 mycotoxins. A QUECHERS approach was used to give a buffering effect to ensure stability of the samples, however the method performance was unsatisfactory for fumonisins and citrinin. Limits of detection ranged from 1 to over 700 µg/kg for individual toxins (Rasmussen *et al*., 2010). Maize silage was analysed in Belgium using a method developed for 26 mycotoxins in silage. The method used clean-up with a solid phase extraction column before LC-MS/MS analysis. The method was validated taking into account the requirements of Commission Decision 2002/657/EC, which prescribes criteria such as retention time drift and ion ratios. The limit of detection and quantitation ranges were 5–348 and 11–695 µg/kg, respectively with apparent recovery ranging from 61 and 116%. Repeatability and reproducibility were within the ranges of 3–45 and 5–49%, respectively, which for the higher end is outside predicted acceptable performance from the Horwitz equation. Another LC-MS/MS method was used to analyse samples of silage in Ireland. Twenty mycotoxins were included in the method, 8 were detected in samples of silage analysed, but not at levels that would give cause for concern. No method validation or performance data were given for this method (McElwhinney *et al.,* 2014).

Table 13. Summary of some recent published analysis methods for mycotoxins in animal feed and animal bedding.

Matrix	Extraction/ cleanup	Detection	Toxin	LOD (µg/kg)	Rec (%)	RSD (%)	Reference
Rice Straw Bermuda Grass Oat hay Animal feed	Water/MeCN with IAC	HPLC LCMS/MS	ZEN a-ZEL b-ZEL	-	84	1.4	Emoto *et al.,* (2008) Fushimi *et al.,* (2014)
Whole wheat plant (above ground)	Water/MeCN Mycotoxin Bond Elute SPE	LCMS/MS	Aflatoxin B1 Aflatoxin B2 Aflatoxin G1 Aflatoxin G2 Aflatoxin M1 Altenuene Tentoxin Alternariol Alternariol methyl ether Ergocornine Ergocryptine Beauvericin Sterigmatocystin Sulochrin Citrinin Patulin DAS HT-2 toxin NEO T-2 toxin Deoxynivalenol FUSX Nivalenol 3AcDON Verrucarin A α-zearelenol β-zearelenol zearalenone	7 4 19 26 20 10 3 20 1 9 9 2 1 14 1 1 1 2 5 2 1 8 8 4 5 2 1 1	69 90 73 101 92 107 107 19 101 107 109 115 99 103 70 99 102 104 82 104 98 102 101 103 103 99 112 98	2 7 4 27 10 2 3 9 10 9 9 6 6 6 2 4 3 6 4 1 10 4 4 2 7 10 8 21	Schenzel *et al.,* (2012)
Maize Silage	QuEChERS MeCN/water/acetic acid, Filter 0.45 µ PTFE	LCMS/MS	Alternariol Alternariol methyl ether Altersetin Andrastin A Citreoisocumarin Deoxynivalenol Gliotoxin Mycophenolic acid Nivalenol Ochratoxin A Patulin Penitrem A ROQ C	10 6 - 1 - 739 71 7 122 10 371 8 158	78 79 91 122 84 83 85 90 68 71 100 107 205	9 5 15 8 7 17 13 11 13 8 17 6 9	Rasmussen *et al.*, 2010

Matrix	Extraction/ cleanup	Detection	Toxin	LOD (µg/kg)	Rec (%)	RSD (%)	Reference
			Tenuazonic acid	121	37	20	
			Zearalenone	9	90	12	
			Cyclopiazonic acid	15	63	22	
			Enniatin B	24	60	21	
			Fumigaclavine A	-	93	12	
			Fumigaclavine C	-	176	11	
			Fumitremorgin A	76	93	18	
			Marcfortine A	-	63	12	
			Marcfortine B	-	61	9	
			Mevinolin	25	68	25	
			PR-toxin	-	56	27	
			ROQ A	-	103	13	
			Sterigmatocystin	8	72	9	
			T-2 toxin	96	55	17	
Corn silage Sorghum silage Wet brewer grains	Dichloromethane extraction. Filter. Evaporation and redissolved in HPLC buffer	HPLC-UV	Gliotoxin	-	-	-	Keller *et al*., 2012
Corn Wheat Barley Distillers grains with solubles	Shake MeCN/water/acetic acid 18h. Centrifuge/filter. Evaporate and redissolve in HPLC buffer	LCMS/MS	Patulin	-	96-42	-	Jackson *et al.,* 2012
			Nivalenol	-	111-68	-	
			Deoxynivalenol	-	107-64	-	
			DON-3GLU	-	78-28	-	
			Penicillic acid	-	122-86	-	
			FUSX	-	93-59	-	
			NEO	-	117-71	-	
			3AcDON	-	97-68	-	
			15AcDON	-	79-66	-	
			Aflatoxin G2	-	95-49	-	
			Aflatoxin G1	-	75-29	-	
			Aflatoxin B2	-	172-55	-	
			Aflatoxin B1	-	185-10	-	
			Gliotoxin	-	96-10	-	
			DAS	-	141-33	-	
			ROQ C	-	31-11	-	
			HT-2 toxin	-	73-56	-	
			Fumonisin B1	-	56-12	-	
			Mycophenolic acid	-	74-58	-	
			Ochratoxin B	-	88-44	-	
			T-2 toxin	-	96-66	-	
			Fumonisin B3	-	72-23	-	
			Zearalenone	-	96-76	-	
			Fumonisin B2	-	63-17	-	
			Ochratoxin A	-	86-29	-	
			Sterigmatocystin	-	79-21	-	
Rice Straw	Acetone/water. Phenyl SPE cleanup	HPLC-FLD	Aflatoxin B1 Aflatoxin B2 Aflatoxin G1	-	-	-	AL-Mamum *et al.,* 2002

Matrix	Extraction/ cleanup	Detection	Toxin	LOD (µg/kg)	Rec (%)	RSD (%)	Reference
		(pre and post column derivatisation)	Aflatoxin G2 Fumonisin B1				
Cereals and bedding straw	MeCN/water/formic acid. Hexane defat. Multisep 226 column cleanup	LCMS/MS	Deoxynivalenol HT-2 toxin T-2 toxin Zearalenone	-	-	-	Nordkvist *et al*., 2014
Animal feed Corn wheat	MeCN/water/formic acid. Hexane defat. Multisep 226 column cleanup	LCMS/MS	Deoxynivalenol HT-2 toxin T-2 toxin Zearalenone Fumonisin B1 Ochratoxin A Fumonisin B2		108-88 148-133 135-129 113-96 132-86 125-89 123-95	14-9 9-7 20-2 10-5 16-2 23-4 14-1	Aberg *et al*., 2013

The Mycotoxins EURL also conducted a multi laboratory validation study of a method for the determination of four *Fusarium* toxins in animal feed. This used isotopically labelled standards and LC-MS/MS detection and was validated for analysis of DON, ZEN, T-2 and HT-2 toxin. Overall the method demonstrated satisfactory performance characteristics, with the exception of low levels of T-2 toxin and ZEN where repeatability values were higher than predicted (Breidbach *et al*., 2013). This method is now undergoing standardisation through the newly formed CEN working group on natural toxins in animal feed, CEN TC 327/WG5.

Two mandates to develop standardised methods for animal feed were granted to CEN TC327 (Animal Feeding Stuffs) by the European Commission last year (M/521 and M/522). In addition to the above method, which is quantitative for a small number of toxins, methods for determination of ergot alkaloids and tropane alkaloids by LC-MS and a multimethod for mycotoxins in feed materials and compound feed by LC-MS are both being developed. The validation studies for these have not taken place yet it so it will be several years before they become available as standard methods.

The move to multi-toxin methods means compromises have to be made in the performance of the method, as the more toxins that are included in the analysis the less targeted and the more generic the method becomes. That said, it does seem possible to achieve acceptable performance for the main classes or most commonly occurring mycotoxins in a single method. The work being carried out through CEN TC327 will also lead to the development of more widely validated methods.

9.2.3. Methods for detection of mycotoxin exposure in animals

Ruminants and non-ruminant animals alike, can often display similar clinical signs when exposed to high levels of mycotoxin in contaminated feed over a prolonged period of time. This can vary from loss of body condition/weight, lameness, diarrhoea, loose faeces with undigested fibres, hyperthermia, feed refusal and reduced milk production (for dairy cattle). There is also an increased incidence of diseases such as displaced abomasum, ketosis, retained placenta, metritis, and mastitis. In the worst case scenario, the death of the animal can result due to a combination of the above effects.

Many of the reported studies into toxicity of mycotoxins have reported differences between administration of pure toxins and feeding studies with naturally contaminated feed containing a similar level of the compound under study.

Applebaum *et al.,* (1982) showed that daily administration of aflatoxin B_1 (13mg) to dairy cattle showed no reduced feed intake and milk production, however treatment with a mixture of aflatoxins and metabolites from *Aspergillus parasiticus* culture significantly reduced the feed intake and milk production. A recent extensive review of the transmission of a range of chemical contaminants to animal products (tissue, milk and eggs) showed that transfer of most mycotoxins was negligible apart from two main mycotoxins. Aflatoxin B_1 is transferred to milk and excreted as aflatoxin M_1 at a rate of 2–6%, while ochratoxin A residues can be found in offal and meat from pigs (MacLachlan, 2011). This means testing for exposure to mycotoxins by analysis of animal products after slaughter, or at post mortem in the case of incidents is difficult, and unreliable for confirming toxin exposure.

In the past, toxicity studies used very high levels of mycotoxins to test for effects and tested for a range of serum biochemistry factors as well as histopathology to assess the effects of the toxin. Residues of the parent compound were also tested in urine and faeces to determine elimination patterns. As most studies were targeted at one toxin, then methods of analysis tended to be targeted to the parent and its known metabolites. Even in cases of historical suspected field outbreaks of mycotoxicoses confirmed diagnosis is not often possible, particularly in cases of chronic disease (Morgavi and Riley, 2007).

More recent studies have tended to use much lower toxin levels and the use of LC-MS/MS now makes it possible to analyse for several toxins in the same study.

Urinary biomarkers for ZEN were initially identified as a result of monitoring for abuse with the steroid zeranol. The occurrence of zeranol in animal urine was reported in New Zealand and Northern Ireland. Investigation revealed the source to be from natural ZEN exposure from pasture (Kennedy *et al.,* 1995, 1998). Subsequently a confirmatory LC-MS/MS method was developed and validated for the analysis zeranol, its epimer taleranol and the mycotoxins ZEN, α-zearalenol and β-zearalenol in bovine urine (Launay *et al*., 2004). As zeranol is a banned substance in Europe its detection in animal products could have serious implications for producers. A European wide study was undertaken by four laboratories. Over 800 bovine urine samples were analysed by immunoassay for zeranol, over 6% screened positive. These samples were subject to confirmatory analysis, of samples identified as true positive 170 / 174 were identified as zeranol positive as a result of in vivo metabolism from *Fusarium* toxins by means of a statistical model that was developed. Thus using this model, and the relative amounts of the metabolites it is possible to differentiate between natural exposure and zeranol abuse (Launay, 2004a). An LC-MS/MS method for detection of zeranol in urine is used routinely at Fera as part of the Veterinary Statutory Monitoring programme. This method is designed to detect zeranol abuse, but is also able to detect ZEN and metabolites α-zearalenol and β-zearalenol. Positive results for these analytes have been found during these analyses indicate natural ZEN exposure occurs in the UK (unpublished data).

In addition *SAC* Consulting: Veterinary Services (*SACCVS*) offers a testing service for ZEN and DON in pig bile, serum or urine. Information about the service is available online, and samples from several aninals may be pooled, to allow larger number so be tested in cases where an investigation of a disease outbreak in a herd is required (SAC, 2015). The analysis of urine or plasma allows testing of live animals for informed remedial action can be taken if mycotoxins are found to be present at significant levels.

Analysis of urinary ZEN and its metabolites has also been reported in sheep. An ELISA method was used to detect ZEN in urine after dosing sheep. The values were ratioed with creatinine levels to adjust for urine volume. As ZEN in pasture in New Zealand is so widespread it was proposed this test could be used to select resistant sheep in ram breeders flocks (Morris *et al.,* 2005, 2006).

More recently an LC-MS/MS method has been developed and used in the study of DON and ZEN metabolism in dairy cattle. Residues of ZEN, α-ZEL, β-ZEL, DON and DOM could be detected in nearly all samples of the *Fusarium* contaminated groups, incubation with enzyme prior to analysis indicated that many of the compounds were present as glucuronides. It was

noted that while toxin concentration in urine correlated with intake, variability between animals was high, however it was proposed that urine measurements, with correlation, could be used to assess dietary intake of DON and ZEN (Winkler *et al.*, 2015).

The trend toward analysis of multi-mycotoxins also applies in urine analysis. A method for the determination of 12 analytes including AFB_1, DON, FB1, OTA, ZEN and T-2 and their metabolites in pig urine has been developed and validated at the University of Ghent in Belgium. The method was used to analyse 28 pig urine samples in a pilot study, and detected residues of DON, FUM, OTA and AFB1. The authors noted that it would be possible to include additional analytes in the method in the future (Song *et al.*, 2013). The method is used in research and does not appear to be available as a commercial service.

There has been a large number of recent publications about multi-mycotoxins analysis in human urine to detect biomarkers of exposure to mycotoxins, as the results of the measurements give a more accurate indication of actual exposure to the compounds of interest. And while it could be a good non-invasive test to determine exposure or identify outbreaks, there is much less published information available about the same type of tests for animals.

However this approach should also be viewed with caution as was pointed out by Danicke and Brezina (2013), as although linear relationships have been derived for DON exposure and DON or de-epoxy-DON concentration in the blood of pigs, dairy cows and sheep, it has to be considered that individual values might markedly deviate from these relationships. This makes interpretation of measured concentrations of DON and its metabolites difficult. They also noted that the situation is further complicated by the lack of established relationships between DON residues in physiological matrices and the adverse effects of DON on the health and performance of farm animals. The same is true for most other mycotoxins.

This was highlighted in a review of the toxicology and state of the art of biomarker development for the most common mycotoxins by Riley *et al.,* (2011). They identified the need to combine information about known exposure, clinical indicators and biomarkers for identifying the cause of disease in the most economical and definitive manner. They noted there is no single diagnostic approach that can identify/pinpoint when a disease outbreak is due to exposure to a mycotoxin. Better definition of the underlying biochemical changes and thresholds that ultimately lead to adverse effects are needed. The conclusion was there is a need for research to validate both disease-specific mechanism-based and exposure

biomarkers for several of the most important mycotoxins including FUM, OTA, DON and other trichothecenes and ZEN (Riley *et al.,* 2011).

In a short article about ZEN toxicoses in pigs the author highlighted the fact it is difficult to make a link between suspected mycotoxicoses of livestock and the presence of mycotoxins, using standard analytical techniques and stated there is an increasing demand for developing innovative, modern, easy and fast tools for correct diagnosis of ZEN toxicoses in pigs. The suggested possible approach to achieve this was “omics” technologies. In this case the author highlighted transcriptomics, that use living cells to study a range of effects (Beev 2014). However, there are a range of other techniques that can be used, for example the use of high resolution mass spectrometry (HRMS) and other methods such as nuclear magnetic resonance spectroscopy (NMR) to carry out metabolomic studies. Using these methods, data sets between control and test animals can be compared and differences found and the specific markers identified.

An example of the use of this technique has been published for OTA in rats where GC-MS and 1H-NMR metabolomics techniques were used to analyse urine and plasma. OTA was found to cause changes in amino acids, pentose and nucleic acid metabolites (Xia, *et al.,* 2014). This demonstrates the possibility to detect changes in metabolism caused by lower concentrations of toxin that may not have caused a visible clinical effect.

10. Grey literature and surveys to obtain information on UK situation on mycotoxins in livestock

10.1. Survey objectives

The objective of the surveys was to describe and evaluate information related to mycotoxin exposure in livestock (cattle, pigs and sheep) on the:

1) Potential effects of mycotoxin exposure
2) Inclusion of mycotoxin binders
3) Availability and use of testing services

10.2. Method

10.2.1. Mycotoxin survey collection

Surveys evaluating mycotoxin exposure in livestock were designed to target veterinary surgeons (VS), farmers, laboratories and technical companies (feed companies); allowing the objectives to be addressed with specific questions to each target group with varying

experiences e.g. farmers using binders vs. feed companies selling binders. Surveys were designed specifically for each of the individual populations, with the aim of maximising compliance and the quantity and quality of the results. Both paper and electronic copies of the surveys were distributed to improve compliance and increase responses by providing the most appropriate response form for different groups. The main context of the surveys was similar with modifications for the target populations. Questions were also included to collect data on perceptions of the significance of mycotoxicosis and the availability of appropriate scientific material to aid decision making. Surveys were restricted geographically to the UK to comply with the project budget. Survey results were collected in Microsoft Excel. Where there were no responses to the questions that contributed to the data set then the response was excluded.

10.2.2. Veterinary survey (See Appendix 1).

The aim of the veterinary survey was to primarily focus on the potential effects of mycotoxin exposure, but to also gather knowledge on binder usage and an update on the availability and use of testing services within the veterinary profession. The surveys were tested on Veterinary Surgeons (VS) at Bishopton Veterinary Group and employees of the University of Nottingham to check for formatting and the time frame necessary to complete the survey. Paper copies of the VS survey were distributed through the British Cattle Veterinary Association (BCVA), Sheep Veterinary Society (SVS) and the Pig Veterinary Society (PVS), to target VS working with cattle, sheep and pigs. The electronic link was advertised on their websites (PVS) and distributed through their mailing lists (BCVA & SVS). The XL Vets forum and emailing network was also used to distribute the survey to VS in 52 practices across the UK. Surveys were also distributed to VS employed by Bishopton Veterinary Group and Synergy Farm Health.

10.2.3. Farmer survey (See Appendix 2).

The aim of the farmer survey was to focus on the potential effects of mycotoxin exposure and binder usage. Questions were also included to gather information on the availability of testing services. The survey was tested by VS at Bishopton Veterinary Group to check formatting and ensure appropriate wording and level of science to promote good farmer response rates. The survey was distributed to all cattle, sheep and pig clients at Bishopton Veterinary Group, North Yorkshire and cattle and sheep clients at Synergy Farm Health, Dorset by post and on-farm visits. This targeted distribution of the surveys aimed to increase the number of responses. The survey was also distributed through AHDB Pork at meetings

and online newsletters. VS were also requested to circulate the farmer survey to their clients when the veterinary surveys were circulated through BCVA, SVS, PVS and XLVets.

10.2.4. Laboratory survey (See Appendix 3).

The primary aim of the laboratory survey was to highlight the availability and appropriateness of testing services available in the UK. Questions were also included to gain opinions from the technical sector on the potential effects of mycotoxin exposure and the availability of binder products. An online search was performed by the Food and Environment Research Agency (FERA) to collate contact details for labs offering mycotoxin testing services in the UK from commercial databases (including; BvD MintGlobal, Research & Market research host and Profound Market Research host) and the web (including; SEO TouchGraph, Google advanced search, World Mycotoxins journal and the Pig Site). Results were collated into a Microsoft Excel spread sheet and included; the lab name, contact details and tests offered. This was subsequently reviewed and supplemented by a search carried out by RAFT to also include organisations providing animal health testing services e.g. post-mortem and animal based tests. The surveys were tested by employees at FERA to check formatting and appropriateness. The electronic survey was emailed to the pre-formed list of laboratories. To improve response rates the laboratories were also contacted by telephone and responses collected over the phone.

10.2.5. Technical survey (See Appendix 4).

The primary aim of the technical survey was to determine the availability of binder products and to further demonstrate the availability of testing services. Questions were also included to gain opinions on the potential effects of mycotoxin exposure. The survey was tested at FERA to ensure appropriateness and correct formatting. An online search using Google was carried out to determine companies that were involved in the production or sale of mycotoxin binder products. These companies were also potentially offering testing services, and therefore the survey was modified and split into two sections; one similar to the laboratory survey regarding testing methods offered and the second more closely related to the veterinary and farmer surveys regarding mycotoxin exposure and availability of binder products. This was distributed in electronic form by email initially, followed by telephone contact to help increase the response rate.

10.3. Survey results

10.3.1. Veterinary survey results

From a total of 98 responses, 69 were analysed. Those not included in the analysis were due to not agreeing to data permission (n=1) or failing to answer an adequate number of questions to meet the inclusion criteria (n=28). Of the total responses analysed, 76% of respondents claimed spending over 50% of their clinical work time with cattle. 7% worked primarily with sheep, and 16% with pigs. The majority of VS had been working with the primary species for over ten years (52%), with 24% and 21% of respondents having 1–5 and 5–10 years' experience, respectively. Just over half of the analysed respondents (52%) claimed they had suspected a case of mycotoxin exposure in an individual animal. The greatest range was seen in pigs (0–30 cases), however the greatest mean value was seen in dairy cattle (3.96) (Table 14). Diarrhoea was the most commonly reported clinical presentation of mycotoxin exposure in dairy cattle (19%), followed by musculoskeletal signs (16%). In pigs, the most commonly observed clinical presentation was a fall in fertility in breeding sows (30%), followed by the presentation of splay legs in piglets (20%) suggesting mycotoxin exposure is observed throughout the production cycle in pigs. The clinical presentation described in beef animals was similar to dairy cattle, with diarrhoea and digestive upset being the most common (18%), followed by a reduced appetite (14%). The clinical presentations described in sheep populations included; abortion, necrosis of the ear tips and lower limbs, demarcation lines and death. The limited number (n=1) of responses to the suspicion of exposure in sheep however makes these results impossible to analyse.

Table 14. Results of suspected cases of mycotoxin exposure in individual animals

Species	Number of responses	Range	Mean
Dairy cattle	28	0-20	3.96
Beef cattle	19	0-6	1
Pigs	21	0-30	2
Sheep	4	0-2	0.2

68% (n=47) of respondents reported a suspected a case of mycotoxicosis on a herd-level basis**.** The most commonly associated clinical presentation on a herd-based level in dairy herds was a fall in milk production (24%), followed by diarrhoea (15%), reduced fertility and overall poor performance (12%). In beef herds, gut disturbances and diarrhoea were the most commonly recorded clinical presentation (30%), followed by poor production and growth rates (20%). A much broader variety of clinical signs were recorded in pigs, the most common being vomiting (15%) and inappetance in growers (15%) and reduced conception

rates/poor fertility in breeding sows (15%). Subcutaneous oedema, diarrhoea and ill thrift were the herd level presentations in sheep. Again however, the low number of responses (n= 2) for sheep makes analysing these results not possible.

31.9% of respondents reported having ever sent samples away for mycotoxin identification. The species split of these responses was 72% cattle, 24% sheep, 3% pigs. It was unclear from the responses whether these were herd-based or individual samples. Of those that did send samples away, the majority (33%) were sent to Scotland's Rural College (SRUC). The Animal and Plant Health Agency (APHA) was the second most commonly used laboratory (17%). Feed was the most common sample sent for analysis (25%), followed by blood (16%). Aflatoxins B_1, B_2, G_1, G_2 and M_1 were the toxins most commonly tested for in cattle (22%), followed by testing for a non-specified combination of toxins (18%). In pigs the most common toxin identification method was to test for a panel of toxins (23%), followed by testing for Zearalenone (20%) or Deoxynivalenol (20%) individually. Aflatoxins (12%) were slightly more commonly tested for in pigs than the other toxins (11%). Of the samples sent away for diagnosis in sheep, no positive results were obtained. Of the results from samples sent away related to dairy cattle, 17% of samples received a positive result, in pigs this was 16%.

72.3% of respondents reported having clients that were using mycotoxin binders, 85% of which were in dairy herds. Drop in yield and poor fertility were the most commonly recorded reasons for advising the inclusion of binder products. The presence of swollen vulvas in piglets (17%) was the most commonly reported reason for including binder products in pig rations, followed by infertility in sows (15%). No respondents recorded the inclusion of binder products in sheep. Mycosorb A+ (Alltech) (37%) was the most commonly used binder product in dairy cattle, followed by Ultrabond (Optivite) (28%) and Ultrasorb (Micron Bio-systems) (18%). Mycosorb A+ (Alltech) was the only binder product reported as used in beef cattle. Of the binders used in pig production, 35% of respondents claimed they were unsure of the product name. After this, Mtox+ (Vetsonic) (18%) was the most commonly used product followed by Mycosorb (Alltech) (17%).

Winter was the most commonly reported time period (29%) when binder products were included in dairy cow rations, followed by inclusion all year round (25%). All the respondents claiming to use binder products in beef animals reported they were using them either during housing and winter or were including them on a risk-based manner. The most common time period of the inclusion of binder products in pig production was all year round (37%),

followed by their inclusion being dependant on the clinical presentation or the harvest quality (18%).

Of the dairy respondents that had used binder products, 31% reported seeing a positive improvement in 1–10% of cases when binders were included. 45% of these said they had seen an improvement within 1–2 weeks. An improvement in yield (30%) was the most commonly reported evidence used to support the observation of a positive response to binder inclusion, followed by the resolution of remaining clinical signs (27%). Of those that did not see any response, 60% reported this was because they did not feel that mycotoxins were the underlying problem. Of the respondents who recorded using binder products in pigs, 40% reported they saw an improvement in 20–50% of cases, 40% in 50–75% of cases and 20% in 10–20% of cases. 60% of these positive responses were reported to have been seen within 2–4 weeks of binder inclusion. 62% of those that reported they saw an improvement following the inclusion of binders said that they had monitored a response through the resolution of clinical signs. Of those that reported they did not see a response, 29% reported that this was because the binders were included pre-emptively and so there were no clinical signs to monitor.

Discarding and avoiding feeding mouldy silage and feeds (37%) was the most commonly advised additional management practice, followed by improved forage and feed management (17%).

36% of respondents claimed they neither agreed nor disagreed that mycotoxins were having a significant impact on livestock production. After this, 34% said that they agreed with this statement. 68% of respondents reported that they did not feel that there was adequate scientific evidence regarding mycotoxins to support their decision making. 7% of respondents thought they had a good level of knowledge of mycotoxins. 54% of responses stated that they thought their clients received the majority of their information from feed companies. After this, 28% reported the main source of information for their clients came from the farming press.

10.3.2. Farmer survey results

From a total of 68 responses, 43 responses were analysed. Those not included in the analysis were due to not agreeing to data permission (n=4) or due to the provision of insufficient answers to meet the inclusion criteria (n=21). Of the adequately completed surveys (n=43), 76% of respondents were herd owners. 29% of respondents were farming

beef animals as the primary species, 27% primarily farmed pigs, 23% dairy cattle and 17% sheep. Within these herds, the breakdown of numbers of animals was that 82% of the animals farmed were pigs, 7% dairy cattle, 3% beef and 4% sheep. 4% also stated they farmed 'other' species. These were all recorded as poultry species. 85% of respondents had worked with the primary species for over 10 years.

28% (n=12) of respondents reported having suspected a case of mycotoxin exposure in an individual animal. The greatest range in number of suspected cases was reported in pigs (Table 15). Swollen vulvas in piglets (25%) and irregular heats in sows (25%) were the most commonly recorded individual clinical signs in pigs. Diarrhoea (29%) and swelling of the hind limb (29%) were the most commonly observed clinical signs in dairy cattle. Blindness was the only recorded clinical sign in beef animals. Coughing was the only reported clinical sign in sheep.

Table 15. Results of suspected cases by farmers of mycotoxin exposure in individual animals.

Species	Number of responses	Range	Mean
Dairy cattle	3	0–25	13
Beef cattle	2	0–5	2.5
Pigs	6	0–250	162
Sheep	2	0–1	0.5

43% of respondents (n=19) reported they had suspected mycotoxicosis on a herd level. Again, this was more commonly reported in pigs than the other species. No herd-level outbreaks were reported in beef or sheep. Of the 15 responses, to the question regarding the clinical presentation in dairy cattle, diarrhoea (27%) was the most commonly recorded presentation, followed by drop in milk yield (20%). Reduced reproductive performance and variable fertility (26%) were the most commonly reported clinical presentations in pigs, followed by splay legs in piglets (18%).

Only 16% of respondents (n=7) claimed they had ever sent samples away for mycotoxin identification. Of the samples sent away, 88% were from pig producers. Of the seven responses to the question, 57% of samples were sent away by the feed companies, 29% were sent away by the vet. The most common sample substrates sent away for analysis were forage samples (45%), followed by compound feedstuffs (33%). SRUC and Alltech were the only two reported laboratories where samples had been sent. Those that reported sending samples to Alltech had been through a University establishment or through the feed company themselves.

In dairy cattle, it was reported that samples had been sent away for testing for AFL, Ochratoxins, ZEN and FUM. In pigs, the only reported toxins tested for were ZEN and DON. One respondent claimed they had had a positive toxin result in dairy cattle, but was unsure of which toxin it was. Positive results were reported in pigs for both ZEN and DON.

51% of respondents claimed they had never used binder products in the animals they farmed. Of those that had, 26% were used in pigs, 20% in dairy cattle and 3% in beef animals. No one reported using binder products in sheep. Of the 12 responses to the question, diarrhoea (34%) and drop in yield (33%) were the most commonly recorded reasons why binders were included in dairy cow rations. Reduced yield, poor fertility and as an insurance policy were the only reasons why binders had been used in beef animals. Irregular heats (23%) and infertility (23%) were the most common reasons why binders were included in pig production. There were a variety of binder products being used in dairy cattle and pigs (Table 16). 33% of respondents said they chose their binder product on the recommendation of a nutritionist, following this 27% chose it based on feed-representative advice (n=15).

Table 16. Binder products used in dairy cattle and pigs

Dairy Cattle (n=10)	**Pigs (n=15)**
Mycosorb (Alltech)	Mycosorb (Alltech)
Mycosorb A+ (Alltech)	Ultrasorb (Micron)
Ultrasorb (Micron)	Mycofix (Biomin)
Other	Mtox+ (Vetsonic)
	Other

From the ten respondents that completed the question, in dairy cattle the most common time period of binder inclusion was non-specific (30%), 20% said they included them either based on clinical signs or just during winter. The one beef respondent claimed they include them either during autumn or following from a wet harvest. There were no responses to this question from sheep farmers. 64% (n=11) of binders used in pig production were being used all year round. The majority of these respondents also stated that this used was primarily in the breeding herd rather than fattening.

The majority of respondents reported not seeing any improvement following binder inclusion in cattle. The most commonly reported reason for no improvement being seen was that mycotoxins were not thought to be the underlying causal factor. Of those that were

monitoring for a response to binder inclusion in dairy cattle, the most commonly recorded method was the resolution of clinical signs (87%). Resolution of clinical signs was also the most common (56%) monitoring technique for a response to binders in pigs (n=9).

Of the 35 respondents, 48% said they neither agreed nor disagreed that mycotoxins were having a significant effect on livestock production, 29% disagreed with the above statement. 63% responded that there was not adequate availability of scientific evidence to support their decision making with regards to mycotoxins (n=22). Of the 35 respondents, 54% thought their knowledge was average, 29% thought it was bad and 17% thought it was good. From the 55 responses, farmers reported to receive most of their information on mycotoxins through the vets (33%), feed companies (25%) and farming press (24%). This was closely correlated to where they would like to receive information from; of the 64 responses to this question, with 42% responding that they would like the main source of information to be from vets, 21% wanting it from the farming press and 19% from feed companies.

10.3.3. Laboratory survey results

Of the 15 responses to the survey, 9 were completed to a level to fit the inclusion criteria for analysis. Of these, 37% of respondents were from commercial laboratories, followed by food safety laboratories (27%). 67% of respondents (n=6) reported offering some form of mycotoxin testing services. One respondent said they offered toxicology based testing, but although this was carried out in house it was done so using a test kit that was externally sourced. This tested for AFL, Ochratoxins, DON, T-2/HT-2 and FUM as either individual tests or as a combination, all of which used an ELISA test that could give either qualitative or quantitative results.

When asked to grade the suitability of the tests, respondents rated the format and test performance at 3/5, and the materials used for the test at 5/5. Two of the respondents said they offered animal based testing services. Only one of these respondents completed the survey with details of the tests offered. The tests they offered were all immuno-affinity columns, which tested for OTA, AFL or ZEN on the urine, blood or tissue of cattle, sheep or pigs. When asked to grade the suitability of these tests, they rated the format as 5/5 and performance and materials used in the test at 4/5. The respondent also commented that at present these tests only test for the parent mycotoxins and so their suitability, with regards to testing for mycotoxin exposure, is limited as it is not always the parent toxins that are involved in the resultant clinical presentation of mycotoxin exposure. At present however these are the only tests that are available.

None of the other respondents offered in house testing facilities. Two respondents reported that they sent samples to external laboratories within both the UK and Europe when necessary. European testing facilities were reportedly most commonly used when more complex testing facilities were required. These respondents also reported that they send either post-mortem tissue samples or stomach contents, but they also reported that they are most commonly requested to send away feed or forage samples for analysis.

Only one respondent reported also offering the sale of a binder product (Mycofix - Biomin).

10.3.4. Technical survey results

Of the 6 responses, only 4 were completed to a level to fit the inclusion criteria. All of the respondents were from animal feed companies/mills and none of them offered mycotoxin testing facilities. 50% of respondents (n=2) said they had experienced cases of mycotoxin exposure on an individual level, both of which were in dairy cattle. Although one respondent commented on the number of cases they see per year, with only one response this is of limited use with regards to determining the impact of mycotoxins within the dairy industry. The clinical signs associated with these cases included; drop in yield, loose faeces, swollen hocks, pink udders and increased mastitis incidence.

50% (n=2) respondents said they had experienced mycotoxin exposure on a herd based level. Unfortunately, only one of these went on to complete the subsequent questions and no clinical signs associated with the outbreak were recorded.

25% (n=1) respondents recorded having ever sent samples away for mycotoxin identification. A forage sample was sent, but the laboratory where these were sent was unknown. It was only tested for ZEN and DON, neither of which came back positive. One consultant working with pigs reported using binder products, but stated that they were only included once all other potential causes had been ruled out. Mtox+ (Engormix) was the binder product used, which was the product they supplied. The binder products were included all year round as they were being used more as a preventative measure than as a treatment.

Only one respondent completed the section regarding their perceptions on the impact mycotoxins are having on livestock production and the degree of their knowledge. They did

however state that they thought that feed companies and binder suppliers were where farmers/clients got the majority of their information from.

10.3.5. Grey literature objectives

The objective of the grey literature review was to collate non peer reviewed information and data on the potential effects of mycotoxin exposure in livestock (cattle/pigs/sheep) and the use of mycotoxin binders.

10.3.6. Summary of results

The criteria for the grey literature review are summarised in Section 3.2.

Grey literature results from the online databases were limited (6.3% of all grey literature results), relative to open access search engines, such as Google Scholar (Table 17). Google Scholar's search criteria limit the results available to view to the first 1000 results. This introduces a degree of bias as the results are automatically sorted through the Google algorithm.

Table 17. Total number and number of relevant grey literature results from each of the sources searched.

Source	Total number of results	Number of relevant grey literature results
OpenGrey	0	0
PubMed	16	0
Medline	10	0
Scopus	36	0
Web of Science	91	0
CAB direct	76	4
Agricola	11	0
Biosis citation index	63	0
ISRCTN directory	0	0
Global health database	120	5
Europe PubMed Central	301	0
GoogleScholar	48390	58
Email contacts	9	1

As the search criteria were split by species, it became apparent there were considerably fewer results regarding sheep (17%). When restricted to our inclusion criteria, the proportion of results relating to pigs increased from 40% to 60% (Figure 1 and Table 18).

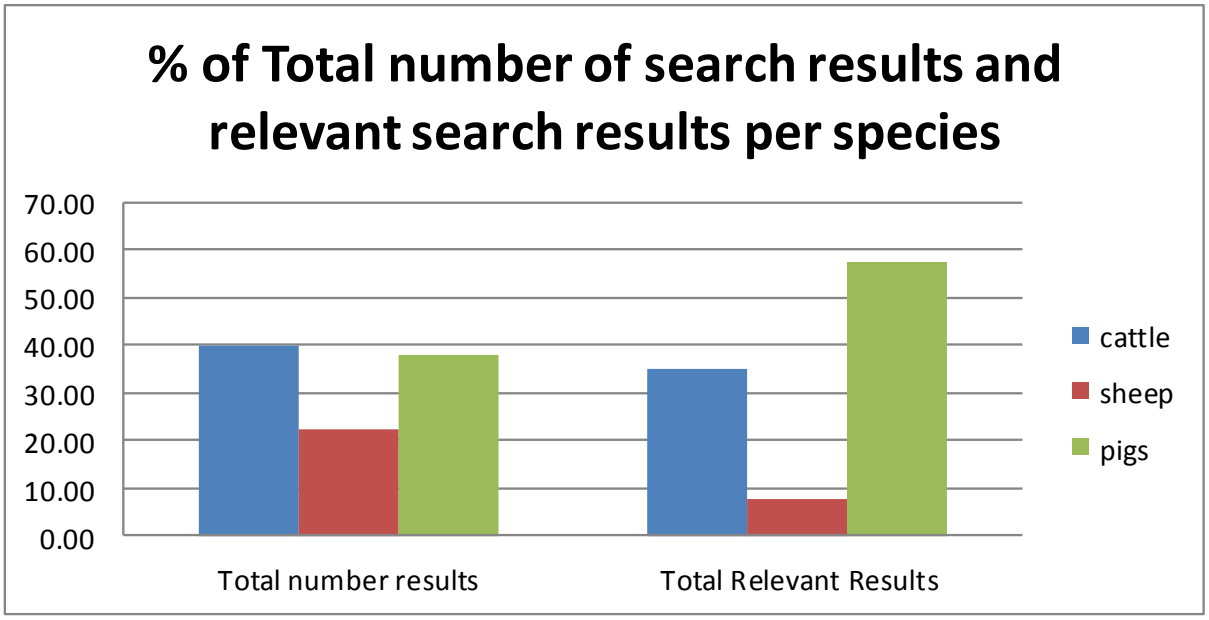

Figure 1. Percentage of the total search results and relevant search results by each species searched for.

Table 18. Total number and number of relevant grey literature results from each of the species searched.

Species	Total number of results	Total relevant grey results
Cattle	16869	14
Sheep	9486	3
Pigs	16098	23

Figure 2 demonstrates that the majority of results, both peer reviewed and non-peer reviewed, were returned through Google Scholar. After this, Europe PubMed Central returned the largest numbers of hits (n=301), but all results were published and so were excluded as they did not fit the grey literature criteria. Global Health database returned the largest number of grey literature results (n=17), after Google Scholar. Only 5 of these fit the inclusion criteria (Table 19).

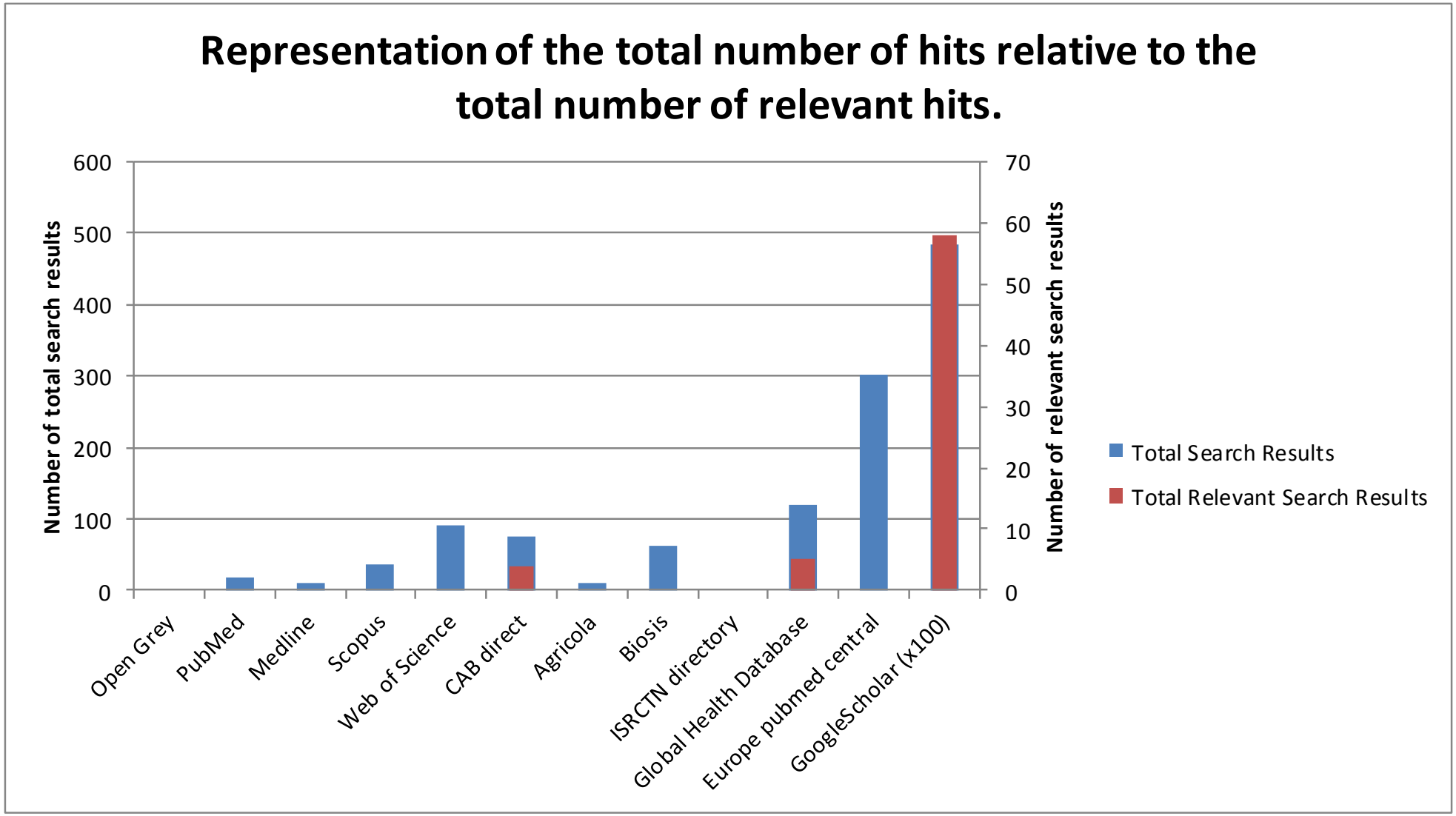

Figure 2. Representation of the total number of hits relative to the total number of relevant hits.

Table 19. Total number and number of relevant grey literature results from each of the search terms searched.

	Total number results	Number relevant grey results
mycotoxin awareness	3861	14
veterinary mycotoxin awareness	2792	6
mycotoxin understanding cattle	7573	6
mycotoxin understanding sheep	4284	1
mycotoxin understanding pigs	7658	8
mycotoxins experience cattle	5834	4
mycotoxin experience sheep	3578	1
mycotoxin experience pigs	5331	11
mycotoxin perception cattle	3464	2
mycotoxin perception sheep	1596	1
mycotoxin perception pigs	3036	4
mycotoxin appreciation cattle	995	0
mycotoxin appreciation sheep	701	0
mycotoxin appreciation pigs	889	0

The proportion of total literature results that fit our inclusion criteria varied between 0.0% and 0.36% (Figure 3). The majority of grey literature results highlighted from the search did not meet our inclusion criteria and were either from outside the UK, or did not fall within the time period 2004–2015.

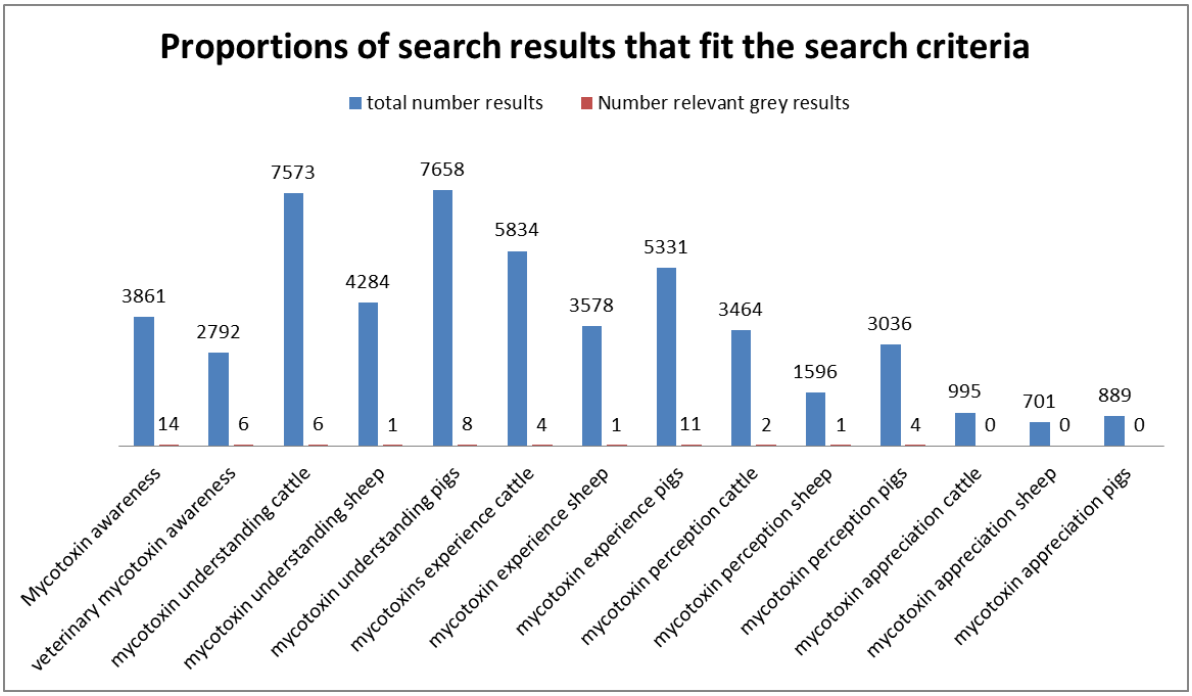

Figure 3. Proportion of grey literature results that fit the search criteria.

10.3.7. Review of the Grey Literature (See appendix 6. for references).

The majority of results were articles that focussed on the effects of fungal and mycotoxin contamination in crops or on human health and referred only briefly to the effects of mycotoxin contamination in livestock. (It did however highlight the need for multifunctional testing mechanisms that allow for the testing of multiple toxins at any one time).

The continued globalisation of agriculture has meant that the awareness surrounding mycotoxicosis in livestock has increased and with it availability and methods for monitoring. This has meant that there has been an increase in the capabilities of prevention and the control of the detrimental effects of clinical mycotoxicosis in livestock (1,4,7). Analytical methods have also therefore had to develop to maintain the accuracy of toxin identification, as rarely is only a single toxin implicated in causing clinical presentation. Contamination of feedstuffs and clinical mycotoxicosis usually involves a complex of mycotoxins that develop due to a multitude of reasons including; housing environment, feed type and substrate condition. The involvement of multiple toxins means there is an ever increasing requirement to maintain analytical sensitivity to limit the potential effect of mycotoxin contamination (1, 2, 3, 4, 8).

Despite the combination of toxins that are usually implicated in contaminated samples, each toxin is thought to be responsible for causing fairly characteristic conditions and it is expected that the variety of associated conditions will continue to increase as testing methods improve.

The Trichothecene family of toxins encompasses two families of toxins that have potentially serious implications for livestock production. They have recently been found to be the most prevalent toxins in finished feeds, grass silage and wheat. This is thought to increase the risk of mycotoxin exposure to pigs at all stages of production, but also to calves and heifers (20). Deoxynivalenol (DON), a type B trichothecene, is reported to be the most commonly identified toxin in swine. It is thought to interfere with DNA replication and hence alter protein synthesis in the affected cells. Clinically, it generally results in vomiting, diarrhoea, reduced appetite and growth rates (7, 10, 17, 18). The specificity of these clinical signs are sometimes regarded enough to diagnose DON toxicosis without the requirement for sampling and further diagnostics (19). Generally it was recorded that monogastric animals were considerably more sensitive to the effects of mycotoxicosis than ruminants, causing DON to cause higher production losses at lower concentrations, hence making them more of a threat in monogastric animals (12, 15). Because of the increased susceptibility of swine relative to other ruminant species, the acceptable EU limit of mycotoxin contamination in pig feed is lower than is acceptable in cattle or sheep feedstuffs (16). It has been suggested that the effect of rumen metabolism of mycotoxins could question their significance with regards to dairy cattle production. There are several other causative factors

resulting in digestive imbalance that cause a reduction in production parameters in dairy cattle, other than clinical mycotoxicosis (19).

T-2 and HT-2 are from the other family of trichothecenes and act via a similar mechanism, resulting in a similar clinical presentation to DON. They are however thought to be more potent than DON and so, despite being less prevalent, exert a similar if not worse clinical effect at lower concentrations (17).

ZEN is another commonly implicated toxin and although it has lower levels of cellular toxicity, it can have massively detrimental effects on swine production. It has been reported to have teratogenic effects, but the most detrimental effect to production is its oestrogenic potency which causes delayed onset of puberty in young piglets but also severe reproductive problems in adult sows. Because of these reproductive effects, young sows are reported to be the most susceptible (16,17). It is also commonly implicated in the contamination of grass silage, posing an increased risk to calves and heifers (20).

Aflatoxins are reported to affect a multitude of species. The level of contamination necessary to cause the variety of clinical presentations observed in livestock species differs (Table 20). This is also demonstrated by the LD50 in swine (0.55mg/kg) relative to sheep (1.4–2.0mg/kg) (15). It should be noted the figure quoted from this source is ten times greater than the maximum level permitted in feed for dairy cows (EC 2014). Practically, the reporting of results with a set unit often leads to misconception of the severity of the problem (19). Aflatoxin infection has been reported to cause depression with a loss of appetite and diarrhoea when due to an acute infection, but has greater interference with nucleic acid binders leading to reduced protein synthesis, liver damage and subsequent reduction in production efficiency when chronically exposed. Interestingly, it was reported that the potential effects of aflatoxin exposure are very similar in animals and humans (11). Aflatoxin M_1 is of increased importance, with regards to its potential public health effects, as it has been reported to be shed in the milk of dairy cows when exposed to aflatoxin B_1 for 3–4 consecutive days (7). A less commonly reported potential effect of aflatoxin exposure is jejunal haemorrhagic syndrome (JHS), seen in adult dairy cattle. Although the true aetiology of the condition is yet to be confirmed, *Aspergillus fumigatus* resulting in the production of genotoxic and cytotoxic products such as gliotoxins is thought to be involved (9).

Table 20. Variation in clinical parameters in different livestock at different levels of contamination of aflatoxin (15).

Parts per billion (µg/kg)	
50	Maximum level without detectable residues shed in milk.
100	Illness seen in calves, chicks and piglets.
200	Adult pigs still don't show any signs of illness.
300	Liver damage in young pigs causing fall in production levels and efficiency.
600	Reduced milk production in dairy cows. Liver damage also seen causing poorer growth rates in beef animals.
1000	Signs of illness seen in adult pigs.

Fumonisins (FUM) are the primary toxins recently reported to be the most common toxin identified in a total mixed ration (TMR) (15, 20). Although they are primarily found in maize forage, they can also be found in grain. They are primarily carcinogenic and hepatotoxic, resulting in reduced production rates in livestock. Similarly, they also result in a very similar clinical presentation in humans (15).

In sheep one of the biggest effects of mycotoxin exposure is the development of facial eczema. This has been a big problem for years in the New Zealand national sheep flock. It is thought that the toxins originate from rye grass causing liver damage and resulting in the inability to excrete the metabolites of chlorophyll. This results in the development of photosensitisation causing the skin to absorb additional energy from the sun causing skin necrosis (7).

To ensure good animal welfare forage crops used as bedding material, such as straw, should be of the best possible quality, i.e. not mouldy or wet. This will also minimise the potential degree of fungal and mycotoxin contamination (19). DON and ZEN appear to be the greatest risk factors for the contamination of bedding material (16). There is a close correlation between wet weather and the potential increased risk for mycotoxin contamination (16,18). Poor storage and damp conditions are recognised to pose an increased risk to mycotoxin contamination (19).

Since the use of antibiotics for growth promotion has been banned, multiple small overviews have been carried out to assess the advantages and disadvantages of other commonly used additives in feed, including mycotoxin binders. Although several of these have shown to have a beneficial effect, the effectiveness was largely dependent on the interaction between the additive and the intrinsic substances within the diet and on overall farm health and hygiene. The interaction between all these factors makes it difficult to determine a true cause and effect relationship following the addition of binder products (19).

Dried distillers grain with solubles is a by-product from the ethanol production industry that several reports suggested as an alternative pig feed, in fact it now has widespread use as a feed for animals including pigs, cattle and poultry. Despite its qualities, it is essential that the mycotoxin levels are monitored. This is due to the severe negative impact mycotoxicosis can have on sow (and other animal) performance, and the fact that during the ethanol production process, a considerable amount of starch is removed from the grain. The reduction in starch results in an, up to three fold, increase in concentration of contaminants, including mycotoxins (13,14).

There are potentially huge economic costs associated with mycotoxin contamination both directly, such as reduced growth rates, costs of treatment and the potential that the animal may not survive to the indirect costs, including; disposal of contaminated feed, investment into further research and the application of control practices to minimise further or future contamination.

10.4. Survey and grey literature discussion

10.4.1. Survey responses

The responses from the vet and farmer surveys highlighted several similarities and some key trends and differences between vet and farmer perceptions. The majority of VS were working with dairy cattle, whereas the largest number of animals and the greatest proportion of herds that were managed by the respondents of the farmer survey were pigs. This could represent the distribution of farm clinical work load within the veterinary profession.

There was close correlation between the perceived effects of mycotoxin exposure from VS and farmers. A greater proportion of VS (52%) suspected cases of mycotoxin exposure on an individual level than farmers (28%). In dairy cattle diarrhoea and musculoskeletal signs were the most commonly observed clinical presentations from both farmers and VS. A fall in fertility and irregular heats were the main clinical presentations recorded by VS and farmers alike in breeding pigs, whilst in piglets, swollen vulvas were the most commonly reported presentation. The observation of clinical presentations in both mature and immature animals, by both VS and farmers, helps to indicate that mycotoxins can have effects on production in all stages of the pig production cycle. There were a limited number of clinical presentations recorded in sheep and beef animals by VS and farmers. This makes drawing accurate conclusions about the potential effects of mycotoxin exposure in these species difficult.

A marginally higher proportion of VS (68%) reported suspected mycotoxin exposure on a herd-level basis than farmers (43%). In farmers this was more commonly recorded by pig producers. VS reported that the most common clinical presentation in dairy cattle was a fall in milk production, whereas farmers perceived this to come second to diarrhoea. A fall in reproductive performance, consisting of a drop in conception rate and variable fertility was the most commonly recorded

clinical presentation in pigs recorded by VS and farmers alike. VS also reported seeing vomiting and inappetance in growing pigs and the splay legs in piglets when suspicious of mycotoxin exposure. Again, this demonstrates how mycotoxins are perceived to be having an effect in multiple stages of the pig production cycle. The lack of experience reported with mycotoxin exposure in sheep meant that analysis of the very small number of results was not appropriate to allow any conclusions to be drawn.

A greater proportion of veterinary respondents (32%) reported having sent samples away for mycotoxin identification compared to farmers (18%). The majority of samples sent away for analysis were feedstuffs, most commonly through feed companies. Both farmers and VS reported that the most commonly used laboratory to send samples to was the SRUC. Farmers also reported sending samples through university establishments. Farmers most commonly reported sending samples away in conjunction with suspected outbreaks in pig production systems. This would correlate with the considerably larger number of suspected herd outbreaks recorded in pigs' systems.

VS primarily reported that Aflatoxins B_1, B_2, G_1, G_2 and M_1 were the toxins most commonly tested for in dairy cattle. Although aflatoxins were also commonly tested for by dairy farmers sending samples away, they also commonly tested for OTA, ZEN and FUM. In pigs, the most commonly tested for toxins by VS were DON and ZEN. Although there were considerably fewer responses to this question from farmers, DON and ZEN were the only toxins that they reported testing for as well.

Conflicting evidence was found between farmers and VS with regarding binder usage. 72% of VS said that they did have clients using binder products, 85% of these were being used by dairy clients. 51% of farmers reported that they did not have experience with using binders. Farmers more commonly reported binders being used in pig systems, whereas VS were more aware of the use in dairy cattle. From dairy farmers' experience with binder inclusion in dairy cattle rations, diarrhoea and drop in yield was the most commonly recorded rationale for inclusion. This differs from the perception of VS who more commonly advised inclusion due to a fall in fertility parameters.

Changes to fertility parameters and performance were reported as the most common reasoning behind the addition of binder products to pig rations by both farmers and VS. Interestingly, none of the farmers commented on the clinical presentation in piglets or growers as a rationale for binder inclusion, potentially highlighting that their focus was more on the adult breeding stock. This hypothesis was backed up as it was repeatedly stated by farmers that they only included the binder products in the ration of the breeding herd. When asked about the clinical presentation of individual

and herd based mycotoxin exposure, farmers reported recognition of the clinical signs seen in weaned and growing animals, but failed to use this as criteria for binder inclusion as a treatment or control measure. This may represent a better understanding of the potential effects and necessity to treat and control mycotoxin exposure in the breeding herd, relative to weaned and growing animals.

A variety of mycotoxin binder products were reported to be used in dairy cattle and pigs, the majority of which were supplied by Alltech. There is, however insufficient evidence from the dataset collected to be able to draw any solid conclusions with regards to product favourability.

VS reported that winter was the most common time that their clients were including binder products. The responses from farmers indicated that binders were included on a more ad-hoc, non-time specific basis, based around the clinical presentation of the animals. This potentially highlights the more preventative mentality for inclusion by VS relative to the attitude from farmers.

The responses from VS and farmers working with pigs highlighted that the majority include binder products on an ongoing basis all year round. This prophylactic approach to use may suggest that the presentation and production effects of mycotoxin exposure in pigs are more clearly understood compared with other species e.g. in dairy cattle. It was again highlighted as well that this inclusion was primarily in the breeding herd ration and not in growers or fatteners. For those not including binder products continually, their requirement was determined based on the potential risk of mycotoxin exposure from that year's harvest. This again emphasises the increased level of appreciation and understanding of mycotoxin contamination in pig production relative to cattle.

When asked about their opinions on the significance of mycotoxin exposure on livestock production, the most common response from both farmers (48%) and VS (36%) was that they neither agreed nor disagreed. A larger proportion of VS (34%) appeared to agree with this comment, while a larger proportion of farmers appeared to disagree (29%). This emphasises the differing attitudes of VS and farmers regarding their perception of the significance of mycotoxin exposure in livestock. It could also potentially be that through the examination of sick animals, VS encounter a higher proportion of animals that they suspect have been exposed to mycotoxins than farmers.

The majority of both VS (68%) and farmer (63%) responses stated that they did not feel there was adequate scientific literature available to support their decision making. This potentially highlights a gap that needs to be addressed to ensure that adequate levels of science are used to reach the correct decisions regarding mycotoxin testing and binder usage.

The proportion of VS who described their knowledge of mycotoxins as “good” (7%) was considerably lower than the result from farmers (17%). It may be that VS and farmers assess the relative importance and presentation of mycotoxin exposure differently, with vets commenting on their knowledge of clinical mycotoxicosis and farmer’s knowledge focussed more on forage production. Interestingly, VS reported that the primary source of information for their clients is through feed companies and the farming press. Similarly, farmers also reported that the farming press was a commonly used source of information, and that their primary source of information is through their VS. When farmers were asked about where they would like to receive information about mycotoxins from, 42% said they would rather this came through their VS. This again highlights a variation in how VS perceive information to be distributed and how farmers would ideally like to get this information. It also potentially highlights where more focus in education from VS to farmers should be focussed.

The quantity of responses gathered from the laboratory and technical parties were far fewer. This is not completely unexpected as there are a limited number of companies in the UK offering testing facilities. The response to the technical survey was potentially poorer than expected despite the same methods of formulation and distribution as the laboratory survey. This could be due to the size of the companies which meant communication with the most appropriate person was difficult. Only a relatively small proportion of VS (31%) and farmers (16%) said they had ever sent samples away for mycotoxin analysis, therefore it is assumed that the market for mycotoxin analysis in the UK is relatively small. Likewise, of the technical companies that completed the survey none offered mycotoxin testing services. This correlates well to where farmers and vets reported sending samples to.

Targeting the surveys to laboratories identified as most commonly used from the VS and farmer surveys was necessary to prevent knowledge gaps. The comments gathered from these targeted survey responses helped to gather further opinions from industry leaders about the perceived effects mycotoxins are having on livestock production in the UK. Despite trying to target the technical surveys more specifically, the number of responses remained low. Of the responses gathered there was good correlation to the responses gathered from VS and farmers, with regards to clinical signs observed and binder usage.

10.4.2. Grey literature search

The availability of information between the different species was consistent with the results from the surveys. The considerably lower availability of information regarding sheep corresponds with less evidence of a clinical problem or use of preventative measures in sheep. The increased numbers of pig results, particularly highlighted when the search was restricted to UK was interesting in comparison to the total number of results, and the total number of grey literature

results. It appears that there is considerably more unpublished information regarding pigs in the UK than other species. This again, reflects what was seen in the survey results, in that there appeared to be increased awareness of clinical problems associated with mycotoxicosis and increased usage and awareness of preventative measures in the pig industry.

The methodology behind the search was staged; the search was gradually refined to become more species specific and encompass multiple different combinations of appropriate and relevant phrasing. However, the capacity of GoogleScholar to only display the first 1000 results is limiting, and potentially means that a degree of bias was introduced as the search was restricted through the GoogleScholar algorithm. The time period and scope of this study limited the comprehensiveness of the grey literature review, but helped to demonstrate that there is scope for future work to further develop the grey literature understanding in this topic area.

10.5. Conclusions from surveys and grey literature

The results of this study helped to demonstrate key trends with regards to the availability and use of testing services, with regards to the demonstration of mycotoxin contamination. Within the industry, from both a veterinary and farmer perspective, there is relative limited uptake of testing services. This in turn results in there being a limited number of key laboratories involved in providing testing facilities in the UK.

The use of binder products appears to be considerably different within the different livestock agricultural sectors. The cattle industry appears to be using binder products as part of treatment based protocols when suspicious of mycotoxin exposure. On the contrary, the use in the pig industry has much more of a prophylactic stance, often being used all year round, and primarily focussed on the adult breeding herd.

There appears to be a considerably greater awareness and understanding of the potential effects of mycotoxin exposure in the pig industry than in the cattle and sheep sectors. The specific clinical signs recognised in pigs are well documented, but this was strongly backed up by the parameters being used to determine suspicion to mycotoxin exposure by both VS and farmers. The potential effects noted in cattle, both dairy and beef, were considerably less specific and raise the question of whether the suspicion of mycotoxin exposure, and the rationale for binder inclusion, can be determined solely from the clinical presentation of these animals.

The work from this project has also helped to demonstrate gaps in knowledge with regards to mycotoxin exposure. The perceived low level of knowledge in both VS and farmer populations could highlight areas where further work and knowledge transfer emphasis should be focused. The

farmer responses have also helped to demonstrate where these knowledge streams would ideally be coming from. The use of this information could help to ensure effective knowledge transfer.

The small number of samples sent away, and the few laboratories involved in testing for mycotoxin exposure could demonstrate gaps in analytical methods used to determine exposure. The lack of specificity of the potential effects mycotoxin exposure can have in cattle could demonstrate an area that would benefit from future research. This could help to solidify the understanding and aid the treatment and control options available in these species. The opinion based responses to the survey also helped to highlight the preferred knowledge transfer streams for the communication of information relating to the potential effects and methods of prevention and control following mycotoxin exposure.

11. Knowledge gaps

11.1. Animal feed surveys

There is no comprehensive, independent, survey of mycotoxins in different feed materials. A recent estimate of feed mycotoxin concentrations in Norway based on the concentration within individual feed ingredients identified the estimate was low compared to actual feed data (VKM, 2013). This would suggest that cereals intended for feed have higher concentration of mycotoxins compared to the total cereal crop harvested. This is highly likely as consignments intended for human consumption are screened for high mycotoxin content and diverted to the feed sector. There is also the possibility of a small additional proportion of the mycotoxin content from protein sources (e.g. soymeal) that were not included in the calculations. An extensive survey of feed ingredients and final compound feeds over a number of years in the UK would clearly identify the range of mycotoxins present within feed and allow relationships between mycotoxin content of crops at harvest and the ultimate concentrations in compound feed to be determined. Such a study should also include bedding material, in particular straw, to identify the range of mycotoxins present within straw as it has been shown to contain significant levels of DON and ZEN in recent studies (Häggblom & Nordkvist, 2014, 2015).

11.2. Animal intake of straw and bedding material

There are very limited studies on the dietary intake of bedding (Mansbridge and Stewart 2012). It would be useful to identify the consumption of straw by pigs of different age groups, different diets and animals on *ad-lib* compared to limited rations so this could be included in the exposure assessments for pigs. In situations where pigs are fed *ad-lib* and may be less inclined to consume the bedding then it would be appropriate to determine the mycotoxin challenge via the respiratory tract through inhalation as it is known very high concentrations of mycotoxins occur in cereal dusts (Sanders *et al*., 2014).

11.3. Mycotoxins in pasture

There is historical evidence of the contamination of pasture with ZEN in the UK and also ZEN and ergot alkaloids in New Zealand and Australia, but there is no recent information about their occurrence, or their possible effects, in the UK in pasture fed animals. It would be informative to conduct sampling and analysis of UK pasture at different times of year to determine if this is an issue in the UK.

11.4. Toxicity studies – natural levels and co-occurrence

Most toxicity studies were conducted in the 1970s and 1980s when analytical techniques used to quantify the mycotoxin present within feed were unable to detect other co-contaminating

mycotoxins present within the test feed. The methods also tended to be poorly validated and non-accredited methods, as such the validity of the results obtained are questionable. This is likely to partly explain the discrepancy in results between naturally contaminated feed and spiked feed samples and the overall discrepancy between calculated NOEL (no observable effect levels) from different studies. This is most critical in pigs as these animals are the most susceptible species and most likely to be exposed to mycotoxins above the NOEL. There is a need to identify the NOEL for reduced feed intake and reduced weight gain for grower pigs using accurate, accredited multi-mycotoxin analysis as these are important economic parameters for the industry that are most likely to occur.

Impact of combinations of key mycotoxins around the NOEL and LOEL concentrations on animal health and welfare should be studied. This will allow the impact of the proposed “cocktail effect” of mycotoxin present in mixtures to be identified and taken into consideration when calculating the impact of multiple mycotoxins around threshold concentrations within feed consignments.

11.5. Diagnostic and field tests

There are no readily available tests for use in the field or the laboratory to diagnose mycotoxicoses incidents, although the increase of more readily available multi mycotoxin methods makes this more achievable. The use of metabolomics methods to study changes caused by the concentrations of mycotoxins encountered in the field to establish biomarkers of exposure would be useful. Rapid tests, based on simple formats such as lateral flow are available but tend to be for single toxins, and are validated for cereal and feed materials. Most still tend to be used in a laboratory setting. Currently no rapid tests are validated for silage, bedding or for diagnosis of symptoms in animals using e.g. urine. Multitoxin tests of this format could be developed, or current tests validated for diagnostic use in the field (for silage, bedding and urine) would also be of a great benefit.

11.6. Mycotoxin deactivation products

There are a range of products available, but only three technological feed additives have been approved by EFSA. Each has very specific activity. Care should be taken to understand the nature of any intoxification before using a deactivation product to ensure it is appropriate for the toxin involved in the incident. Further research, in independent controlled studies with feed at levels found normally, would provide reassurance and allow objective advice to be developed for farmers as it is apparent there is no clear consensus on the use or effectiveness of these products at the present time.

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Key individuals with regards to work on mycotoxins were contacted in the following establishments;
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AB Agri
Ag Industries
Alltech