Is early detection the solution to ensure damage by mycotoxins is reduced?

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Mycotoxins are a group of naturally occurring toxins produced by fungi, commonly known as moulds, which are harmful to humans, domestic animals and livestock.

Why mycotoxins attract so much attention throughout the world is because of the possible significant economic losses with their impact on human health, animal productivity and trade.

Mycotoxins are found in a wide range of foods and feeds, particularly in areas with climates of high temperature and humidity.

Routes of contamination

They can enter the food or feed chain through contaminated crops, in particular cereals, but also nuts, beans, spices, dried fruit, oilseeds, coffee and cocoa, poultry meat and kidneys, pig kidneys and pork sausages.

Contamination may also occur post-harvest during storage, transport, and processing stages of the food or feed supply chain. Factors of both a scientific and socio-economic nature largely influence the establishment of mycotoxin limits and regulations, therefore various mycotoxin limits and regulations in feed have been set by multiple food agencies worldwide.

For example, the EU 2002-32 Directive sets maximum permitted levels (MPLs) for substances that are present in animal feed that have the potential to pose danger to animal or human health, to the environment, or could have an undesirable affect to livestock production.

Pig feed contaminated with mycotoxins can cause serious risk to pig health.

Types of mycotoxins

Aflatoxins consumed by swine, for example, can expose non-clinical characteristics with low level exposure (20-200ppb), inducing symptoms displayed such as feed avoidance, gastrointestinal disturbances, paleness and slower growth.

It can also suppress the immune system and cause young piglets to become more susceptible to bacterial, viral or parasitic diseases. Prolonged exposure causes a greater risk of cancer, liver damage and jaundice.

High concentrations of aflatoxin (1,000-5,000ppb) result in acute effects, including death. Aflatoxin B1 is the only mycotoxin with MPLs under this order. It is a genotoxic carcinogen and suitably its levels have been set as low as realistically possible in complete feedingstuffs for pigs and poultry with a maximum content value of 0.02ppm.

Ochratoxin A is listed as another of the five mycotoxins under Commission Recommendation 2006/576/EC which is a nephrotoxic and nephrocarcinogenic compound referring specifically to complementary and complete feedingstuffs for pigs.

The guidance value assigned to ochratoxin A in complementary and complete feedingstuffs for pigs is suggested at a level of 0.05ppm. Over an extended period of ingestion, kidney damage can occur and it has the potential to contaminate most of the edible tissue encouraging enough damage to the carcase for it to be condemned. This toxin can induce acute renal failure in acute cases and furthermore, death of the animal.

Biochip Carrier.

Zearalenone called F2 is produced by a strain of Fusarium graminearum and has been listed under the Directive with a guidance value.

It has an oestrogenic action and is significantly toxic to the reproductive system of animals with the potential to cause rectal and vaginal prolapses in gilts (young sows).

Zearalenone has been allocated a suggested guidance value of 0.1ppm in complementary and complete feedingstuffs for piglets and gilts and 0.25ppm in feedstuffs for sows and fattening pigs.

The final two toxins listed with recommended maximum values are Deoxynivalenol (DON) and Fumonisin B1 and B2. These have both been suggested as acceptable with higher levels.

DON is suggested to have a maximum value of 0.9ppm in complementary and complete feedingstuffs for pigs and a value

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Table 1. Mycotoxins detected simultaneously with biochip arrays.

Mycotoxins detected	
Aflatoxin B1 (including B2)	Fumonisins (including B1/B2/B3)
Aflatoxin G1 (including G2)	Ochratoxin A
Deoxynivalenol (including 3-acetyldeoxynivalenol/ 15-acetyldeoxynivalenol)	Paxilline
Diacetoxyscirpenol	T-2 toxin (including HT-2 toxin)
Ergot alkaloids (including their related -inines)	Zearalenone (including metabolites)

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of 5ppm for the latter. In high enough doses they have been known to cause adverse effects such as a decrease in feed intake and impairment of the immune system.

With the risk from multiple mycotoxins in pig feed it is important to be able to detect dangerous levels of each listed in the EU Directive in order to reduce instances of damage to pig health.

Mycotoxin	FAPAS Assigned value (µg/kg)	Randox Food Diagnostics result (µg/kg)	z-score
Ochratoxin A	4.72	3.71	-1.00
Zearalenone	245	192.87	-1.10
Deoxynivalenol	1981	1537.94	-1.60
Aflatoxin BI	9.45	5.90	-1.70
Total T-2 & HT-2	693	496.61	-1.70

Table 2. Data from test material from proficiency testing scheme assessed with mycotoxin biochip arrays.

Detecting mycotoxins

Currently chromatographic, spectrometric and immunoassay based techniques are used for the detection of these toxins. However, Biochip Array Technology (BAT) enables simultaneous quantitative determination of multiple analytes from a single sample reducing the time it takes to result.

With BAT from Randox Food Diagnostics, multiple mycotoxins can be screened from a single feed sample, as multiple immunoassays take place at the same time in discrete test sites on the biochip surface. This increases the output of test results.

Furthermore, this methodology is flexible. With MycoFlex the user can customise the mycotoxins to be screened according to the most prevalent in a particular geographical region for example.

Simple sample preparation

The feed sample preparation for the mycotoxins biochip arrays is simple, fast, highly robust and generic for all the mycotoxins on the array.

It does not require the use of:

- Multiple sample extraction methods – one extraction method is suitable for all of the mycotoxins on the array.
- Immunoaffinity columns for sample clean up.
- Solid Phase Extraction (SPE) for sample clean up.
- Filters.
- Large volumes of organic solvents.

Note that 45 samples can typically be extracted for all mycotoxins within 45 minutes equating to approximately one minute per sample.

Evidence Investigator.

Simultaneous detection

For the quantitative detection of mycotoxins (and their cross-reactants), simultaneous competitive chemiluminescent immuno-assays, arrayed on the biochip surface, are employed. The biochip (9mm x 9mm) is also the vessel for the immunoassays.

The assays are applied to the well established semi-automated Evidence Investigator analyser. With this system, the immunoassay steps (i.e. reagent loading and washing) are manually performed under controlled incubation conditions as a customised Thermoshaker unit is provided.

The biochips are supplied in carriers (3 x 3 biochips per carrier = 9 reaction vessels per carrier). A handling tray with the capacity to accommodate six carriers (54 biochips) is also provided with the system. Once the biochip carrier is inserted in the image station of the analyser the dedicated software processes, reports and archives the data generated for retrospective access.

This system enables the quantitative screening of up to 10 mycotoxins (and their cross-reactants) in less than three hours for a batch of 45 samples. Mycotoxins that can be detected simultaneously with Randox Food Diagnostics mycotoxin biochip arrays are shown in Table 1.

Optimal performance

With over 2000 participants in over 100 countries, FAPAS proficiency testing scheme is the largest and most comprehensive in the food sector. It provides an independent check of a laboratory's procedures ensuring the delivery of quality results and Randox Food Diagnostics is a regular participant in this scheme. The participants' data are analysed statistically providing assigned values to the test sample and individual z-scores for the sample result submitted from each participant (in normal circumstances about 95% of z-scores will lie in the range $-2 \leq z \leq 2$ over time).

Test material from the scheme was assessed with Randox Food Diagnostics mycotoxin biochip arrays. Table 2 shows data for aflatoxin BI (AFBI), deoxynivalenol (DON), ochratoxin A (OTA) zearalenone (ZON) and total T-2 and HT-2. The results from the mycotoxin biochip assays lie comfortably within the range and are fit for purpose when compared with other testing methods.

Conclusion

The mycotoxin biochip arrays allow for the simultaneous quantitative detection of up to 10 mycotoxins and their cross-reactants from a single sample. With MycoFlex it is possible for the user to customise the number of toxins that are screened as per the user's individual needs.

BAT is a labour saving, multi-analytical solution for the accurate quantifiable screening of all the most prevalent mycotoxins to comply with global regulations.

Combining confidence with convenience Randox Food Diagnostics is breaking the mould of laborious mycotoxin test methods with the aim to improve food security. ■

References are available from enquiries@randoxfood.com on request

Thermoshaker.