# Biomarkers for exposure of mycotoxins in pigs and broiler chickens

In the field (pre-harvest) and during storage, transport and processing (post-harvest) agricultural commodities can be contaminated by fungi. Depending on environmental circumstances, such as temperature and humidity, these fungi can flourish and produce toxic secondary metabolites, called mycotoxins. Research by the Food and Agriculture Organisation has estimated that these toxins are present in 25% of all food crops all worldwide.

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The presence of certain fungi and their corresponding mycotoxins in general is mostly typical for a specific part of the world. Nevertheless, worldwide trade and climate change mean that these fungi can occur almost everywhere.

Over 400 different mycotoxins are identified but only a small fraction of these are known to have distinct toxic effects. The main producers are species from the aspergillus, penicillum and fusarium genus. They produce a number of mycotoxins that are important from an economical and safety point of view. The greatest attention goes to aflatoxins, ochratoxins and fusarium mycotoxins. Aflatoxins, especially aflatoxin B1, are notorious because they are considered the most potent naturally occurring carcinogen, especially targetting the liver. The worldwide occurrence and its nephrotoxic properties also make ochratoxin A a toxin of significant importance.

The fusarium mycotoxins can be divided into five different groups: type A and type B trichothecenes (deoxynivalenol (DON) and T2-toxin, respectively), fumonisins, zearalenone (ZEN) and the emerging mycotoxins such as enniatins.

Based on large scale feed surveys, several fusarium mycotoxins are highly prevalent with contamination levels for DON and ZEN of 58% and 26%, respectively. Apart from these major fusarium mycotoxins, other 'emerging' fusarium mycotoxins, such as enniatins (ENNs) and beauvericin also frequently contaminate feed commodities.

Contamination incidence can be as high as 96% with enniatin (ENN) A, A1, B and B1 as the most prevalent ENNs, namely 87%, 95%, 92% and 92%, respectively (83 investigated feed samples).

In addition to the frequent contamination, feed will often be contaminated with more than one mycotoxin. There are multiple reasons for this co-contamination.

Firstly, some fungi can produce more than one mycotoxin, for example Fusarium graminearum species can produce both DON and ZEN. Secondly, the crops can be infected with different fungal species at the same time or successively leading to contamination with multiple mycotoxins. Thirdly, animal feed is often prepared using a combination of different grains originating from different sources also contributing to co-contamination.

This may lead to potential additive or synergistic effects, enhancing the toxicity of the toxins. Consequently, it is important to make use of multi-mycotoxin detection methods for assessing the contamination level.

## Research programme

Innovad and Ghent University have embarked on a four year research programme, the aim of which is to develop multi-mycotoxin analysis methods to detect and quantify mycotoxins in samples from animal origin (not just in feed samples).

This will be done using an ultra-high performance liquid chromatographic instrument (UPLC) coupled to a high resolution mass spectrometer (HRMS type Synapt G2-SiHDMS) for screening purposes, and an UPLC-tandem mass spectrometer (LC-MS/MS type Xevo) for confirmation purposes offering a more sensitive detection.

These multi methods will be developed for plasma, urine and faeces of pigs and plasma and excreta of broiler chickens.

Pigs and broiler chickens are chosen because these animals are highly exposed

to mycotoxins due to their mainly cereal based diet. Moreover, pigs are much more sensitive to most mycotoxins than chickens. This makes the comparison between these two animal species very interesting.

Furthermore, acute and chronic exposure to mycotoxin contaminated feed can cause deleterious effects on the performance and well being of the animal and leads to economic losses. Both animal species are economically important which makes it valuable to develop methods to assess their exposure to mycotoxins, so that economic losses can be minimised.

The mycotoxins will be detected in plasma, urine and faeces or excreta of the animal. This can be considered complementary to the analysis of feed, and avoids the difficulty of sampling feed.

In biological matrices the concentration of mycotoxins will be more constant, while in feed the toxins are often not proportionally spread. This is caused by the uneven distribution of fungi throughout the field, leading to spots with a high concentration of mycotoxins (so-called 'hot spots'), while the rest of the feed is free of mycotoxins.

## Assessing mycotoxin exposure

By measuring the concentration of the mycotoxins in biological fluids, the mycotoxin exposure in livestock can be

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assessed. In this research project a large field trial will be executed to collect samples. These samples will be used to correlate the concentration with the health status of the flock using biomarkers as an indicator of exposure.
Typical biomarkers for exposure are parent compounds and their phase I and II metabolites. To determine these biomarkers, feed and selected feedstuffs will first be screened for mycotoxin contamination.

Following this, toxicokinetic studies of the most prevalent mycotoxins will be performed in pigs and broiler chickens to evaluate species dependent differences in absorption, distribution, metabolisation and excretion (ADME) processes of these mycotoxins in both animal species.

Consequently, the most appropriate biomarkers for exposure can be identified for each mycotoxin in both animal species.

To counteract the negative impact of mycotoxins on animal health it is important to minimise the exposure to mycotoxins. This can be done by using pre and post harvest strategies. The pre-harvest strategy is mostly the implementation of good agricultural practice (GAP).

Application of GAP tries to minimise the mycotoxin contamination in the field. This is done by choosing the correct variety of crops, rotating crops between highly and lowly susceptible hosts for a certain fungus and correct time of harvesting. The post-harvest strategies consist of well controlled storage with regard to moisture, temperature and insect control and the separation of diseased from unaffected raw material.

Despite all these measures, the presence of mycotoxins cannot be completely avoided and can still lead to disorders in animals and to economic losses. Thus, to minimise the effects of the mycotoxins on animal health, extra measures need to be taken. This can be done using mycotoxin detoxifying agents.

These feed additives act in the gastro-intestinal tract and ideally minimise the oral bioavailability of the mycotoxins. Two kinds of detoxifying agents exist: mycotoxin binders and mycotoxin modifiers.

The first category adsorbs the mycotoxins to their surface to reduce the gastro-intestinal absorption. They are often large molecular weight molecules and can be silica-based inorganic clay minerals or carbon-based organic compounds.

In both cases they should be able to bind the toxins and to eliminate these as a complex via the faeces/excreta.

The second category will transform the toxin into a safer, less toxic form. These modifiers are often bacteria, yeasts, fungi or enzymes. The efficacy of these additives needs to be tested in vivo. The proposed endpoints for these trials is the determination of the concentration of mycotoxins and/or their metabolites in plasma/excreta of the target species. This concentration will ideally be lower with a detoxifier and will depend on its efficacy.

This research forms the second part of this project. The effect of candidate mycotoxin detoxifiers on the toxicokinetic behaviour of the mycotoxins and the selected biomarkers will be evaluated.

Finally, the most potent mycotoxin detoxifier(s) will be retained and assessed in long-term feeding trials where a mycotoxin contaminated diet will be fed to the animals with and without detoxifier.

## Conclusion

In conclusion, by developing screening and confirmation methods for the detection of multiple mycotoxins, information will be gathered about the presence and the toxicokinetic behaviour of these mycotoxins in animals.

Biomarkers will be selected to assess exposure in pigs and poultry, minimising economic losses. Finally, the effect of detoxifying agents will be evaluated and the most promising agent(s) will be used to counteract the effects of mycotoxins in animals. ■

**References are available from the author on request**