What are the potential pitfalls of mycotoxin biomarkers?

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An important part of effective mycotoxin risk management is the regular analysis of feed material for mycotoxins. Contaminated feed ingested by farm animals leads to mycotoxin exposure and further to impaired health effects.

For more than 30 years scientists have been working on the development of so called 'biomarkers' to link health effects and exposure to contamination – by measuring one crucial parameter in blood or other physiological samples.

Mycotoxins are toxic metabolites produced by filamentous fungi and can be found in almost all types of grains. Despite the widespread use of preventive measures in good agricultural practice, 81% of more than 4,200 feed samples tested positive for mycotoxins in 2013 (Biomin Mycotoxin Survey, 2013).

As the consequences and health effects of mycotoxins differ greatly between animals, scientists, veterinarians and farmers have been on a persistent search for diagnostically conclusive biomarkers.

What are biomarkers?

● Biomarker of exposure

It is important to differentiate between biomarkers of exposure and effect. A good example of a biomarker of exposure is aflatoxin M_1 in the milk of cows (see Table 1). Biomarkers of exposure measure the mycotoxin or their metabolites in the blood, milk, urine, faeces or other physiological samples. To some extent, the mycotoxin can be detected unchanged in physiological samples, the rest is being metabolised.

Depending on the milk production yield among other factors, it is estimated that 1-6% of ingested aflatoxin B_1 (AfB_1) can be found in the form of aflatoxin M_1 (AfM_1), the hydroxylated metabolite in the milk of cows. Roughly calculated, 0.05ppb of AfM_1 (EU maximum level for milk) would correlate to a range of AfB_1 contamination from 0.8- 5ppb in compound feed (5ppb is the EU maximum level for compound feed in dairy cattle). This example shows that conducting mycotoxin analyses on feed is recommended in order to prevent the economic risk of aflatoxin contaminated milk close to the EU maximum level.

● Biomarker of effect

Biomarkers of effect, also called mechanism-based biomarkers, should be directly linked to a specific step in the disruption of metabolic and cellular processes.

For instance, the first step leading towards porcine pulmonary oedema in pigs, is the disruption of the sphingolipid metabolism by fumonisin B_1 (FB_1). This compound inhibits the ceramide synthase resulting in an elevated ratio of sphinganine to sphingosine (Sa/So). The Sa/So ratio is a scientifically well recognised biomarker of effect for fumonisins (FUM) in pigs, but not in humans.

Practical challenges

In the case of FUM, the Sa/So ratio applies to scientific trials but not at the farm level. The lack of non-exposed groups on farms makes it difficult to define the cut-off and provide controlled feeding.

Mycotoxin	Biomarker of exposure	Biomarker of effect
Aflatoxin B_1 (AfB_1)	AfM_1 in milk	• AfB_1-albumin adducts in blood • AfB_1-DNA adducts in urine, tissue
Fumonisin B_1 (FB_1)	FB_1 in blood, urine, faeces	Sa/So ratio in blood, tissue
Deoxynivalenol (DON)	DON, deepoxy-DON and other metabolites in urine, tissue, faeces	Pro-inflammatory cytokines in blood, tissue
Zearalenone (ZEN)	ZEN, zearalenol, zearalanol and other metabolites in blood, urine, faeces	• Glucuronic acid-conjugates in urine, faeces • Endocrine disruption in tissue
Ochratoxin A (OTA)	OTA and its metabolites in blood, urine, tissue (kidney)	OTA-DNA adducts in tissue

Table 1. Potential biomarkers of exposure and effect for the main mycotoxins used in scientific studies (Adapted from Baldwin et al, 2011).

In addition, for a biomarker to have practical relevance there must be a linear correlation between the exposure and ingestion of the mycotoxin. In some published trials, a linear relationship could be found for DON and its metabolites measured in the blood or urine of swine; however, there are also limitations.

Nonetheless, the deviation of individual levels does not allow a conclusion to be made on the amount of ingested mycotoxins and their health effects in single animals. These are the reasons for the lack of established guidance levels on critical concentrations of DON or other mycotoxins in the blood of animals, which renders the interpretation of results impossible.

The situation is further complicated by the need for a precise time when sampling for a representative analysis. This is because of the peak in DON and its metabolites in the blood within two hours after ingestion, which is followed by a rapid depletion afterwards. ZEN takes longer to deplete due to the enterohepatic circulation (absorption in the blood, excretion via bile and reabsorption in the blood). Farm animals are usually fed ad libitum which makes sampling time unpredictable, thereby yielding results that are not representative.

Another important aspect is the fact that DON, like other mycotoxins, is converted into metabolites such as DON-glucuronide, deepoxy-DON and also unknown metabolites. The proportion depends on the species, life cycle, gut microbiota and health status of the animal. Furthermore, the toxicity of DON metabolites may differ from the parental compound; for example, deepoxy-DON is non-toxic. ZEN can be found as alpha- and beta-zearalenol, alpha- and beta-zearalanol and their glucuronated forms in physiological specimens. The transformation of ZEN into alpha-zearalenol increases oestrogenicity. As a result, analysing for only one individual mycotoxin is not enough.

The use of biomarkers as a diagnostic tool is only possible within scientific trials due to the range of resulting metabolites and their differences in toxicity. It must also be considered that there are no guidelines for risk levels in physiological specimens.

Analysing biomarkers

A trend in recent years has been the development of LC-MS/MS (liquid chromatography-mass spectrometry/mass spectrometry) based methods, which are highly selective and sensitive enough to detect mycotoxins at very low concentrations. LC-MS/MS offers the possibility to quantify several metabolites in parallel.

In contrast, ELISA (enzyme-linked immunosorbent assay) methods can only serve as a rough screening method as the matrix effects caused by body fluids alter the results. Antibodies used in ELISA tests to quantify mycotoxins have a wide cross-reactivity to related metabolites. For example, most ELISA kits for ZEN also detect alpha-zearalenol but cannot differentiate between the metabolites. The cross-reactivity for the different metabolites is often not evaluated nor specified precisely in the user manual.

While validated methods to analyse mycotoxins in feed exist, there are hardly any for biomarkers. In contrast to feed, quality control for mycotoxin analyses of physiological samples has yet to be established for commercial laboratories.

Although biomarkers are valuable tools in scientific studies, more knowledge is needed on the factors influencing the bioavailability, kinetics and metabolic profile of mycotoxins in animals before biomarkers could be used in practice on farms. There is still a lack of linear correlation for biomarkers. The use of control groups and elaborate sampling is indispensable, which makes the procedure very costly. The well established analysis of mycotoxins in feed is a reliable approach to assess possible risks and is therefore the method of choice. ■

References are available from the author on request.