Biomarkers shed new light on mycotoxin issues

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Hundreds of different mycotoxins have been detected during the last few decades. For each mycotoxin, there are differences in toxicity, symptoms, incidence in feedstuffs, species impact, etc.

When considering 'biomarkers', the numbers are further multiplied from hundreds to thousands. Why do scientists further complicate the issue?

What are (some of) the advantages of distinguishing different biomarkers?

What are biomarkers?

Each medical sign that can be measured accurately and reproducibly as an influence or prediction for the incidence or outcome of disease could be defined as a 'biological marker'.

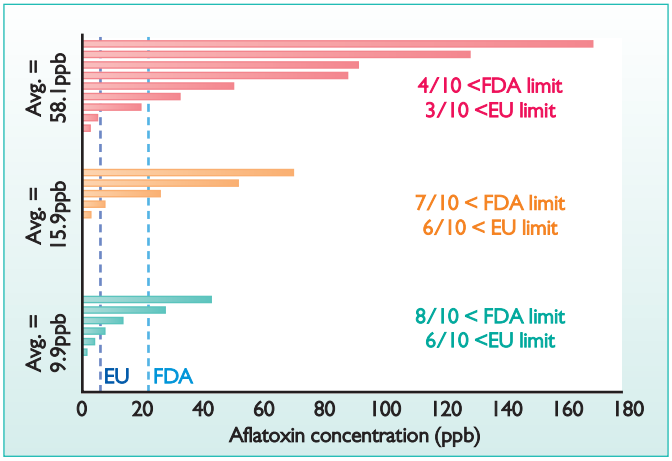

Fig. 2. Low sampling reliability

Such signs are observed from outside the patients, in contrast to medical symptoms which are perceived by the patients themselves.

The list of possible biomarkers is endless: from jaundice, over basic measurements such as blood pulse or breathing rate to blood or urine components. However, the validity of biomarkers depends on:

- The accuracy and reproducibility of the measuring.
- The correlation with the medical aspects.

Although jaundice as observed in the white of the eyes could be a sign of liver impairment caused by aflatoxicosis, it is hard to measure.

Blood bilirubin levels are already more accurate since they can be measured; but since there are too many other possible causes or aetiologies, the correlation between jaundice or bilirubin and aflatoxicosis would be too poor to use such signs as mycotoxin biomarkers.

More reliable biomarkers are, for example, the levels of the different aflatoxins found in urine or the levels of aflatoxin-conjugates in serum.

Such figures are measured more accurately while showing a far superior correlation with the disease than is the case for bilirubins.

Stress genes test

More than a decade ago now, Impextraco used a 'Stress Genes Test' for the development of its Elitox range.

This test was developed in co-operation with the University of Antwerp and the Institute De Nayer in Mechelen, both in Belgium.

Fig. 1 shows some of the effects obtained in a human liver cell line (CAT-Tox test).

The impact of increasing doses (5ppm in dilutions of 1:32, 1:8, 1:4, 1:2, 1:1) of AFB1 on the expression of selected genes (the UmuDC gene being involved in carcinogenesis) was verified.

The left side represents the effects without an additive, while the right side represents the effects with supplementation of the concerned feed additive. Obviously, the feed additive makes a difference in the effects induced by the mycotoxins.

Such testing protocol helps explain why biomarkers currently receive a lot of focus in mycotoxin research.

Some of those genes encode for proteins which can be determined in blood or other body fluids; such compounds are true biomarkers. They are helpful to understand and predict the mechanisms by which mycotoxins induce certain effects (for example carcinogenicity).

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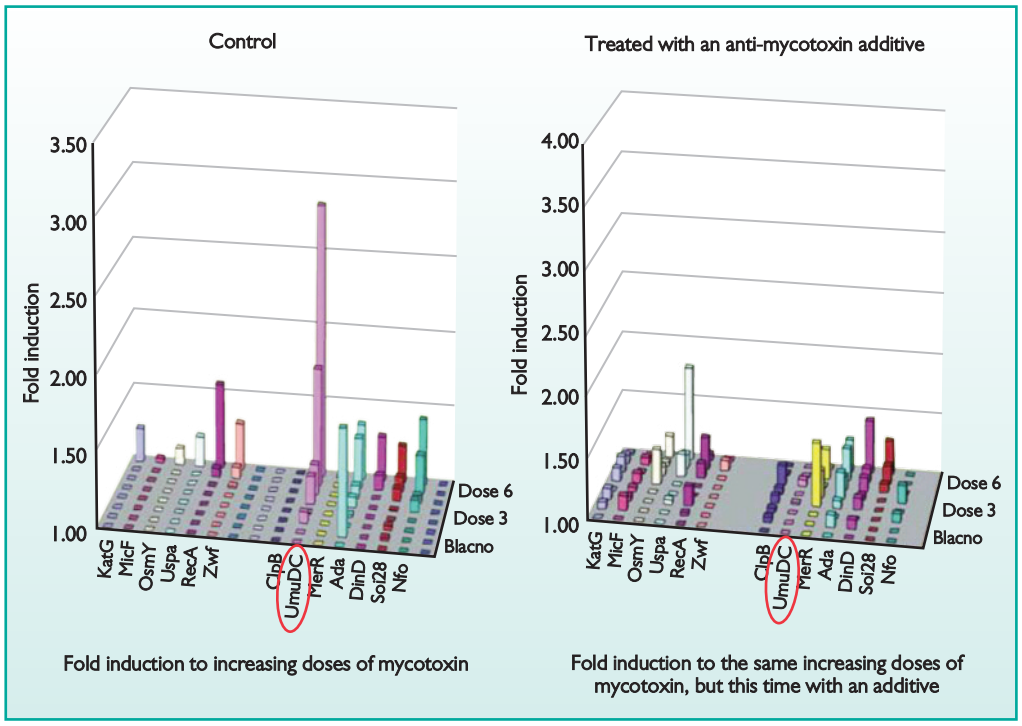
Fig. 1. Fold induction pattern showing an intracellular stress response to mycotoxins.



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It is common knowledge that most feedstuffs are to some extent contaminated with mycotoxins; in most cases, more than one mycotoxin is present.

Less commonly known is that feedstuffs classified as safe are often 'false negatives'; when the contamination level is low to moderate, around half of the samples show a nil assay even though the 'average' surpasses the safety limit. Fig. 2. shows this aspect for moderate aflatoxin contamination levels.

Sampling strategies

Sampling strategies to improve the assay outcome have been implemented: enlarging the number of samples in line with the size of the feedlot, while suggesting distribution patterns to take the subsequent samples at separate locations within the feedlot.

Regardless of how carefully you perform the sampling, nature may still trick you by presenting mycotoxins in hotspots. At certain points in the storage bin, a localised mould growth may induce a mycotoxin concentration that reaches a multitude of the average figure. This is influential: a more than thousand-fold mycotoxin concentration in such hotspots compared to the average can have a dramatic impact.

The size of such a hotspot can be very small: literature reports a 25g sample (some 200 kernels) in which 98% of aflatoxin contamination was found in one single kernel: a 10,000 times higher concentration in the concerned kernel compared to the average.

During grinding and mixing, the hotspot mycotoxins are spread over the entire batch of feed, thus raising the average. But, what happens when a single animal ingests such a highly contaminated kernel in intact form? Clinical mycotoxicosis in an individual animal?

Part of the mycotoxins is already produced when the crops are still in the field. Checks for mycotoxin contamination are mostly performed by the feed mill at the moment of feedstuff reception because contaminated batches can still be rejected at that moment. However, during storage, moulding and mycotoxin production also remains an issue. Such storage mycotoxins contribute to underestimation in routine mycotoxin surveys.

Average storage time at the feed mill lasts several months after which the feedstuffs are grinded and mixed (thus spreading the possible hotspots over the entire batch of feed). At the farm level, storage time is limited to weeks only, but possible hotspots are presented to the animals in a more concentrated form. Individual animals may or may not reject the highly contaminated fractions; the question remains: how often is clinical mycotoxicosis missed as a diagnosis?

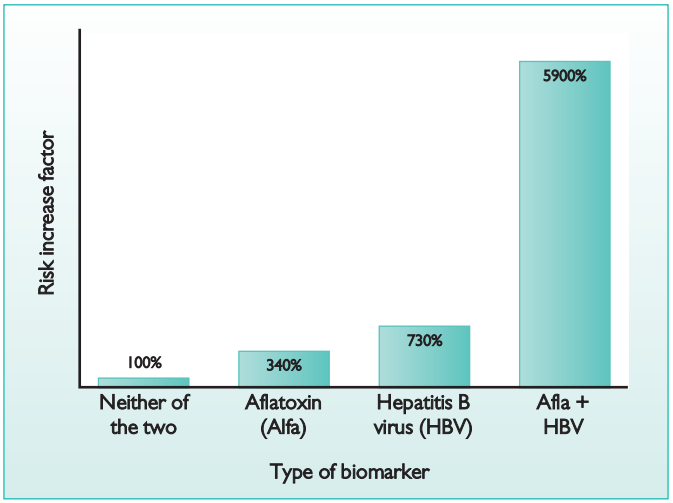

Fig. 3. Hepatocellular carcinoma.

The above information highlights that the average figures obtained from mycotoxin surveys, albeit useful for understanding the mycotoxin problem, do not accurately predict or explain the disease risk when considering individual animals or individual farms. Biomarkers remedy this issue, albeit with a post-fact approach.

Masked mycotoxins

Plants also cope with fungi and the resulting mycotoxins. Therefore, cereals and other crops develop defence mechanisms which are different from those observed in humans or animals. Such defence mechanisms modify part of the mycotoxin molecules (for example conjugation), thus rendering those undetectable by the assay techniques used for mycotoxin detection in feedstuffs.

Research revealed that digestive processes partly restore the original mycotoxin (for example hydrolysis of the concerned conjugation).

Mycotoxins that remain hidden during assay, while regaining toxicity in the animal are 'masked' mycotoxins. Masked mycotoxins obviously contribute to underestimating the problems.

Verifying assumptions

Traditionally, mycotoxins have been seen as contaminants of food and feed. Based on clinical findings, limitations have been implemented for a few mycotoxins. However, visions are often incoherent: in the US, <500ng/L aflatoxin M_1 in milk for human consumption remains acceptable, while it must be below 50ng/L in the EU.

Therefore more extensive contamination surveys have been organised in both food and feedstuffs. Such approach reveals that 'average' contamination levels are not sufficiently conclusive to predict or explain disease risks; more accurate parameters are required, such as biomarkers.

The former belief that the rumen detoxifies mycotoxins has been largely questioned in more recent years. Although detoxification theories remain valid for certain mycotoxins (OTA, T-2, DON), reverse reactions were observed for others (AFB1), while it also happens that toxicity is intensified in the rumen (ZEA -> α-Zearalenol). Obviously, a more profound understanding of metabolic processes is required. Biomarkers are tools to comprehend such mechanisms.

Most feedstuffs contain more than one mycotoxin. Determination of the contamination level for each mycotoxin separately will provide a range of figures, which are difficult to interpret. Many recent research documents highlight synergistic effects when more than one mycotoxin is present.

However, the effects of multiple contaminations may be synergistic, additive, indifferent and even antagonistic. Assaying feedstuffs does not provide means to differentiate such mechanisms; biomarkers are clues to do so.

In human medicine, cancer research is well funded, thus it produces a lot of innovative findings. The debate on aflatoxin carcinogenicity is a good example as hepatocellular carcinoma is one of the predominant cancers in many developing countries. Both AFB1 and HBV (hepatitis B virus) are involved, which has been food for discussion about the primary cause.

Elucidation of the mechanisms leading to cancer showed that the body converts part of the ingested AFB1 into a more reactive AFB1-epoxide; this epoxide binds covalently to DNA and serum albumin, thus producing AFB1-N7-guanine and lysine-adducts respectively.

Reaction of the AFB1-epoxide with guanine in codon 249 of tumor suppressor gene p53 produces mutations involved in HCC; since HBV affects other genes, synergism between the two factors becomes evident.

Animal nutrition benefits

This kind of research indirectly benefits animal nutrition: AFB1-N7-guanine concentration in urine appears to be a good measure for AFB1 ingestion during the preceding 1-2 day period, while the AFB1-lysine in serum indicates a 2-3 month exposure. Differentiating several biomarkers thus leads to a better understanding of mycotoxicoses.

Apart from mycotoxin synergies, more and more mechanisms are elucidated showing that mycotoxins are detrimental at lower contamina-

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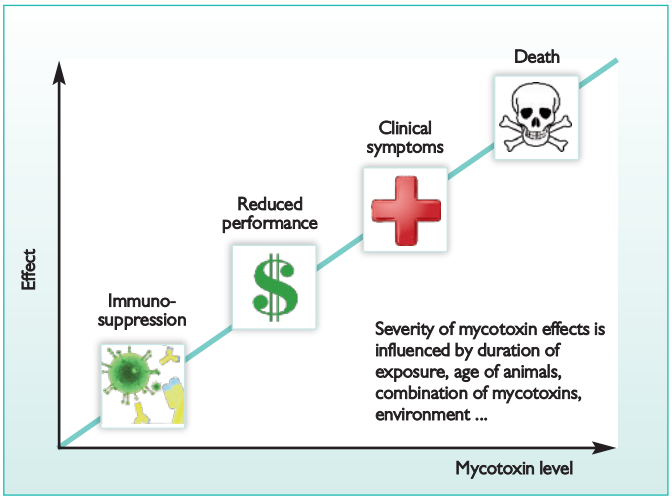
Fig. 4. Over the years, fine-tuning assay techniques allowed the impact of aflatoxins to be elucidated on a continuously expanding number of parameters: death at >5000 ppb (1960s), liver impairment at >500ppb (1980s), poor DWG and FCR at >200ppb (1990s), reduced vaccination efficiency at >50ppb (2000's).



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tion levels. As an example: previously unknown effects on immunity were recently highlighted in several studies. Nevertheless, many issues in animal husbandry still remain obscure. Human health authorities, for example, put pressure on salmonella occurrence, while veterinarians can only partially explain why latent infections resurge to the clinical stage.

A 2012 postdoctorate at Ghent University revealed that the mycotoxin DON may be one of the factors triggering salmonella relapses; also in this kind of research, biomarkers play a vital role.

So, biomarkers not only shed extra light on mycotoxin issues, but even on topics that previously appeared unrelated.

Biomarker disadvantages

Obviously, there is a cost factor involved in using biomarkers. One single mycotoxin is often transformed to many metabolites; AFB1 is, for example metabolised to AFB-8,9-epoxide, AFB1-N7-guanine and AFB-lysine in blood and tissues, is secreted in the form of AFM1 in urine and milk or AFQ1 and AFP1 in urine and faeces, while still many other metabolites or intermediates can be detected.

Differentiating assay methods for each of those compounds is more expensive compared to focusing exclusively on the original AFB1; thus there must be some kind of added value to substantiate such an approach.

Metabolites or intermediates are not necessarily detoxified: some are less toxic, while others are not.

For milk products, a limit has been determined on the metabolite AFM1 instead of the original AFBs or AFGs; this is due to the fact that AFM1 is more readily secreted in milk than AFB1. However, AFM1 also remains toxic. Therefore, conversion rates must be determined as well as the toxicity of the separate intermediates or metabolites. So, the extra information comes at a cost. Biomarkers are an indirect method of research. This means that misinterpretations are a risk factor. Certain biomarkers are highly correlated with disease or reduced performance, while others are not. Therefore, the use of biomarkers must be carefully validated.

Expectations for the future

A lot of research on mycotoxins is performed with an eye on human health issues. The above described topic of hepatocellular carcinoma is only one example.

Authorities more and more recognise the presence of mycotoxins in products for human consumption as a concern.

Continued efforts to improve the general health status of the human population also motivate extra research funding.

Indirectly, animal husbandry will benefit from those research efforts. Elucidating toxicity mechanisms in humans will lead to transposing this information to the animals.

So, we may expect an improvement in understanding the different mycotoxin related problems.

Already today, Impextraco uses biomarkers for research purposes. A few examples are:

- Blood parameters as an indirect measure for liver function.
- A flow cytometer to observe immunologic changes (occurring already at very low mycotoxin contamination levels).
- Vulva measuring to observe oestrogenic effects.

Laboratory techniques and assay procedures that are developed by human medicine will be useful for animal husbandry.

While today batch processing of feedstuffs is performed to check the level of several mycotoxins during assay, we may expect a shift from feedstuffs to body fluids of diseased animals (blood, serum, urine or faeces).

Surely, more accurate diagnostic procedures are to be expected.

Occasionally, human health authorities also check animal products for human consumption on their mycotoxin content.

Even though crops for animal consumption are prone to higher mycotoxin contaminations compared to those for human consumption, meat generally appears to be safer than, for example, breakfast cereals. So, animals detoxify mycotoxins rather than accumulating them.

Will this improve the consumer's appreciation of animal products? Or, with current research in mind, should we prepare for more stringent limitations on mycotoxin contamination in animal products?

Conclusion

Although the variety of research in which biomarkers are used seems to complicate things, a lot of extra information is obtained thanks to using biomarkers.

This has already improved our methods to cope with mycotoxins, while the major progress still lies ahead of us. In the meantime, the widely occurring variety of mycotoxins in feedstuffs urges us to use products with the widest possible spectrum of mycotoxin eliminating mechanisms. ■