FIGHTING THE THREAT OF EMERGING MYCOTOXINS WITH EXCENTIAL TOXIN BINDERS

Jolien van Soest, Central Technical Manager

Mycotoxins are secondary metabolites that are produced by certain filamentous fungi. They are found to be common contaminants of food and feed sources. The most commonly detected mycotoxins, with relevance to animal production, are aflatoxins, fumonisins, zearalenone, ochratoxin A and trichothecenes (DON, HT-2, T-2). However, there is another group of mycotoxins, the emerging mycotoxins, which are more recently detected metabolites that pose an increasing threat. The danger of this group is that they are not controlled by legislation, limited toxicity information is available and they are often not determined during feed analysis. This highlights the importance of more research into these mycotoxins, to unravel toxicity mechanisms and be able to set maximum inclusion levels in feed.

Commercially available mycotoxin adsorbents often focus only on binding the 'common' mycotoxins, while data on the binding efficacy for the emerging mycotoxins remains limited. Many of the emerging mycotoxins are not yet standardized and are therefore difficult to include in analysis, even though it is important to know the effects of mycotoxin adsorbents against these toxins. Orffa continues to focus on fighting the threat of emerging mycotoxins, by performing trials to study the binding capacity of Orffa's mycotoxin adsorbents **Excential Toxin A** and **Excential Toxin Plus** for the emerging mycotoxins alternariol, enniatin B, fusaric acid, beauvericin and sterigmatocystin.

The emerging mycotoxin threat; little information but high prevalence

Alternariol, one of the members of the emerging mycotoxins group, is produced by *Alternaria* fungi. Data on alternariol toxicity in animals is still limited and mostly originates from *in vitro* trials. It was suggested that this mycotoxin possesses genotoxic, cytotoxic and mutagenic properties. Alternariol has been shown to interact with the DNA and induce several DNA damage responses. Besides, it affects macrophages and reduces immune functioning. In 2021, alternariol was already shown to have a global prevalence of 35% in feed. This indicates the importance of more research into alternariol.

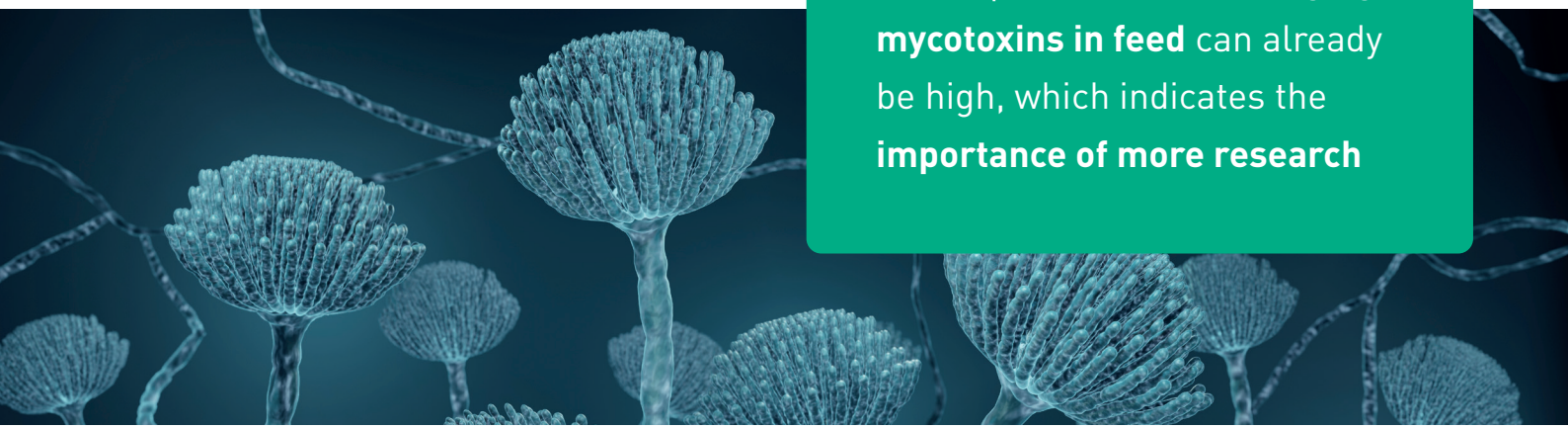
Another emerging mycotoxin is enniatin B, produced by *Fusarium* fungi. The main toxicity mechanism is hypothesized to be related to enniatin B's ionophoric properties. Also, it depolarizes mitochondria and has negative effects on cellular metabolism. Mycotoxin analysis from 2021 shows that approximately 74% of the tested feed samples were contaminated with enniatin B.

Fusaric acid is a toxic metabolite of *Fusarium* fungi and also for this mycotoxin, relevant *in vivo* data on toxicity is scarce. It is expected that fusaric acid toxicity is related to oxidative stress, mitochondrial disfunction, and inhibition of cell proliferation, DNA synthesis and dopamine beta hydroxylase. Also for this mycotoxin, the prevalence in 2021 was shown to be already quite high with the occurrence of approximately 97%, 6% and 95% in corn, forage or grain samples respectively.

Beauveria bassiana and several *Fusarium* fungi are producers of beauvericin. Again, the toxicity mechanism is not yet fully understood but it is likely related to its functioning as a potassium-specific ionophore, inducing reactive oxygen species (ROS) and increasing oxidative stress which can result in apoptosis of cells. Also, beauvericin has been shown to activate various cellular signalling pathways, such as mitogen-activated protein kinase (MAPK), internal mitochondrial pathway, NF-κB, and p53, which can also result in apoptosis. However, besides some toxic effects, beauvericin has also been shown to have some antibacterial and antifungal properties. In 2021, the occurrence of beauvericin was already high, with a level of around 70% in the tested feed samples.

Sterigmatocystin is a metabolite of *Aspergillus* fungi. This emerging mycotoxin is considered to be closely related to aflatoxins and its toxic effects seem to resemble those of aflatoxin B1; carcinogenicity, mutagenicity and cytotoxicity. Information on toxicity is based on both *in vitro* and *in vivo* trials, which show toxic effects across species, with the liver and kidney being the most affected. The occurrence of this mycotoxin seems to be around 19%, based on analysis in 2021.

> Global prevalence of **emerging mycotoxins in feed** can already be high, which indicates the **importance of more research**



Binding efficacy of the Excential Toxin Binders for emerging mycotoxins

Several trials have been performed, in collaboration with the University of Ghent in Belgium, to determine the binding efficacy *in vitro* towards alternariol (100 ng/ml), enniatin B (20 ng/ml), fusaric acid (15 ng/ml), beauvericin (0.75 ng/ml) and sterigmatocystin (25 ng/ml). Three different conditions were tested for each separate mycotoxin sample;

1. with buffer and mycotoxin standard mixture;
2. with buffer, mycotoxin standard mixture and the binder product;
3. with buffer and the binder product.

The solutions at pH 3 were incubated for one hour at 37 °C. A sample was collected at pH 3, after which the mycotoxins were extracted. The remaining buffer was then adjusted to pH 7 for another three hours of incubation, after which a sample was collected at pH 7 and the mycotoxins were extracted. All samples were analysed via liquid chromatography-tandem mass spectrometry (LC-MS/MS), after which the binding efficacies (%) were calculated.

It is essential to analyse the binding efficacy of mycotoxin binders in the pH range that represents the entire gastrointestinal tract, pH 3 and pH 3-7 (pH3; simulating the stomach environment, pH7; simulating the intestinal environment), because the intestine is the key link between ingested mycotoxins and their negative effects on the animal, since this is the location where the mycotoxins will be absorbed.

Regarding Excential Toxin A, complete binding was shown for enniatin B at both pH 3 as well as pH 3-7. It was shown for fusaric acid that there is partial binding at pH 3 and limited binding at pH 3-7. For beauvericin, there was partial binding at pH 3 and complete binding at pH 3-7. Sterigmatocystin is shown to be completely bound by the mycotoxin adsorbent at both pH 3 as well as pH 3-7 (Table 1).

> The **Excential Toxin Binders** play an important role in **fighting the threat** of emerging mycotoxins

For Excential Toxin Plus, it was shown that there is complete binding towards alternariol at both pH 3 and pH 3-7. For enniatin B, for Excential Toxin Plus, there was partial binding (88%) at pH 3 and complete binding at pH 3-7. Fusaric acid was shown to be completely bound at pH 3 and partially bound at pH 3-7. For beauvericin, there was partial binding at pH 3 and complete binding at pH 3-7. Excential Toxin Plus completely binds sterigmatocystin, both at pH 3 as well as in the range of pH 3-7 (Table 1).

Table 1: Mycotoxin binding efficacy of Excential Toxin A and Excential Toxin Plus
0 (<10%) no significant proven effect, + (10-50%) limited effect, ++ (51-90%) partial binding, +++ (>91%) complete binding

	Excential Toxin A	Excential Toxin Plus
Alternariol pH 3	N.A.*	+++
Alternariol pH 3-7	N.A.*	+++
Enniatin B pH 3	+++	++
Enniatin B pH 3-7	+++	+++
Fusaric acid pH 3	++	+++
Fusaric acid pH 3-7	+	++
Beauvericin pH 3	++	++
Beauvericin pH 3-7	+++	+++
Sterigmatocystin pH 3	+++	+++
Sterigmatocystin pH 3-7	+++	+++

*Not analysed during the binding efficacy studies.

The Excential Toxin Binders provide protection against emerging mycotoxins

Based on these results, it can be stated that both Excential Toxin A and Excential Toxin Plus can provide complete protection in the pH range of the gastrointestinal tract (pH 3-7) for enniatin B, beauvericin and sterigmatocystin. For alternariol (not analysed for Excential Toxin A) and fusaric acid (limited binding for Excential Toxin A), it is advised to use Excential Toxin Plus, which offers complete protection.

It is important to note that Excential Toxin A is a single spectrum solution, based on clinoptilolite, and focused on adsorption of the mycotoxins in the gastrointestinal tract of animals. Excential Toxin Plus, on the other hand, is a broad spectrum solution, not only focussed on adsorption but also on prevention, hepatoprotection, and supporting immune functioning and intestinal integrity. Sometimes the mycotoxin threat in the feed is not yet clear, for example when emerging mycotoxins are present that have not yet been evaluated in binding efficacy studies. In such cases, it is advised to supplement the feed with a broad spectrum product that allows for additional support towards the health of the animal.

Excential Toxin Plus improves mycotoxin defences via a combined approach, by using five synergistically working ingredients. Besides aluminosilicates and yeast aimed at the adsorption of mycotoxins, Excential Toxin Plus also contains ingredients aimed at reducing the growth of fungi in stored feed and supporting intestinal integrity and liver health. Excential Toxin Plus therefore provides a broad spectrum solution, and it can be stated that Excential Toxin Plus can be used against the full range of mycotoxins including the emerging mycotoxins.

Overall, it can be concluded that the Excential Toxin Binders play an important role in fighting the threat of emerging mycotoxins!