Toxin Mitigation 101: Essentials for Animal Production



By **Monish Raj**, Assistant Manager-Technical Services, EW Nutrition **Inge Heinzl**, Editor, EW Nutrition

Mycotoxins, toxic secondary metabolites produced by fungi, are a constant and severe threat to animal production. They can contaminate grains used for animal feed and are highly stable, invisible, and resistant to high temperatures and normal feed manufacturing processes. Mycotoxin-producing fungi can be found during plant growth and in stored grains; the prevalence of fungi species depends on environmental conditions, though in grains, we find mainly three genera: Aspergillus, Penicillium, and Fusarium. The most critical mycotoxins for poultry production and the fungi that produce them are detailed in Fig 1.



Figure 1: Fungi species and their mycotoxins of worldwide importance for poultry production (adapted from Bryden, 2012).

The effects of mycotoxins on the animal

are manifold

When, usually, more than one mycotoxin enters the animal, they "cooperate" with each other, which means that they combine their effects in different ways. Also, not all mycotoxins have the same targets.

The synergistic effect: When $1+1 \ge 3$

Even at low concentrations, mycotoxins can display <u>synergistic effects</u>, which means that the toxicological consequences of two or more mycotoxins present in the same sample will be higher than the sum of the toxicological effects of the individual mycotoxins. So, disregarded mycotoxins can suddenly get important due to their additive or synergistic effect.

Synergistic interactions					
	DON	ZEN	T-2	DAS	
FUM	*	*	*		
NIV	*	*	*		
AFL			*	*	

Table 1: Synergistic effects of mycotoxins in poultry

Table 2: Additive effects of mycotoxins in poultry

Additive interactions					
	AFL	T2	DAS	MON	
FUM	+	+	+	+	
DON	+	+			
OTA	+	+			

Recognize the effects of mycotoxins in animals is not easy

The mode of action of mycotoxins in animals is complex and has many implications. Research so far could identify the main target organs and effects of high levels of individual mycotoxins. However, the impact of low contamination levels and interactions are not entirely understood, as they are subtle, and their identification requires diverse analytical methods and closer observation.

With regard to the gastrointestinal tract, mycotoxins can inhibit the absorption of nutrients vital for maintaining health, growth, productivity, and reproduction. The nutrients affected include amino acids, lipid-soluble vitamins (vitamins A, D, E, and K), and minerals, especially Ca and P (<u>Devegowda and Murthy, 2005</u>). As a result of improper absorption of nutrients, egg production, eggshell formation, fertility, and hatchability are also negatively influenced.

Most mycotoxins also have a negative impact on the immune system, causing a higher susceptibility to disease and compromising the success of vaccinations. Besides that, organs like kidneys, the liver, and lungs, but also reproduction, endocrine, and nervous systems get battered.

Mycotoxins have specific targets

Aflatoxins, fumonisins, and ochratoxin impair the liver and thus the physiological processes modulated and performed by it:

lipid and carbohydrate metabolism and storage

- synthesis of functional proteins such as hormones, enzymes, and nutrient transporters
- metabolism of proteins, vitamins, and minerals.

For trichothecenes, the gastrointestinal tract is the main target. There, they hamper digestion, absorption, and intestinal integrity. T-2 can even produce necrosis in the oral cavity and esophagus.



Figure 2: Main target organs of important mycotoxins

How to reduce mycotoxicosis?

There are two main paths of action, depending on whether you are placed along the crop production, feed production, or animal production cycle. Essentially, you can either prevent the formation of mycotoxins on the plant on the field during harvest and storage or, if placed at a further point along the chain, mitigate their impact.

Preventing mycotoxin production means preventing mold growth

To minimize the production of mycotoxins, the development of molds must be inhibited already during the cultivation of the plants and later on throughout storage. For this purpose, different measures can be taken:

Selection of the suitable crop variety, good practices, and optimal harvesting conditions are half of the battle

Already before and during the production of the grains, actions can be taken to minimize mold growth as far as possible:

- Choose varieties of grain that are area-specific and resistant to insects and fungal attacks.
- Practice crop rotation
- Harvest proper and timely
- Avoid damage to kernels by maintaining the proper condition of harvesting equipment.

Optimal moisture of the grains and the best hygienic conditions are essential

The next step is storage. Here too, try to provide the best conditions.

- Dry properly: grains should be stored at <13% of moisture
- Control moisture: minimize chances of moisture to increase due to condensation, and rain-water leakage
- Biosecurity: clean the bins and silos routinely.
- Prevent mold growth: organic acids can help prevent mold growth and increase storage life.

Mold production does not mean that the war is lost

Even if molds and, therefore, mycotoxins occur, there is still the possibility to change tack with several actions. There are measures to improve feed and support the animal when it has already ingested the contaminated feed.

1. Feed can sometimes be decontaminated

If a high level of mycotoxin contamination is detected, removing, replacing, or diluting contaminated raw materials is possible. However, this is not very practical, economically costly, and not always very effective, as many molds cannot be seen. Also, heat treatment does not have the desired effect, as mycotoxins are highly heat stable.

2. Effects of mycotoxins can be mitigated

Even when mycotoxins are already present in raw materials or finished feed, you still can act. Adding products adsorbing the mycotoxins or mitigating the effects of mycotoxins in the organism has been considered a highly-effective measure to protect the animals (<u>Galvano et al., 2001</u>).

This type of mycotoxin mitigation happens at the animal production stage and consists of suppressing or reducing the absorption of mycotoxins in the animal. Suppose the mycotoxins get absorbed in the animal to a certain degree. In that case, mycotoxin mitigation agents help by promoting the excretion of mycotoxins, modifying their mode of action, or reducing their effects. As toxin-mitigating agents, the following are very common:

Aluminosilicates: inorganic compounds widely found in nature that are the most common agents used to mitigate the impact of mycotoxins in animals. Their layered (phyllosilicates) or porous (tectosilicates) structure helps "trap" mycotoxins and adsorbs them.

- Bentonite / Montmorillonite: classified as phyllosilicate, originated from volcanic ash. This
 absorbent clay is known to bind multiple toxins in vivo. Incidentally, its name derives from the
 Benton Shale in the USA, where large formations were discovered 150 years ago.
 Bentonite mainly consists of smectite minerals, especially montmorillonite (a layered silicate
 with a larger surface area and laminar structure).
- <u>Zeolites</u>: porous crystalline tectosilicates, consisting of aluminum, oxygen, and silicon. They
 have a framework structure with channels that fit cations and small molecules. The name
 "zeolite" means "boiling stone" in Greek, alluding to the steam this type of mineral can give off
 in the heat). The large pores of this material help to trap toxins.

Activated charcoal: the charcoal is "activated" when heated at very high temperatures together with gas. Afterward, it is submitted to chemical processes to remove impurities and expand the surface area. This porous, powdered, non-soluble organic compound is sometimes used as a binder, including in cases of treating acute poisoning with certain substances.

Yeast cell wall: derived from *Saccharomyces cerevisiae*. Yeast cell walls are widely used as adsorbing agents. Esterified glucomannan polymer extracted from the yeast cell wall was shown to bind to aflatoxin, ochratoxin, and T-2 toxin, individually and combined (<u>Raju and Devegowda 2000</u>).

Bacteria: In <u>some studies</u>, Lactic Acid Bacteria (LAB), particularly *Lactobacillus rhamnosus*, were found to have the ability to reduce mycotoxin contamination.

Which characteristics are crucial for an effective toxin-mitigating solution

If you are looking for an effective solution to mitigate the adverse effects of mycotoxins, you should keep some essential requirements:

- 1. The product must be safe to use:
 - a. safe for the feed-mill workers.
 - b. does not have any adverse effect on the animal
 - c. does not leave residues in the animal
 - d. does not bind with nutrients in the feed.
- 2. It must show the following effects:
 - a. effectively adsorbs the toxins relevant to your operation.
 - b. helps the animals to cope with the consequences of non-bound toxins.
- 3. It must be practical to use:
 - a. cost-effective
 - b. easy to store and add to the feed.

Depending on

- the challenge (one mycotoxin or several, aflatoxin or another mycotoxin),
- the animals (short-cycle or long-living animals), and
- the economical resources that can be invested,

different solutions are available on the market. The more cost-effective solutions mainly contain clay to adsorb the toxins. Higher-in-price products often additionally contain substances such as phytogenics supporting the animal to cope with the consequences of non-bound mycotoxins.

Solis - the cost-effective solution

In the case of contamination with only aflatoxin, the cost-effective solution Solis is recommended. Solis consists of well-selected superior silicates with high surface area due to its layered structure. Solis shows high adsorption of aflatoxin B1, which was proven in a trial:



Initial SOLIS concentration 200 ppb, decreasing

Figure 3: Binding capacity of Solis for Aflatoxin

Even at a low inclusion rate, Solis effectively binds the tested mycotoxin at a very high rate of nearly 100%. It is a high-efficient, cost-effective solution for aflatoxin contamination.

Solis Max 2.0: The effective mycotoxin solution for sustainable profitability

Solis Max 2.0 has a synergistic combination of ingredients that acts by chemi- and physisorption to prevent

toxic fungal metabolites from damaging the animal's gastrointestinal tract and entering the bloodstream.



Figure 4: Composition and effects of Solis Max 2.0

Solis Max 2.0 is suitable for more complex challenges and longer-living animals: in addition to the pure mycotoxin adsorption, Solis Max 2.0 also effectively supports the liver and, thus, the animal in its fight against mycotoxins.

In an in vitro trial, the adsorption capacity of Solis Max 2.0 for the most relevant mycotoxins was tested. For the test, the concentrations of Solis Max 2.0 in the test solutions equated to 1kg/t and 2kg/t of feed.



Figure 5: Efficacy of Solis Max 2.0 against different mycotoxins relevant in poultry production

The test showed a high adsorption capacity: between 80% and 90% for Aflatoxin B1, T-2 Toxin (2kg/t), and Fumonisin B1. For OTA, DON, and Zearalenone, adsorption rates between 40% and 80% could be achieved at both concentrations (Figure 5). This test demonstrated that Solis Max 2.0 could be considered a valuable tool to mitigate the effects of mycotoxins in poultry.

Broiler trial shows improved performance in broilers

Protected and, therefore, healthier animals can use their resources for growing/laying eggs. A trial showed improved liver health and performance in broilers challenged with two different mycotoxins but supported with Solis Max 2.0.

For the trial, 480 Ross-308 broilers were divided into three groups of 160 birds each. Each group was placed in 8 pens of 20 birds in a single house. Nutrition and management were the same for all groups. If the birds were challenged, they received feed contaminated with 30 ppb of Aflatoxin B1 (AFB1) and 500 ppb of Ochratoxin Alpha (OTA).

Negative control:	no challenge	no mycotoxin-mitigating product
Challenged group:	challenge	no mycotoxin-mitigating product
Challenge + Solis Max 2.0	challenge	Solis Max 2.0, 1kg/t

The body weight and FCR performance parameters were measured, as well as the blood parameters of alanine aminotransferase and aspartate aminotransferase, both related to liver damage when increased.

Concerning performance as well as liver health, the trial showed partly even better results for the challenged group fed with Solis Max 2.0 than for the negative, unchallenged control (Figures 6 and 7):

- 6% higher body weight than the negative control and 18.5% higher body weight than the challenged group
- 12 points and 49 points better FCR than the negative control and the challenged group, respectively

• Lower levels of AST and ALT compared to the challenged group, showing a better liver health

The values for body weight, FCR, and AST, even better than the negative control, may be owed to the content of different gut and liver health-supporting phytomolecules.



Figure 6: Better performance data due to the addition of Solis Max 2.0



Figure 7: Healthier liver shown by lower values of AST and ALT

Effective toxin risk management: staying power is required

Mycotoxin mitigation requires many different approaches. Mycotoxin mitigation starts with sewing the appropriate plants and continues up to the post-ingestion moment. From various studies and field experience, we find that besides the right decisions about grain crops, storage management, and hygiene, the use of effective products which mitigate the adverse effects of mycotoxins is the most practical and effective way to maintain animals healthy and well-performing. According to Eskola and co-workers (2020), the worldwide contamination of crops with mycotoxins can be up to 80% due to the impact of climate change and the availability of sensitive technologies for analysis and detection. Using a proper mycotoxin mitigation program as a precautionary measure is, therefore, always recommended in animal production.

Toxin Risk Management



EW Nutrition's Toxin Risk Management Program supports farmers by offering a tool (<u>MasterRisk</u>) that helps identify and evaluate the risk and gives recommendations concerning using toxin solutions.

Price hikes = more cereal byproducts in animal feed. What about mycotoxin risk?



By Marisabel Caballero, Global Technical Manager Poultry, EW Nutrition

Most grains used in feed are susceptible to <u>mycotoxin contamination</u>, causing severe economic losses all along feed value chains. As skyrocketing raw material prices force producers to

include a higher proportion of economical cereal byproducts in the feed, the risks of mycotoxin contamination likely increase. In this article, we review why mycotoxins cause the damage they do - and how effective toxin-mitigating solutions prevent this damage.



Mycotoxin contamination of cereal byproducts requires solutions

Cereal byproducts may become more important feed ingredients as grain prices increase. But also from a sustainability point of view and considering population growth, using cereal byproducts in animal feed <u>makes a lot of sense</u>. Dried distiller's grains with solubles (DDGS) are a good example of how byproducts from food processing industries can become <u>high-quality animal feed</u>.



Figure 1: Byproducts are a crucial protein source (data from <u>FEFAC Feed & Food 2021</u> report)

Still, research on what happens to mycotoxins during food processing shows that mycotoxins are concentrated into fractions that are commonly used as animal feed (cf. <u>Pinotti et al., 2016 + link to article IH+MC</u>). To safeguard animal health and performance when feeding lower-quality cereals, it is essential to monitor mycotoxin risks through regular testing and to use <u>toxin-mitigating solutions</u>.

Problematic effects of mycotoxins on the intestinal epithelium

Most mycotoxins are absorbed in the proximal part of the gastrointestinal tract. This absorption can be high, as in the case of aflatoxins (ca. 90%), but also very limited, as in the case of fumonisins (< 1%); moreover, it depends on the species. Importantly, a significant portion of unabsorbed toxins remains within the lumen of the gastrointestinal tract.

Importantly, studies based on realistic mycotoxin challenges (e.g., <u>Burel et al., 2013</u>) show that the mycotoxin levels necessary to trigger damaging processes are lower than the <u>levels reported as safe</u> by EFSA, the Food Safety Agency of the European Union. The ultimate consequences range from diminished nutrient absorption to inflammatory responses and pathogenic disorders in the animal (Figure 2).



Figure 2: Mycotoxins' impact on the GIT and consequences for monogastric animals

1. Alteration of the intestinal barrier's morphology and functionality

Several studies indicate that mycotoxins such as aflatoxin B1, DON, fumonisin B1, ochratoxin A, and T2, can increase the permeability of the intestinal epithelium of poultry and swine (e.g. <u>Pinton & Oswald, 2014</u>). This is mostly a consequence of the inhibition of protein synthesis.

As a result, there is an increase in the passage of antigens into the bloodstream (e.g., bacteria, viruses, and toxins). This increases the animal's susceptibility to infectious enteric diseases. Moreover, the damage that mycotoxins cause to the intestinal barrier entails that they are also being absorbed at a higher rate.

2. Impaired immune function in the intestine

The intestine is a very active immune site, where several immuno-regulatory mechanisms simultaneously defend the body from harmful agents. <u>Immune cells are affected by mycotoxins</u> through the initiation of apoptosis, the inhibition or stimulation of cytokines, and the induction of oxidative stress.

For poultry production, one of the most severe enteric problems of bacterial origin is <u>necrotic</u> <u>enteritis</u>, which is caused by *Clostridium perfringens* toxins. Any agent capable of disrupting the gastrointestinal epithelium – e.g. mycotoxins such as DON, T2, and ochratoxin – promotes <u>the</u> <u>development of necrotic enteritis</u>.

3. Alteration of the intestinal microflora



Recent studies on the effect of various

mycotoxins on the intestinal microbiota show that <u>DON and other trichothecenes favor the</u> <u>colonization of coliform bacteria in pigs</u>. DON and ochratoxin A also induce a <u>greater invasion of</u> <u>Salmonella</u> and their translocation to the bloodstream and vital organs in birds and pigs – even at non-cytotoxic concentrations.

It is known that fumonisin B1 may induce changes in the balance of sphingolipids at the cellular level, including for gastrointestinal cells. This facilitates the adhesion of pathogenic bacteria, increases in their populations, and prolongs infections, <u>as has been shown for the case of *E. coli*</u>. The colonization of the intestine of food-producing animals by pathogenic strains of *E. coli* and *Salmonella* also poses a risk for human health.

4. Interaction with bacterial toxins

When mycotoxins induce changes in the intestinal microbiota, this can lead to an increase in the endotoxin concentration in the intestinal lumen. <u>Endotoxins promote the release of several</u> <u>cytokines</u> that induce an enhanced immune response, causing inflammation, thus reducing feed consumption and animal performance, damage to vital organs, sepsis, and death of the animals in some cases.

The synergy between mycotoxins and endotoxins can result in an overstimulation of the immune system. The interaction between endotoxins and estrogenic agents such as zearalenone, for example, generates <u>chronic inflammation and autoimmune disorders</u> because immune cells have estrogen receptors, which are stimulated by the mycotoxin.

Increased mycotoxin risks through byproducts? Invest in mitigation solutions

To prevent the detrimental consequences of mycotoxins on animal health and performance, proactive solutions are needed that support the intestinal epithelium's digestive and immune functionality and help maintain a balanced microbiome in the GIT. As the current market conditions will likely engender a long-term shift towards the inclusion of more cereal byproducts in animal diets, this becomes even more important.

Trial data shows that EW Nutrition's toxin-mitigating solution SOLIS MAX provides effective protection against feedborne mycotoxins. The synergistic combination of ingredients in SOLIS MAX mycotoxins from damaging the animals' gastrointestinal tract and entering the blood stream:



In-vitro study shows SOLIS MAX' strong mitigation effects against wide range of mycotoxins

Animal feed is often contaminated with two or more mycotoxins, making it important for an anti-mycotoxin agent to be effective against a wide range of different mycotoxins. A dose response evaluation of SOLIS MAX was conducted a at an independent laboratory in Spain, for inclusion levels of 0.10%, 0.15%, and 0.20% (equivalent to 1 kg, 1.5 kb, and 2 kg per ton of feed). A phosphate buffer solution at pH 7 was prepared to simulate intestinal conditions in which a portion of the mycotoxins may be released from the binder (desorption).

Mycotoxin	Challenge type	
Aflatoxin B1	800	
DON	800	
Fumonisin B1	2000	
T-2	300	
Ochratoxin A	400	
ZEA	1200	

Each mycotoxin was tested separately by adding a challenge to buffer solutions, incubating for one hour at 41°C, to establish the base line (see table). At the same time a solution with the toxin challenge and SOLIS MAX was prepared, incubated, and analyzed for the residual mycotoxin. All analyses were carried out by high performance liquid chromatography (HPLC) with standard detectors.



Figure 3: SOLIS MAX adsorption capacity against different mycotoxins (%)

The results demonstrate that SOLIS MAX is a very effective solution against the most common mycotoxins found in raw materials and animal feed, showing clear dose-response effects.

Mycotoxin risk management for better animal

feed

A healthy gastrointestinal tract is crucial to animals' overall health: it ensures that nutrients are optimally absorbed, it provides effective protection against pathogens through its immune function, and it is key to maintaining a well-balanced microflora. Even at levels considered safe by the European Union, mycotoxins can compromise different intestinal functions, resulting in lower productivity and susceptibility to disease.

The globalized feed trade, which spreads mycotoxins beyond their geographical origin, climate change and raw material market pressures only escalates the problem. On top of rigorous testing, producers should mitigate unavoidable mycotoxin exposures through the use of solutions such as SOLIS MAX – for stronger animal health, welfare, and productivity.

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