Mycotoxins in layer and breeder feed impact hens, eggs, hatchery, and chicks

By Technical Team EW Nutrition

As the planet's climate experiences changes, new patterns affect the microbial communities colonizing crops. Recently, several areas of the planet have experienced extreme temperatures, drought, changes in the humid/dry cycles, and an increase in atmospheric carbon dioxide (1,2). As a response, the fungi affecting the crops have shifted their geographical distribution, and with this, the pattern of mycotoxin occurrence also changed. For instance, in Europe, we are looking at higher frequencies and levels of Aflatoxins (AF), Ochratoxins (OT), and Fumonisins (FUM) than ten or even five years ago (2-4).

This affects animal production, as mycotoxin challenges show increased frequency, quantity, and variety. Mainly long-living animals, such as laying hens and breeders, can have a higher risk. Moreover, mycotoxins can also be carried over to the eggs, potentially risking human health in the case of layers (table eggs) and in the case of breeder hens, hatchery performance and day-old chick (DOC) quality.

Laying hens and breeders: carryover of

mycotoxins into eggs

Most mycotoxins are absorbed in the proximal part of the gastrointestinal tract (Table 1). This absorption can be high, as in the case of aflatoxins (~90%), but also very limited, as in the case of fumonisins (<1%), with a significant portion of unabsorbed toxins remaining within the lumen of the gastrointestinal tract (5).

Once mycotoxins are ingested, detoxification and excretion processes are started by the body, and at the same time, organ damage ensues. The detoxification of mycotoxins is mainly carried out by the liver (6), and their accumulation happens primarily in the liver and kidneys. However, accumulation in other tissues, such as the reproductive organs and muscles, has also been found (7-9). The detoxification process' objective is the final excretion of the toxins, which occurs through urine, feces, and bile; often, the toxins can also reach the eggs (7-20).

Table 1: mycotoxin absorption rates for poultry and their carry-over rate into eggs

Mycotoxin	Main absorption sites	Absorption rate in poultry	Carry-over rate into eggs
Aflatoxins	Duodenum, jejunum	≈90%	≈0.55%
DON	Duodenum, jejunum	≈20%	≈0.001%
Fumonisins	Duodenum, jejunum	≈1%	≈0.001%
Ochratoxin	Jejunum	≈40%	≈0.15%
T-2	Duodenum, jejunum	≈20%	≈0.10%
Zearalenone	Small & large intestine	≈10%	≈0.30%

(Adapted from 5, 7-17, 19-21)

Table 1 shows carry-over rates of mycotoxins into eggs, resulting from diverse studies (7-10, 14, 16, 19). However, the same studies indicate that results can vary broadly due to different factors, as reviewed by Völkel and collaborators (26). This variability is related to the amount and source of contamination, way of application, period, and the possible co-occurrence of various mycotoxins or several metabolites. Other factors to consider are animal-related, such as species, breed, sex, age group, production level, and health status. Environmental and management factors can play a role in carry-over rates, and finally, detection limits and analytical procedures also influence these results. In summary, highly varying carry-over has been demonstrated, and the risk needs to be considered when animals are exposed.

Mycotoxins in breeder's feed impact hatchery performance and day-old chick quality

When hens are exposed to mycotoxins, their effects on the intestine, liver, and kidney decrease egg production and quality (10, 14, 27), and, in the case of breeders, consequently, affect hatchery performance, DOC production, and DOC quality (28-30). The main effects of mycotoxins, when we speak about DOC production, are exerted in the gastrointestinal tract, the liver, and the kidneys, affecting embryos and young chicks:

- **Intestine and kidneys**: Mycotoxins harm the intestinal epithelium and have nephrotoxic effects, affecting calcium and vitamin D3 absorption and metabolism, necessary for eggshell quality (31). Thin and fragile shells can increase embryonic mortality, lower embryonic weight gain, and hinder hatchability (32).
- **Liver:** The liver plays a central role in egg production as it is responsible for vitamin D3

metabolism, the production of nutrient transporters, and the synthesis of the lipids that make up the yolk. Thus, when liver function is impaired, the internal and external quality of the egg declines, which affects DOC production (31-34).

- **Embryo and young chicks:** Studies (33-38) have found how mycotoxins affect the embryos. In general, there are two possibilities: the direct one, when the mycotoxin is transferred into the egg, and the indirect one, when the mycotoxin impacts egg quality and, therefore, leads to disease or death of the embryo. The result is a higher embryonic mortality or lower DOC quality. These, among others, result from the lower transfer of antioxidants and antibodies from the hen, low viability of the chick's immune cells, and higher bacterial contamination. A lower relative weight of the bursa of Fabricio and the thymus is often found.

Qreshi's team (29) studied the effects on the progeny of broiler breeders consuming feed highly contaminated with AFB1, finding suppression in antibody production and macrophage function in chicks after ten days. Similar results were found by other researchers (36, 37) evaluating the effects of AF and OTA as single and combined contamination. When both mycotoxins are present in the feed, the effect on hatchability and DOC quality are synergistic.

Due to mycotoxin contamination, the reproduction and immune response are impaired, resulting in decreased DOC production and increased early chick mortality, as they are more susceptible to bacterial and viral infections.

Mycotoxins impair table egg production and quality

Studies (22-24) have found mycotoxin contamination in commercial table eggs. A meta-analysis of mycotoxins' concentration based on 11 published papers was completed recently (22): counting with data from 9509 samples, the meta-analysis reveals an overall presence of mycotoxins in 30% of the samples, being Beauvericin in the first place, followed by DON as well as AF and OTA in third and fourth place, respectively. The risk for humans depends on the intake of contaminated foods in terms of amount and frequency (25), and so far, it has not been estimated in most parts of the world.

Natural contamination in laying hens: a case report

Giancarlo Bozzo's team (39) reported and published a veterinary case regarding natural mycotoxin contamination in commercial egg production: up to week 47 of age, production parameters were on top of the genetic standards. However, a drop in egg production started at around week 47, and at week 50, egg production was only 68% (figure 1).

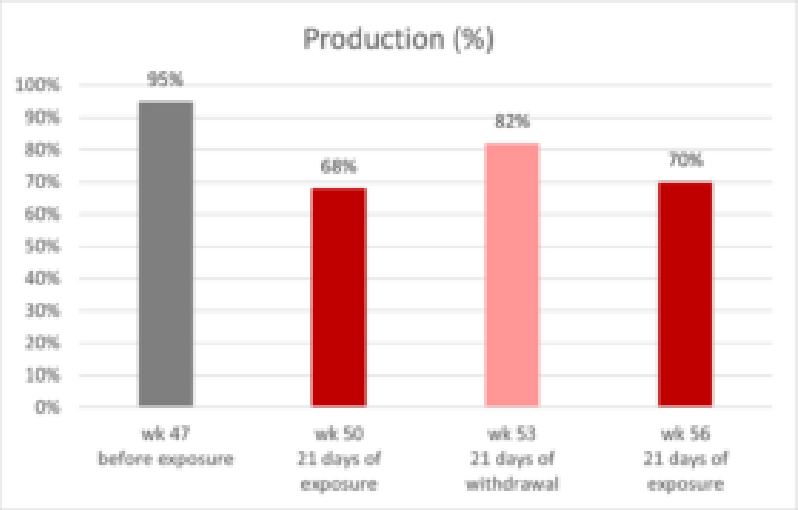


Figure 1: production of laying hens fed naturally contaminated feed with AFB1 and OTA

The house with the reduced performance received feed with linseed. In other houses of the same complex, which did not include linseed in the feed, production

was unaffected. Therefore, this raw material was considered a possible cause of the issue. Linseed was removed from the formula, and three weeks after (53 weeks of age), egg production was at 84%. Afterward, linseed got back into the formulation, and the laying rate dropped again to 70% (week 56), this time accompanied by a significant increase in mortality.

Samples were collected at week 56, and AFB1 and OTA were detected in feed and the kidneys and livers of the hens consuming it (table 2). While the levels in the feed were not considered high risk, evidence from necropsy and histopathology suggested either a higher or a prolonged exposure; a synergistic effect of both mycotoxins on hen's health and productivity can be inferred.

Table 2: mycotoxin analysis results for feed and organs

HPLC analysis results in samples of:				
toxin	Feed 1 (n=5)	Feed 2 (n=5)	Kidney (n=10)	Liver (n=10)
OTA	1.1 ± 0.1 ppb	31 ± 3 ppb	47 ± 3 ppb	24 ± 2 ppb
AFB1	ND	5.6 ± 0.3 ppb	1.4 ± 0.3 ppb	3.6 ± 0.4 ppb

The liver and kidneys were enlarged and showed signs of damage. Furthermore, urate crystals in the peritoneum and the abdominal air sac were observed, indicating renal failure. This limited the excretion of both toxins in the urine, increasing their half-life in the organism and enhancing the effects in target organs, contributing to the synergistic effect observed.

After using mycotoxin-free certified linseed, the problem receded. Though this is the best option to keep animals healthy and productive, it may not be practical in the long term due to the ubiquitous nature of the toxins and the cost and availability constraints of feed raw materials. Moreover, the mycotoxin levels present in the feed were relatively low and fell under recommended guidelines. For these reasons, in-feed toxin mitigation solutions must also be considered to reduce exposure for production animals.

In-feed intervention mitigates the effects of intermittent exposure to multiple mycotoxins

EW Nutrition conducted a study with Hy-Line W-36 layer-breeders intercalating three 10-day cycles of feed with 100ppb AFB1 + 100ppb OTA, with two 21-day cycles of non-challenged feed. An in-feed intervention (Solis Max 2.0, displayed as IFI) containing bentonite, yeast cell wall components, and a mixture of phytogenic components mitigated all effects.

Table 3: experimental groups and mycotoxin challenge

Treatment	Group	100 ppb AFB1+ 100 ppb OTA	IFI (2 kg/ton)
T-1	Control (C)		
T-2	C+IFI		X
T-3	Challenge (Ch)	X	
T-4	Ch+IFI	X	X

Trial design:

A total of 576 hens (18 replicates per diet, 8 hens each) and 58 roosters were randomly assigned to four diets at 28 weeks of age, as shown in Table 3. The 72-day experimental period included alternating 10-day challenge and 21-day non-challenge intervals (Figure 2). During the challenge intervals, the breeders in T-3 and T-4 were fed the mycotoxin-contaminated feed with and without the IFI.

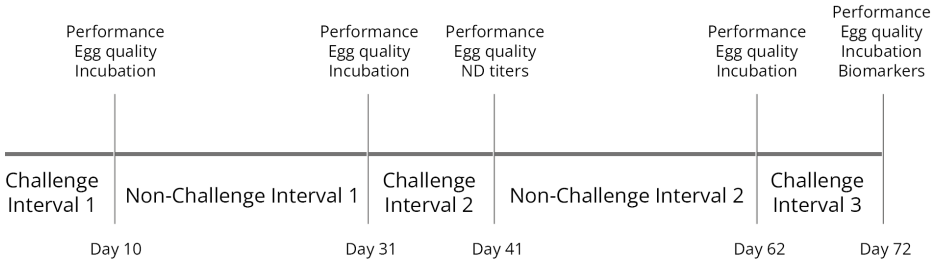
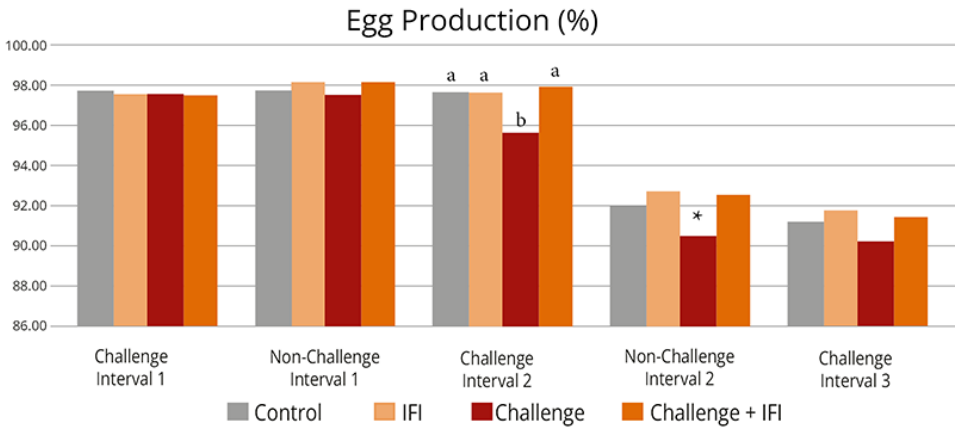


Figure 2: trial timeline showing challenge and non-challenge intervals and days of data collection and sampling.

Mitigated effects on egg production and egg quality

The challenge decreased overall egg production (Figure 3), egg mass, and shell thickness (Table 4). The first challenge interval did not affect production, but days later, from the first non-challenge period, all parameters were lower for the challenged group.



Different letters indicate significant differences ($p<0.05$). Statistical tendencies ($p<0.1$) are indicated by (*).

***Figure 3**: Egg production of hens intermittently challenged with AFB1 and OTA, with and without in-feed Solis Max*

The adverse effects on productivity and egg quality started after the first challenged feed was withdrawn and persisted through the following intervals until the end of the experiment. Similar effects in chronic mycotoxin challenges have been previously found (37, 39).

***Table 4**: Average egg quality parameters of hens intermittently challenged with AFB1+OTA, with and without an in-feed intervention (IFI)*

Group	Eggshell strength (N)	Eggshell thickness (mm)	Haugh Units
Control	21,02^{a}	0,3661^{ab}	70,88
IFI	21,16^{a}	0,3702^{a}	71,68
Challenge	20,05^{b}	0,3630^{b}	70,07*
Ch+IFI	21,06^{a}	0,3698^{a}	71,06

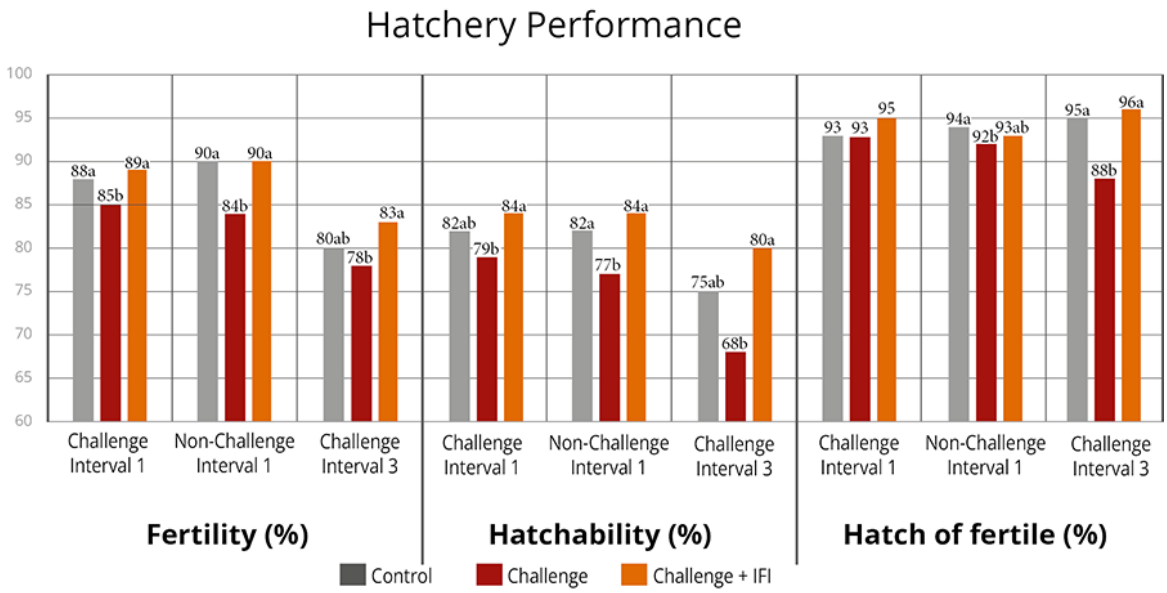
Different letters indicate significant differences ($p<0.05$). Statistical tendencies ($p<0.1$) are indicated by (*).

Mitigated effects on the progeny in incubation

trials

Three incubation trials were performed: after the first challenge and non-challenge interval and at the end of the trial period after the third challenge interval. A significant decrease in fertility and hatchability was observed for the challenged group in all incubation trials. As mycotoxins affect egg quality (22-24) and can be transferred to the eggs (10, 14, 27), the effects were also shown in the case of hatchability and offspring performance. Fertility was affected from the first challenge interval onwards, continuing to be low for the challenge group until the end of the trial. However, the hatchability of fertile eggs dropped after the withdrawal of the contaminated feed and showed the lowest value during the third challenge interval.

The in-feed supplementation of Solis Max 2.0 (IFI) resulted in the consistent recovery of egg production and egg quality throughout the whole experimental period, achieving the same levels of productivity as the non-challenged control.



Letters indicate significant differences ($p<0.05$). Statistical tendencies ($p<0.1$), indicated by (*).

Figure 4: Hatchery parameters of eggs from breeders intermittently challenged with AFB1 and OTA, with and without an in-feed intervention (IFI).

Results in hatch of fertile can be related to egg quality, as the thickness of the eggshell influences the egg's moisture loss and exchange with the environment during the incubation period. Thinner eggshells lead to higher embryo mortality (31, 32). The group having the challenge with Solis Max showed the same performance as the non-challenged control regarding hatchery performance.

Day-old chick weight was not affected. However, weight gain and mortality after ten days were hindered for the chicks from breeders taking the mycotoxin-contaminated feed (Table 5).

***Table 5**: Average day- and 10-day-old chick parameters from hens intermittently challenged with AFB1+OTA, with and without an in-feed intervention (IFI)*

Parameter	Control	Challenge	Ch + IFI
DOC body weight (g)	36,67	36,24	36,80
10-day body weight (g)	76,30[a]	75,94[b]	79,50[a]
10-day mortality (%)	0,94	1,26	0,97

Letters indicate significant differences ($p<0.05$). Statistical tendencies ($p<0.1$) indicated by (*)

At the end of the experiment, oxidative stress biomarkers were measured in the blood serum of 15 hens per treatment, showing significantly lower GPx, and SOD (figure 5) in the challenged group, which indicates a depletion of the mechanisms to fight oxidative stress (40), the hens taking the in-feed product

did not show this depletion.

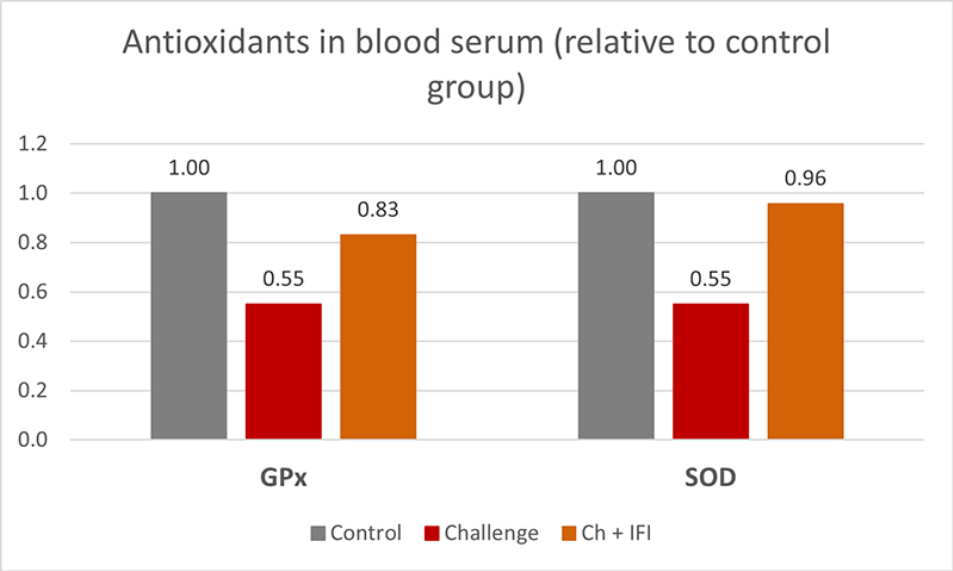


Figure 5: Antioxidants in blood serum, glutathione peroxidase (GPx), and superoxide dismutase (SOD) from breeders intermittently challenged with AFB1 and OTA, with and without an in-feed intervention (IFI).

Intermittent exposure to AFB1 and OTA negatively affected layer breeder productivity, egg quality, and hatchability and promoted oxidative stress in the birds. Intermittent mycotoxin challenges may affect animals even after the contamination is withdrawn. In-feed interventions showed effectiveness in mitigating these effects.

Climate changes bring new mycotoxin challenges – the right in-feed solutions can help

Today's mycotoxin scenario shows increased frequency, quantity, and variety. Mainly long-living animals, such as laying hens and breeders, can be at more risk. Additionally, the contamination can be carried over to the eggs, potentially risking human health in the case of table eggs and hatchery performance and DOC quality in the case of breeders.

From case reports, we learn the consequences of real challenges and struggles in commercial production; from scientific trials based on possible commercial situations, we realize the advantages of interventions designed to tackle those challenges.

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Masked mycotoxins – particularly dangerous for dairy cows

*By **Si-Trung Tran**, SEAP Regional Technical Manager, EW Nutrition*

***Technical Team** and **Dr. Inge Heinzl**, Editor, EW Nutrition*

Mycotoxins are secondary metabolites of fungi, commonly found as contaminants in agricultural products. In some cases, these compounds are used in medicine or industry, such as penicillin and patulin. In most cases, however, they are considered xenobiotics that are toxic to animals and humans, causing the disease collectively known as mycotoxicosis. The adverse effects of mycotoxins on human and animal health have been documented in many publications. Aflatoxins (AFs) and deoxynivalenol (DON, vomitoxin) are amongst the most critical mycotoxins affecting milk production and -quality.

Aflatoxins do not only affect cows

Aflatoxins (AFs) are highly oxygenated, heterocyclic difuranocoumarin compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They colonize crops, including many staple foods and feed ingredients. Within a group of over 20 AFs and derivatives, aflatoxin B1 (AFB1), B2, G1, and G2 are the most important naturally occurring compounds.

Among the aflatoxins, AFB1 is the most widespread and most toxic to humans and animals. Concern about mycotoxin contamination in dairy products began in the 1960s with the first reported cases of contamination by aflatoxin M1 (AFM1), a metabolite of AFB1 formed in the liver of animals and excreted in the milk.

There is ample evidence that lactating cows exhibit a significant reduction in feed efficiency and milk yield

within a few days of consuming aflatoxin-contaminated feed. At the cellular level, aflatoxins cause degranulation of endoplasmic membranes, loss of ribosomes from the endoplasmic reticulum, loss of nuclear chromatin material, and altered nuclear shapes. The liver, as the organ mainly dealing with the decontamination of the organism, gets damaged, and performance drops. Immune cells are also affected, reducing immune competence and vaccination success (Arnold and Gaskill, 2023).

DON reduces cows' performance

Another mycotoxin that can also reduce milk quality and affect metabolic parameters, as well as the immune function of dairy cows, is DON. DON is produced by different fungi of the *Fusarium* genus that infect plants. DON synthesis is associated with rainy weather from crop flowering to harvest. Whitlow and co-workers (1994) reported the association between DON and poor performance in dairy herds and showed decreased milk production in dairy cows fed 2.5 mg DON/kg. However, in cows fed 6 to 12 mg DON/kg dry matter for 10 weeks, no DON or its metabolite DOM-1 residues were detected in milk.

Masked mycotoxins hide themselves during analysis

Plants suffering from fungal infestations and thus confronted with mycotoxins convert the harmful forms of mycotoxins into less harmful or harmless ones for themselves by conjugation to sulfates, organic acids, or sugars. Conjugated mycotoxins cannot always be detected by standard analytical methods. However, in animals, these forms can be released and transformed into parent compounds by enzymes and microorganisms in the gastrointestinal tract. Thus, the feed may show a concentration of mycotoxins that is still below the limit value, but in the animal, this concentration is suddenly much higher. In dairy cows, the release of free mycotoxins from conjugates during digestion may play an important role in understanding the silent effects of mycotoxins.

Fusarium toxins, in particular, frequently occur in this "masked form". They represent a serious health risk for animals and humans.

Aflatoxins first show up in the milk

Masked aflatoxins may also play a role in total aflatoxin contamination of feed materials. Research has harvested little information on masked aflatoxins that may be present in TMR ingredients. So far, metabolites such as Aflatoxin M2 have been identified (Righetti, 2021), which may reappear later in milk as AFM1.

DON-related symptoms without DON?

Sometimes, animals show DON-related symptoms, with low levels detected in the feed or raw materials. Besides sampling errors, this enigma could be due to conjugated or masked DON, which is structurally altered DON bound to various compounds such as glucose, fatty acids, and amino acids. These compounds escape conventional feed analysis techniques because of their modified chemical properties but can be released as their toxic precursors after acid hydrolysis.

Masked DON was first described in 1984 by Young and co-workers, who found that the DON content of yeast-fermented foods was higher than that of the contaminated wheat flour used in their production. The most plausible reason for this apparent increase was that the toxin from the wheat had been converted to a compound other than DON, which could be converted back to DON under certain conditions. Since this report, there has been much interest in conjugated or masked DON.

Silage: masked DON is a challenge for dairy producers

Silage is an essential feed for dairy cows, supporting milk production. Most silage is made from corn and other grains. The whole green plant is used, which can be infected by fungi. Since infection of corn with Fusarium spp. and subsequent DON contamination is usually a major problem in the field worldwide, a relatively high occurrence of this toxin in silage must be expected. The ensiling process may reduce the amount of Fusarium fungi, but the DON formed before ensiling is very stable.

Silage samples show DON levels of concern

It is reasonable to assume that the DON biosynthesized by the fungi was metabolized by the plants to a new compound and thus masked DON. Under ensiling conditions, masked DON can be hydrolyzed, producing free DON again. Therefore, the level of free DON in the silage may not reflect the concentration measured in the plants before ensiling.

A study analyzed 50 silage samples from different farms in Ontario, Canada. Free DON was found in all samples, with levels ranging from 0.38 to 1.72 µg/g silage (unpublished data). Eighty-six percent of the samples contained DON at concentrations higher than 0.5 µg/g. Together with masked DON, it poses a potential threat to dairy cattle.

Specific hydrolysis conditions allow detection

However, in the natural ensiling process, the conditions for hydrolysis of masked DON are not optimal. The conditions that allow improved analysis of masked DON were recently described. This method detected masked DON in 32 of 50 silage samples (64%) along with free DON, increasing DON concentration by 23% in some cases (unpublished data).

Mycotoxins impact humans and animals

Aflatoxins, as well as DON, have adverse effects. In the case of DON, the impact on the animal is significant; in the case of aflatoxin, the possible long-term effects on humans are of higher relevance.

DON has more adverse effects on the animal and its performance

Unlike AFs, DON may be found in milk at low or trace concentrations. It is more associated with negative effects in the animal, altered rumen fermentation, and reduced flow of usable protein into the duodenum. For example, milk fat content was significantly reduced when cows were fed 6 µg DON/kg. However, the presence of DON also indicates that the feed probably contains other mycotoxins, such as zearalenone (ZEA) (estrogenic mycotoxin) and fusaric acid (pharmacologically active compound). All these mycotoxins may interact to cause symptoms that are different or more severe than expected, considering their individual effects. DON and related compounds also have immunosuppressive effects, resulting in increased somatic cell counts in milk. The U.S. FDA has established an action level for DON in wheat and wheat-derived products intended for cows, which is 5µg DON/g feed and the contaminated ingredient must not exceed 40% of the ration.

Aflatoxins decrease milk quality and pose a risk to humans

Aflatoxins are poorly degraded in the rumen, with aflatoxicol being the main metabolite that can be reconverted to AFB1. Most AFs are absorbed and extensively metabolized/hydrolyzed by enzymes found mainly in the liver. This results in the formation of AFM1, a part of which is conjugated to glucuronic acid and subsequently excreted in the bile. The other part enters the systemic circulation. It is either excreted in urine or milk. AFM1 appears within 12-48 hours after ingestion in cow’s milk. The excreted amount of AFM1 in milk from dairy cows usually ranges from 0.17% to 3% of the ingested AFB1. However, this carryover rate may vary from day to day and from one milking to the next in individual animals, as it is influenced by various factors, such as feeding regime, health status, individual biotransformation capacity, and, of course, by actual milk production. Carryover rates of up to 6.2% have been reported in high-yielding dairy cows producing up to 40 liters of milk per day.

In various experiments, AFM1 showed both carcinogenic and immunosuppressive effects. Accordingly, the International Agency for Research on Cancer (IARC) classified AFM1 as being in Group 2B and, thus, possibly carcinogenic in humans. The action level of 0.50 ppb and 0.05 ppb for AFM1 in milk is strictly adhered to by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), respectively.

Trials show the high adsorption capacity of Solis Max

A trial was conducted at an independent laboratory located in Spain. The evaluation of the performance of Solis Max was executed with the following inclusion levels:

- 0.10% equivalent to 1.0 kg of Solis Max per ton of feed
- 0.20% equivalent to 2.0 kg of Solis Max per ton of feed

A phosphate buffer solution at pH 7 was prepared for the trial to simulate rumen conditions. Each mycotoxin was tested separately, preparing solutions with known contamination (final concentration described in the table below). The contaminated solutions were divided into 3 parts: A positive control, 0.10% Solis Max and 0.20% Solis Max. All samples were incubated at 41°C for 1 hour, centrifuged, and the supernatant was analyzed for the mycotoxin added to determine the binding efficacy. All analyses were carried out by high-performance liquid chromatography (HPLC) with standard detectors.

Mycotoxin	Contamination Level (ppb)
Aflatoxin B1	800

DON	800
Fumonisin B1	2000
ZEA	1200

Results:

The higher concentration of Solis max showed a higher adsorption rate for most mycotoxins. The high dose of Solis Max adsorbed 99% of the AFB1 contamination. In the case of DON, more than 70% was bound. For fumonisin B1 and zearalenone, Solis max showed excellent binding rates of 87.7% and 78.9%, respectively (Figure 1).

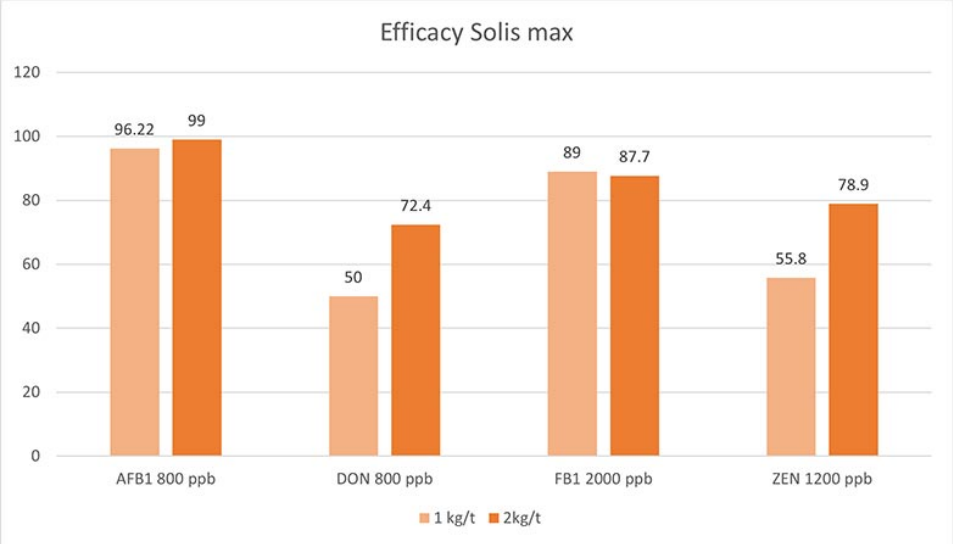


Figure 1: Solis Max showed a high binding capacity for the most relevant mycotoxins

Another trial was conducted at an independent laboratory serving the food and feed industry and located in Valladolid, Spain.

All tests were carried out as duplicates and using a standard liquid chromatography/mass spectrometry (LC/MS/MS) quantification. Interpretation and data analysis were carried out with the corresponding software. The used pH was 3.0, toxin concentrations and anti-mycotoxin agent application rates were set as follows (Table 1):

Mycotoxin	Challenge level	Challenge (ppb)	Solis Plus 2.0 inclusion	Assay time
Aflatoxin	Low	150	0.2%	30 min.
	High	1500	0.2%	30 min.
Fumonisin	Low	500	0.2%	30 min.
	High	5000	0.2%	30 min.
Ochratoxin	Low	150	0.2%	30 min.
	High	1500	0.2%	30 min.

Table 1: Trial set-up testing the binding capacity of Solis Plus 2.0 for several mycotoxins in different contamination levels

Results:

Under acidic conditions (pH3), Solis Plus 2.0 effectively adsorbs the three tested mycotoxins at low and high levels. 100% binding of aflatoxin was achieved at a level of 150ppb and 98% at 1500ppb.In the case of fumonisin, 87% adsorption could be reached at 500ppb and 86 for a challenge with 5000ppb. 43% ochratoxin was adsorbed at the contamination level of 150ppb and 52% at 1500ppb.

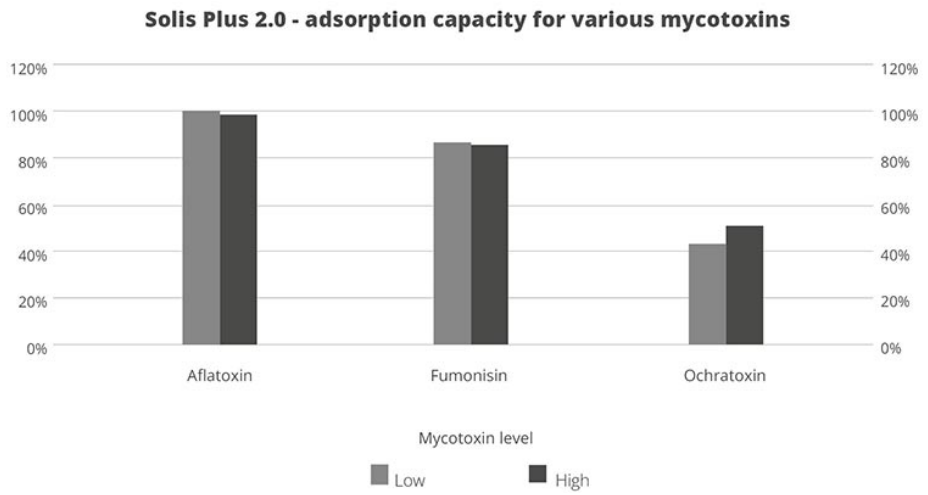


Figure 2: The adsorption capacity of Solis Plus 2.0 for three different mycotoxins at two challenge levels

Mycotoxins – Effective risk management is of paramount importance

Although the rumen microflora may be responsible for conferring some mycotoxin resistance to ruminants compared to monogastric animals, there are still effects of mycotoxins on rumen fermentation and milk quality. In addition, masked mycotoxins in feed present an additional challenge for dairy farms because they are not readily detectable by standard analyses.

Feeding dairy cows with feed contaminated with mycotoxins can lead to a reduction in milk production. Milk quality may also deteriorate due to an adverse change in milk composition and mycotoxin residues, threatening the innocuousness of dairy products. Dairy farmers should therefore have feed tested regularly, consider masked mycotoxins, and take action. EW Nutrition's MasterRisk tool provides a risk evaluation and corresponding recommendations for the use of products that mitigate the effects of mycotoxin contamination and, in the end, guarantee the safety of all of us.

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.

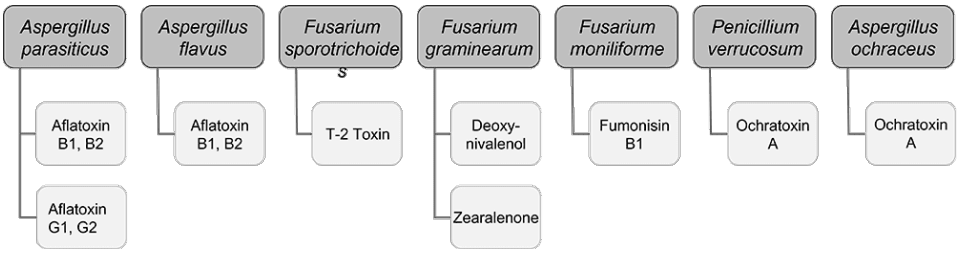


Figure 1: Fungi species and their mycotoxins of worldwide importance for poultry production (adapted from Bryden, 2012).

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 ≥3

Even at low concentrations, mycotoxins can display synergistic effects, 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.

Table 1: Synergistic effects of mycotoxins in poultry

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

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 (Devegowda and Murthy, 2005). 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.

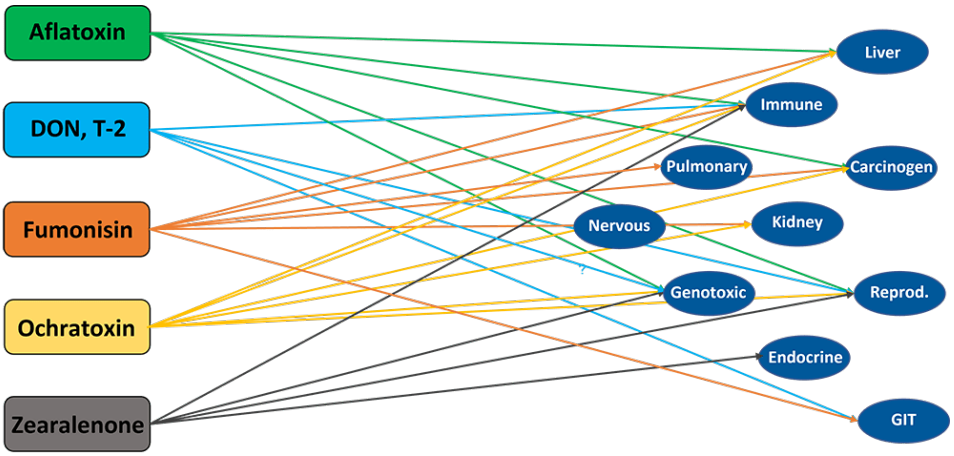


Figure 2: Main target organs of important mycotoxins

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 (Galvano et al., 2001).

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).

- Zeolites: 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 (Raju and Devegowda 2000).

Bacteria: In some studies, 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:

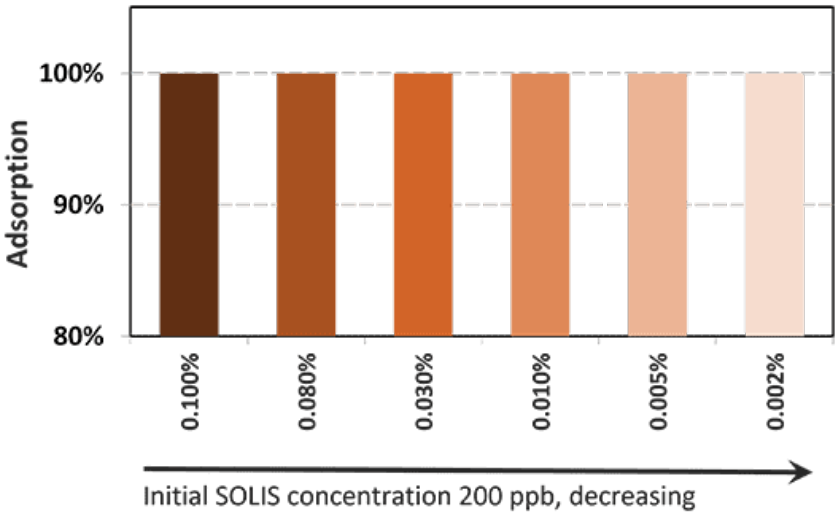


Figure 3: Binding capacity of Solis for Aflatoxin

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.

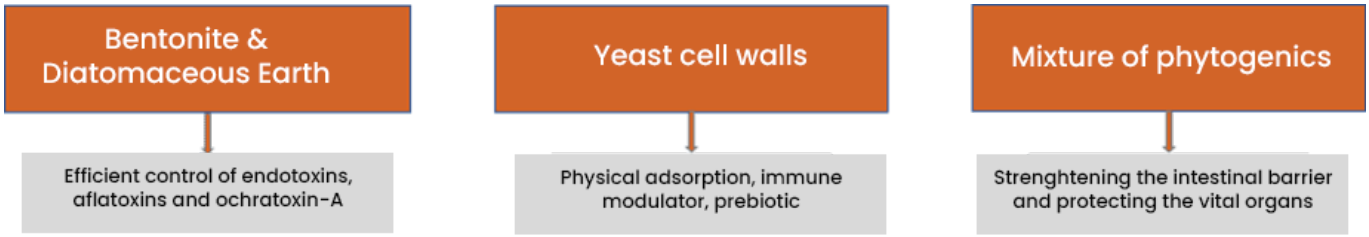


Figure 4: Composition and effects of Solis Max 2.0

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.

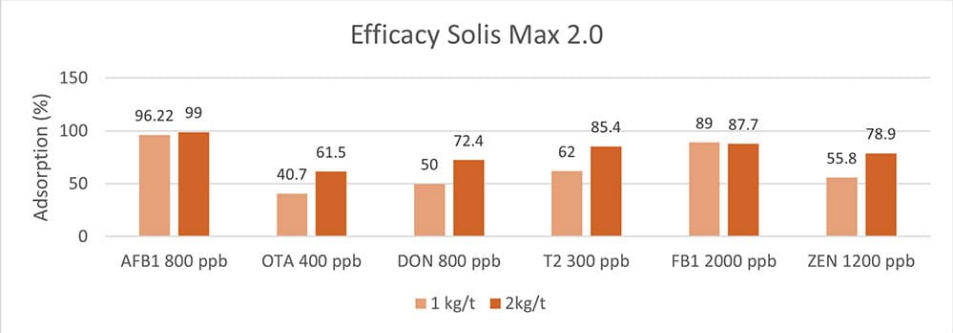


Figure 5: Efficacy of Solis Max 2.0 against different mycotoxins relevant in poultry production

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.

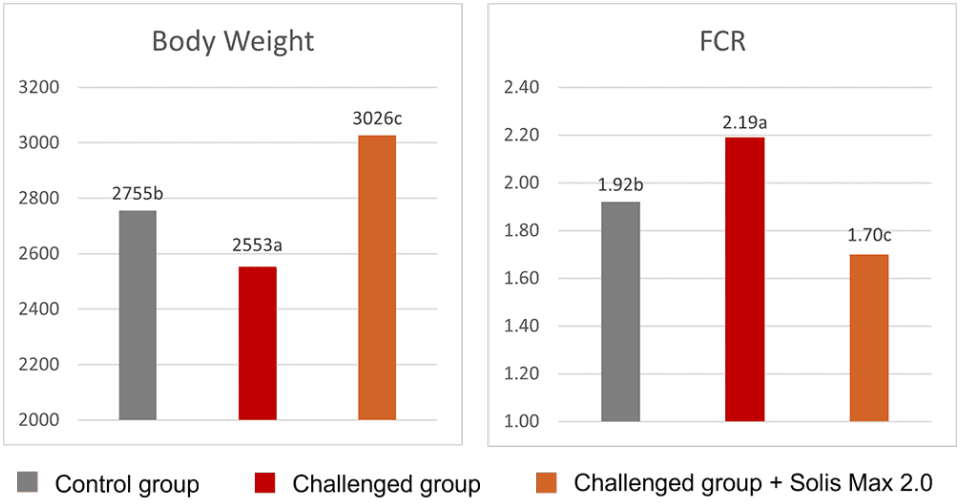


Figure 6: Better performance data due to the addition of Solis Max 2.0

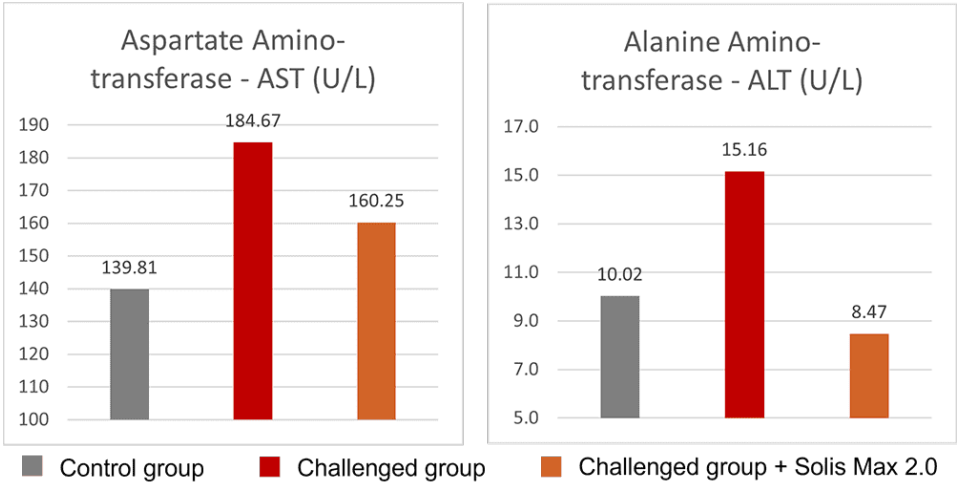


Figure 7: Healthier liver shown by lower values of AST and ALT

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 (MasterRisk) 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 Technical Team, EW Nutrition

Most grains used in feed are susceptible to mycotoxin contamination, 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 makes a lot of sense. Dried distiller's grains with solubles (DDGS) are a good example of how byproducts from food processing industries can become high-quality animal feed.

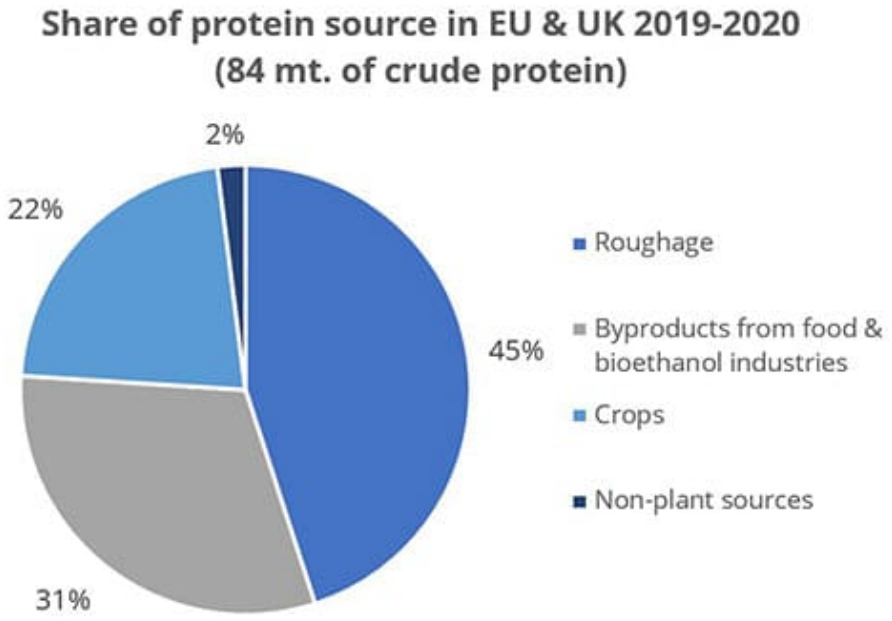


Figure 1: Byproducts are a crucial protein source (data from FEFAC Feed & Food 2021 report)

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. Pinotti et al., 2016 + link to article IH+MC). To safeguard animal health and performance when feeding lower-quality cereals, it is essential to monitor mycotoxin risks through regular testing and to use toxin-mitigating solutions.

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., Burel et al., 2013) show that the mycotoxin levels necessary to trigger damaging processes are lower than the levels reported as safe 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).

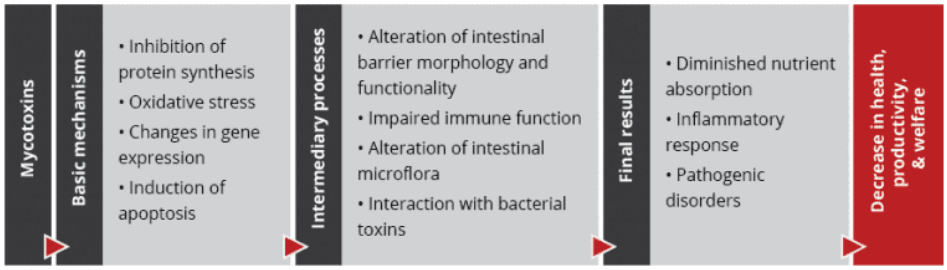


Figure 2: Mycotoxins' impact on the GIT and consequences for monogastric animals

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. Pinton & Oswald, 2014). 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. Immune cells are affected by mycotoxins 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 necrotic enteritis, 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 the development of necrotic enteritis.

3. **Alteration of the intestinal microflora**

Recent studies on the effect of various mycotoxins on the intestinal microbiota show that DON and other trichothecenes favor the colonization of coliform bacteria in pigs. DON and ochratoxin A also induce a greater invasion of *Salmonella* 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, as has been shown for the case of *E. coli*. 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**

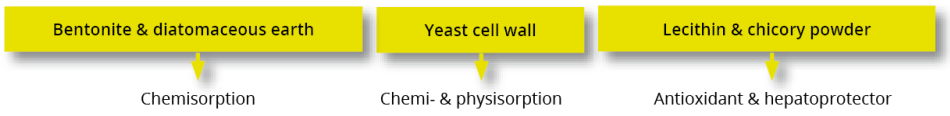
   When mycotoxins induce changes in the intestinal microbiota, this can lead to an increase in the endotoxin concentration in the intestinal lumen. Endotoxins promote the release of several cytokines that induce an enhanced immune response, causing inflammation, thus reducing feed consumption and animal 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 chronic inflammation and autoimmune disorders 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.

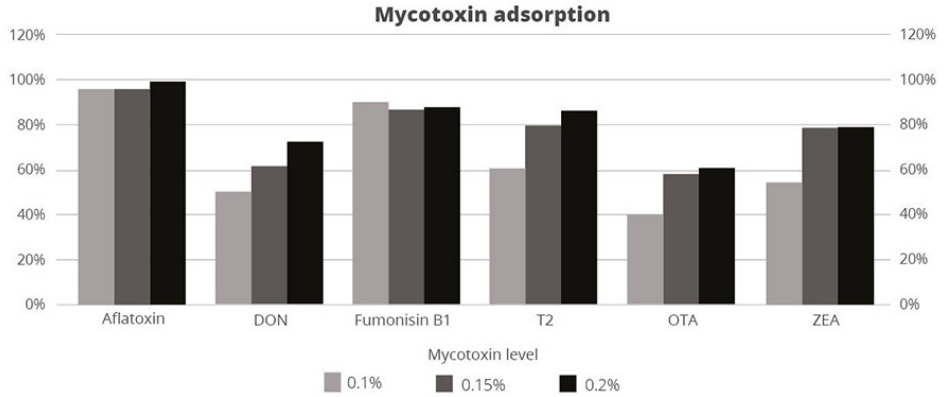


Figure 3: SOLIS MAX adsorption capacity against different mycotoxins (%)

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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