Mycotoxins as contributors to antibiotic resistance?

By Dr. Inge Heinzl, Editor EW Nutrition and
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Antibiotic resistance is a growing global health concern, making infections more complicated to treat and increasing the risk of disease spread, severe illness, and death. While overuse and misuse of antibiotics are the primary causes, recent research has uncovered another unexpected contributor: mycotoxins. Among these, deoxynivalenol (DON), a toxin commonly found in contaminated grains, has been shown to significantly alter gut microbiota and promote antibiotic resistance. This article examines how DON impacts gut bacteria, influences antibiotic resistance, and highlights why this issue warrants urgent attention.

Mycotoxins – originators of antimicrobial resistance?

Actually, it would be logical…

Alexander Fleming discovered Penicillin when he returned after the summer holidays and saw that a mold had grown on the agar plate he had prepared. Around the mold, Staphylococcus was unable to proliferate. The reason was a substance produced by the mold – penicillin, which, like other toxins produced by molds, is a mycotoxin. In his article about the origin of antibiotics and mycotoxins, Shier (2011)

stated that antibiotics and mycotoxins share considerable similarities in structure, metabolic roles, and biosynthesis.

A short excursus to antimicrobial resistance

In general, the primary mechanisms of resistance involve the prevention or limitation of the antimicrobial substance's uptake, modifying the drug target, inactivating the drug, or facilitating its discharge with efflux pumps.

There are two types of resistance: natural resistance, which is further divided into intrinsic and induced resistance, and acquired resistance.

Intrinsic resistance is a "characteristic" of a bacterial species and is not dependent on antibiotic exposure. An example is the reduced permeability of the outer membrane of gram-negative bacteria, which prevents certain antibiotics from entering.

Induced resistance, however, needs to be initiated by antibiotics. Here, multidrug-efflux pumps can be mentioned.

The third one, acquired resistance, refers to the process by which bacteria acquire genetic material, the resistance genes, from other bacteria that are resistant. The mechanisms include vertical transfer to daughter cells and horizontal transfer, such as the transfer from dead bacteria to living ones, by viruses, or the transfer of plasmids (Reygaert, 2018).

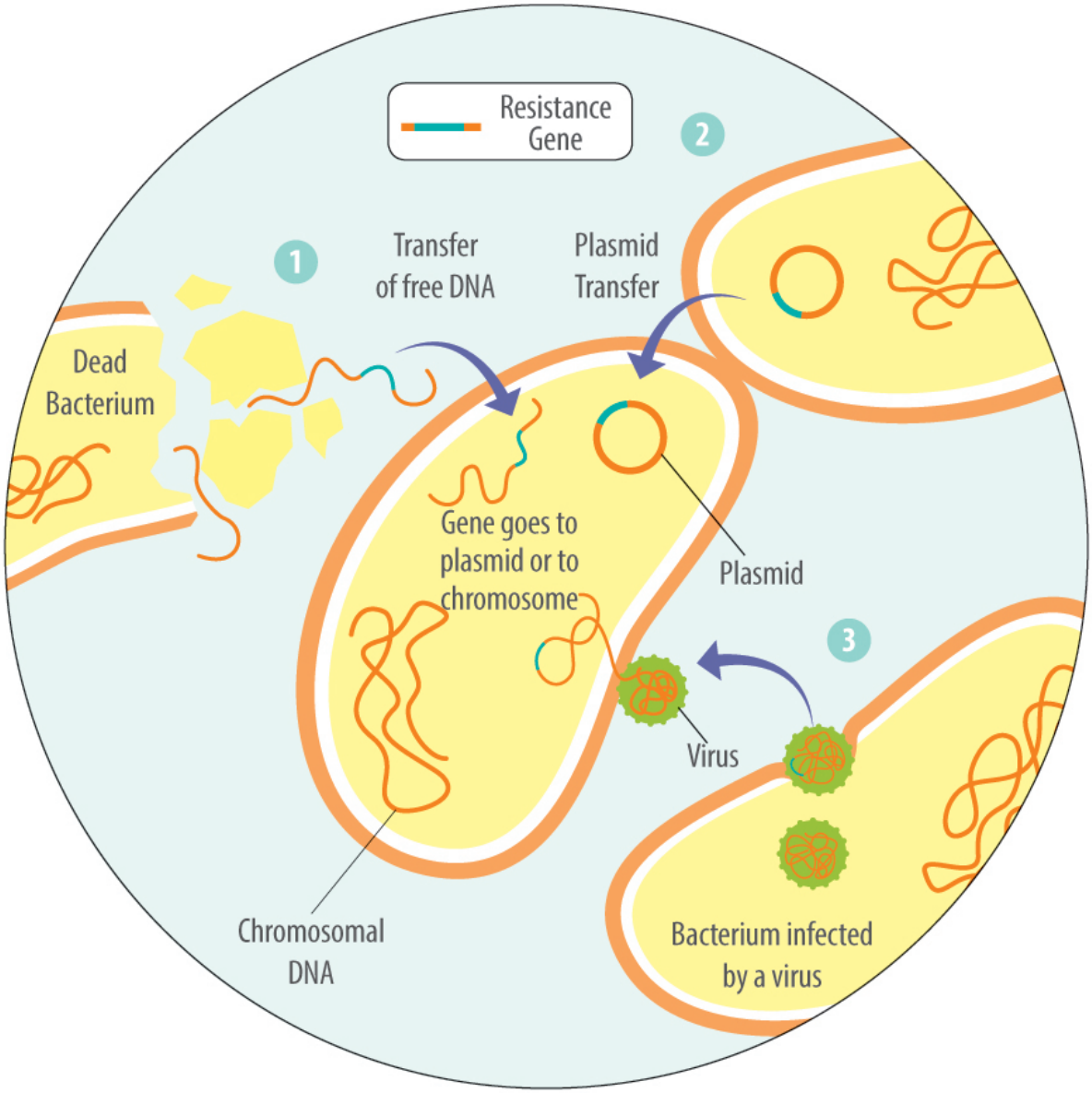

Figure 1: Different possibilities of transfer of resistance genes

Deoxynivalenol (DON) promotes resistance in gut microbiota

A Chinese group of researchers (Deng et al., 2025) examined for the first time the influence of DON on the intestinal microbiota of chickens. One of the most alarming findings is DON's ability to enhance antibiotic resistance. It contributes to this issue in several ways:

1. Encouraging resistant bacteria – By disrupting microbial balance, DON provides a survival advantage to bacteria that carry resistance genes.
2. Activating resistance genes – Studies suggest that DON can increase the expression of genes that help bacteria withstand antibiotics.
3. Enhancing gene transfer – Bacteria can share resistance genes through horizontal gene transfer. DON appears to promote this process, making antibiotic-resistant strains spread more rapidly.
4. Weakening antibiotic effectiveness – DON-induced changes in the gut environment can reduce the effectiveness of antibiotics, making treatments less successful.

A further indication that mycotoxins can enhance resistance is the significant overlap in the geographical distribution of antimicrobial-resistant bacteria and genes with that of mycotoxins, as noted by Deng et al.

Which protection mechanisms do bacteria have against mycotoxins?

In the case of mycotoxins, bacteria employ similar molecular mechanisms to those used against antibiotics. In an in vitro experiment, Hassan et al. (2019) challenged Devosia mutans, a gram-negative bacterium, with DON in the growth medium. DON inhibits protein synthesis, induces oxidative stress, and compromises cell membrane integrity in eucaryotic cells. Hassan et al. asserted three adaptive mechanisms as the response to the challenge:

1. Activation of cellular membrane proteins (adenosine 5'-triphosphate-binding cassette -ABC-transporters) responsible for the unidirectional transport of substrates, either outward or inward. These ABC transporters can work as drug efflux pumps.
2. Production of DON-specific deactivation enzymes, thereby engaging a toxin-specific pyrroloquinoline quinone-dependent detoxification pathway. This enables the bacterial isolate to transform DON to a non-toxic stereoisomer.
3. Upregulation of auxiliary coping proteins, such as porins (transmembrane proteins involved in metabolite exchange), glutathione S-transferases, and phosphotransferases, both of which are likely involved in the detoxification of xenobiotics.

Public health implications and preventive measures

Given the widespread presence of DON in food and animal feed, its potential role in antibiotic resistance poses a serious threat. The combination of increased bacterial resistance and weakened antibiotic efficacy could lead to more difficult-to-treat infections. This is particularly concerning in hospital settings, where antibiotic-resistant infections already cause high mortality rates.

To address the issue, several strategies can be implemented:

1. Reducing DON contamination: Implementing improved agricultural practices, such as crop rotation, the use of fungal-resistant crop varieties, and maintaining proper storage conditions, can help limit fungal growth and DON production.
2. Monitoring food and feed supply – Strict regulations and testing for DON contamination in grains and animal feed are essential to minimize human and animal exposure.
3. Effective mycotoxin risk management at feed mill and farm levels: Using tools such as MasterRisk and effective products combatting mycotoxins.
4. Maintaining gut health: A healthy diet rich in fiber, probiotics, and gut health-supporting feed supplements, such as Ventar D or products from the Activo line, may help counteract some of the adverse effects of DON on gut microbiota.
5. Developing new treatments: Research into alternative therapies and new antibiotics is crucial to combat the rise of antibiotic resistance.

Antimicrobial resistance: Be aware of the mycotoxins!

The connection between mycotoxins, such as DON, and antibiotic resistance underscores the need for a broader perspective on public health and food safety and once again brings the "One Health Concept" into focus. While antibiotic overuse remains the primary driver of resistance, environmental factors, such as

exposure to mycotoxins, should not be overlooked. By increasing awareness, enhancing food safety regulations, and investing in research, we can take steps to mitigate this emerging threat and safeguard the effectiveness of antibiotics for future generations.

References:

Deng, Fengru, Chuying Yao, Linyu Ke, Meichan Chen, Mi Huang, Jikai Wen, Qingmei Chen, Jun Jiang, and Yiqun Deng. “Emerging Threat to Antibiotic Resistance: Impact of Mycotoxin Deoxynivalenol on Gut Microbiota and Clonal Expansion of Extensively Drug-Resistant Enterococci.” Environment International 197 (March 2025): 109353. https://doi.org/10.1016/j.envint.2025.109353.

Hassan, Yousef I., Jian Wei He, Dion Lepp, and Ting Zhou. “Understanding the Bacterial Response to Mycotoxins: The Transcriptomic Analysis of Deoxynivalenol-Induced Changes in Devosia Mutans 17-2-E-8.” Frontiers in Pharmacology 10 (November 14, 2019). https://doi.org/10.3389/fphar.2019.01098.

Reygaert, Wanda C. “An Overview of the Antimicrobial Resistance Mechanisms of Bacteria.” AIMS Microbiology 4, no. 3 (2018): 482–501. https://doi.org/10.3934/microbiol.2018.3.482.

Shier, W. Thomas. “On the Origin of Antibiotics and Mycotoxins.” Toxin Reviews 30, no. 1 (January 28, 2011): 6–30. https://doi.org/10.3109/15569543.2011.550862.

Smith, William P., Benjamin R. Wucher, Carey D. Nadell, and Kevin R. Foster. “Bacterial Defences: Mechanisms, Evolution and Antimicrobial Resistance.” Nature Reviews Microbiology 21, no. 8 (April 24, 2023): 519–34. https://doi.org/10.1038/s41579-023-00877-3.

Mycotoxins in poultry – External signs can give a hint

Part 4: Paleness

By Dr. Inge Heinzl, Editor and Marisabel Caballero, Global Technical Manager Poultry

We already showed bad feathering, mouth and beak lesions, bone issues, and foot pad lesions as signs of mycotoxin contamination in the feed, but there is another indicator: paleness. Paleness can signify a low count of red blood cells resulting from blood loss or inadequate production of these cells. Other possibilities are higher bilirubin levels in the blood due to an impaired liver, leading to jaundice or missing pigmentation.

Hen with pale comb and wattles (adapted from Bozzo et al., 2023)

The mycotoxins mainly causing anemia are Aflatoxins, Ochratoxin, DON, and T-2 toxin

Anemia can be diagnosed using parameters such as red blood cell count, hemoglobin levels, and hematocrit/packed cell volume (PCV). Numerous studies have examined the impact of mycotoxins on hematological parameters. They reveal their propensity to affect red blood cell production by impairing the function of the spleen and inducing hematological alterations. On the other hand, anemia can be caused by blood loss. Due to affecting coagulation factors, mycotoxins can lead to internal hemorrhages. The gut wall damage, probably due to secondary infections such as coccidiosis and necrotic enteritis, can entail bloody diarrhea in various animal species.

Impact on the production of blood cells

Low values of blood parameters such as red blood cells, hemoglobin, and hematocrit can result from inadequate production due to impacted production organs. The World Health Organization (WHO, 1990) and European Commission (European Commission, 2001) have identified hematopoietic tissues as targets for necrosis caused by T-2 toxin. Chu (2003) even stated that “the major lesion of T-2 toxin is its devastating effect on the hematopoietic system in many mammals, including humans”. Pande et al. (2006) suggested that reduced hemoglobin values result from decreased protein synthesis due to mycotoxin contamination, a notion supported by Pronk et al. (2002), who described trichothecenes as potent inhibitors of protein, DNA, and RNA synthesis, particularly affecting tissues with high cell division rates. Additionally, the European Commission (2001) highlighted the sensitivity of red blood cell progenitor cells (in this trial, the cells of mice, rats, and humans) to the toxic effects of T-2 and HT-toxins. DAS also seems to attack the hematopoietic system, as shown in humans (WHO, 1990). A further cause for anemia might be low feed intake or nutrient absorption, which inhibits adequate iron absorption and leads to iron deficiency. In their case report, Bozzo et al. (2023) assumed that renal failure and a resulting impaired excretion capacity caused by OTA might even increase the half-life of the toxins. This would enhance their effects on their target organs, such as the liver and bone marrow, and lead to anemia.

Several studies utilizing different animal species and mycotoxin dosages have been conducted to assess the effects of Aflatoxins, Ochratoxin, and T-2 Toxin on hematological parameters. The following table provides a summary of some of these studies.

Animal species	Dosage	Impact	Reference
T-2 Toxin and other Trichothecenes			
Broilers	T-2 – 0, 1, 2, and 4 mg T-2 toxin/kg n=30 per group	Significant reduction in hemoglobin at 1, 2, and 4 ppm; PCV significantly reduced at 4 ppm	Pande et al., 2006
Broilers	T-2 – 0 and 4 mg/kg diet n=60 per group	Decrease in hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration	Kubena et al., 1989a
Broilers	4, 16, 50, 100, 300 ppm for seven days n=5-20 chickens per group	Anemia; significant reduction of hematocrit (50 and 100 ppm); survivors had atrophied lymphoid organs and were anemic	Hoerr et al., 1982
Yangzhou goslings	0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 mg/kg; n=6 per group	Red blood cell count decreased in the 2.0 mg/kg group along with an increase in mean corpuscular hemoglobin ($p<0.05$) and reduced mean platelet volume ($P<0.05$)	Gu et al., 2023

Broilers	2 ppm; 32 birds per group	Anemia, as indicated by significantly (P<0.05) lower total erythrocyte count (TEC) values, lower hemoglobin levels, and packed cell volume; additional thrombocytopenia could be the cause of bleeding	Yohannes et al., 2013
DON			
Broilers	5 and 15 mg/kg of feed for 42 days	Decrease in erythrocytes, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) at 15 mg/kg; decrease in hematocrit and hemoglobin at both levels of DON.	Riahi, 2021
Piglets	0.6 mg/kg and 2.0 mg/kg	Significant decrease in mean corpuscular volume	Modrá et al., 2013
Broilers	16 mg/kg diet n=60 per group	Significant decrease in mean corpuscular volume	Kubena et al., 1989c
Ochratoxin			
Broilers	2 mg/kg diet singly or combined with DAS 6 mg/kg	Reduced mean corpuscular hemoglobin values	Kubena et al., 1994
Broilers	2 mg/kg diet	Significant decrease in hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration	Kubena et al., 1989b
Aflatoxins			
Broilers	2.5 µg/g	Decrease in red blood cell count	Huff et al., 1988
Broilers	≥1.25 µg/g	Significant decrease in hemoglobin and erythrocyte count	Tung et al., 1975
AFB1 + OTA			
Laying hens	Natural feed contamination OTA – 31 ± 3.08 µg/kg and AFB1 – 5.6 ± 0.33 µg/kg dry weight	Anemia signs (pale appearance of combs and wattles), evidenced by the discoloration of the content of the femoral medullary cavity.	Bozzo et al., 2023

Table 1: The effects of different mycotoxins on hematological parameters – hematopoiesis

In their meta-analysis, Andretta et al. (2012) reported that the presence of mycotoxins in broiler diets decreased the hematocrit and the hemoglobin concentration by 5% and 15%, and aflatoxin alone decreased the parameters by 6% and 20%.

It should be evident that a simultaneous occurrence of several mycotoxins even aggravates the situation. In an experiment involving Sprague Dawley rats, administering T-2, DON, NIV, ZEA, NEO, and OTB decreased hematocrit and red blood cell counts across all mycotoxins. However, for DON, NIV, ZEN, and OTB, red blood cell values showed partial recovery after 24 hours (Chattopadhyay, 2013). Perhaps the organism learns to cope with the mycotoxins.

The examples show that Trichothecenes, such as T-2 toxin, DON, and others, as well as Ochratoxins and Aflatoxins, impact blood parameters such as hematocrit, hemoglobin, red blood cell count, and mean corpuscular volume. All these changes might lead to paleness of the skin and birds' feet and combs.

Blood loss caused by bleeding or destruction of erythrocytes

The second possibility for anemia is blood loss due to injuries or lesions. In addition to directly causing hemorrhages, mycotoxins can promote secondary infections such as coccidiosis, which damages the gut

and may produce bloody feces.

Parent-Massin (2004) e.g. reports on rapidly progressing coagulation problems after the ingestion of trichothecenes leading to septicemia and massive hemorrhages. Table 2 shows more examples of mycotoxins causing paleness due to blood loss.

Animal species	Dosage	Impact	Reference
T-2 Toxin and other Trichothecenes			
Cats	T-2 toxin – 0.06-0.1 mg/kg body weight/day	Bloody feces, hemorrhages	Lutsky et al., 1978
Cats	T-2 toxin – 0.08 mg/kg BW every 48 h until death	Bloody feces	Lutzky and Mor, 1981
Pigeon	DAS in oat, sifting	Emesis and bloody stools	Szathmary (1983)
Calves	0.08, 0.16, 0.32, or 0.6 mg/kg BW per day for 30 days; 1 calf per treatment	Bloody feces at doses ≥0.32 mg/kg BW per day	Pier et al., 1976
Ochratoxin			
Rats	Single dosages of 0, 17, or 22 mg/kg BW in 0.1 Mol/L $NaHCO_3$, gavage	Multifocal hemorrhages in many organs	Albassam et al., 1987
DON			
Broilers	0, 35, 70, 140, 280, 560, and 1120 mg/kg body weight	Ecchymotic hemorrhages throughout the intestinal tract, liver, and musculature; relationship to hemorrhagic anemia syndrome seems warranted	Huff et al., 1981
Sterigmatocystin (ST)			
10-12-day old chicks (93-101 g)	10 and 14 mg/kg BW intraperitoneal	Hemorrhages and foci of necrosis in the liver	Sreemannarayana et al., 1987
Aflatoxins			
Broiler chickens	100 µg/kg feed	Hemorrhages in the liver	Abdel-Sattar, 2019
Turkeys	500 and 1000 ppb in the diet	Bloody diarrhea, spleens with hemorrhages, petechial hemorrhages in the small intestine	Giambrone et al., 1984
Broilers	0, 0.625, 1.25, 2.5, 5.0, and 10.0 mg/kg of diet combined with Infectious Bursal Disease	Slight hemorrhages in the skeletal muscles; decreased hematocrit and hemoglobin due to hemolytic anemia.	Chang and Hamilton, 1981
Broilers	0, 1, and 2 mg AFB1/kg of diet	Downregulation of the genes involved in blood coagulation (coagulation factor IX and X) and upregulation of anticoagulant protein C precursor, an inactivator of coagulation factors Va and VIIIa, and antithrombin-III precursor with 2 mg/kg	Yarru, 2009

Pigs	1-4 mg/kg, 4 weeks 0.4-0.8 mg/kg, 10 weeks	Hemorrhages	Henry et al., 2001

Table 2: The effects of different mycotoxins on hematological parameters – blood loss

Poor pigmentation

The fourth reason for paleness can be inadequate pigmentation. According to Hy Line (2021), the so-called pale bird syndrome is characterized by poor skin and egg yolk pigmentation and is caused by reduced absorption of fat and carotenoid pigments in compromised birds. This is also the case when the diets contain pigment supplements. Tyczkowski and Hamilton (1986) observed in their experiment with chickens exposed to doses of 1-8 µg of Aflatoxins/g of diet for three weeks that aflatoxins can cause poor pigmentation in chickens, probably by impairing carotenoids absorption but also transport and deposition. Osborne et al. (1982) asserted that carotenoids were significantly ($P<0.05$) depressed by 2 ppm ochratoxin as well as by 2.5 ppm aflatoxin in the diet.

Another possibility is oxidative stress due to the mycotoxin challenge. As pigments also serve as antioxidants, they may be expended for this purpose and are no longer available for pigmentation.

Paleness in poultry – a reason to think about mycotoxins

Paleness can have different causes, some of which are influenced by mycotoxins. If your chickens or hens are pale, checking the feed concerning mycotoxins is always recommended. A feed analysis can give information about possible contamination (see our tool MasterRisk).

In the case of contamination, effective products binding the mycotoxins and mitigating the adverse effects of these harmful substances can help protect your birds. As paleness is usually not the only effect of mycotoxins but also a decrease in growth, toxin binders can help maintain the performance of your animals.

References:

Abdel-Sattar, Ward Masoud, Kadry Mohamed Sadek, Ahmed Ragab Elbestawy, and Disouky Mohamed Mourad. “The Protective Role of Date Palm (Phoenix Dactylifera Seeds) against Aflatoxicosis in Broiler Chickens Regarding Carcass Characterstics, Hepatic and Renal Biochemical Function Tests and Histopathology.” *Journal of World’s Poultry Research* 9, no. 2 (June 25, 2019): 59–69. https://doi.org/10.36380/scil.2019.wvj9.

Albassam, M. A., S. I. Yong, R. Bhatnagar, A. K. Sharma, and M. G. Prior. “Histopathologic and Electron Microscopic Studies on the Acute Toxicity of Ochratoxin a in Rats.” *Veterinary Pathology* 24, no. 5 (September 1987): 427–35. https://doi.org/10.1177/030098588702400510.

Andretta, I., M. Kipper, C.R. Lehnen, and P.A. Lovatto. “Meta-Analysis of the Relationship of Mycotoxins with Biochemical and Hematological Parameters in Broilers.” *Poultry Science* 91, no. 2 (February 2012): 376–82. https://doi.org/10.3382/ps.2011-01813.

Bhat, RameshV, Y Ramakrishna, SashidharR Beedu, and K.L Munshi. “Outbreak of Trichothecene Mycotoxicosis Associated with Consumption of Mould-Damaged Wheat Products in Kashmir Valley, India.” *The Lancet* 333, no. 8628 (January 1989): 35–37. https://doi.org/10.1016/s0140-6736(89)91684-x.

Bozzo, Giancarlo, Nicola Pugliese, Rossella Samarelli, Antonella Schiavone, Michela Maria Dimuccio, Elena Circella, Elisabetta Bonerba, Edmondo Ceci, and Antonio Camarda. “Ochratoxin A and Aflatoxin B1 Detection in Laying Hens for Omega 3-Enriched Eggs Production.” *Agriculture* 13, no. 1 (January 5, 2023): 138. https://doi.org/10.3390/agriculture13010138.

Chang, Chao-Fu, and Pat B. Hamilton. “Increased Severity and New Symptoms of Infectious Bursal Disease during Aflatoxicosis in Broiler Chickens.” *Poultry Science* 61, no. 6 (June 1982): 1061–68. https://doi.org/10.3382/ps.0611061.

Chattopadhyay, Pronobesh, Amit Agnihotri, Danswerang Ghoyary, Aadesh Upadhyay, Sanjeev Karmakar, and Vijay Veer. “Comparative Hematoxicity of Fusarium Mycotoxin in Experimental Sprague-Dawley Rats.” *Toxicology International* 20, no. 1 (2013): 25. https://doi.org/10.4103/0971-6580.111552.

European Commission. “Opinion of the Scientific Committee on Food on Fusarium Toxins Part 5: T-2 Toxin and HT-2 Toxin.” Food.ec.europa. Accessed May 30, 2001. https://food.ec.europa.eu/document/download/a859c348-a38e-404c-a2af-c3e29a3a8777_en?filename=sci-com_scf_out88_en.pdf.

Giambrone, J.J., U.L. Diener, N.D. Davis, V.S. Panangala, and F.J. Hoerr. “Effect of Purified Aflatoxin on Turkeys.” *Poultry Science* 64, no. 5 (May 1985): 859–65. https://doi.org/10.3382/ps.0640859.

Gu, Wang, Qiang Bao, Kaiqi Weng, Jinlu Liu, Shuwen Luo, Jianzhou Chen, Zheng Li, et al. “Effects of T-2 Toxin on Growth Performance, Feather Quality, Tibia Development and Blood Parameters in Yangzhou Goslings.” *Poultry Science* 102, no. 2 (February 2023): 102382. https://doi.org/10.1016/j.psj.2022.102382.

Henry, H., T. Whitaker, I. Rabban, J. Bowers, D. Park, W. Price, F.X. Bosch, et al. “Aflatoxin M1.” Aflatoxin M1 (JECFA 47, 2001). Accessed July 29, 2024. https://inchem.org/documents/jecfa/jecmono/v47je02.htm.

Hoerr, F., W. Carlton, B. Yagen, and A. Joffe. “Mycotoxicosis Caused by Either T-2 Toxin or Diacetoxyscirpenol in the Diet of Broiler Chickens.” *Fundamental and Applied Toxicology* 2, no. 3 (May 1982): 121–24. https://doi.org/10.1016/s0272-0590(82)80092-4.

Huff, W.E., J.A. Doerr, P.B. Hamilton, and R.F. Vesonder. “Acute Toxicity of Vomitoxin (Deoxynivalenol) in Broiler Chickens,” *Poultry Science* 60, no. 7 (July 1981): 1412–14. https://doi.org/10.3382/ps.0601412.

Huff, W.E., R.B. Harvey, L.F. Kubena, and G.E. Rottinghaus. “Toxic Synergism between Aflatoxin and T-2 Toxin in Broiler Chickens.” *Poultry Science* 67, no. 10 (October 1988): 1418–23. https://doi.org/10.3382/ps.0671418.

Hy-Line. “Mycotoxins: How to deal with the threat of mycotoxicosis.” Hy-Line International. Accessed July 29, 2024. https://www.hyline.com/.

Klein, P. J., T. R. Vleet, J. O. Hall, and R. A. Coulombe. “Dietary Butylated Hydroxytoluene Protects against Aflatoxicosis in Turkey.” *Poisonous plants and related toxins*, November 24, 2003, 478–83. https://doi.org/10.1079/9780851996141.0478.

Kubena, L.F., R.B. Harvey, T.S. Edrington, and G.E. Rottinghaus. “Influence of Ochratoxin A and Diacetoxyscirpenol Singly and in Combination on Broiler Chickens.” *Poultry Science* 73, no. 3 (March 1994): 408–15. https://doi.org/10.3382/ps.0730408.

Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Corrier, T.D. Philipps, and G.E. Rottinghaus. “Influence of Ochratoxin A and T-2 Toxin Singly and in Combination on Broiler Chickens.” *Poultry Science* 68, no. 7 (July 1989): 867–72. https://doi.org/10.3382/ps.0680867.

Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Corrier, T.D. Phillips, and G.E. Rottinghaus. “Influence of Ochratoxin A and T-2 Toxin Singly and in Combination on Broiler Chickens.” *Poultry Science* 68, no. 7 (July 1989): 867–72. https://doi.org/10.3382/ps.0680867.

Kubena, L.F., W.E. Huff, R.B. Harvey, T.D. Phillips, and G.E. Rottinghaus. “Individual and Combined Toxicity of Deoxynivalenol and T-2 Toxin in Broiler Chicks.” *Poultry Science* 68, no. 5 (May 1989): 622–26. https://doi.org/10.3382/ps.0680622.

Lutsky, I.I., and N. Mor. “Alimentary Toxic Aleukia (Septic Angina, Endemic Panmyelotoxicosis, Alimentary Hemorrhagic Aleukia): T-2 Toxin-Induced Intoxication of Cats.” The American journal of pathology, 1980. https://pubmed.ncbi.nlm.nih.gov/6973281/.

Lutsky, Irving, Natan Mor, Boris Yagen, and Avraham Z. Joffe. “The Role of T-2 Toxin in Experimental Alimentary Toxic Aleukia: A Toxicity Study in Cats.” *Toxicology and Applied Pharmacology* 43, no. 1 (January 1978): 111–24. https://doi.org/10.1016/s0041-008x(78)80036-2.

MEJ, Pronk, Schothorst RC, and H.P. van Egmond. “Toxicology and Occurrence of Nivalenol, Fusarenon X, Diacetoxyscirpenol, Neosolaniol and 3- and 15- Acetyldeoxynivalenol; a Review of Six Trichothecenes.” Home – Web-based Archive of RIVM Publications, November 7, 2002. https://rivm.openrepository.com/handle/10029/9184.

Modra, Helena, Jana Blahova, Petr Marsalek, Tomas Banoch, Petr Fictum, and Martin Svoboda. “The Effects of Mycotoxin Deoxynivalenol (DON) on Haematological and Biochemical Parameters and Selected Parameters of Oxidative Stress in Piglets.” *Neuro Endocrinol Lett.* 34, no. Suppl 2 (2013): 84–89.

Osborne, D.J., W.E. Huff, P.B. Hamilton, and H.R. Burmeister. “Comparison of Ochratoxin, Aflatoxin, and T-2 Toxin for Their Effects on Selected Parameters Related to Digestion and Evidence for Specific Metabolism of Carotenoids in Chickens,” *Poultry Science* 61, no. 8 (August 1982): 1646–52. https://doi.org/10.3382/ps.0611646.

Pande, Vivek, Nitin Kurkure, and A.G. Bhandarkar. “Effect of T-2 Toxin on Growth, Performance and Haematobiochemical Alterations in Broilers .” *Indian Journal of Experimental Biology* 44, no. 1 (February 2006): 86–88.

Pier , A.C., S.J. Cysewski, J.L. Richard , A.L. Baetz, and L. Mitchell. “Experimental Mycotoxicoses in Calves with Aflatoxin, Ochratoxin, Rubratoxin, and T-2 Toxin.” Proceedings, annual meeting of the United States Animal Health Association, 1976. https://pubmed.ncbi.nlm.nih.gov/1078072/.

Resanovic, Radmila, Ksenija Nesic, Vladimir Nesic, Todor Palic, and Vesna Jacevic. “Mycotoxins in Poultry Production.” *Zbornik Matice srpske za prirodne nauke*, no. 116 (2009): 7–14. https://doi.org/10.2298/zmspn0916007r.

Riahi, Insaf, Virginie Marquis, Anna Maria Pérez-Vendrell, Joaquim Brufau, Enric Esteve-Garcia, and Antonio J. Ramos. “Effects of Deoxynivalenol-Contaminated Diets on Metabolic and Immunological Parameters in Broiler Chickens.” *Animals* 11, no. 1 (January 11, 2021): 147. https://doi.org/10.3390/ani11010147.

Sreemannarayana, O., A. A. Frohlich, and R. R. Marquardt. “Acute Toxicity of Sterigmatocystin to Chicks.” *Mycopathologia* 97, no. 1 (January 1987): 51–59. https://doi.org/10.1007/bf00437331.

Stack, Jim, and Mike Carlson. “Fumonisins in Corn.” DigitalCommons@University of Nebraska – Lincoln, 2003. https://core.ac.uk/download/pdf/188054556.pdf.

Szathmary, C.I. “Trichothecene Toxicoses and Natural Occurrence in Hungary.” Essay. In *Ueno, Y: Developments in Food Science IV. Trichothecenes*, 229–50. New York: Elsevier, 1983.

Tung, Hsi-Tang, F.W. Cook, R.D. Wyatt, and P.B. Hamilton. “The Anemia Caused by Aflatoxin.” *Poultry Science* 54, no. 6 (November 1975): 1962–69. https://doi.org/10.3382/ps.0541962.

Tyczkowski, Juliusz K., and Pat B. Hamilton. “Altered Metabolism of Carotenoids during Aflatoxicosis in Young Chickens,” *Poultry Science* 66, no. 7 (July 1987): 1184–88. https://doi.org/10.3382/ps.0661184.

WHO. “Selected Mycotoxins : Ochratoxins, Trichothecenes, Ergot / Published under the Joint Sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization.” World Health Organization, January 1, 1990. https://apps.who.int/iris/handle/10665/39552.

Yohannes, T., A. K. Sharma, S. D. Singh, and V. Sumi. “Experimental Haematobiochemical Alterations in Broiler Chickens Fed with T-2 Toxin and Co-Infected with IBV.” *Open Journal of Veterinary Medicine* 03, no. 05 (2013): 252–58. https://doi.org/10.4236/ojvm.2013.35040.

Mycotoxins in poultry – External

signs can give a hint

Part 3: Bone disorders and foot pad lesions

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Bone health is essential for animals and humans. Besides giving structural support, allowing movement, and protecting vital organs, the bones release hormones that are crucial for mineral homeostasis and acid balance and serve as reservoirs of energy and minerals (Guntur & Rosen, 2012; Rath, N.C. & Durairaj, 2022; Suchacki et al., 2017).

Bone disorders and foot pad lesions are considerable challenges in poultry production, especially for fast-growing birds with high final weights. Due to pain, the animals do not move, and dominant, healthy birds may restrict lame birds' access to feed and water. In consequence, these birds are often culled. Moreover, processing these birds is problematic, and often, they must be discarded or downgraded.

Foot pad lesions, another common issue in poultry production, can also have significant economic implications. On the one hand, pain restricts birds from eating and drinking and reduces weight gain. On the other hand, for many producers, chicken feet constitute a substantial part of the economic value of the bird; therefore, discarding them represents a significant financial loss. Additionally, to push poultry production in the right direction concerning animal health and welfare, a foot pad scoring system at the processing plant is in place in European countries.

Mycotoxins affect bones in different ways

Mycotoxins, depending on their target organs, can have diverse effects on the skeleton of birds. For example, mycotoxins that target the liver can disrupt calcium metabolism, which in turn affects the mineralization of the bones (rickets) and the impairment of chondrocytes can slow down bone growth (e.g., tibial dyschondroplasia). When the kidneys are impacted, urate clearance decreases, plasma uric acid consequently increases, and urate crystals form in the synovial fluid and tendon sheaths of various joints, particularly the hock joints. These examples highlight the complex and varied ways mycotoxins can impact poultry bone health.

Inadequate bone mineralization and strength – Rickets and layer cage fatigue

Sufficient bone mineralization is essential for the stability of the skeleton. Calcium (Ca), Vitamin D, and Phosphorous (P) deficiency leads to inadequate mineralization, weakens the bone, and can cause soft and bent bones or, in the case of layers, cage fatigue – a collapse of the spinal bone- and paralysis. Inadequate bone mineralization can be caused in different ways, among them:

1. Decrease in the availability of the nutrients necessary for mineralization. This can occur if the digestibility of these nutrients deteriorates
2. Impact on the Ca/P ratio—A ratio of 1 – 2:1 is vital for adequate bone development (Loughrill et al., 2016). Mycotoxins can alter absorption and transporters for one or both elements, altering their ratio.
3. Impact on the Vitamin D receptor, affecting its expression or the transporters for Ca and P.

Aflatoxins can impair bone mineralization by different modes of action. An important one is the impairment of the digestibility of Ca and P: Kermanshahi et al. (2007) fed broilers diets with high levels of aflatoxins (0.8 to 1.2 mg AFB1/kg feed) for three weeks, which resulted in a significant reduction of Ca and P digestibility. Other researchers, however, did not find an effect on Ca and P digestibility with lower aflatoxin levels: Bai et al. (2014) feeding diets contaminated with 96 (starter) and 157 µg Aflatoxins (grower) per kg of feed to broilers and Han et al. (2008) saw no impact on cherry valley ducks with levels of 20 and 40 µg AFB1/kg diet.

Indirectly, a decrease in the availability of Ca and P due to aflatoxin-contaminated feed can be shown by blood or tibia levels of these minerals, as demonstrated by Zhao et al. (2010): They conducted a trial with broilers, resulting in blood serum levels of Ca and P levels significantly ($P<0.05$) dropped with feed contaminated with 2 mg/kg of AFB1. Another trial conducted by Bai et al. (2014) showed decreased Ca in the tibia and reduced tibial break strength.

To get more information about the effect of mycotoxins on bone mineralization and the utilization of Ca, P, and Vit. D in animal organisms, Costanzo et al. (2015) challenged osteosarcoma cells with 5 and 50 ppb of aflatoxin B1. They asserted a significant down-modulation of the expression of the Vitamin D receptor. Furthermore, they assumed an interference of AFB1 with the actions of vitamin D on calcium-binding gene expression in the kidney and intestine. Paneru et al. (2024) could confirm this downregulation of the Vit D receptor and additionally of the Ca and P transporters in broilers with levels of ≥75 ppb AFB1. They also saw a significant reduction in tibial bone ash content at AFB1 levels >230 ppb, a decreased trabecular bone mineral content and density at AFB1 520 ppb, and a reduced bone volume and tissue volume of the cortical bone of the femur at the level of 230 ppb (see Figure 1). They concluded that AFB1 levels of already 230 ppb contribute to bone health issues in broilers.

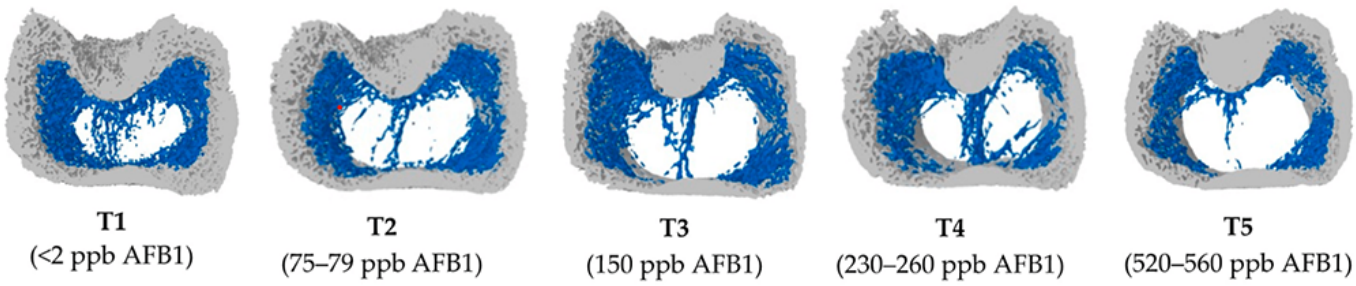


Figure 1: Increasing doses of AFB1 (<2 ppb – 560 ppb) deteriorate bone quality (Paneru, 2024): Cross-sectional images of femoral metaphysis with increasing AFB1 levels (left to right). The outer cortical bone is shown in light grey, and the inner trabecular bone in blue. Higher levels of AFB1 (T4 and T5) show a disruption of the trabecular bone pattern (less dense blue pattern with thinner and more fragmented bone strands and with wide spaces between the trabecular bone) (shown in white).

All experiments strongly suggest that aflatoxins harm bone homeostasis. Additional liver damage, oxidative stress, and impaired cellular processes can exacerbate bone health issues**.**

Trichothecenes also negatively impact bone mineralization. Depending on the mycotoxin, they may affect the gut, decreasing the absorption of Ca and P and probably provoking an imbalance in the Ca/P ratio.

For instance, when **T-2 toxin** was fed to Yangzhou goslings at 0.4, 0.6, and 0.8 mg/kg of diet, it decreased the Ca levels (halved at 0.8 mg/kg) and increased the P levels in the blood serum, so the Ca/P ratio decreased from the adequate ratio of 1 – 2 to 0.85, 0.66, and 0.59 ($P<0.05$) (Gu et al., 2023). The alterations of the Ca and P levels, the resulting decreasing Ca/P ratio, and an additional increase in alkaline phosphatase (ALP) suggest that T-2 toxin negatively impacts Ca absorption, increases ALP, and, therefore, disturbs calcification and bone development.

Other studies show that serum P levels decreased in broilers fed DON-contaminated feed with levels of only 2.5 mg/kg (Keçi et al., 2019). One reason for the lower P level is probably the lower dry matter intake, affecting Ca and P intake. Ca serum level is not typically reduced, which can be explained by the fact that Ca plays many critical physiological roles (e.g., nerve communication, blood coagulation, hormonal regulation), so the body keeps the blood levels by reducing bone mineralization. Another explanation is delivered by Li et al. (2020): After their trial with broilers, they stated that dietary P deficiency is more critical for bone development than Ca deficiency or Ca & P deficiency. The results of the trial conducted by Keçi et al. with DON (see above) were reduced bone mineralization, affected bone density, ash content, and ash density in the femur and tibiotarsus with a stronger impact on the tibiotarsus than on the femur.

In line with trichothecenes effects in Ca and P absorption, Ledoux et al. (1992) suppose that diarrhea caused by intake of fumonisins leads to malabsorption or maldigestion of vitamin D, calcium and phosphorus, having birds with rickets as a secondary effect.

Ochratoxin A (OTA) impairs kidney function, negatively affects vitamin D metabolism, reduces Ca absorption, and contributes to deteriorated bone strength (Devegowda and Ravikiran, 2009). Indications from Huff et al. (1980) show decreased tibia strength after feeding chickens OTA levels of 2, 4, and 8 µ/g, and Duff et al. (1987) report similar results also in turkey poults.

A further mycotoxin possibly contributing to leg weakness is cyclopiazonic acid produced by Aspergillus and Penicillium. This mycotoxin is known for leading to eggs with thin or visibly racked shells, indicating an impairment of calcium metabolism (Devegowda and Ravikiran, 2009). Tran et al. (2023) also showed this fact with multiple mycotoxins.

The co-occurrence of different mycotoxins in the feed – the standard in praxis – increases the risk of leg issues. A trial with broiler chickens conducted by Raju and Devegowda (2000) showed a bone ash-decreasing effect of AFB1 (300 µg/kg), OTA (2 mg/kg), and T-2 toxin (3 mg/kg), fed individually but an incomparable higher effect when fed in combination.

Impairment of bone growth – tibial

dyschondroplasia (TD)

In TD, the development of long bones is impaired, and abnormal cartilage development occurs. It is frequent in broilers, with a higher incidence in males than females. It happens when the bone grows, as the soft cartilage tissue is not adequately replaced by hard bone tissue. Some mycotoxins have been related to this condition: According to Sokolović et al. (2008), actively dividing cells such as bone marrow are susceptible to T-2 toxin, including the tibial growth plates, which regulate chondrocyte formation, maturation, and turnover.

T-2 toxin: In a study with primary cultures of chicken tibial growth plate chondrocytes (GPCs) and three different concentrations of T-2 toxin (5, 50, and 500 nM), He et al. (2011) found that T-2 toxin decreased cell viability, alkaline phosphatase activity, and glutathione content ($P < 0.05$). Additionally, it increased the level of reactive oxygen species and malondialdehyde in a dose-dependent way, which could be partly recompensated by adding an antioxidant (N-acetyl-cysteine). They concluded that T-2 toxin inhibits the proliferation and differentiation of GPCs and contributes, therefore, to the development of TD, altering cellular homeostasis. Antioxidants may help to reduce these effects.

Gu et al. (2023) investigated the closely bodyweight-related shank length and the tibia development in Yangzhou goslings fed feed with six different levels (0 to 2.0 mg/kg) of T-2 toxin for 21 days. They determined a clear dose-dependent slowed tibial length and weight growth ($p<0.05$), as well as abnormal morphological structures in the tibial growth plate. As tibial growth and shank length are closely related to weight gain (Gu et al., 2023; Gao et al., 2010; Ukwu et al., 2014; Yu et al., 2022), their slowdown indicates lower growth performance.

Fumonisin B1 is also a potential cause of this kind of leg issue. Feeding 100 and 200 mg/kg to day-old turkey poults for 21 days led to the development of TD (Weibking et al., 1993). Possible explanations are the reduced viability of chondrocytes, as found by Chu et al. (1995) after 48 h of exposure, or the toxicity of FB1 to splenocytes and chondrocytes, which was shown in different primary cell cultures from chicken (Wu et al., 1995).

Bacterial chondronecrosis with osteomyelitis lameness (BCO) can be triggered by DON and FUM

BCO presents a highly critical health and welfare issue in broiler production worldwide, and it is estimated that 1-2 % of condemnations in birds at the marketing age result from this disease. What is the reason? Today’s fast-growing broilers are susceptible to stress. This enables pathogenic bacteria to compromise epithelial barriers, translocate from the gastrointestinal tract or the pulmonary system into the bloodstream, and colonize osteochondrotic microfractures in the growth plate of the long bone. This can lead to bone necrosis and subsequent lameness.

In their experiment with DON and FUM in broilers, Alharbi et al. (2024) showed that these mycotoxins reduce the gut’s barrier strength and trigger immunosuppressive effects. They used contaminations of 0.76, 1.04, 0.94, and 0.93 mg DON/kg of feed and 2.40, 3.40, 3.20, and 3.50 mg FUM/kg diet in the starter, grower, finisher, and withdrawal phases, respectively. The team observed lameness on day 35; the mycotoxin groups always showed a significantly ($P<0.05$) higher incidence of cumulative lameness.

The increase in uric acid leads to gout

In general, mycotoxins, which damage the kidneys and, therefore, impact the renal excretion of uric acid, are potentially a factor for gout appearance.

One of these mycotoxins is T-2 toxin. With the trial mentioned before (Yangzhou goslings, 21 days of exposure), Gu et al. (2023) showed that the highest dosage of the toxin (2.0 mg/kg) significantly increased uric acid in the blood ($P<0.05$), possibly leading to the deposit of uric acid crystals in the joints and to gout.

Huff et al. (1975) applied Ochratoxin to chicks at 0, 0.5, 1.0, 2.0, 4.0, and 8.0 µg/g of feed during the first three weeks of life. They found ochratoxin A as a severe nephrotoxin in young broilers as it caused damage to the kidneys with doses of 1.0 µg/g and higher. At 4.0 and 8.0 µg/g doses, uric acid increased by 38 and 48%, respectively (see Figure 2). Page et al. (1980) also reported increased uric acid after feeding 0.5 or 1.0 mg/kg of Ochratoxin A to adult white Leghorn chickens.

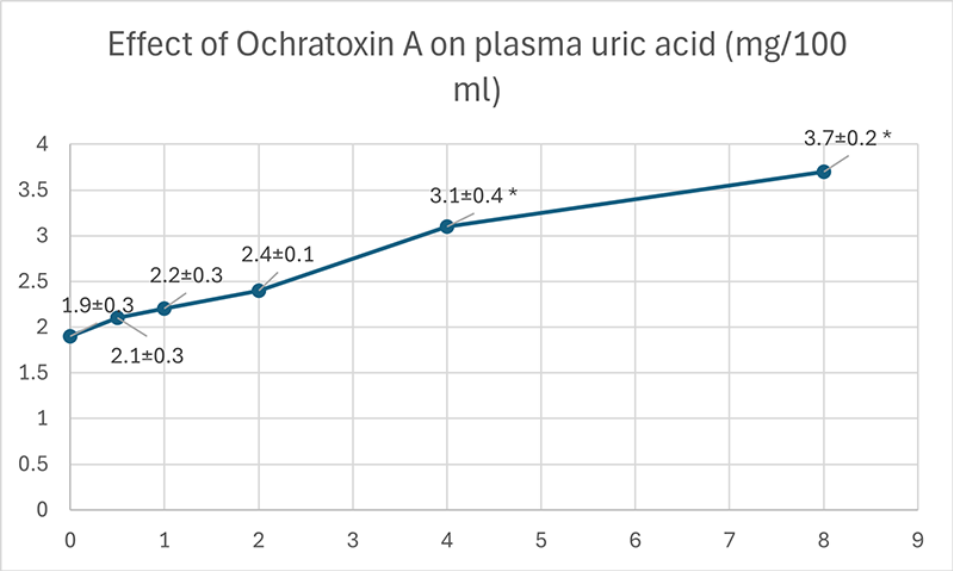


Figure 2: Effect of Ochratoxin A on plasma uric acid (mg/100 ml) (according to Huff et al., 1975)

Foot pad lesions – a further hint of mycotoxicosis

Foot pad lesions often result from wet litter, originating from diarrhea due to harmed gut integrity. Frequently, mycotoxins impact the intestinal tract and create ideal conditions for the proliferation of diarrhea-causing microorganisms and, therefore, secondary infections. Some also negatively impact the immune defense system, allowing pathogens to settle down or aggravate existing bacterial or viral parasitic diseases. In general, mycotoxins affect the physical (intestinal cell proliferation, cell viability, cell apoptosis), chemical (mucins, AMPs), immunological, and microbial barriers of the gut, as reported by Gao et al. (2020). Here are some examples of the adverse effects of mycotoxins leading to intestinal disorders and diarrhea:

- Mycotoxins can modulate intestinal epithelial integrity and the renewal and repair of epithelial cells, negatively impacting the intestinal barrier's intrinsic components; for instance, DON can significantly reduce the transepithelial electrical resistance (TEER)(Grenier and Applegate, 2013). A higher permeability of the epithelium and a decreased absorption of dietary proteins can lead to higher protein in the digesta in the small intestine, which serves as a nutrient for pathogens including *perfringens* (Antonissen et al., 2014; Antonissen et al., 2015).
- The application of Ochratoxin A (3 mg/kg) increased the number of S. typhimurium in the duodenum and ceca of White Leghorn chickens (Fukata et al., 1996). Another trial with broiler chicks at a concentration of 2 mg/kg aggravated the symptoms due to an infection by S. gallinarum (Gupta et al., 2005).
- In a trial by Grenier et al., 2016, feed contaminated with DON (1.5 mg/kg), Fumonisin B (20 mg/kg), or both mycotoxins aggravated lesions caused by coccidia.
- DON impacts the mucus layer composition by downregulating the expression of the gene coding for MUC2, as shown in a trial with human goblet cells (Pinton et al., 2015). The mucus layer prevents pathogenic bacteria in the intestinal lumen from contacting the intestinal epithelium (McGuckin et al., 2011).
- Furthermore, DON and other mycotoxins decrease the populations of lactic acid-producing bacteria, indicating a shift in the microbial balance (Antonissen et al., 2016).
- FB1 causes intestinal disturbances such as diarrhea, although it is poorly absorbed in the intestine. According to Bouhet and Oswald (2007), the main toxicological effect ascertained in

vivo and in vitro is the accumulation of sphingoid bases associated with the depletion of complex sphingolipids. This negative impact on the sphingolipid biosynthesis pathway could explain other adverse effects, such as reduced intestinal epithelial cell viability and proliferation, modification of cytokine production, and impairment of intestinal physical barrier function.
- T-2 toxin can disrupt the immune response, enhance the proliferation of *coli* in the gut, and increase its efflux (Zhang et al., 2022).

All these mycotoxins can cause foot pad lesions by impacting gut integrity or damaging the gut mucosa. They promote pathogenic organisms and, thus, provoke diarrhea and wet litter.

Mitigating the negative impact of mycotoxins on bones and feet is crucial for performance

Healthy bones and feet are essential for animal welfare and performance. Mycotoxins can be obstructive. Consequently, the first step to protecting your animals is to monitor their feed. If the analyses show the occurrence of mycotoxins at risky levels, proactive measures must be taken to mitigate the issues and ensure the health and productivity of your poultry.

References

Alharbi, Khawla, Nnamdi Ekesi, Amer Hasan, Andi Asnayanti, Jundi Liu, Raj Murugesan, Shelby Ramirez, Samuel Rochell, Michael T. Kidd, and Adnan Alrubaye. “Deoxynivalenol and Fumonisin Predispose Broilers to Bacterial Chondronecrosis with Osteomyelitis Lameness.” *Poultry Science* 103, no. 5 (May 2024): 103598. https://doi.org/10.1016/j.psj.2024.103598.

Antonissen, Gunther, Filip Van Immerseel, Frank Pasmans, Richard Ducatelle, Freddy Haesebrouck, Leen Timbermont, Marc Verlinden, et al. “The Mycotoxin Deoxynivalenol Predisposes for the Development of Clostridium Perfringens-Induced Necrotic Enteritis in Broiler Chickens.” *PLoS ONE* 9, no. 9 (September 30, 2014). https://doi.org/10.1371/journal.pone.0108775.

Antonissen, Gunther, Filip Van Immerseel, Frank Pasmans, Richard Ducatelle, Geert P. Janssens, Siegrid De Baere, Konstantinos C. Mountzouris, et al. “Mycotoxins Deoxynivalenol and Fumonisins Alter the Extrinsic Component of Intestinal Barrier in Broiler Chickens.” *Journal of Agricultural and Food Chemistry* 63, no. 50 (December 10, 2015): 10846–55. https://doi.org/10.1021/acs.jafc.5b04119.

Antonissen, Gunther, Venessa Eeckhaut, Karolien Van Driessche, Lonneke Onrust, Freddy Haesebrouck, Richard Ducatelle, Robert J Moore, and Filip Van Immerseel. “Microbial Shifts Associated with Necrotic Enteritis.” *Avian Pathology* 45, no. 3 (May 3, 2016): 308–12. https://doi.org/10.1080/03079457.2016.1152625.

Bai, Shiping, Leilei Wang, Yuheng Luo, Xumei Ding, Jun Yang, Jie Bai, Keying Zhang, and Jianping Wang. “Effects of Corn Naturally Contaminated with Aflatoxins on Performance, Calcium and Phosphorus Metabolism, and Bone Mineralization of Broiler Chicks.” *The Journal of Poultry Science* 51, no. 2 (2014): 157–64. https://doi.org/10.2141/jpsa.0130053.

Bouhet, Sandrine, and Isabelle P. Oswald. “The Intestine as a Possible Target for Fumonisin Toxicity.” *Molecular Nutrition & Food Research* 51, no. 8 (August 2007): 925–31. https://doi.org/10.1002/mnfr.200600266.

Chi, M.S., C.J. Mirocha, H.J. Kurtz, G. Weaver, F. Bates, W. Shimoda, and H.R. Burmeister. “Acute Toxicity of T-2 Toxin in Broiler Chicks and Laying Hens ,.” *Poultry Science* 56, no. 1 (January 1977): 103–16. https://doi.org/10.3382/ps.0560103.

Chu, Qili, Weidong Wu, Mark E. Cook, and Eugene B. Smalley. “Induction of Tibial Dyschondroplasia and Suppression of Cell-Mediated Immunity in Chickens by Fusarium Oxysporum Grown on Sterile Corn.” *Avian Diseases* 39, no. 1 (January 1995): 100. https://doi.org/10.2307/1591988.

Costanzo, Paola, Antonello Santini, Luigi Fattore, Ettore Novellino, and Alberto Ritieni. “Toxicity of Aflatoxin B1 towards the Vitamin D Receptor (VDR).” *Food and Chemical Toxicology* 76 (February 2015): 77–79.

https://doi.org/10.1016/j.fct.2014.11.025.

Costanzo, Paola, Antonello Santini, Luigi Fattore, Ettore Novellino, and Alberto Ritieni. “Toxicity of Aflatoxin B1 towards the Vitamin D Receptor (VDR).” *Food and Chemical Toxicology* 76 (February 2015): 77–79. https://doi.org/10.1016/j.fct.2014.11.025.

Debouck, C., E. Haubruge, P. Bollaerts, D. van Bignoot, Y. Brostaux, A. Werry, and M. Rooze. “Skeletal Deformities Induced by the Intraperitoneal Administration of Deoxynivalenol (Vomitoxin) in Mice.” *International Orthopaedics* 25, no. 3 (March 24, 2001): 194–98. https://doi.org/10.1007/s002640100235.

Devegowda, G., and D. Ravikiran. “Mycotoxins and Skeletal Problems in Poultry.” *World Mycotoxin Journal* 2, no. 3 (August 1, 2009): 331–37. https://doi.org/10.3920/wmj2008.1085.

Duff, S.R.I., R.B. Burns, and P. Dwivedi. “Skeletal Changes in Broiler Chicks and Turkey Poults Fed Diets Containing Ochratoxin a.” *Research in Veterinary Science* 43, no. 3 (November 1987): 301–7. https://doi.org/10.1016/s0034-5288(18)30798-7.

Fukata, T., K. Sasai, E. Baba, and A. Arakawa. “Effect of Ochratoxin A on Salmonella Typhimurium-Challenged Layer Chickens.” *Avian Diseases* 40, no. 4 (October 1996): 924. https://doi.org/10.2307/1592318.

Gao, Y., Z.-Q. Du, C.-G. Feng, X.-M. Deng, N. Li, Y. Da, and X.-X. Hu. “Identification of Quantitative Trait Loci for Shank Length and Growth at Different Development Stages in Chicken.” *Animal Genetics* 41, no. 1 (January 6, 2010): 101–4. https://doi.org/10.1111/j.1365-2052.2009.01962.x.

Grenier, Bertrand, Ilse Dohnal, Revathi Shanmugasundaram, Susan Eicher, Ramesh Selvaraj, Gerd Schatzmayr, and Todd Applegate. “Susceptibility of Broiler Chickens to Coccidiosis When Fed Subclinical Doses of Deoxynivalenol and Fumonisins—Special Emphasis on the Immunological Response and the Mycotoxin Interaction.” *Toxins* 8, no. 8 (July 27, 2016): 231. https://doi.org/10.3390/toxins8080231.

Gu, Wang, Qiang Bao, Kaiqi Weng, Jinlu Liu, Shuwen Luo, Jianzhou Chen, Zheng Li, et al. “Effects of T-2 Toxin on Growth Performance, Feather Quality, Tibia Development and Blood Parameters in Yangzhou Goslings.” *Poultry Science* 102, no. 2 (February 2023): 102382. https://doi.org/10.1016/j.psj.2022.102382.

Guntur, Anyonya R., and Clifford J. Rosen. “Bone as an Endocrine Organ.” *Endocrine Practice* 18, no. 5 (September 2012): 758–62. https://doi.org/10.4158/ep12141.ra.

Gupta, S., N. Jindal, R.S. Khokhar, A.K. Gupta, D.R. Ledoux, and G.E. Rottinghaus. “Effect of Ochratoxin A on Broiler Chicks Challenged with*Salmonella Gallinarum*.” *British Poultry Science* 46, no. 4 (August 2005): 443–50. https://doi.org/10.1080/00071660500190850.

Han, Xin-Yan, Qi-Chun Huang, Wei-Fen Li, Jun-Fang Jiang, and Zi-Rong Xu. “Changes in Growth Performance, Digestive Enzyme Activities and Nutrient Digestibility of Cherry Valley Ducks in Response to Aflatoxin B1 Levels.” *Livestock Science* 119, no. 1–3 (December 2008): 216–20. https://doi.org/10.1016/j.livsci.2008.04.006.

He, Shao-jun, Jia-fa Hou, Yu-yi Dai, Zhen-lei Zhou, and Yi-feng Deng. “*N*-acetyl-cysteine Protects Chicken Growth Plate Chondrocytes from T-2 Toxin-induced Oxidative Stress.” *Journal of Applied Toxicology* 32, no. 12 (July 28, 2011): 980–85. https://doi.org/10.1002/jat.1697.

Hou, Hai-Feng, Jin-Ping Li, Guo-Yong Ding, Wen-Jing Ye, Peng Jiao, and Qun-Wei Li. “The Cytotoxic Effect and Injury Mechanism of Deoxynivalenol on Articular Chondrocytes in Human Embryo.” *Zhonghua Yu Fang Yi Xue Za Zhi* 45, no. 7 (July 2011): 629–32.

Huff, W. E., R. D. Wyatt, and P. B. Hamilton. “Nephrotoxicity of Dietary Ochratoxin A in Broiler Chikens1.” *Applied Microbiology* 30, no. 1 (1975): 48–51. https://doi.org/10.1128/aem.30.1.48-51.1975.

Huff, William E., John A. Doerr, Pat B. Hamilton, Donald D. Hamann, Robert E. Peterson, and Alex Ciegler. “Evaluation of Bone Strength during Aflatoxicosis and Ochratoxicosis.” *Applied and Environmental Microbiology* 40, no. 1 (July 1980): 102–7. https://doi.org/10.1128/aem.40.1.102-107.1980.

Kermanshahi, H., M.R. Akbari, M. Maleki, and M. Behgar. “Effect of Prolonged Low Level Inclusion of Aflatoxin B1 into Diet on Performance, Nutrient Digestibility, Histopathology and Blood Enzymes of Broiler Chickens.” *J of Anim and Vet Adv* 6, no. 5 (2007): 686–92.

Keçi, Marsel, Annegret Lucke, Peter Paulsen, Qendrim Zebeli, Josef Böhm, and Barbara U. Metzler-Zebeli.

"Deoxynivalenol in the Diet Impairs Bone Mineralization in Broiler Chickens." *Toxins* 11, no. 6 (June 18, 2019): 352. https://doi.org/10.3390/toxins11060352.

Ledoux, David R., Tom P. Brown, Tandice S. Weibking, and George E. Rottinghaus. "Fumonisin Toxicity in Broiler Chicks." *Journal of Veterinary Diagnostic Investigation* 4, no. 3 (July 1992): 330–33. https://doi.org/10.1177/104063879200400317.

Li, Tingting, Guanzhong Xing, Yuxin Shao, Liyang Zhang, Sufen Li, Lin Lu, Zongping Liu, Xiudong Liao, and Xugang Luo. "Dietary Calcium or Phosphorus Deficiency Impairs the Bone Development by Regulating Related Calcium or Phosphorus Metabolic Utilization Parameters of Broilers." *Poultry Science* 99, no. 6 (June 2020): 3207–14. https://doi.org/10.1016/j.psj.2020.01.028.

Loughrill, Emma, David Wray, Tatiana Christides, and Nazanin Zand. "Calcium to Phosphorus Ratio, Essential Elements and Vitamin D Content of Infant Foods in the UK: Possible Implications for Bone Health." *Maternal & Child Nutrition* 13, no. 3 (September 9, 2016). https://doi.org/10.1111/mcn.12368.

McGuckin, Michael A., Sara K. Lindén, Philip Sutton, and Timothy H. Florin. "Mucin Dynamics and Enteric Pathogens." *Nature Reviews Microbiology* 9, no. 4 (March 16, 2011): 265–78. https://doi.org/10.1038/nrmicro2538.

Morishita, Y., K. Nagasawa, Naoko Nakano, and Kimiko Shiromizu. "Bacterial Overgrowth in the Jejunum of ICR Mice and Wistar Rats Orally Administered with a Single Lethal Dose of Fusarenon-x, a Trichothecene Mycotoxin." *Journal of Applied Bacteriology* 66, no. 4 (April 1989): 263–70. https://doi.org/10.1111/j.1365-2672.1989.tb02478.x.

Paneru, Deependra, Milan Kumar Sharma, Hanyi Shi, Jinquan Wang, and Woo Kyun Kim. "Aflatoxin B1 Impairs Bone Mineralization in Broiler Chickens." *Toxins* 16, no. 2 (February 2, 2024): 78. https://doi.org/10.3390/toxins16020078.

Pegram, R.A., and R.D. Wyatt. "Avian Gout Caused by Oosporein, a Mycotoxin Produced by Chaetomium Trilaterale." *Poultry Science* 60, no. 11 (November 1981): 2429–40. https://doi.org/10.3382/ps.0602429.

Persico, Marco, Raffaele Sessa, Elena Cesaro, Irene Dini, Paola Costanzo, Alberto Ritieni, Caterina Fattorusso, and Michela Grosso. "A Multidisciplinary Approach Disclosing Unexplored Aflatoxin B1 Roles in Severe Impairment of Vitamin D Mechanisms of Action." *Cell Biology and Toxicology* 39, no. 4 (September 6, 2022): 1275–95. https://doi.org/10.1007/s10565-022-09752-y.

Pinton, Philippe, Fabien Graziani, Ange Pujol, Cendrine Nicoletti, Océane Paris, Pauline Ernouf, Eric Di Pasquale, Josette Perrier, Isabelle P. Oswald, and Marc Maresca. "Deoxynivalenol Inhibits the Expression by Goblet Cells of Intestinal Mucins through a PKR and MAP Kinase Dependent Repression of the Resistin-like Molecule β." *Molecular Nutrition & Food Research* 59, no. 6 (April 27, 2015): 1076–87. https://doi.org/10.1002/mnfr.201500005.

Raju, M.V.L.N., and G. Devegowda. "Influence of Esterified-Glucomannan on Performance and Organ Morphology, Serum Biochemistry and Haematology in Broilers Exposed to Individual and Combined Mycotoxicosis (Aflatoxin, Ochratoxin and T-2 Toxin)." *British Poultry Science* 41, no. 5 (December 2000): 640–50. https://doi.org/10.1080/713654986.

Rath, Narayan C., and Vijay Durairaj. "Avian Bone Physiology and Poultry Bone Disorders." *Sturkie's Avian Physiology*, 2022, 549–63. https://doi.org/10.1016/b978-0-12-819770-7.00037-2.

Siller, W.G. "Renal Pathology of the Fowl — a Review." *Avian Pathology* 10, no. 3 (July 1981): 187–262. https://doi.org/10.1080/03079458108418474.

Suchacki, Karla J, Fiona Roberts, Andrea Lovdel, Colin Farquharson, Nik M Morton, Vicky E MacRae, and William P Cawthorn. "Skeletal Energy Homeostasis: A Paradigm of Endocrine Discovery." *Journal of Endocrinology* 234, no. 1 (July 2017). https://doi.org/10.1530/joe-17-0147.

Tran, Si-Trung, Y. Ruangpanit, K. Rassmidatta, K. Pongmanee, K. Palanisamy, and M. Caballero. "The World Mycotoxin Forum, 14th Conference." In *WMF Meets Belgium – Abstracts of Lectures and Posters*, 120–21. Antwerp: Conference Secretariat Bastiaanse Communication, 2023.

Ukwu, H.O, V.M.O. Okoro, and R.J. Nosike. "Statistical Modelling of Body Weight and Linear Body Measurements in Nigerian Indigenous Chicken." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, Ver. V, 7, no. 1

(2014): 27–30.

Wright, G.C., Walter F.O. Marasas, and Leon Sokoloff. “Effect of Fusarochromanone and T-2 Toxin on Articular Chondrocytes in Monolayer Culture in Monolayer Culture.” *Toxicological Sciences* 9, no. 3 (1987): 595–97. https://doi.org/10.1093/toxsci/9.3.595.

Wu, Weidong, Mark E. Cook, Qili Chu, and Eugene B. Smalley. “Tibial Dyschondroplasia of Chickens Induced by Fusarochromanone, a Mycotoxin.” *Avian Diseases* 37, no. 2 (April 1993): 302. https://doi.org/10.2307/1591653.

Wu, Weidong, Tianxing Liu, and Ronald F. Vesonder. “Comparative Cytotoxicity of Fumonisin B1 and Moniliformin in Chicken Primary Cell Cultures.” *Mycopathologia* 132, no. 2 (November 1995): 111–16. https://doi.org/10.1007/bf01103783.

Yu, Jun, Yu Wan, Haiming Yang, and Zhiyue Wang. “Age- and Sex-Related Changes in Body Weight, Muscle, and Tibia in Growing Chinese Domestic Geese (Anser Domesticus).” *Agriculture* 12, no. 4 (March 25, 2022): 463. https://doi.org/10.3390/agriculture12040463.

Zhang, Jie, Xuerun Liu, Ying Su, and Tushuai Li. “An Update on T2-Toxins: Metabolism, Immunotoxicity Mechanism and Human Assessment Exposure of Intestinal Microbiota.” *Heliyon* 8, no. 8 (August 2022). https://doi.org/10.1016/j.heliyon.2022.e10012.

Zhao, J., R.B. Shirley, J.D. Dibner, F. Uraizee, M. Officer, M. Kitchell, M. Vazquez-Anon, and C.D. Knight. “Comparison of Hydrated Sodium Calcium Aluminosilicate and Yeast Cell Wall on Counteracting Aflatoxicosis in Broiler Chicks.” *Poultry Science* 89, no. 10 (October 2010): 2147–56. https://doi.org/10.3382/ps.2009-00608.

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

By Marisabel Caballero, Global Technical Manager Poultry

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).

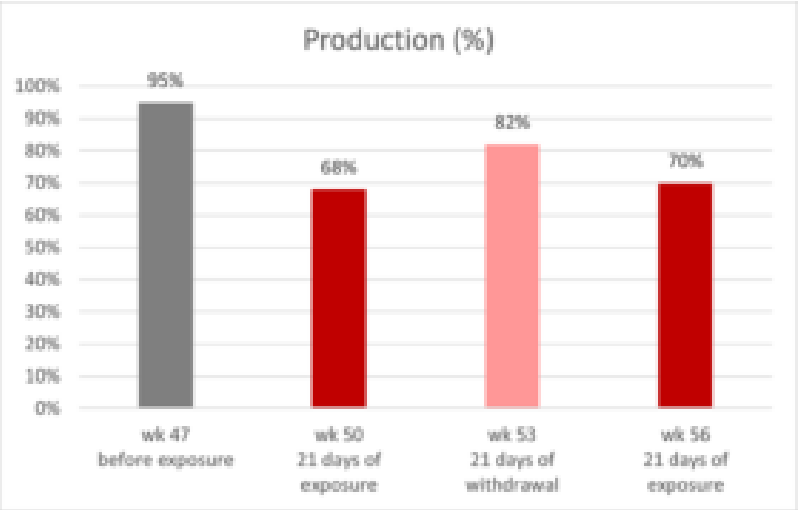


Figure 1: production of laying hens fed naturally contaminated feed with AFB1 and OTA

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.

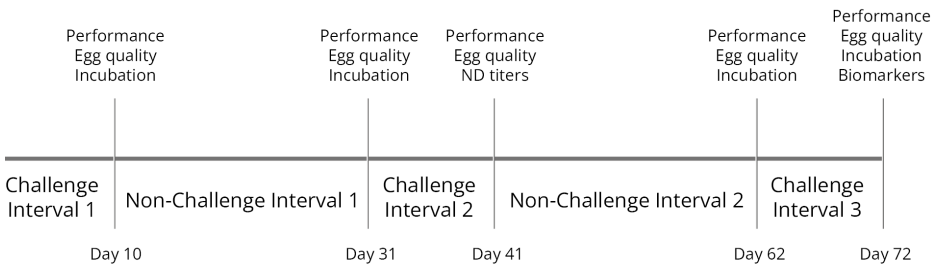
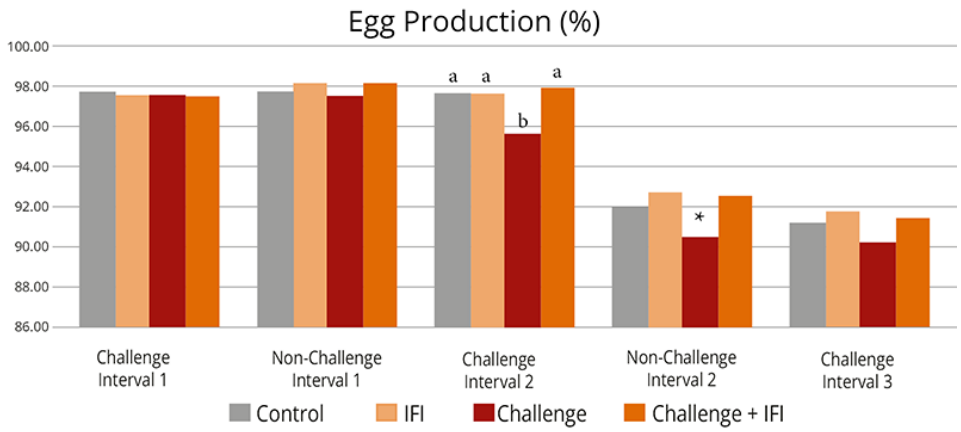


Figure 2: trial timeline showing challenge and non-challenge intervals and days of data collection and sampling.

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)*

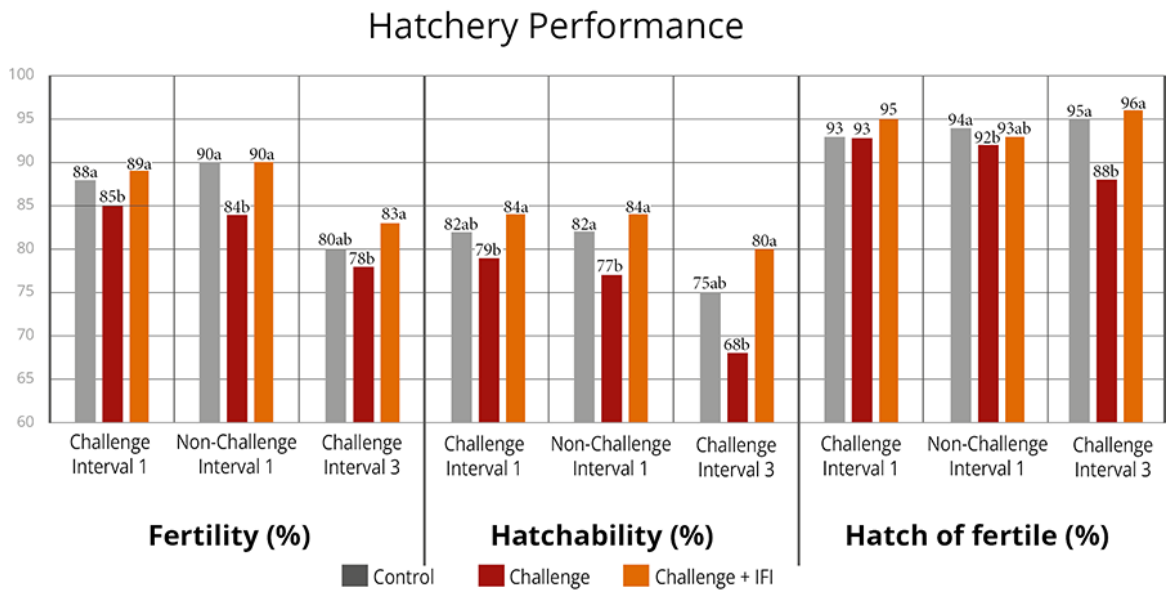
Group	Eggshell strength (N)	Eggshell thickness (mm)	Haugh Units
Control	21,02^{a}	0,3661^{ab}	70,88
IFI	21,16^{a}	0,3702^{a}	71,68
Challenge	20,05^{b}	0,3630^{b}	70,07*
Ch+IFI	21,06^{a}	0,3698^{a}	71,06

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.

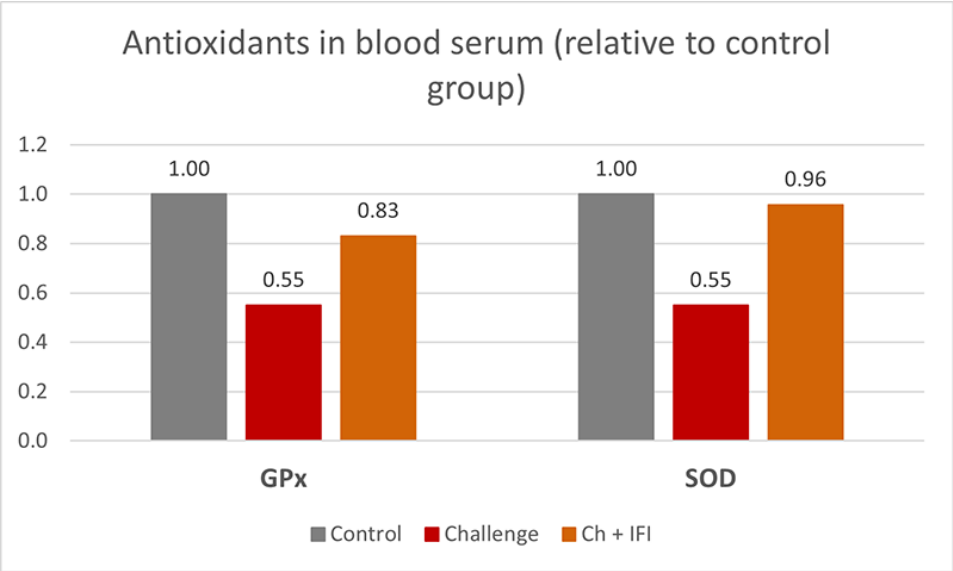


Figure 5: Antioxidants in blood serum, glutathione peroxidase (GPx), and superoxide dismutase (SOD) from breeders intermittently challenged with AFB1 and OTA, with and without an in-feed intervention (IFI).

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.

References

1. Kos, Jovana, Mislav Anić, Bojana Radić, Manuela Zadravec, Elizabet Janić Hajnal, and Jelka Pleadin. "Climate Change—a Global Threat Resulting in Increasing Mycotoxin Occurrence." *Foods* 12, no. 14 (July 14, 2023): 2704. https://doi.org/10.3390/foods12142704.
2. Zingales, Veronica, Mercedes Taroncher, Piera Anna Martino, María-José Ruiz, and Francesca Caloni. "Climate Change and Effects on Molds and Mycotoxins." *Toxins* 14, no. 7 (June 30, 2022): 445. https://doi.org/10.3390/toxins14070445.
3. Loi, Martina, Antonio F. Logrieco, Tünde Pusztahelyi, Éva Leiter, László Hornok, and István Pócsi. "Advanced Mycotoxin Control and Decontamination Techniques in View of an Increased Aflatoxin Risk in Europe Due to Climate Change." *Frontiers in Microbiology* 13 (January 10, 2023). https://doi.org/10.3389/fmicb.2022.1085891.
4. Medina, Ángel, Jesús M González-Jartín, and María J Sainz. "Impact of Global Warming on Mycotoxins." *Current Opinion in Food Science* 18 (December 2017): 76–81. https://doi.org/10.1016/j.cofs.2017.11.009.
5. Grenier, Bertrand, and Todd Applegate. "Modulation of Intestinal Functions Following Mycotoxin Ingestion: Meta-Analysis of Published Experiments in Animals." *Toxins* 5, no. 2 (February 21, 2013):

396–430. https://doi.org/10.3390/toxins5020396.
6. Filazi, Ayhan, Begum Yurdakok-Dikmen, Ozgur Kuzukiran, and Ufuk Tansel Sireli. “Mycotoxins in Poultry.” *Poultry Science*, February 15, 2017. https://doi.org/10.5772/66302.
7. Amirkhizi, Behzad, Seyed Rafie Arefhosseini, Masoud Ansarin, and Mahboob Nemati. “Aflatoxin B1in Eggs and Chicken Livers by Dispersive Liquid–Liquid Microextraction and HPLC.” *Food Additives & Contaminants: Part B*, August 27, 2015, 1–5. https://doi.org/10.1080/19393210.2015.1067649.
8. Emmanuel K, Tangni, Van Pamel Els, Huybrechts Bart, Delezie Evelyne, Van Hoeck Els, and Daeseleire Els. “Carry-over of Some Fusarium Mycotoxins in Tissues and Eggs of Chickens Fed Experimentally Mycotoxin-Contaminated Diets.” *Food and Chemical Toxicology* 145 (November 2020): 111715. https://doi.org/10.1016/j.fct.2020.111715.
9. Ebrahem, Mohammad, Susanne Kersten, Hana Valenta, Gerhard Breves, and Sven Dänicke. “Residues of Deoxynivalenol (Don) and Its Metabolite de-Epoxy-Don in Eggs, Plasma and Bile of Laying Hens of Different Genetic Backgrounds.” *Archives of Animal Nutrition* 68, no. 5 (August 20, 2014): 412–22. https://doi.org/10.1080/1745039x.2014.949029.
10. Salwa, A. Aly, and W. Anwer. “Effect of Naturally Contaminated Feed with Aflatoxins on Performance of Laying Hens and the Carryover of Aflatoxin B1 Residues in Table Eggs.” *Pakistan Journal of Nutrition* 8, no. 2 (January 15, 2009): 181–86. https://doi.org/10.3923/pjn.2009.181.186.
11. Devreese, Mathias, Gunther Antonissen, Nathan Broekaert, Siegrid De Baere, Lynn Vanhaecke, Patrick De Backer, and Siska Croubels. “Comparative Toxicokinetics, Absolute Oral Bioavailability, and Biotransformation of Zearalenone in Different Poultry Species.” *Journal of Agricultural and Food Chemistry* 63, no. 20 (May 19, 2015): 5092–98. https://doi.org/10.1021/acs.jafc.5b01608.
12. Galtier, P. “Biotransformation and Fate of Mycotoxins.” *Toxin Reviews* 18, no. 3 (August 1, 1999): 295–312. https://doi.org/10.3109/15569549909162648.
13. Galtier, P., M. Alvinerie, and J.L. Charpenteau. “The Pharmacokinetic Profiles of Ochratoxin A in Pigs, Rabbits and Chickens.” *Food and Cosmetics Toxicology* 19 (January 1981): 735–38. https://doi.org/10.1016/0015-6264(81)90528-9.
14. Hassan, Zahoor Ul, Muhammad Z Khan, Ahrar Khan, Ijaz Javed, and Zahid Hussain. “Effects of Individual and Combined Administration of Ochratoxin A and Aflatoxin B1 in Tissues and Eggs of White Leghorn Breeder Hens.” *Journal of the Science of Food and Agriculture* 92, no. 7 (December 16, 2011): 1540–44. https://doi.org/10.1002/jsfa.4740.
15. Li, Shao-Ji, Guangzhi Zhang, Bin Xue, Qiaoling Ding, Lu Han, Jian-chu Huang, Fuhai Wu, Chonggao Li, and Chunmin Yang. “Toxicity and Detoxification of T-2 Toxin in Poultry.” *Food and Chemical Toxicology* 169 (November 2022): 113392. https://doi.org/10.1016/j.fct.2022.113392.
16. Prelusky, D.B., R.M.G. Hamilton, and H.L. Trenholm. “Transmission of Residues to Eggs Following Long-Term Administration of 14 C-Labelled Deoxynivalenol to Laying Hens.” *Poultry Science* 68, no. 6 (June 1989): 744–48. https://doi.org/10.3382/ps.0680744.
17. Ringot, Diana, Abalo Chango, Yves-Jacques Schneider, and Yvan Larondelle. “Toxicokinetics and Toxicodynamics of Ochratoxin A, an Update.” *Chemico-Biological Interactions* 159, no. 1 (January 2006): 18–46. https://doi.org/10.1016/j.cbi.2005.10.106.
18. Osselaere, Ann, Mathias Devreese, Joline Goossens, Virginie Vandenbroucke, Siegrid De Baere, Patrick De Backer, and Siska Croubels. “Toxicokinetic Study and Absolute Oral Bioavailability of Deoxynivalenol, T-2 Toxin and Zearalenone in Broiler Chickens.” *Food and Chemical Toxicology* 51 (January 2013): 350–55. https://doi.org/10.1016/j.fct.2012.10.006.
19. Sudhakar, BV. “A Study on Experimentally Induced Aflatoxicosis on the Carryover of Aflatoxin B1 into Eggs and Liver Tissue of White Leghorn Hens.” *The Pharma Innovation Journal* 11, no. 2S (2022): 213–17.
20. Yiannikouris, Alexandros, and Jean-Pierre Jouany. “Mycotoxins in Feeds and Their Fate in Animals: A Review.” *Animal Research* 51, no. 2 (March 2002): 81–99. https://doi.org/10.1051/animres:2002012.
21. Bouhet, Sandrine, and Isabelle P. Oswald. “The Intestine as a Possible Target for Fumonisin Toxicity.” *Molecular Nutrition & Food Research* 51, no. 8 (August 2007): 925–31. https://doi.org/10.1002/mnfr.200600266.
22. Fakhri, Yadolah, Mansour Sarafraz, Amene Nematollahi, Vahid Ranaei, Moussa Soleimani-Ahmadi, Van Nam Thai, and Amin Mousavi Khaneghah. “A Global Systematic Review and Meta-Analysis of Concentration and Prevalence of Mycotoxins in Birds’ Egg.” *Environmental Science and Pollution Research* 28, no. 42 (September 9, 2021): 59542–50. https://doi.org/10.1007/s11356-021-16136-y.
23. Osaili, Tareq M., Akram R. Al-Abboodi, Mofleh AL. Awawdeh, and Samah Aref Jbour. “Assessment of Mycotoxins (Deoxynivalenol, Zearalenone, Aflatoxin B1 and Fumonisin B1) in Hen’s Eggs in Jordan.” *Heliyon* 8, no. 10 (October 2022). https://doi.org/10.1016/j.heliyon.2022.e11017.
24. Wang, Lan, Qiaoyan Zhang, Zheng Yan, Yanglan Tan, Runyue Zhu, Dianzhen Yu, Hua Yang, and Aibo Wu. “Occurrence and Quantitative Risk Assessment of Twelve Mycotoxins in Eggs and Chicken Tissues in China.” *Toxins* 10, no. 11 (November 16, 2018): 477. https://doi.org/10.3390/toxins10110477.

25. Tolosa, J., Y. Rodríguez-Carrasco, M.J. Ruiz, and P. Vila-Donat. “Multi-Mycotoxin Occurrence in Feed, Metabolism and Carry-over to Animal-Derived Food Products: A Review.” *Food and Chemical Toxicology* 158 (December 2021): 112661. https://doi.org/10.1016/j.fct.2021.112661.
26. Völkel, Inger, Eva Schröer-Merker, and Claus-Peter Czerny. “The Carry-over of Mycotoxins in Products of Animal Origin with Special Regard to Its Implications for the European Food Safety Legislation.” *Food and Nutrition Sciences* 02, no. 08 (2011): 852–67. https://doi.org/10.4236/fns.2011.28117.
27. Yuan, Tao, Junyi Li, Yanan Wang, Meiling Li, Ao Yang, Chenxi Ren, Desheng Qi, and Niya Zhang. “Effects of Zearalenone on Production Performance, Egg Quality, Ovarian Function and Gut Microbiota of Laying Hens.” *Toxins* 14, no. 10 (September 21, 2022): 653. https://doi.org/10.3390/toxins14100653.
28. Song, Bin, Teng Ma, Damien P. Prévéraud, Keying Zhang, Jianping Wang, Xuemei Ding, Qiufeng Zeng, et al. “Research Note: Effects of Feeding Corn Naturally Contaminated with Aflatoxin B1, Deoxynivalenol, and Zearalenone on Reproductive Performance of Broiler Breeders and Growth Performance of Their Progeny Chicks.” *Poultry Science* 102, no. 11 (November 2023): 103024. https://doi.org/10.1016/j.psj.2023.103024.
29. Qureshi, MA, J Brake, PB Hamilton, WM Hagler, and S Nesheim. “Dietary Exposure of Broiler Breeders to Aflatoxin Results in Immune Dysfunction in Progeny Chicks.” *Poultry Science* 77, no. 6 (June 1998): 812–19. https://doi.org/10.1093/ps/77.6.812.
30. Ul-Hassan, Zahoor, Muhammad Zargham Khan, Ahrar Khan, and Ijaz Javed. “Immunological Status of the Progeny of Breeder Hens Kept on Ochratoxin a (OTA)- and Aflatoxin B1(Afb1)-Contaminated Feeds.” *Journal of Immunotoxicology* 9, no. 4 (April 24, 2012): 381–91. https://doi.org/10.3109/1547691x.2012.675365.
31. Devegowda, G., and D. Ravikiran. “Mycotoxins and Eggshell Quality: Cracking the Problem.” *World Mycotoxin Journal* 1, no. 2 (May 1, 2008): 203–8. https://doi.org/10.3920/wmj2008.1037.
32. Onagbesan, O., V. Bruggeman, L. De Smit, M. Debonne, A. Witters, K. Tona, N. Everaert, and E. Decuypere. “Gas Exchange during Storage and Incubation of Avian Eggs: Effects on Embryogenesis, Hatchability, Chick Quality and Post-Hatch Growth.” *World’s Poultry Science Journal* 63, no. 4 (December 1, 2007): 557–73. https://doi.org/10.1017/s0043933907001614.
33. Ebrahem, Mohammad, Susanne Kersten, Hana Valenta, Gerhard Breves, Andreas Beineke, Kathrin Hermeyer, and Sven Dänicke. “Effects of Feeding Deoxynivalenol (Don)-Contaminated Wheat to Laying Hens and Roosters of Different Genetic Background on the Reproductive Performance and Health of the Newly Hatched Chicks.” *Mycotoxin Research* 30, no. 3 (April 11, 2014): 131–40. https://doi.org/10.1007/s12550-014-0197-z.
34. Yegani, M., T.K. Smith, S. Leeson, and H.J. Boermans. “Effects of Feeding Grains Naturally Contaminated with Fusarium Mycotoxins on Performance and Metabolism of Broiler Breeders.” *Poultry Science* 85, no. 9 (September 2006): 1541–49. https://doi.org/10.1093/ps/85.9.1541.
35. Calini, F, and F Sirri. “Breeder Nutrition and Offspring Performance.” *Revista Brasileira de Ciência Avícola* 9, no. 2 (June 2007): 77–83. https://doi.org/10.1590/s1516-635×2007000200001.
36. Hassan, ZU, MZ Khan, A Khan, I Javed, U Sadique, and A Khatoon. “Ochratoxicosis in White Leghorn Breeder Hens: Production and Breeding Performance.” *Vet. J.* 32, no. 4 (2012): 557–61.
37. Verma, J., T. S. Johri, and B. K. Swain. “Effect of Varying Levels of Aflatoxin, Ochratoxin and Their Combinations on the Performance and Egg Quality Characteristics in Laying Hens.” *Asian-Australasian Journal of Animal Sciences* 16, no. 7 (January 1, 2003): 1015–19. https://doi.org/10.5713/ajas.2003.1015.
38. Johnson-Dahl, M.L., M.J. Zuidhof, and D.R. Korver. “The Effect of Maternal Canthaxanthin Supplementation and Hen Age on Breeder Performance, Early Chick Traits, and Indices of Innate Immune Function.” *Poultry Science* 96, no. 3 (March 2017): 634–46. https://doi.org/10.3382/ps/pew293.
39. Bozzo, Giancarlo, Nicola Pugliese, Rossella Samarelli, Antonella Schiavone, Michela Maria Dimuccio, Elena Circella, Elisabetta Bonerba, Edmondo Ceci, and Antonio Camarda. “Ochratoxin A and Aflatoxin B1 Detection in Laying Hens for Omega 3-Enriched Eggs Production.” *Agriculture* 13, no. 1 (January 5, 2023): 138. https://doi.org/10.3390/agriculture13010138.
40. Surai, Peter F., Ivan I. Kochish, Vladimir I. Fisinin, and Michael T. Kidd. “Antioxidant Defence Systems and Oxidative Stress in Poultry Biology: An Update.” *Antioxidants* 8, no. 7 (July 22, 2019): 235. https://doi.org/10.3390/antiox8070235.

Masked mycotoxins – particularly dangerous for dairy cows

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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).

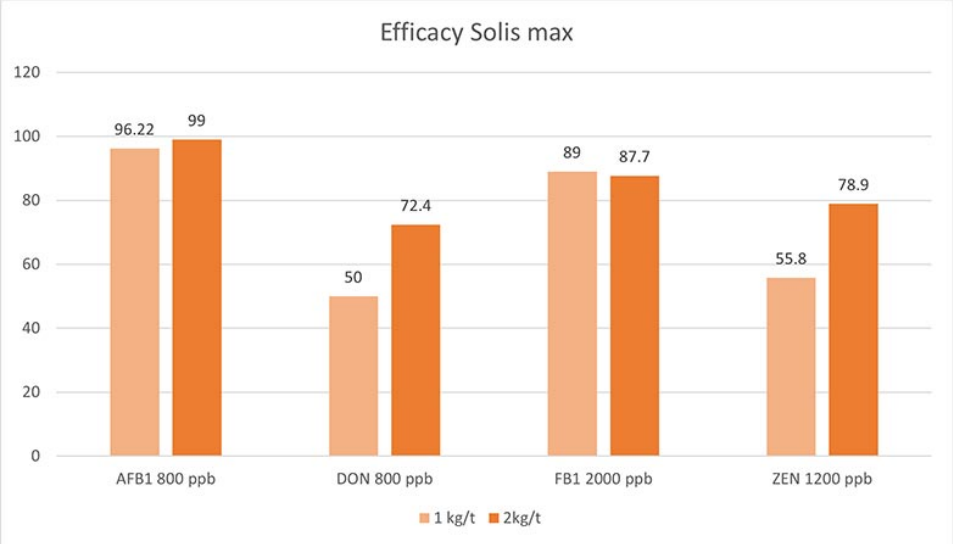

Figure 1: Solis Max showed a high binding capacity for the most relevant mycotoxins

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.

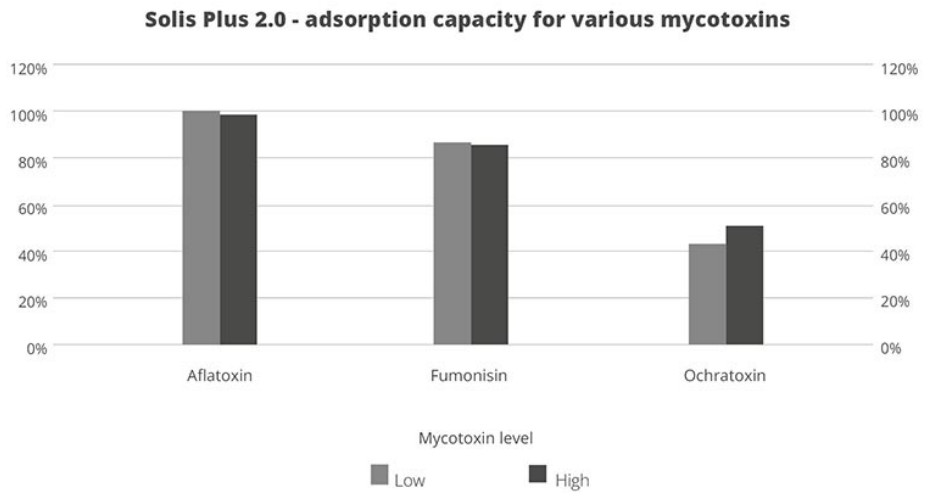

Figure 2: The adsorption capacity of Solis Plus 2.0 for three different mycotoxins at two challenge levels

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.

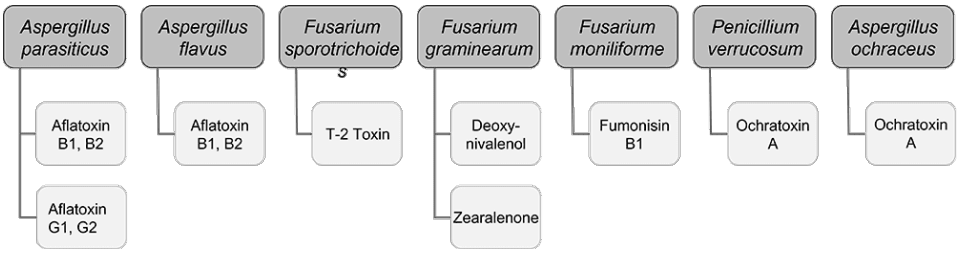

Figure 1: Fungi species and their mycotoxins of worldwide importance for poultry production (adapted from Bryden, 2012).

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.

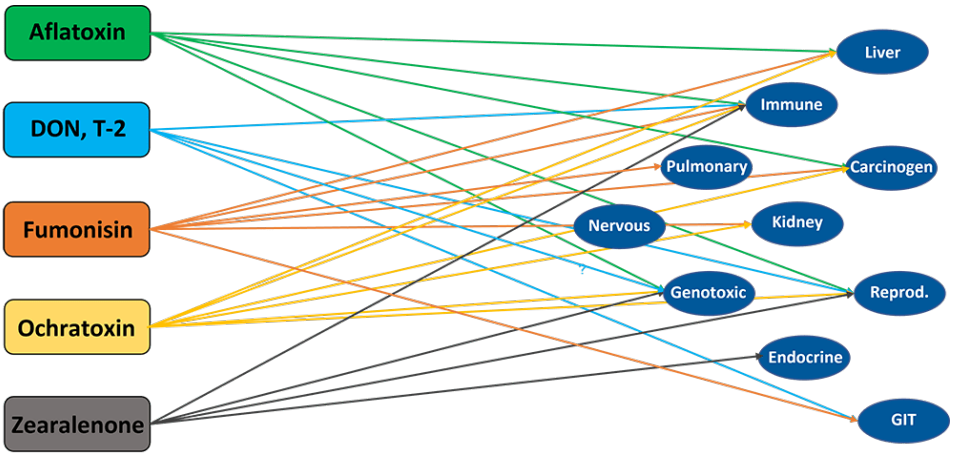

Figure 2: Main target organs of important mycotoxins

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:

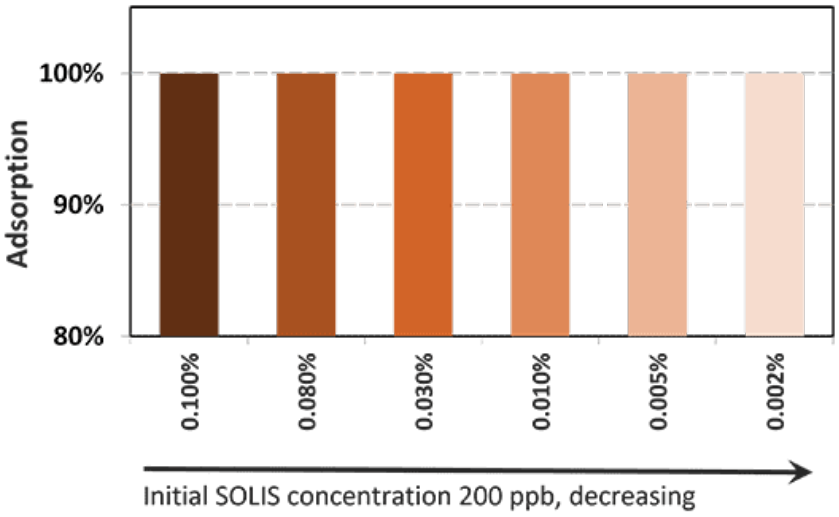

Figure 3: Binding capacity of Solis for Aflatoxin

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.

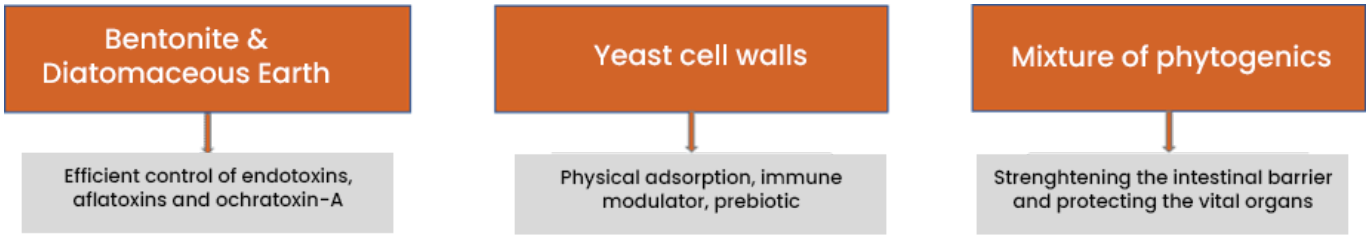

Figure 4: Composition and effects of Solis Max 2.0

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.

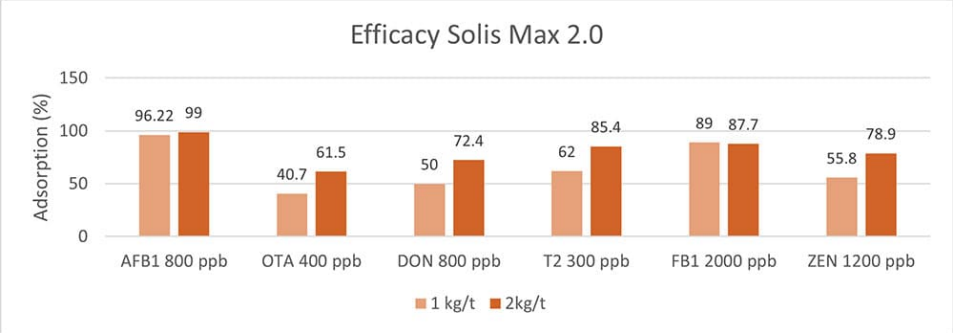

Figure 5: Efficacy of Solis Max 2.0 against different mycotoxins relevant in poultry production

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.

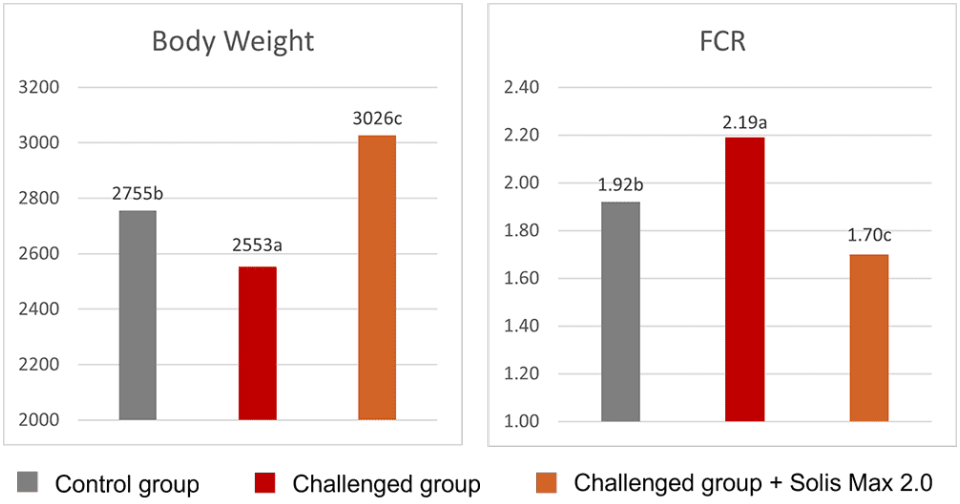

Figure 6: Better performance data due to the addition of Solis Max 2.0

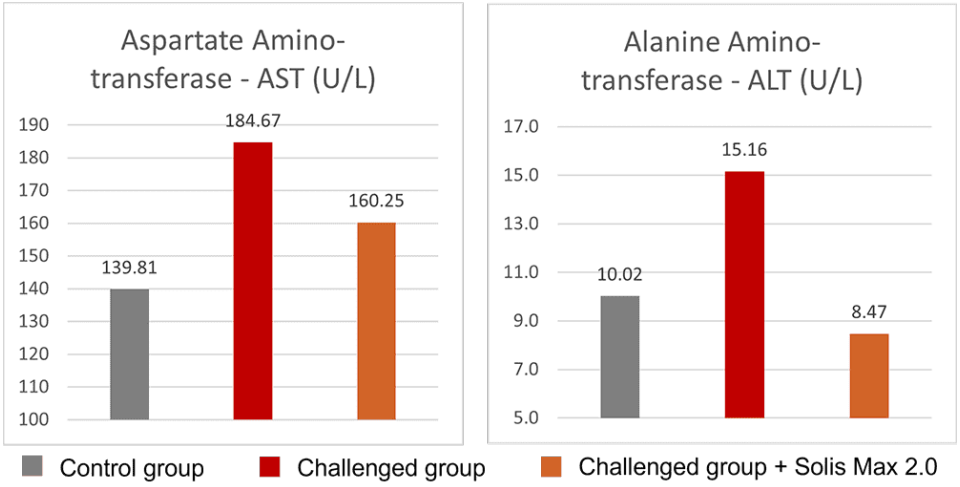

Figure 7: Healthier liver shown by lower values of AST and ALT

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.