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

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

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

Laying hens and breeders: carryover of

mycotoxins into eggs

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

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

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

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

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

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

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

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

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

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

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

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

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

Mycotoxins impair table egg production and quality

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

Natural contamination in laying hens: a case report

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

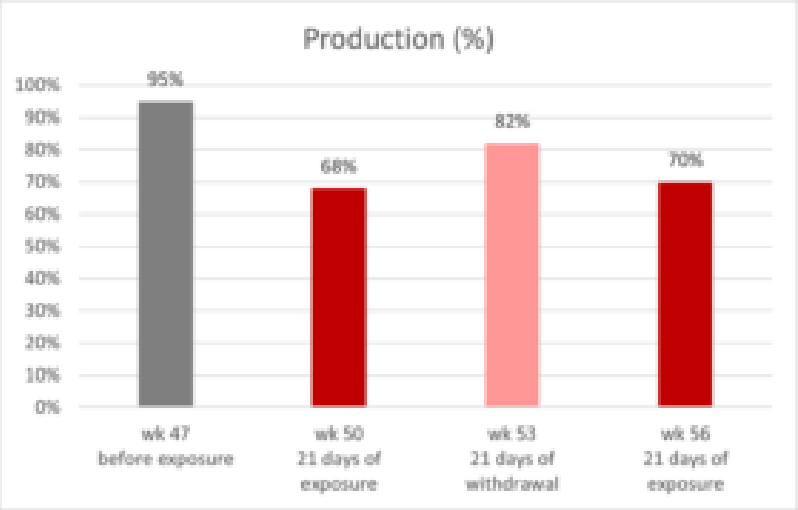


Figure 1: production of laying hens fed naturally contaminated feed with AFB1 and OTA

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

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

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

Table 2: mycotoxin analysis results for feed and organs

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

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

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

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

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

Table 3: experimental groups and mycotoxin challenge

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

Trial design:

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

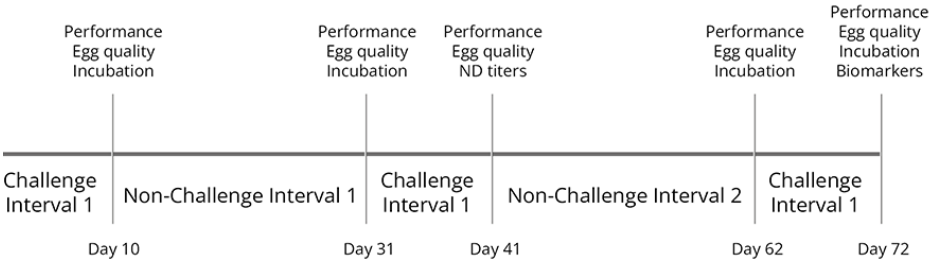
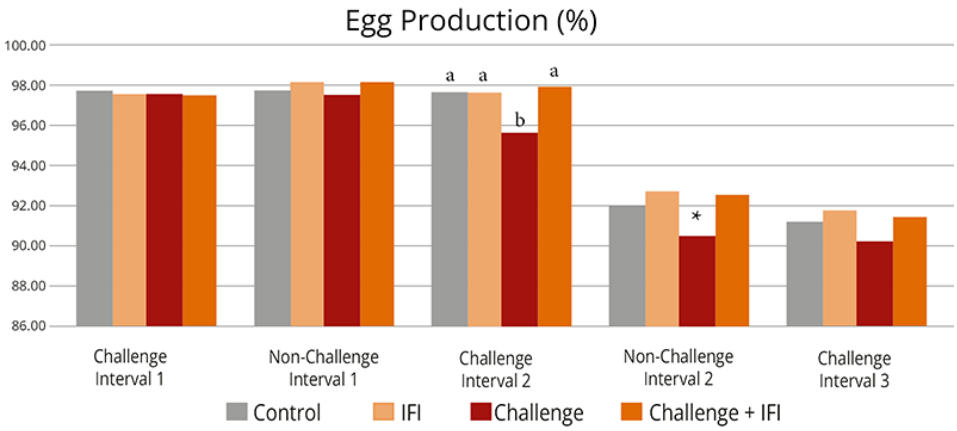


Figure 2: trial timeline showing challenge and non-challenge intervals and days of data collection and sampling.

Mitigated effects on egg production and egg quality

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



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

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

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

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

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

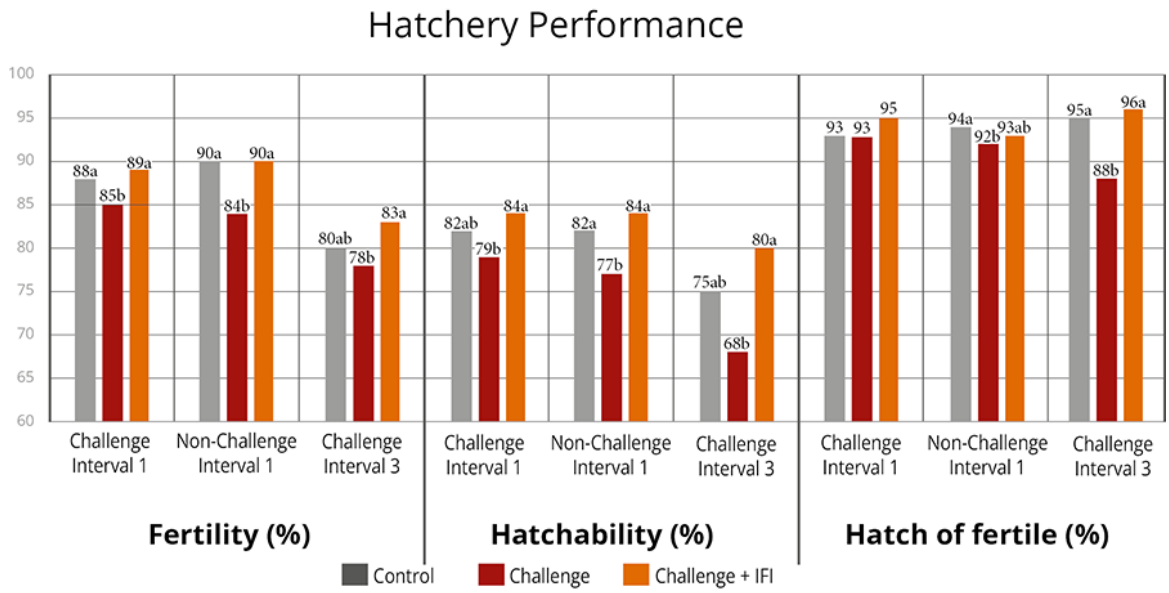
Different letters indicate significant differences ($p<0.05$). Statistical tendencies ($p<0.1$) are indicated by (*).

Mitigated effects on the progeny in incubation

trials

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

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



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

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

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

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

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

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

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

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

did not show this depletion.

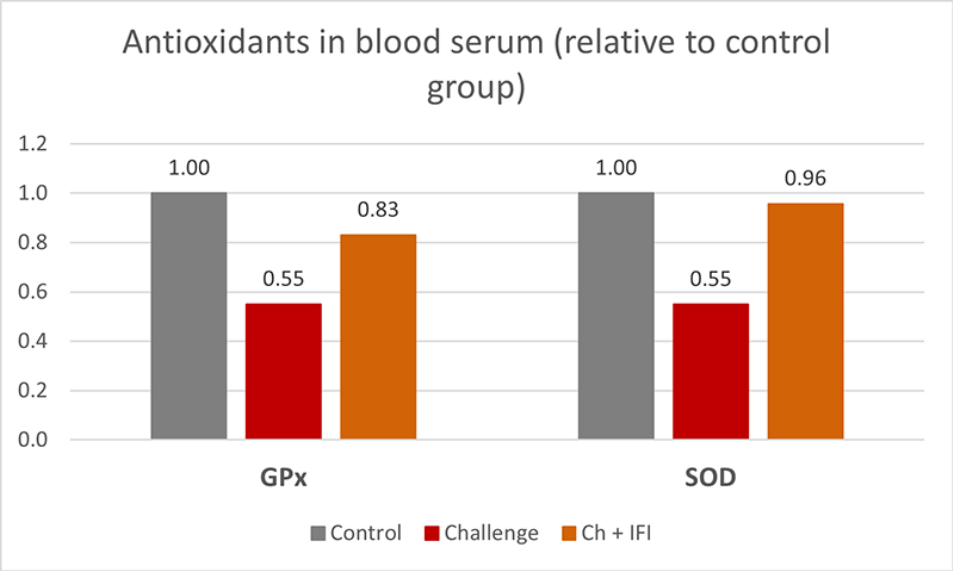


Figure 5: Antioxidants in blood serum, glutathione peroxidase (GPx), and superoxide dismutase (SOD) from breeders intermittently challenged with AFB1 and OTA, with and without an in-feed intervention (IFI).

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

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

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

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

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