

# Feed hygiene protects animals and humans



By **Vaibhav Gawande**, Assistant Manager Technical Services, **Dr. Inge Heinzl**, Editor, and **Marisabel Caballero**, Global Technical Manager Poultry, EW Nutrition

The utility value of feed consists of the nutritional value and the quality. The first covers all characteristics concerning the essential nutrients and is important for feed formulation and the adequate supply of the animals.

Feed quality comprises all characteristics of a feed influenced by treatment, storage, conservation, hygiene, and its content of specific substances. For many factors, guidance and threshold values are available which should be met to guarantee animal health and welfare, as well as to protect public health, since some undesirable substances can be transferred to animal products such as meat, eggs, and milk.

In this article, we will focus on feed hygiene. We will talk about the consequences of low feed quality, how to understand it, its causes, and possible solutions.

## What are the effects of deficient feed hygiene?

The consequences of deficient feed hygiene can be divided into two parts, impurities and spoilage.

Impurities comprise:

- the presence of soil, sand, or dust
- contamination with or residues of heavy metals, PCB, dioxins, pesticides, fertilizers, disinfectants, toxic plants, or banned feed ingredients

In the case of spoilage, we see:

- degradation of organic components by the action of molds and bacteria
- growth of pathogens such as E. coli, salmonella, etc.
- accumulation of toxins such as mycotoxins or bacterial toxins ([Hoffmann, 2021](#))

Bad feed hygiene can also negatively impact the feed's nutritional value by leading to a loss of energy as well as decreasing the bioavailability of vitamins A, D3, E, K, and B1.

But, how can all signs of deficient feed hygiene be recognized? Soil, sand, and probably dust can be seen in well-taken samples and impurities can be analyzed. But is it possible to spot spoilage? In this case, agglutinated particles, rancid odor, moisture, and discoloration are indicators. Sometimes, also the temperature of the feed or ingredient increases. However, spoilage is not always obvious and an analysis of the feed can give more information about the spoilage-related organisms present. It also helps to decide if the feed is safe for the animals or not. In the case of obvious alterations, the feed should not be consumed by any animal.

## Different organisms decrease feed quality and impact health

Several organisms can be responsible for a decrease in feed quality. Besides the visible pests such as rats, mice, or beetles, which can easily be noticed and combatted, there are organisms whose mastering is much more difficult. In the following part, the different harmful organisms and substances are described and solutions are presented.

## Enteropathogens can cause diarrhea and production losses

In poultry, different bacteria responsible for high production losses can be transferred via the feed. The most relevant of them are Clostridium perfringens, Escherichia coli, and some strains of Salmonella.

### Clostridium perfringens, the cause of necrotic enteritis

Clostridium perfringens is a Gram-positive, anaerobic bacterium that is extremely resistant to environmental influences and can survive in soil, feed, and litter for several years and even reproduce. Clostridium perfringens causes [necrotic enteritis](#) mainly in 2-16 weeks old chickens and turkeys, being more critical in 3-6 weeks old chicks.

There is a clinical and a subclinical form of necrotic enteritis. The clinical form can be detected very well due to clear symptoms and mortality rates up to 50%. The subclinical form, while harder to detect, also raises production costs due to a significant decrease in performance. The best prophylaxis against clostridia is the maintenance of gut health, including feed hygiene.

Clostridia can be found in animal by-products, as can be seen in table 1.

Sr. No.	Sample details	Clostridium perfringens contamination		Total number of samples	Positivity %
		Positive	Negative		
1	Meat and bone meal	39	52	91	42.86
2	Soya meal	0	3	3	0
3	Rape seed meal	0	1	1	0
4	Fish meal	21	17	38	55.26

5	Layer Feed	21	71	93	22.58
6	Dry fish	5	8	13	38.46
7	De-oiled rice bran	0	2	2	0
8	Maize	0	2	2	0
9	Bone meal	13	16	29	44.83

Table 1: Isolation of *Clostridium perfringens* from various poultry feed ingredients in Tamil Nadu, India (Udhayavel et al., 2017)

## Salmonella is harmful to animals and humans

Salmonella is a gram-negative enterobacterium and can occur in feed. There are only two species – *S. enterica* and *S. bongori* (Lin-Hui and Cheng-Hsun, 2007), but almost 2700 serotypes. The most known poultry-specific Salmonella serotypes are *S. pullorum* affecting chicks and *S. gallinarum* affecting adult birds. The other two well-known serotypes, *S. enteritidis* and *S. typhimurium* are the most economically important ones because they can also infect humans.

*Salmonella enteritidis*, in particular, can be transferred via table eggs to humans. The egg content can be infected vertically as a result of a colonization of the reproductive tract of the hen (De Reu, 2015). The other possibility is a horizontal infection, as some can penetrate through the eggshell from a contaminated environment or poor egg handling.

Salmonella can also be transferred through meat. However, as there are more production steps where contamination can happen (breeder and broiler farm, slaughterhouse, processing plants, food storage...), traceability is more complicated. As feed can be vector, feed hygiene is crucial.

Moreover, different studies have found that the same Salmonella types found in feed are also detected – weeks later – in poultry farms and even further in the food chain, as reviewed by Ricke and collaborators (2019). Other researches even imply that Salmonella contamination of carcasses and eggs could be significantly reduced by minimizing the incidence of Salmonella in the feed (Shirota et al., 2000).

## E. coli - some are pathogenic

*E. coli* is a gram-negative, not acid-resistant bacterium and most strains are inhabitants of the gut flora of birds, warm-blooded animals, and humans. Only some strains cause disease. To be infectious, the bacteria must have fimbriae to attach to the gut wall or the host must have an immune deficiency, perhaps due to stress. *E. coli* can be transmitted via contaminated feed or water as well as by fecal-contaminated dust.

*Escherichia coli* infections can be found in poultry of all ages and categories and nearly everywhere in the bird. *E. coli* affects the navel of chicks, the reproductive organs of hens, several parts of the gut, the respiratory tract, the bones and joints, and the skin and are part of the standard control.

The feed microbiome can contribute to a balanced gut microbial community. The origins of pathogenic *E. coli* in a flock can also be traced to feed contamination (Stanley & Bajagai, 2022). Especially in pre-starter/starter feeds, *E. coli* contamination can be critical as the day-old chick's gut is starting to be colonized. Especially in this phase, maintaining a low microbial count in feed is crucial.

## Molds cause feed spoilage and reduce nutritional value

Molds contaminate grains, both in the field and during storage, and can also grow in stored feed and even in feed stored or accumulated in storage facilities in animal production farms.

The contamination of feed by molds and their rapid growth can cause heating of the feed. As molds also need nutrients, their growth results in a reduction of energy and the availability of vitamins A, D3, E, K, and B1, thus decreasing the feed's nutritional value. This heating occurs in most feeds with a moisture content higher than 15 /16%. Additionally, mold-contaminated feed tends to be dusty and has a bad taste

impacting palatability and, as a consequence, feed intake and performance.

Molds produce spores that can, when inhaled, cause chronic respiratory disease or even death if the animals are exposed to contaminated feed for a longer time. Another consequence of mold contamination is the production of mycotoxins by several mold species. These mycotoxins can affect the animal in several ways, from decreasing performance to severe disease (Esmail, 2021; Government of Manitoba, 2023).

With effective feed hygiene management, we want to stop and prevent mold growth, as well as all its negative consequences.

## **Prevention is better than treatment**

It is clear that when the feed is spoiled, it must be removed, and animal health supporting measures should take place. However, it is better to prevent the consequences of low feed hygiene on animals. Proper harvest and adequate storage of the feed are basic measures to stop mold growth. Additionally, different tools are available to protect the animals from feed bacterial load and other risk factors.

## **Solutions are available to support feed hygiene**

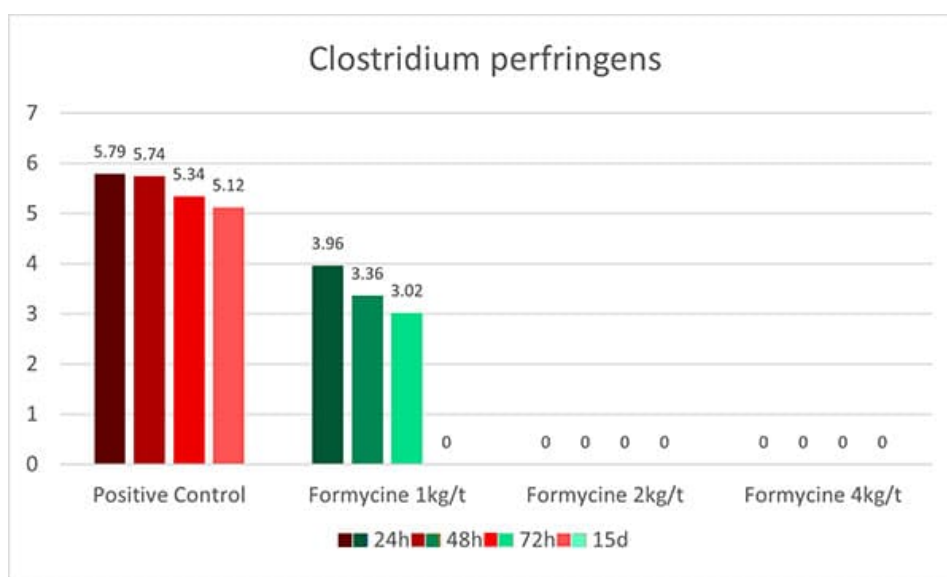
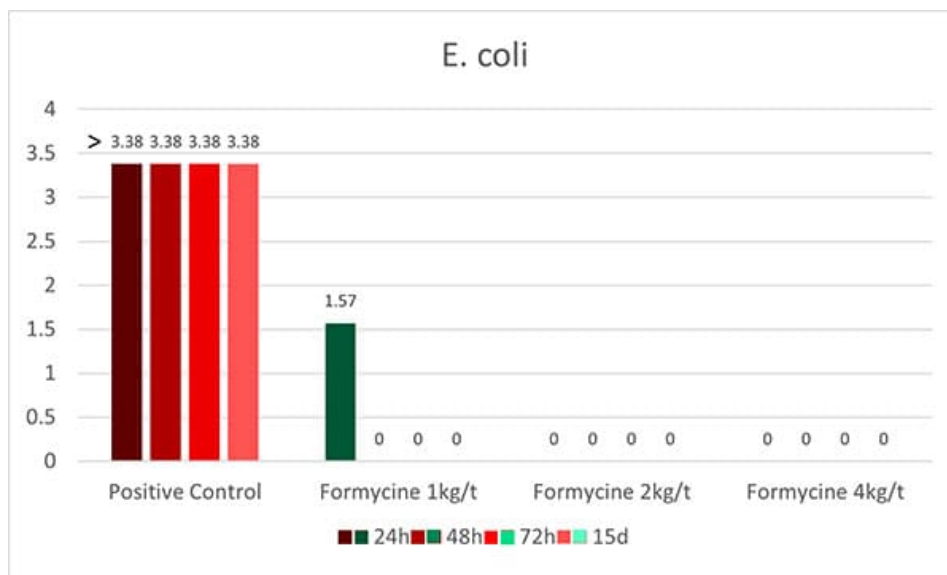
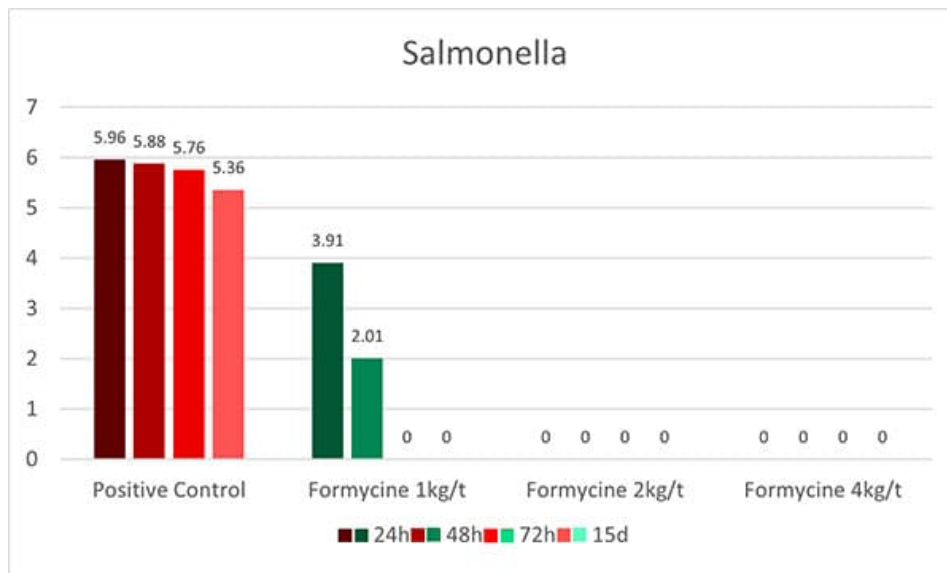
There are several solutions to fight the organisms which decrease feed quality. Some directly act against the harmful substances / pathogens, and others act indirectly, meaning that they change the environment to a non-comfortable one for the organism.

## **Formaldehyde and propionic acid – an unbeatable team against bacteria**

A combination of formaldehyde and propionic acid is perfect to sanitize feed. Formaldehyde results in bacterial DNA and protein damage, and propionic acid is active against bacteria and molds. Together, they improve the microbiological quality of the feed and reduce the risk of secondary diseases such as necrotic enteritis or dysbiosis on the farm. In addition to the pure hygienic aspect, organic acids support digestion.

An in-vitro trial was conducted to evaluate the effect of such a combination (Formycine Gold Px) against common poultry pathogens. Poultry feed was spiked with three different bacteria, achieving very high initial contamination of 1,000,000 CFU/g per pathogen. One batch of the contaminated feed served as a control (no additive). To the other contaminated batches, 1, 2, or 4 kg of Formycine per ton of feed were added. The results (means of triplicates) are shown in figures 1 a-c.





Figures 1 a-c: Reduction of bacterial count due to the addition of Formycine

Formycine Gold Px significantly reduced the bacterial counts in all three cases. A clear dose-response-effect can be seen and by using 2 kg of Formycine / t of feed, pathogens could not be detected anymore in the feed.

A further trial showed the positive effects of feeding Formycine Gold Px treated feed to the animals. Also here, the feed for both groups was contaminated with 1,000,000 CFU of Clostridium/g. The feed of the control group was not treated and to the treatment group, 2 kg of Formycine per t was added.

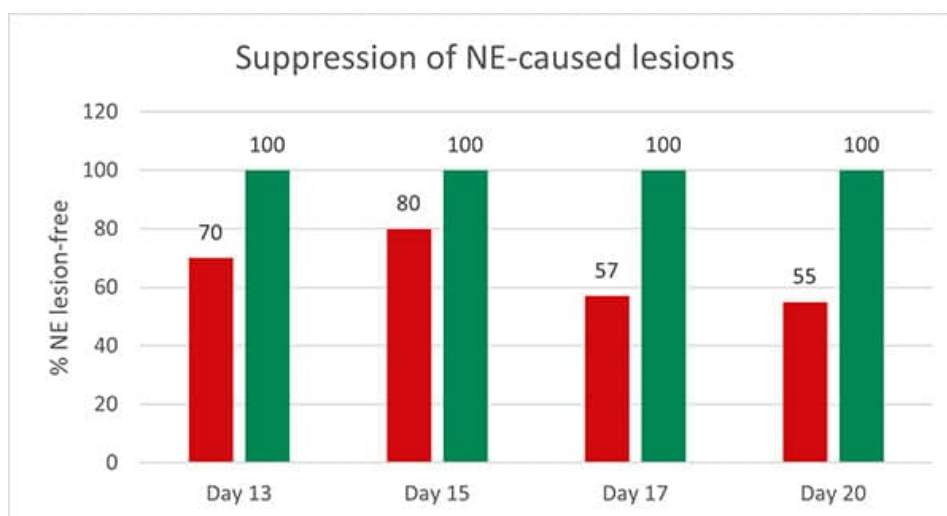


Figure 2: Preventive effect of Formycine Gold Px concerning necrotic enteritis gut lesions

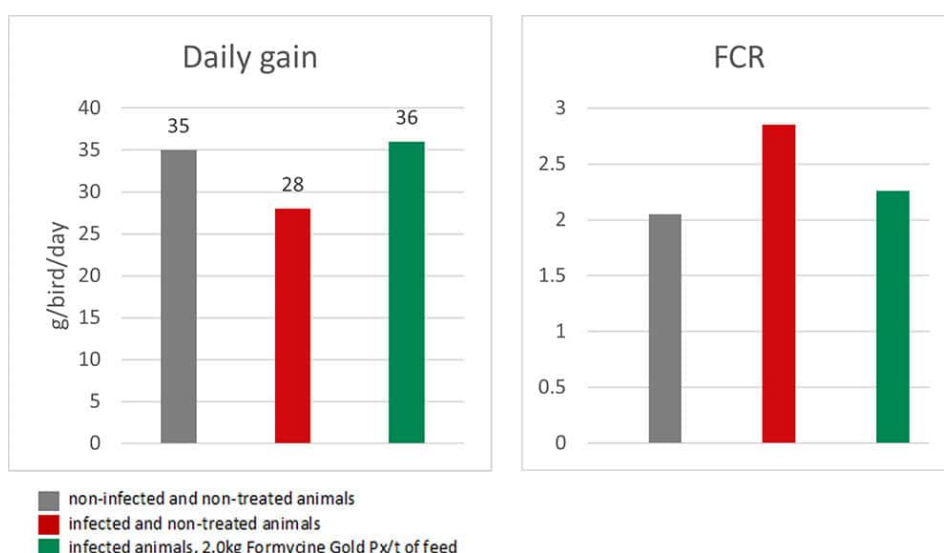


Figure 3a and 3b: Performance-maintaining effect of Formycine Gold Px

The trial showed that Formycine Gold Px reduced the ingestion of the pathogen, and thus could prevent the lesions caused by necrotic enteritis (Fig. 2). The consequence of this improved gut health is a better feed conversion and higher average daily gain (Fig.3a and 3b).

Products containing formaldehyde may represent a risk for humans, however, the adequate protection equipment helps to reduce/avoid exposure.

## A combination of free acids and acid salts provides optimal hygienic effects

Additionally, another blend of organic acids (Acidomix AFG) shows the best effects against representatives of relevant feed-borne pathogens in poultry. In a test, 50 µl solution containing different microorganisms (reference strains of *S. enterica*, *E. coli*, *C. perfringens*, *C. albicans*, and *A. niger*; concentration  $10^5$  CFU/ml, respectively) were pipetted into microdilution plates together with 50 µl of increasing concentrations of a mixture of organic acids (Acidomix). After incubation, the MIC and MBC of each pathogen were calculated.

The test results show (figure 4, Minimal Bactericidal Concentration) that 0.5% of Acidomix AFG in the

medium ( $\pm$  5kg/t of feed) is sufficient to kill *S. enterica*, *C. albicans*, and *A. niger* and even only 2.5kg/t in the case of *E. coli*. If the pathogens should only be prevented to proliferate, even a lower amount of product is requested (figure 5, Minimal Inhibitory Concentration – MIC)

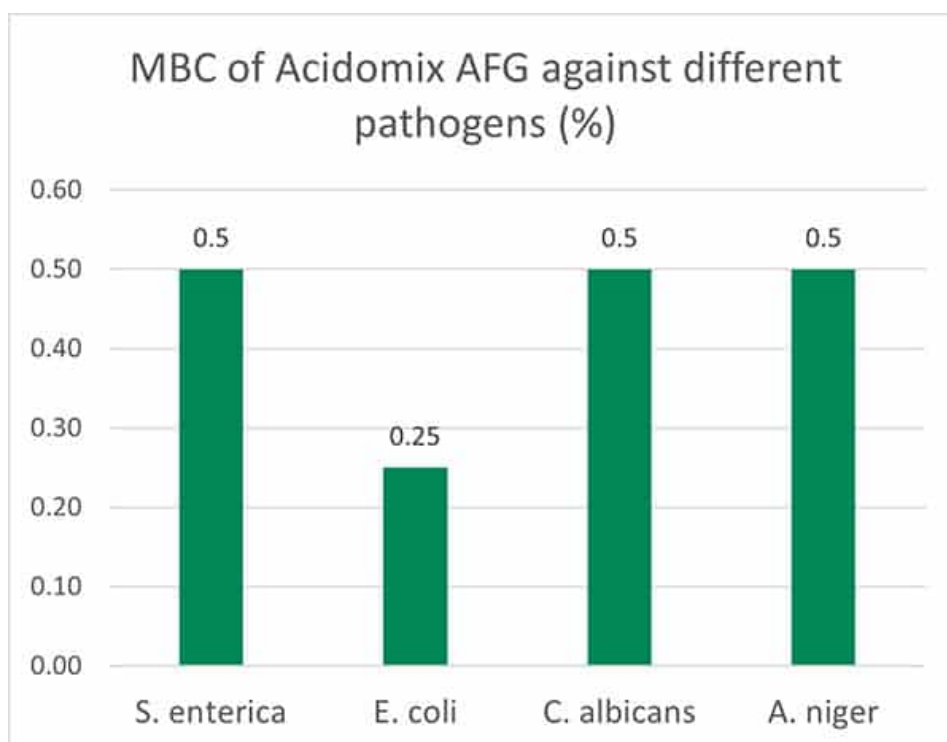


Figure 4: MBC of Acidomix AFG against different pathogens (%)

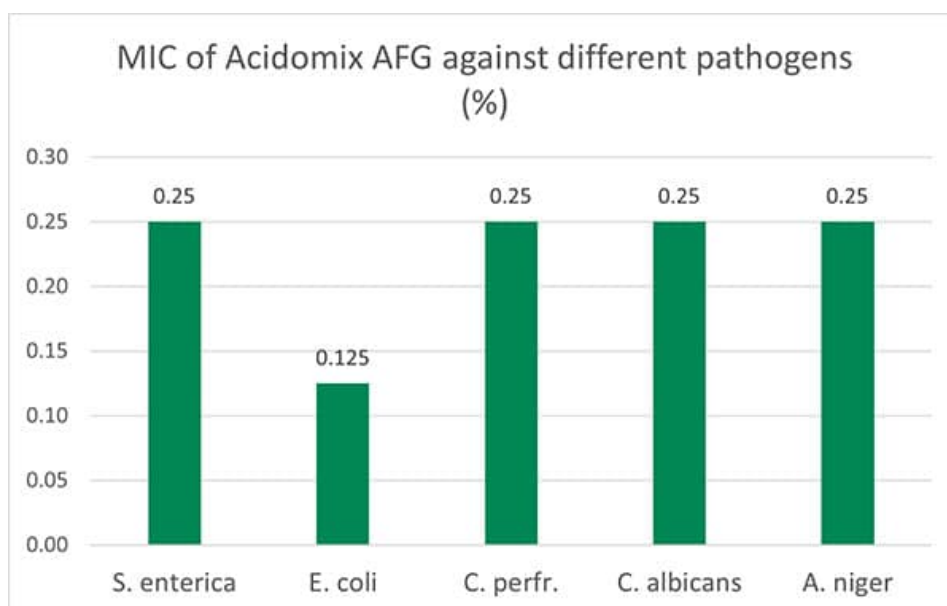


Figure 5: MIC of Acidomix AFG against different pathogens (%)

In addition to the direct antimicrobial effect, this product decreases the pH of the feed and reduces its buffering capacity. The combination of free acids and acid salts provides prompt and long-lasting effects.

## Feed hygiene: a critical path to animal

# performance

Feed accounts for 65-70% of broiler and 75-80% of layer production costs. Therefore, it is essential to use the available feed to the utmost. The quality of the feed is one decisive factor for the health and performance of the animals. Proper harvesting and storage are in the hands of the farmers and the feed millers. The industry offers products to control the pathogens causing diseases and the molds producing toxins and, therefore, helps farmers save feed AND protect the health and performance of their animals.

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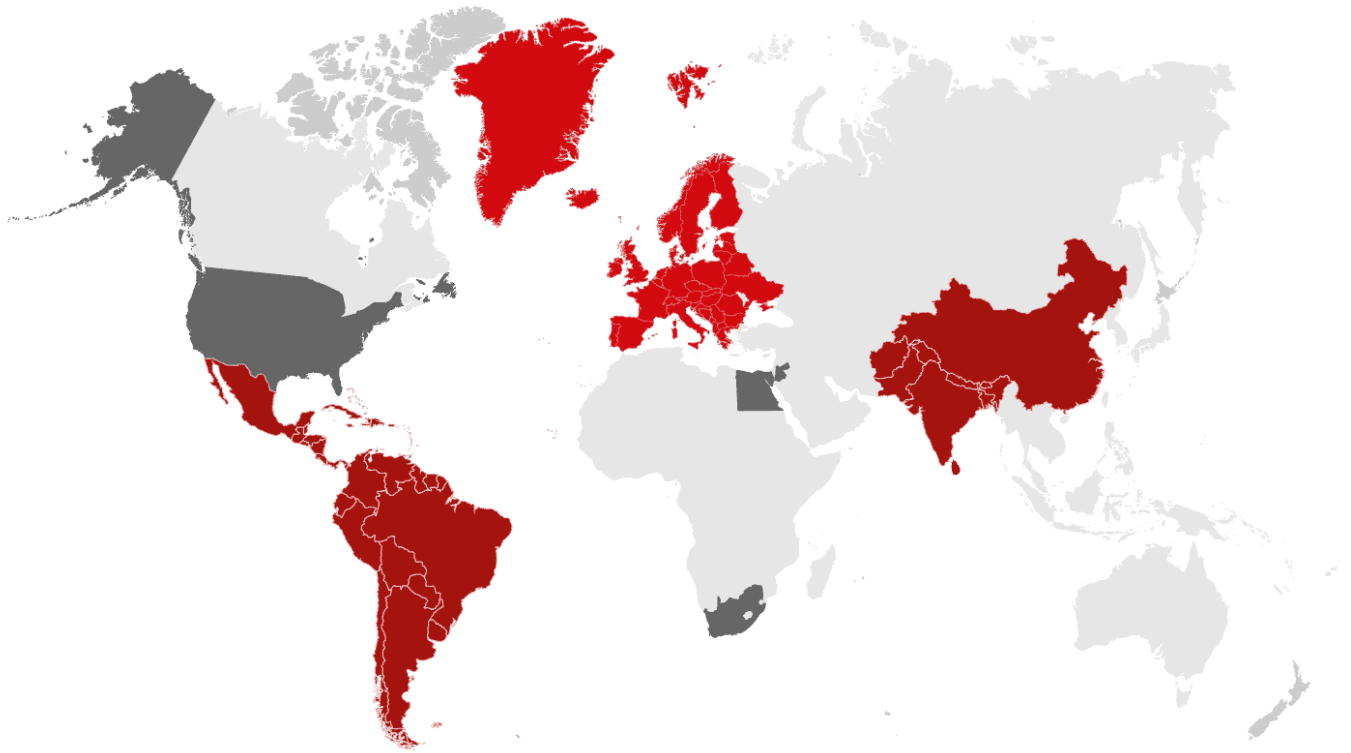
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## Global mycotoxin report: Jan-June

# 2022 | Find the pain points



By **Marisabel Caballero**, Global Technical Manager Poultry, and **Vinil Samraj Padmini**, Global Category Manager Feed Quality, EW Nutrition

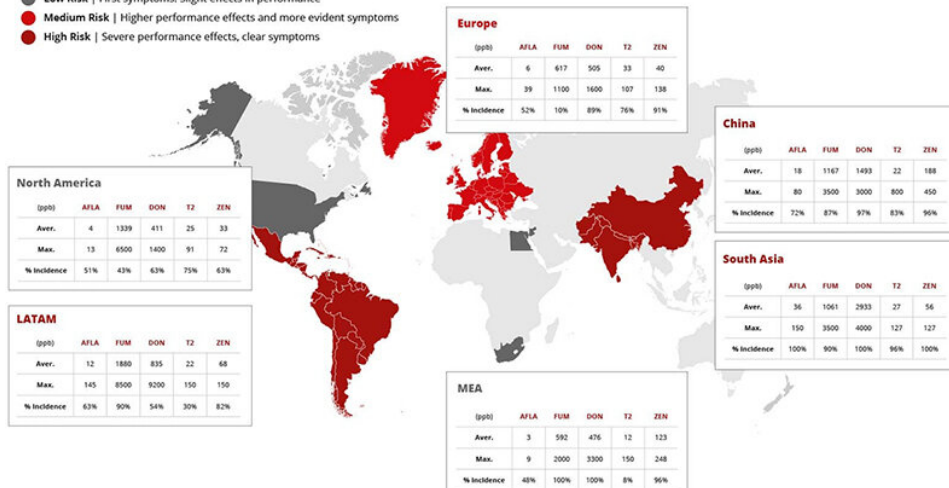
The pressure of climate change is taking a severe toll – not just on weather-dependent industries, but already on society in general. For feed and food, the impact is already dramatic. Extreme weather events, increased temperatures, and rising carbon dioxide levels are facilitating the growth of toxigenic fungi in crops, severely increasing the risk of mycotoxin contamination. Once feed is contaminated, animal health can be impacted, with chain reactions affecting productivity for animal farming, as well as, ultimately, the quality and availability of food.

**\*\*\* Download the full report for an analysis of mycotoxin contamination risks around the world**

## Global mycotoxin challenges Q1-Q2 2022

### Risk Categories

- No data
- Low Risk | First symptoms, Slight effects in performance
- Medium Risk | Higher performance effects and more evident symptoms
- High Risk | Severe performance effects, clear symptoms





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# Mycotoxin interactions amplify damages - What are the right solutions?



*By Technical Team, EW Nutrition*

**Contamination with multiple mycotoxins is the rule for animal feeds, rather than the exception. Trial data shows that producers can prevent negative effects on animal health and performance by using high-performing toxin binders.**



# Multiple mycotoxins contaminate animal feed - problems and solutions

Mycotoxins pose an exceptional challenge for feed and animal producers. Generated by common molds, they occur in a great variety and numbers. Difficult to diagnose, mycotoxicosis in farm animals shows in a range of acute and chronic symptoms: decreased performance, feed refusal, poor feed conversion, reduced body weight gain, immune suppression, reproductive disorders, and residues in animal food products.

## Regulatory mycotoxin thresholds don't account for interactions

Regulatory thresholds for permissible mycotoxin levels in feed are derived from toxicological data on the effects of exposure of a certain species, at a certain production stage, to a single mycotoxin. This makes practical sense: while aflatoxins are carcinogens, fumonisins attack the pulmonary system in swine, for example. Mycotoxins also affect poultry in a different way than cattle, and broilers in a different way than breeders or laying hens, to mention more cases.

The problem is that, in reality, individual mycotoxin challenges are the exception. Animal diets are usually contaminated by multiple mycotoxins at the same time (Monbaliu et al., 2010; Pierron et al., 2016). Since 2014, EW Nutrition has conducted more than 50,000 mycotoxin tests on both raw material and finished feeds samples, across the globe. 85% of these samples were contaminated with more than one mycotoxin and one third positive for four or more mycotoxins.

## How does contamination with multiple mycotoxins occur in animal feed?

The concurrent appearance of mycotoxins in feed can be explained as follows: each mold species has the capacity to produce several mycotoxins simultaneously. Each species, in turn, may infest several raw materials, leaving behind one or more toxic residue. In the end, a complete diet is made up of various raw materials with individual mycotoxin loads, resulting in a multitude of toxic challenges for the animals.

If animals were exposed to only one mycotoxin at a time, following the regulatory guidelines on maximum challenge levels would usually be enough to keep them safe. However, several studies have shown that the effects of exposure to multiple mycotoxins can differ greatly from the effects observed in animals exposed to a single mycotoxin (Alassane-Kpembi et al., 2015 & 2017). The simultaneous presence of mycotoxins may be more toxic than one would predict based on the known effects of the individual mycotoxins involved. This is because mycotoxins interact with each other. The interactions can be classified into three main different categories: *antagonistic*, *additive*, and *synergistic* (Grenier and Oswald, 2011).

## Types of mycotoxin interactions

- **Additivity** occurs when the effect of the combination equals the expected sum of the individual effects of the two toxins.



- **Synergistic** interactions of two mycotoxins lead to a greater effect of the mycotoxin combination than would be expected from the sum of their individual effects. Synergistic actions may occur when the single mycotoxins of a mixture act at different stages of the same mechanism. A special form of synergy, sometimes called **potentiation**, occurs when one or both of the mycotoxins do not induce significant effects alone but their combination does. Fumonisin alone, for example, requires high levels to exert effects on broiler performance. When aflatoxin is also in the feed, the effects are higher than those of aflatoxin alone (Miazzo et al., 2005)
- **Antagonism** can be observed when the effect of the mycotoxin combination is lower than expected from the sum of their individual effects. Antagonism may occur when mycotoxins compete with one another for the same target or receptor site. In an *in-vitro* study using human colon carcinoma cells (HCT116), Bensassi and collaborators (2014), found that DON and Zearalenone individually caused a marked decrease of cell viability in a dose-dependent manner; when combined, the effect was drastically reduced.

Most of the mycotoxin mixtures lead to additive or synergistic effects. The actual consequences for the animal will depend on its species, age, sex, nutritional status, the dose and duration of exposure as well as environmental factors. What is clear is that mycotoxin interactions pose a significant threat to animal health and critically impede risk assessment.

## From awareness to action: risk assessment and toxin binders

Given their complex interactions, the toxicity of [combinations of mycotoxins](#) cannot merely be predicted based upon their individual toxicities. Mycotoxin risk assessments have to consider that even low levels of mycotoxin combinations can harm animal productivity, health, and welfare. Feed and animal producers need to be aware of which raw materials are likely to be contaminated with which mycotoxins, be able to accurately [link them to the risk they pose](#) for the animal and consequently take actions before the problems appear in the field.

## Trials demonstrate effectiveness of toxin mitigation solutions

Toxin binders that are effective against a broad spectrum of mycotoxins significantly reduce the risks of mycotoxin exposure. *In vitro* trial data shows that EW Nutrition's cost-effective toxin-mitigating product



[Solis Max](#) shows a high mitigation capacity, even at low inclusion rates (Figure 1). Importantly, Solis Max helps to reduce various mycotoxins' negative effects on performance without any negative effects on nutrient absorption.

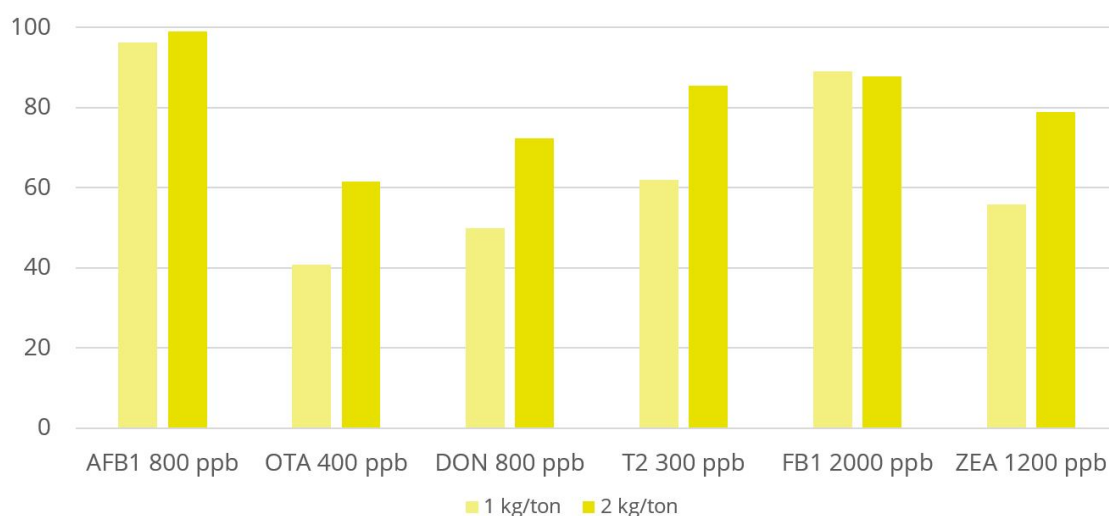


Figure 1: Solis Max shows mitigation capacity in in vitro trial (%)

In a recent trial of 416 day-old Vencobb-430 broilers, premium product [Mastersorb Gold](#) has demonstrated its ability to support animals coping with multiple mycotoxin challenges. For broilers challenged with 200 ppb AFB1 and 350 ppb OTA, Mastersorb Gold supplementation resulted in 4.3% higher average daily weight gain than the challenged group, a higher body weight on day 42 and a 2% better feed conversion (Figure 2), which means a total recovery of the performance when compared with the non-challenged control.

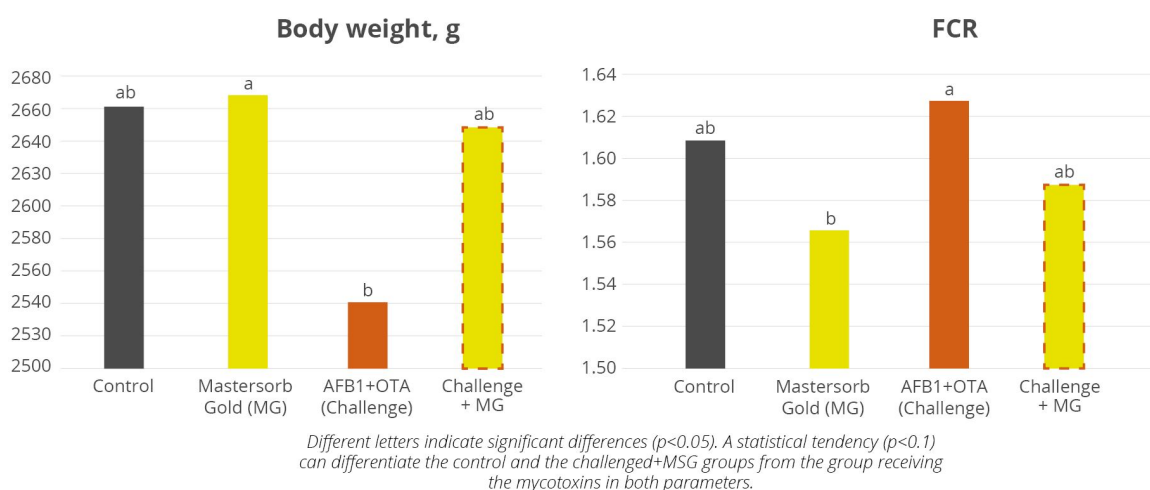


Figure 2: Mastersorb Gold improves body weight and FCR of broilers challenged with AFB1 and OTA

Liver health also improved: after 21 days, broilers receiving Mastersorb Gold showed lower AST (-20%) and ALT (-50%) levels compared to the challenged group. Mycotoxin-induced stress was also lower, as evidenced by a 25% lower H/L ratio and 20% reduced white blood cell count for the Mastersorb Gold group. All of the mentioned biomarkers were similar to the non-challenged control, showing the preventive effects of Mastersorb Gold on health and performance.

# Proactive management: tackle multiple mycotoxin challenges head on

Mycotoxins interactions are the norm, not the exception. Yet, regulatory standards currently only cover the effects of individual mycotoxins, leaving productions exposed to risks of additive and synergistic mycotoxin interactions animals' health and performance. Luckily, management options are available: Careful [risk evaluation](#) explicitly includes the threat of multiple contaminations. And producers can proactively ensure better health, welfare and productivity of their animals by investing in the right toxin mitigation solution for their business.

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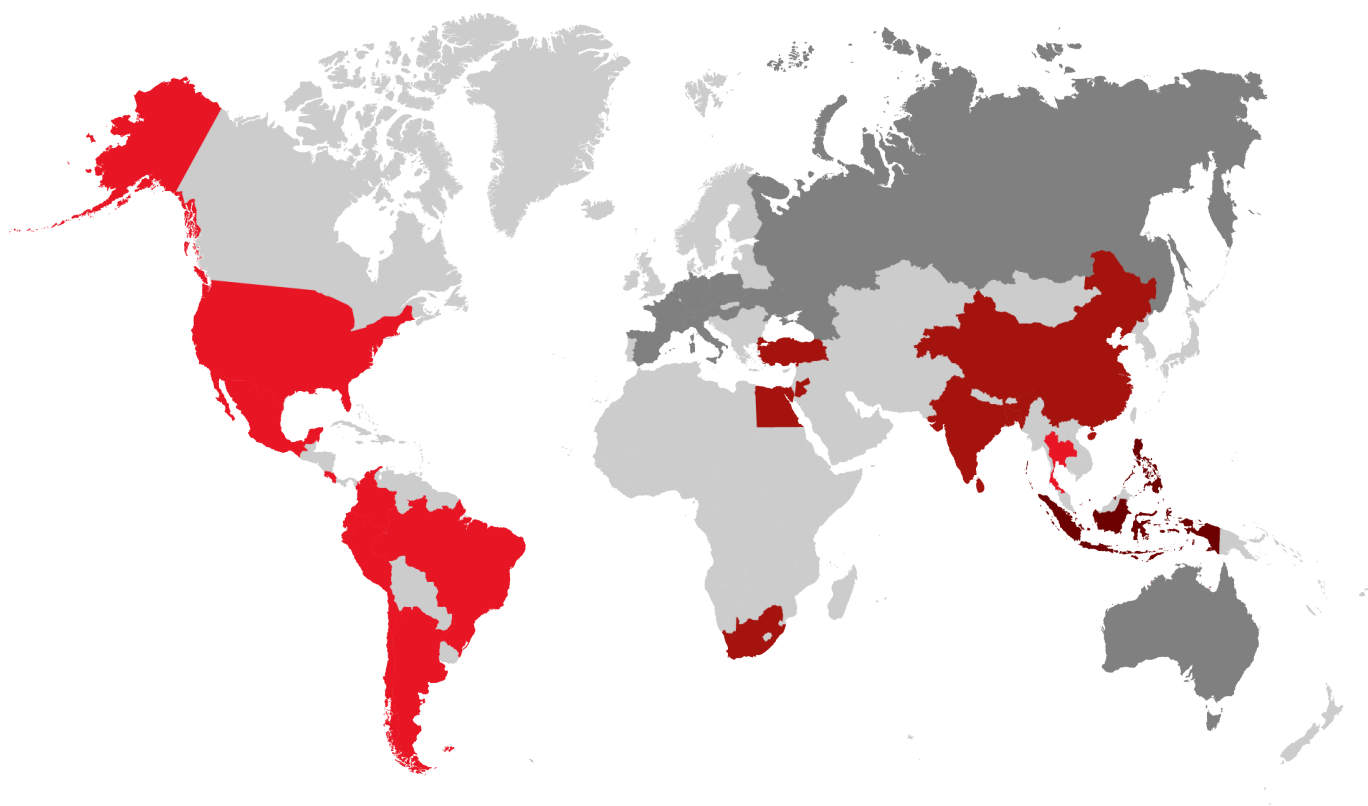
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# Global mycotoxin challenges: 2021 report



By **Marisabel Caballero**, Global Technical Manager Poultry, EW Nutrition

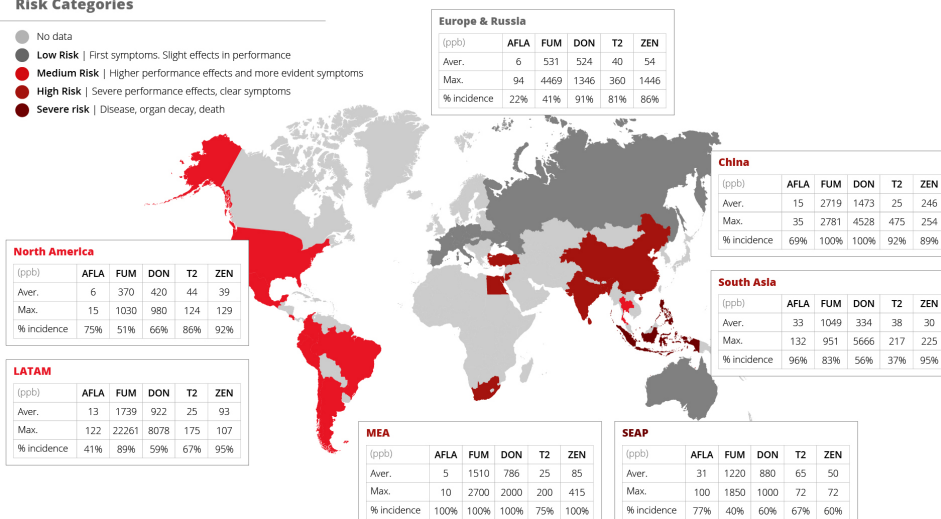
Climate around the globe has changed, increasing atmospheric temperatures and carbon dioxide levels. This change favors the growth of toxigenic fungi in crops and thus increases the risk of mycotoxin contamination. When contaminating feed, mycotoxins exert adverse effects in animals and could be transferred into products such as milk and eggs.

**\*\*\* Please download the full article for detailed information**

## Global mycotoxin challenges Q1-Q4 2021

### Risk Categories

- No data
- Low Risk | First symptoms. Slight effects in performance
- Medium Risk | Higher performance effects and more evident symptoms
- High Risk | Severe performance effects, clear symptoms
- Severe risk | Disease, organ decay, death



[Click to see the full-size image](#)

# **Mycotoxins: a worldwide challenge in 2021**

Amongst naturally occurring mycotoxins, the five most important ones are aflatoxin, ochratoxin, deoxynivalenol, zearalenone, and fumonisin. Their incidence varies with the different climates, the prevalence of plant cultures, the occurrence of pests, and the handling of harvest and storage. Worldwide, farmers faced various and sometimes extremely high mycotoxin contamination in their feed materials in 2021. In the following, we show the major challenges in five main regions.

## **Asia faced high aflatoxin contamination**

In Asia, high temperatures and humidity favor *Aspergillus* growth in grains. As a result, 95 % of the samples in South Asia and three-quarters of the samples in the China and the SEAP region (Indonesia, Philippines, Vietnam) showed aflatoxin contamination. The average contamination being higher than the threshold for all farm animals represents an increased risk for their health and performance. In China and the SEAP region, also DON and T-2 were highly prevalent. Showing an incidence of more than 60%, they pose a severe risk when combined with aflatoxin.

## **Fumonisin afflicted the LATAM region**

In Mexico, Central and South America, fumonisin contamination prevailed with an incidence of almost 90% at average levels that can be considered risky for swine and dairy. Together with incidence levels of around 60% found for DON and T2, fumonisin may act synergically in the animals, raising the risk for health and performance.

The *Fusarium* species linked to these mycotoxin contaminations occur in the grains on the field. Amongst others, insect damage, droughts during growing, and rain at silking favor their development.

## **Trichothecenes prevailed in North America**

Contamination with trichothecenes (DON and T2) is the rule in the United States. The interaction of these mycotoxins is at least additive. The damage they cause to the gut opens the door to dysbiosis and disease, decreasing performance and profitability.

Also in this case, the responsible molds for the contamination are *Fusarium* species that develop when grains are in the field. As with fumonisins, the molds are favored by insect damage, moderate to warm temperatures and rainfall.

## **Fusarium toxins contaminated grain in the MEA region**

*Fusarium* toxins such as Fumonisin, DON, and T2 prevail in the region of Egypt, Jordan, and South Africa. In combination, these mycotoxins have additive effects at the intestinal level, which increases the risk of

dysbiosis in poultry.

## **A challenging year with long-term repercussions**

Since mycotoxin contamination affects animal health, measures must be taken to provide the best protection. Besides improving agricultural practices in the field, smart in-feed solutions and mold inhibitors can be used in stored grain. These measures help producers preserve feed quality after a troubled year for crops around the world.

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## **Harvest to bring significant quality challenges for feed, says EW Nutrition [Press Release]**



**VISBEK, GERMANY, 23 August - Bad news for feed producers: after supply chain disruptions and raw material unavailability, now weather-related challenges in Europe will most likely affect this year's crop quantity and quality. Cold temperatures, heatwaves, tornados, and hailstorms are expected to adversely affect the quality and quantity of the harvest.**

The moisture brought by the rainfalls is generally expected to affect the quality of the crops. The torrential rains in France, Germany, etc. have darkened Central and Western farmers' prospects: while the quantity may be there, the quality of wheat and corn is under question. Sprouting grains, diseased crops, and fungi may dampen the optimism brought by numbers alone.

Further east, droughts have posed different issues. Still, countries such as Romania and Bulgaria seem to have weathered the challenges somewhat better and are seeing YoY increases in their wheat and corn crop output.

In Great Britain, rainfall has not caused dramatic drops in crop output but has nevertheless greatly increased mycotoxin risk up to a "moderate to high" level.

Depending on the type of mycotoxin, weather challenges and storage conditions are the most common contributors to severe infestation. This year's intemperate weather has, in fact, been ideal for a large spectrum of fungi. Fungal risks can be calculated at the two critical times: at flowering and at harvest and baling, when there is an increased risk of storage molds and mycotoxin production.

Preliminary analysis shows Europe's wheat crops at potential risk of DON, as well as potentially Aflatoxin and Fumonisin infestation and more. Specialists continue to collect and monitor harvest results and adjust recommendations; however, we can definitely expect the presence of moderate, if not quite high levels of mycotoxin risk this year.

[info@ew-nutrition.com](mailto:info@ew-nutrition.com)

<https://ew-nutrition.com/>

+49 4445 9868-0

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## **From sub-acute ruminal acidosis to endotoxins: Prevention for lactating cows**





*by Technical Team, EW Nutrition*

Sub-acute acidosis (SARA) is linked to high levels of ruminal LPS. The LPS cause inflammation and contribute to different metabolic conditions and diseases. Various strategies and solutions can be applied to modulate the rumen microbiota and prevent this risk.



In sub-acute rumen acidosis (SARA), the quantity of free lipopolysaccharides (LPS) coming from Gram- bacteria increases considerably. These LPS cross the ruminal wall and intestine, passing into the bloodstream. The negative consequences on the health of the animal are then reflected in decreased productive and reproductive performance.



The LPS are released during the lysis of GRAM- bacteria which die due to the low pH, and these bacteria are mainly responsible for the production of propionic acid for the energy yield that is obtained. It is essential to preserve ruminal balance between Gram+ and Gram- such that there is no excess of LPS.

# Nutritional needs of lactating cows with SARA

In the first phase of lactation (from 1 week after calving to 80 – 100 days of lactation), the cow needs a high energy level to meet the large demand for milk production. This energy demand is often not fully satisfied and feed intake falls short. This deficit leads to the need to provide as much energy as possible per feed ration.

Imagine a 650 kg live weight cow, producing about 35 kg of milk per day with a fat percentage of 3.7 and a protein percentage of 3.2. To achieve this production level and fulfill its maintenance requirements, this animal needs a feed intake of 22 kg of dry matter (DM) per day, with an energy level of 21 UFL equal to 36,000 Kcal/day of NE I (Net Energy Lactation)).

To obtain an energy supply of this type, it is necessary to provide rations with a high content of cereals rich in nonstructured carbohydrates (NSC). This will allow the animals to obtain the maximum efficacy in getting the NE I from the metabolizable energy (ME) expressed as kI\*.

\* kI expresses the effectiveness in passing from EM to EN I net of the heat dissipated by the animal, therefore  $kI = ENI/EM$  (Van Es 1978).

Compared to a diet rich in NDF (Neutral Detergent Fiber), this type of diet promotes and stimulates certain strains of bacteria to the detriment of others, shifting the balance towards a greater population of bacteria that produce propionic acid instead those which produce acetic acid. This change also determines a greater share of Gram- compared to Gram+.

## What is rumen acidosis?

Rumen acidosis is that “pathology” whereby the volume of SCFA (Short Chain Fatty Acids) produced by the rumen bacteria is greater than the ability of the rumen itself to absorb and neutralize them. Rumen acidosis is mainly caused by the amylolytic and saccharolytic bacteria (*Streptococcus bovis*; *Selenomonas ruminantium*, *Bacteroides amylophilus*, *Bacteroides rumenicola* and others) responsible for the production of lactic acid. Unlike the other most representative volatile fatty acids (acetic, butyric and propionic), lactic acid has a lower pKa: 7 (3.9 versus 4.7).

This means that for the same amount of molecules produced, lactic acid releases a number of ions  $H^+$  in the fluid ten times greater than other VFAs, with evident effects on the pH.

Ruminal acidosis can be characterized as acute or subacute. During acute ruminal acidosis, the pH in the rumen drops below 4.8 and remains low for an extended period of time. Acute acidosis leads to complete anorexia, abdominal pain, diarrhea, lethargy, and eventually death. However, the prevalence of acute acidosis in dairy is very low.

## Consequences of rumen acidosis

In such situations, a series of negative consequences can be triggered in the lactating cow. Investigations (for instance, using fistulated cows) can reveal, among others, the following alteration in the rumen:

- Shift in total microbiome rumen profile (density; diversity; community structure)
- Shift in protozoa population (increase in ciliates protozoa after 3 weeks of SARA; increase in the GNB population)
- Shift in fungi population (decreasing the fungi population with high fibrolytic enzymes, which are sensitive to low pH)
- Rise in LPS rumen concentration (increasing the GNB strain and their lysis)
- Influence on the third layer of Stratified Squamous Epithelium (SSE) (desmosomes and tight junctions)
- Lower ruminal fiber degradation (reduction in the number of cellulolytic bacteria which are less resistant to acid pH)
- Reduction of the total production of fatty acids (propionic, acetic, butyric), therefore less available energy

- Lower rumen motility (also as a consequence of the smaller number of protozoa)
- The increased acid load damages the ruminal epithelium
- Acid accumulation increases the osmotic pressure of the rumen inducing an higher flux of water from the blood circulation into the rumen, causing swelling and rupture of rumen papilla as well as a greater hemoconcentration

The last points are extremely important, as it enables an easier passage of fluids from the blood to the pre-stomachs, greatly influencing the fermentation processes.

Furthermore, with diets low in NDF, the level of chewing and salivation is certainly lower, with a consequent lower level of salivary buffers that enter the rumen and which would maintain an appropriate pH under normal conditions.

## Rumen sub-acute and acute acidosis: a fertile ground for LPS

Studies inducing SARA in dairy cows have shown that feeding high levels of grain causes the death and cell lysis of Gram- bacteria, resulting in higher concentration of free LPS in the rumen. In a trial conducted by Ametaj et al., in 2010 (Figure 1), a lower ruminal pH and an increase in the concentration of LPS in the rumen fluid -measured as ng / ml (nanograms / milliliter)-, was the result of increasing of NSC present in the diet (% of grains).

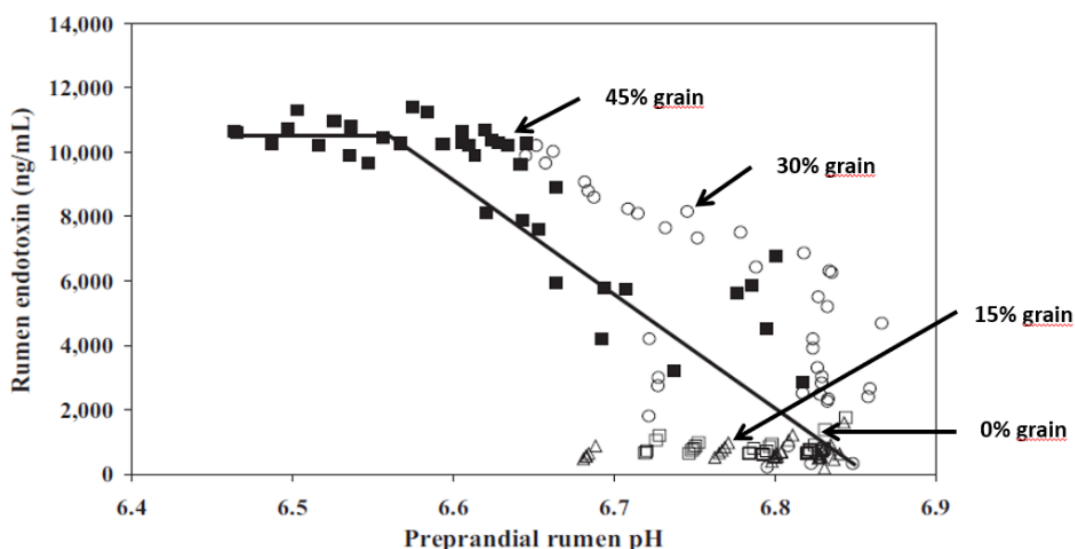


Figure 1. The increase in the level of endotoxins in the rumen is directly correlated with an increase in ration concentrates

In the rumen, the presence of Gram- is very significant, however the dietary changes towards high energy concentrates, reduce the substrates necessary for them to thrive, leading to their lysis and favoring gram-positive bacteria (Gram+). Gram+ also produce bacteriocins against a wide variety of bacteria, including many Gram-. Figure 2 shows the influence of ruminal pH in the population of different bacteria, many of which are crucial for the production of SCFA and therefore of energy.

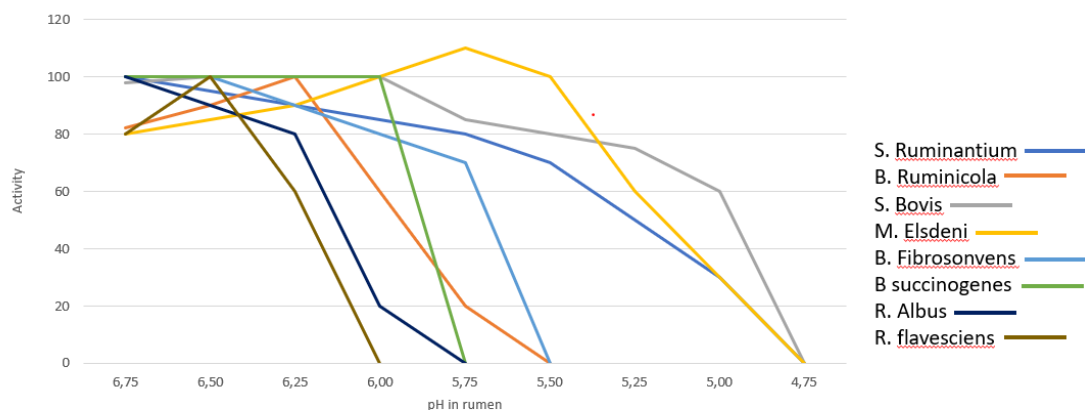


Figure 2. Activity of main bacteria in the rumen in function of pH (Daniele Cevolani Edizioni Agricole di New Business Media srl 2020)

It is therefore necessary to pay close attention to the energy level of the ration as an energy input (generally around 1500 – 1700 Kcal/kg of DM intake). At the same time, we need to ensure that the animal does receive and ingest that daily amount of DM. If ingestion is negatively influenced by acidosis (clinical or sub-clinical), this can lead to endotoxemia, with harmful consequences for the animal's health and production performance.

We can therefore note that the level of LPS (endotoxins) present in the rumen is directly correlated with the pH of the rumen itself and with a symptomatologic picture dating back to SARA. This occurs when the mortality and lysis of Gram- bacteria (GNB) is high and through the consequent imbalance created with diets containing excess fermentable starches, compared to diets with higher fiber content.

In fact, it was shown that the transition from a concentrated fodder ratio of 60:40 to a more stringent ratio of 40:60 caused the level of free LPS in the rumen to go from 410 to 4.310 EU / ml.

## Endotoxemia: Pathological consequences in dairy cows

Once the LPS enter the bloodstream, they are transported to the liver (or other organs) for the detoxification. However, sometimes this is not enough to neutralize all the endotoxins present in blood. The remaining excess can cause issues such as the modification of the body's homeostasis or cause that cascade of inflammatory cytokines responsible for the most common pathologies typical in cows in the first phase of lactation. The most common symptoms are the increase of somatic cells in milk or claws inflammation.

Pro-inflammatory cytokines as TNF, IL6 and IL8 induced by LPS-related inflammation are able to stimulate the production of ACTH (adrenocorticotrophic hormone).

ACTH, together with cortisol and the interleukins, inhibit the production of GnRH and LH, with serious effects on milk production. The productivity and the fertility of the animal are thus compromised.

Moreover, prostaglandins are as well stimulated by LPS, and are linked with fever, anorexia and ruminal stasis. This not only limits the amount of energy available for production and maintenance functions, but also induces a higher susceptibility to disease and adds-up to the emergence of other metabolic conditions, such as laminitis and mastitis.

## Preventing rumen acidosis

The solution to these massive risks is a prudent and proactive approach by the nutritionist towards all situations that can cause a rapid increase of Gram- in the rumen. It is therefore necessary to avoid cases of clinical and sub-clinical acidosis (SARA) in order to avoid the issues listed above. This would also help avoid stressful conditions for the animal that would lead to decreased performance and health.

To maintain balance and a healthy status of the animal, the use of additives such as phytomolecules and binders is suggested in the first phase of

lactation, starting from 15 days before giving birth.

Activo Premium (a mix of phytogetic substances) has given excellent results in decreasing the acetic/propionic acid ratio, while safeguarding the population of Gram+ bacteria. This is in contrast to treatments with ionophores, which, as is well known, interfere with the Gram+ population.

## Case study. Acetic acid:propionic acid ratio with Activo Premium

In a study conducted at the the University of Lavras and the Agr. Res. Comp. of Minas Gerais (both Brazil), 30 Holstein cows were allocated to two groups considering parity and milk production. One group was fed the standard feed (control), the other group received standard feed containing 150mg of Activo Premium/kg of dietary dry mass (DM). The following parameters were measured or calculated: intake of DM and milk production, milk ingredients such as fat, protein, lactose every week, body weight and body condition score every two weeks, and ruminal constituents (ph and SCFAs) through oesophageal samples at day 56.

Activo Premium was able to decrease the ratio between acetic acid and propionic acid, and at the same time maintain the level of Gram+ bacteria in the rumen, thus reducing the risk of endotoxins. The same trial carried out at the University of Lavras demonstrated how the performance of the animals was superior in the group fed with Activo Premium compared to the control group (see below).

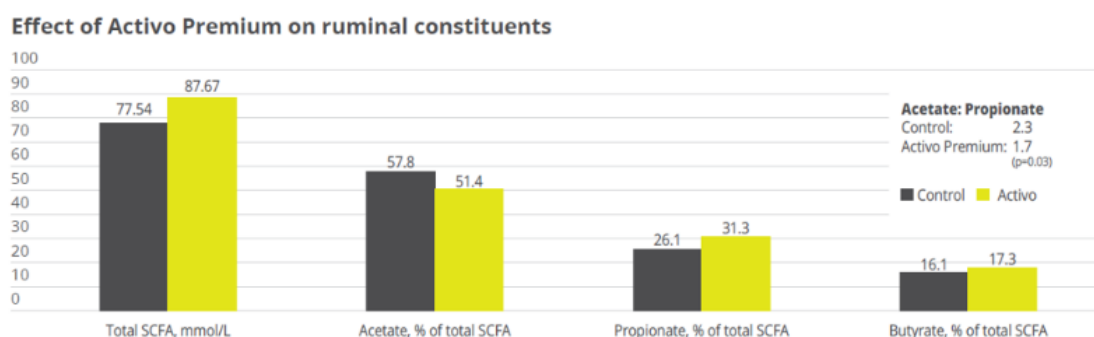


Figure 3. Effect of Activo Premium on ruminal constituents

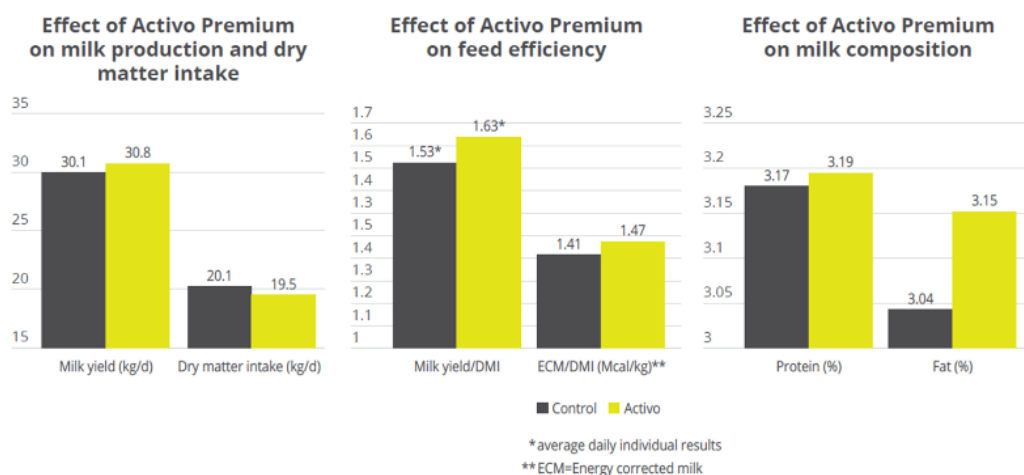


Figure 4. Effect of Activo Premium on animal performance

## Solution: Preserve Gram+ bacteria levels while decreasing free LPS

We have therefore seen how important it is to decrease the acetic:propionic ratio in the rumen to obtain a greater share of available energy. However, the level of endotoxins in the rumen must remain low in order to avoid those problems of endotoxemia linked to very specific pathologies typical of “*super productive cows*”. These pathologies (always linked to inflammatory manifestations) can be prevented by decreasing the level of free LPS in the rumen with a product that can irreversibly bind the LPS and thus make them inactive.

In a trial with porcine intestinal cells (IPEC-J2) challenged by *E. coli* LPS, a decrease in the intensity of inflammation was observed when Mastersorb Gold was added. This decrease could be shown through a lower amount of phosphorylated NF- $\kappa$ B in an immunofluorescence trial, as well as through the reduced production of Interleukin (IL)-8 in the cells measured by ELISA.

The fact that pig intestine tissue was used does not affect the adsorption concept. In this case, these intestinal cells are only a vehicle to demonstrate that in an aqueous solution containing 50 ng of LPS / ml and in the same solution with the addition of Mastersorb Gold, the level of LPS actually active is decreased, as a part of the LPS was tied up by Mastersorb. The solution with a lower level of LPS gave minor “inflammatory” reactions to intestinal cells, and this can be statistically reported in dairy cows.

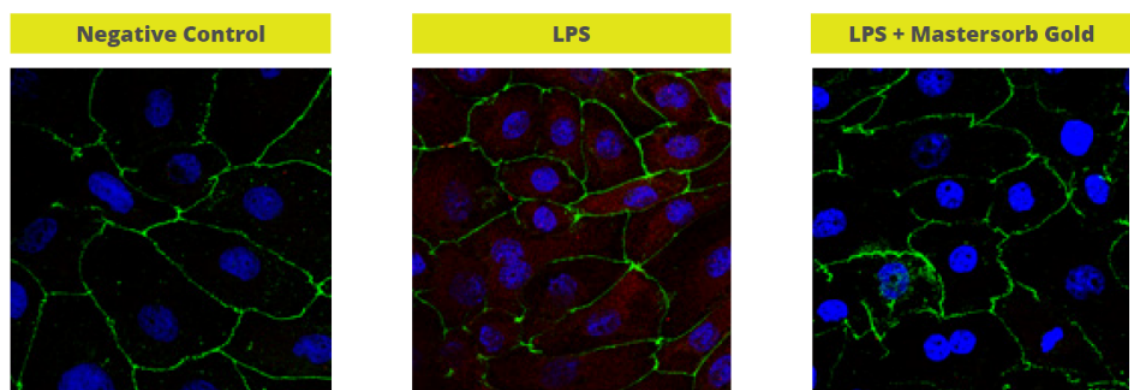


Figure 5. Immunofluorescence in IPEC-J2: Challenge with LPS without (in the middle) and with Mastersorb Gold (right)

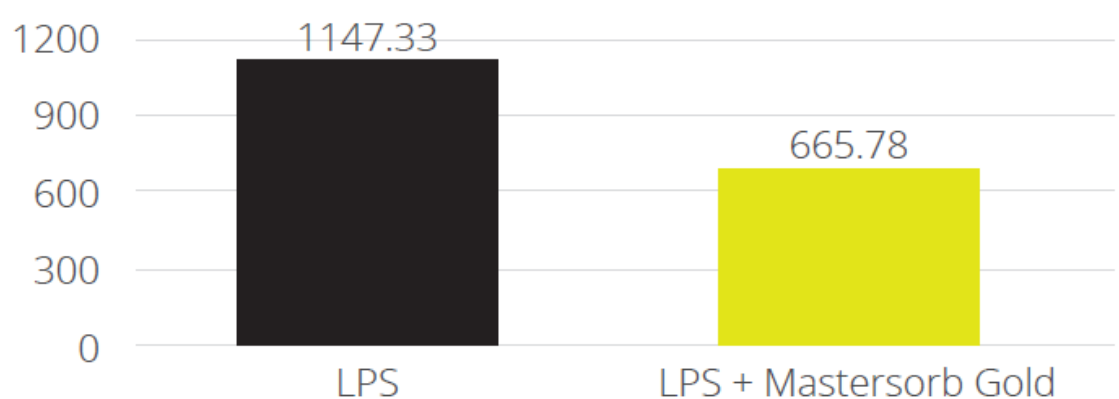


Figure 6. IL-8 AP secretion after incubation with LPS 0111:B4 for 24h without and with Mastersorb Gold

## Conclusions

To demonstrate how the decrease in the level of LPS in the rumen is directly correlated with inflammatory states in general, a trial with a total of 60 dairy cows shows that the inclusion of 25g of Mastersorb Premium/animal/day increases milk yield and improves milk quality by decreasing



somatic cell count. Adsorbing substances contained in Mastersorb Premium tie up the LPS produced in the rumen in different cow lactation phases.

Normally, the rise in the level of somatic cells in milk depends on etiological agents such as *Streptococcus spp*, *Staphylococcus spp*, *mycoplasma* and more. LPS stress is not the sole agent responsible for raising somatic cell counts, but also other factors among which:

- Lactation stage and age of the animal
- Season of the year (in summer the problem is increased)
- Milking plant (proper maintenance)
- General management and nutrition

However, by reducing the level of LPS, Mastersorb provides an important aid to decrease somatic cell count.

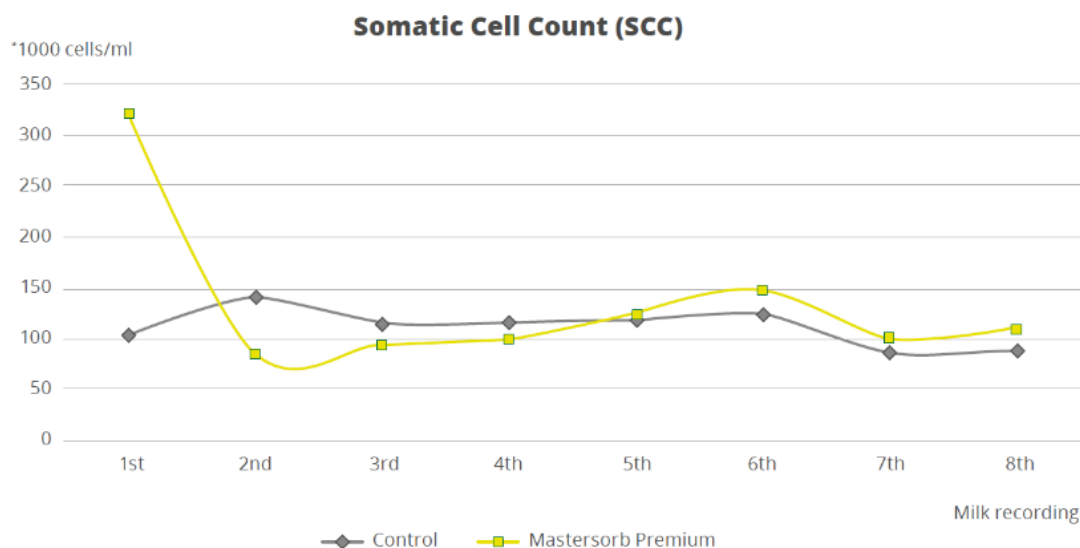


Figure 7. Effect of Mastersorb Premium on somatic cell count

## Prevent escalation with rumen balance

In the end, ruminant producers are, like all livestock operations, interested in producing healthy animals that can easily cope with various stressors. Ensuring a proper diet, adjusted to the energy requirements of various production stages, is a first step. Providing the animal with the ingredients that modulate the microbiota and reduce the negative impact of stress in the rumen is the next essential step in efficient production.

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## Mycotoxin interactions: An

# obstacle to risk assessment



**In animal feed, multi-mycotoxin contamination is found quite frequently and seems to be the rule rather than the exception in practical diets. Here is a quick overview of the known interactions.**

## What are the most common mycotoxins in feed?

Mycotoxins represent an exceptional challenge for feed and animal producers: they are produced by common molds, occur in a great variety and number, are sporadic or heterogeneous in their distribution, and their effects on farm animals are seldom recognized as mycotoxicosis. Among hundreds of known mycotoxins, aflatoxins, mainly produced by *Aspergillus* species, ochratoxin A, produced by *Aspergillus* and *Penicillium* species, as well as fumonisins, trichothecenes (especially DON and T-2 toxin) and zearalenone, primarily produced by many *Fusarium* species stand out as the most common contaminants.

## Consequences of mycotoxin contamination

Ingestion of these mycotoxins may cause an acute toxicity or chronic disorders, depending on the concentration and duration of exposure. In farm animals, this might manifest as decreased performance, feed refusal, poor feed conversion, reduced body weight gain, immune suppression, reproductive disorders, and residues in animal food products.

Due to their frequent occurrence and their severe toxic properties, many countries appointed legal regulations or guidance for the major mycotoxins to protect animals and human consumers. The current regulations are typically very specific in terms of animal species and even for the production stage considering that mycotoxins affect for example poultry in a different way than cattle and broilers in a different way than breeders or laying hens. The threshold and/or guidance values for each species, however, were determined based on toxicological data from studies investigating a monoexposure leaving out the possibility of any combined effects of mycotoxins.

# Multi-contamination: the rule, not the exception

If we were able to ensure that the animals were exposed to only one mycotoxin at a time, following the regulatory guidelines would allow us to protect our animals in most of the cases. Several worldwide surveys show, however, that mycotoxin multicontamination of animal feed is found very frequently\* and seems to be the rule rather than the exception in practical diets. The concurrent appearance of mycotoxins in feed can be explained as follows: each mold species has the capacity to produce a number of mycotoxins simultaneously. Each species, in turn, may infest several raw materials leaving behind one or more toxic residue. In the end, a complete diet is made up of various raw materials with individual mycotoxin loads resulting in a multitude of toxic challenges for the animals.

Several researchers showed that the effects observed during multiple mycotoxin exposure can differ greatly from the effects observed in animals exposed to a single mycotoxin, indicating that the simultaneous presence of mycotoxins may be more toxic than predicted from the mycotoxins alone. This is because mycotoxins interact with each other. The interactions can be classified into three main different categories: *antagonistic*, *additive*, and *synergistic*.

## Types of mycotoxin interactions

**Additivity** occurs when the effect of the combination equals the expected sum of the individual effects of the two toxins (*Figure 1a*).

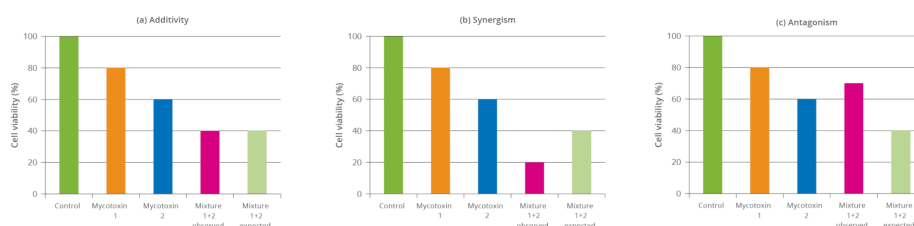
**Synergistic** interactions of two mycotoxins lead to a greater effect of the mycotoxin combination than would be expected from the sum of their individual effects (*Figure 1b*). A special form of synergy, sometimes called potentiation, occurs when one or both of the mycotoxins do not induce effects whereas the combination induces a significant effect.

When the effect of the mycotoxin combination is lower than expected from the sum of their individual effects, **antagonism** can be observed (*Figure 1c*). In general, most of the mycotoxin mixtures lead to additive or synergistic effects, highlighting a significant threat to animal health and being the major reason that impedes risk assessment. Synergistic actions may occur when the single mycotoxins of a mixture act at different stages of the same mechanism, e.g. T-2 increases ROS production while AFB1 decreases its clearance when the presence of one mycotoxin increases the absorption of another or decreases its metabolic degradation.

## Be aware of contaminations

Given their complex interactions, the toxicity of [combinations of mycotoxins](#) cannot merely be predicted based upon their individual toxicities. Knowing that even low levels of mycotoxin combinations can harm animal productivity, health, and welfare, it is useful for feed and animal producers to be aware of present contaminations, to be able to [link them to the risk they pose](#) for the animal and consequently take actions before the problems appear in the field.

Figure 1 - Characterisation of the interaction between mycotoxins (Smith et al., 2016).



\*References are available on request.

**By Marisabel Caballero, Global Technical Manager, Poultry**  
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