Decoding the connection between stress, endotoxins, and poultry health



By Technical Team, EW Nutrition

Stress can be defined as any factor causing disruptions to homeostasis, which triggers a biological response to <u>regain equilibrium</u>. We can distinguish four major types of stressors in the poultry industry:

- Technological: related with management events and conditions
- Nutritional: involving nutritional disbalances, feed quality and feed management
- Pathogenic: comprising health challenges.
- Environmental: changes in environment conditions

In practical poultry production, multiple stress factors occur simultaneously. Their effects are also additive, leading to chronic stress. The animals are not regaining homeostasis and continuously deviate the use of resources through inflammation and the gut barrier-function, thus leading to microbiome alteration. As a consequence, welfare, health, and productivity are compromised.

What are endotoxins?

Bacterial lipopolysaccharides (LPS), also known as endotoxins, are the main components of the outer membrane of all Gram-negative bacteria and are essential for their survival. LPS have direct contact with the bacteria's surroundings and function as a protection mechanism against the host's immunological

response and chemical attacks from bile salts, lysozymes, or other antimicrobial agents.

Gram-negative bacteria are part of animals' microbiota; thus, there are always LPS in the intestine. Under optimal conditions, this does not affect the animals, because intestinal epithelial cells are not responsive to LPS when stimulated from the apical side. In stress situations, the intestinal barrier function is impaired, allowing the passage of endotoxins into the blood stream. When LPS are detected by the immune system either in the blood or in the basolateral side of the intestine, inflammation and changes in the gut epithelial structure and functionality occur.

The gut is critically affected by stress

Even when there is no direct injury to the gut, signals from the brain can modify different functions of the intestinal tract, including immunity. Stress can lead to functional disorders, as well as to inflammation and infections of the intestinal tract. Downstream signals act via the brain-gut axis, trigger the formation of reactive oxygen and nitrogen species as well as local inflammatory factors, and circulating cytokines, affecting intestinal homeostasis, microbiome, and barrier integrity.

Stress then results in cell injury, apoptosis, and compromised tight junctions. For this reason, luminal substances, including toxins and pathogens, leak into the bloodstream. Additionally, under stress, the gut microbiome shows and increment on Gram-negative bacteria (GNB). For instance, a study by Minghui Wang and collaborators (2020) found an increase of 24% in GNB and lower richness, in the cecum of pullets subjected to mild heat stress (increase in ambient temperature from 24 to 30°C).

Both these factors, barrier damage and alterations in the microbiome, facilitate the passage of endotoxins into the blood stream, which promotes systemic chronic inflammation.

What categories of stress factors trigger luminal endotoxins' passage into the bloodstream?

Technological stress

Various management practices and events can be taken as stressors by the animals' organism. One of the most common examples is **stocking density**, defined as the number of birds or the total live weight of birds in a fixed space. High levels are associated with stress and loss of performance.

A study from the Chung-Ang University in 2019 found that broilers with a stocking density of 30 birds/m² presented two times more blood LPS than birds kept at half of this stocking density. Moreover, the body weight of the birds in the high-density group was 200g lower than the birds of the low-density group. The study concluded that high stocking density is a factor that can disrupt the intestinal barrier.

Nutritional stress

The feed supplied to production animals is designed to contribute to express their genetic potential, though some feed components are also continuous inflammatory triggers. **Anti-nutritional factors, oxidized lipids, and mycotoxins** induce a low-grade inflammatory response.

For instance, when mycotoxins are ingested and absorbed, they trigger stress and impair immunity in animals. Their effects start in gastrointestinal tract and extend from disrupting immunity to impairing the intestinal barrier function, prompting secondary infections. Mycotoxins can increase the risk of endotoxins

in several ways:

- By inducing changes in the intestinal microbiota that increase gram-negative bacteria
- By <u>disrupting the intestinal barrier function</u>, allowing endotoxins (as well as other toxins and pathogens) to cross the gut barrier and pass into the bloodstream
- By <u>alterations in the immune response</u>, low doses of mycotoxins, such as trichothecenes, induce the upregulation of pro-inflammatory cytokines. A <u>possible synergy</u> can be inferred as when they are together, the effects may be prolonged and require a lower dosage to be triggered.

A study conducted by EW Nutrition (Figure 1) shows an increase in intestinal lesions and blood endotoxins after a mycotoxin challenge of 200pbb of Aflatoxin B1 + 360ppb Ochratoxin in broilers at 21 days of age. The challenged birds show two times more lesions and blood endotoxins than the ones in the unchallenged control. The use of the right mitigation strategy, a product based on bentonite, yeast cell walls, and phytogenics (EW Nutrition GmbH) successfully prevented these effects as it not only mitigates mycotoxins, but also targets endotoxins in the gut.

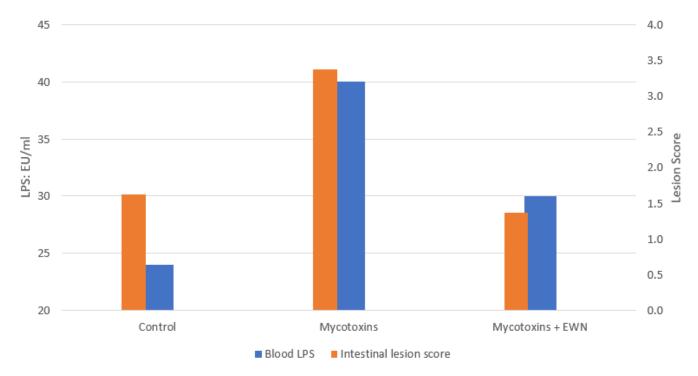


Figure 1 Blood LPS and intestinal lesion score of broilers challenged with 200ppb AFB1 + 350 ppb OTA from 1 to 21 days of age without and with an anti-toxin product from EW Nutrition GmbH (adapted from Caballero et al., 2021)

Pathogenic stress

Intestinal disease induces changes in the microbiome, reducing diversity and allowing pathogens to thrive. In clinical and subclinical necrotic enteritis (NE), the intestinal populations of GNB, <u>including Salmonella and E.coli</u> also increases. The lesions associated with the pathogen compromise the epithelial permeability and the intestinal barrier function, resulting in <u>translocation of bacteria and LPS</u> (Figure 5) into the bloodstream and internal organs.

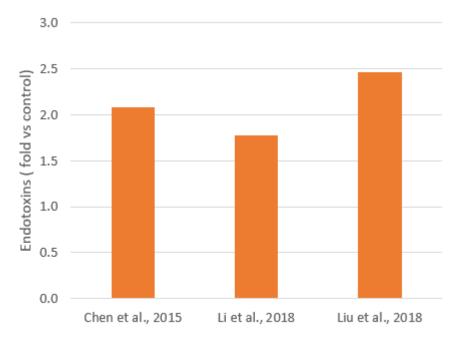


Figure 2 Increase in systemic LPS (vs a healthy control) after a NE challenge (adapted from Chen et al., 2015, Li et al., 2018 & Liu at al., 2018)

Environmental stress

Acute and chronic heat and cold stress increases gut permeability, by <u>increasing intestinal oxidative</u> <u>stress</u> and <u>disrupting the expression of tight junction proteins</u>. This results in the damage and destruction of intestinal cells, inflammation, and imbalance of the microbiota. An increased release and passage of endotoxins has been demonstrated in heat stress (Figure 3), as well as a higher expression of TLR-4 and inflammation.

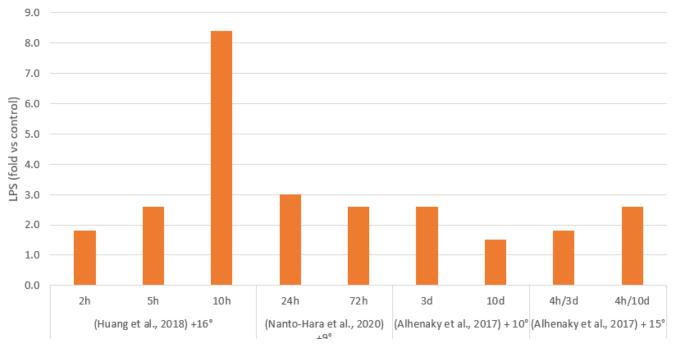
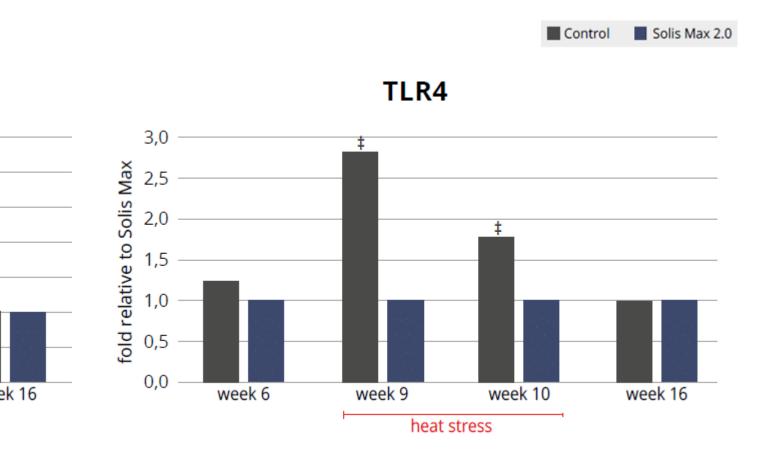


Figure 3 Systemic LPS increase (in comparison with a non-stressed control) after different heat stress challenges in broilers: 16°C increased for 2, 5 and 10 hours (Huang et al., 2018); 9°C increased for 24 and 72 hours (Nanto-Hara et al., 2020); 10°C continuously for 3 and 10 days, and 15°C 4 hours daily for 3 and 10 days (Alhenaky et al., 2017)

Zhou and collaborators (2021) showed that 72 hours of low temperature treatment in young broilers increased intestinal inflammation and expression of tight junction proteins, while higher blood endotoxins indicate a disruption of the intestinal barrier. As a consequence, the stress decreased body gain and increased the feed conversion rate.

An experiment conducted by EW Nutrition GmbH with the objective of evaluating the ability of a toxin mitigation product to ameliorate heat-stress induced LPS. For the experiment, 1760 Cobb 500 pullets were divided into two groups, and each was placed in 11 pens of 80 hens, in a single house. One of the groups received feed containing 2kg/ton of the product from the first day. From week 8 to week 12, the temperature of the house was raised 10°C for 8 hours every day.

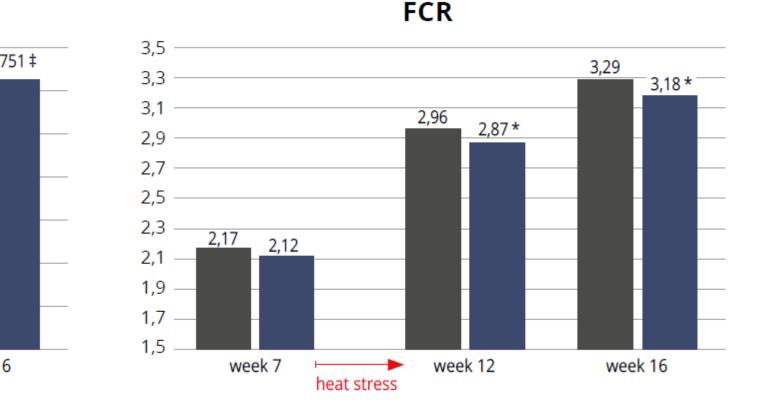
Throughout the heat stress period, blood LPS (Fig 4) was lower in the pullets receiving the product, which allowed lower inflammation, as evidenced by the lower expression of TLR4 (Fig. 5). Oxidative stress was also mitigated with the help of the combination of phytomolecules in the product, obtaining 8.5% improvement on serum total antioxidant capacity (TAC), supported by an increase in in superoxide dismutase (SOD glutathione peroxidase (GSH) and a decrease in malondialdehyde (MDH).



es of pullets before (wk 6) and during heat stress (wk 9 and 10). (*) indicates significant differences (P<0,05), and

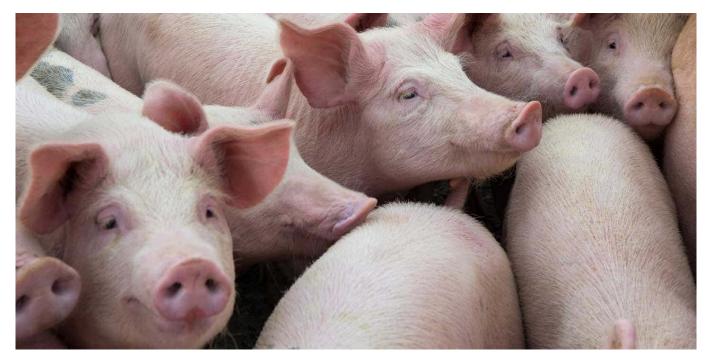
In practice: there is no silver bullet

In commercial poultry production, a myriad stressors may occur at the same time and some factors trigger a chain of events that work to the detriment of animal health and productivity. Reducing the solution to the mitigation of LPS is a deceitfully simplistic approach. However, this should be part of a strategy to achieve better animal health and performance. In fact, EW Nutrition's toxin mitigation product alone helped the pullets to achieve 3% improvement in body weight and 9 points lower cumulative feed conversion (Figure 6).



Keeping the animals as free of stress as possible is a true priority for poultry producers, as it promotes animal health as well as the integrity and function of the intestinal barrier. Biosecurity, good environment, nutrition and good management practices are crucial; the use of feed additives to reduce the consequences of unavoidable stress also critically supports the profitability of poultry operations.

Salmonella in pigs: a threat for humans and a challenge for pig producers



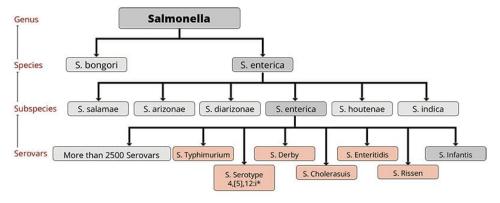
By **Dr. Inge Heinzl**, Editor, EW Nutrition

Salmonellosis is third among foodborne diseases leading to death (Ferrari, 2019). More than 91,000 human cases of Salmonellosis are reported by the EU each year, generating overall costs of up to €3 billion a year (EFSA, 2023), 10-20% of which are attributed to pork consumption (Soumet, 2022). The annual costs arising from the resulting human health losses in 2010 were about €90 million (FCC Consortium, 2010). Take the example of Ireland, where a high prevalence of Salmonella in lymph nodes still shows a severe issue pre-slaughter and a big challenge for slaughterhouses to stick to the process hygiene requirements (Deane, 2022).

Several governments already have monitoring programs in place, and the farms are categorized according to the salmonella contamination of their pigs. In some countries, e.g., Denmark, an economic penalty of 2% of the carcass value must be paid if the farm has level 2 (intermediate seroprevalence) and 4-8% if the level is 3. Other countries, e.g., Germany, the UK, Ireland, or the Netherlands, use quality assurance schemes. The farmers can only sell their carcasses under this label if their farm has a certain level.

Let's take a quick look at the genus of Salmonella

Salmonellas are rod-shaped gram-negative bacteria of the family of enterobacteria that use flagella for their movement. They were named after the American vet Daniel Elmer Salmon. The genus of Salmonella consists of two species (S. bongori and S. enterica with seven subspecies) with in total more than 2500 serovars (see Figure 1). The effects of the different serovars can range from asymptomatic carriage to severe invasive systemic disease (Gal-Mor, 2014). All Salmonella serovars generally can cause disease in humans; the rosa-marked ones already showed infections.



*a monophasic variant of S. Typhimurium (Elnecave, 2018)

Figure 1: the genus of Salmonella with Salmonella serovars relevant for pigs (according to Bonardi, 2017: Salmonella in the pork production chain and its impact on human health in the European Union)

Within the group of Salmonella, some serovars can only reside in one or few species, e.g., S. enterica spp. enterica Serovar Dublin (S. Dublin) in bovines (<u>Waldron, 2018</u>) or S. Cholerasuis in pigs (<u>Chiu, 2004</u>). An infection in humans with these pathogens is often invasive and life-threatening (<u>WHO, 2018</u>). On the contrary, serovars like S. Typhimurium and S. Enteritidis are not host-specific and can cause disease in various species.

The serotypes S. Typhi and S. Paratyphi A, B, or C are highly adapted to humans and only for them pathogenic; they are responsible for the occurrence of typhus.

Serovars occurring in pigs and relevant for humans are, for example, S. Typhimurium (<u>Hendriksen, 2004</u>), S. Serotype 4,[5],12:I (<u>Hauser et al., 2010</u>), S. Cholerasuis (<u>Chiu, 2004</u>), S. Derby (<u>Gonzalez-Santamarina, 2021</u>), S. Agona (<u>Brenner Michael, 2006</u>) and S. Rissen (<u>Elbediwi, 2021</u>).

Transmission of Salmonella mostly happens via contaminated food

The way of transmission to humans depends on the serovar:

Human-specific and, therefore, only in humans and higher primates residing serovars S. Typhi and Paratyphi A, B, or C (typhoidal) are excreted via feces or urine. Therefore, any food or water contaminated with the feces or urine of infected people can transmit this disease (<u>Government of South Australia, 2023</u>). Typhoid and paratyphoid Salmonellosis occur endemic in developing countries with the lack of clean water and, therefore, inadequate hygiene (<u>Gal-Mor, 2014</u>).

Serovars which can cause disease in humans **and** animals (non-typhoidal), can be transmitted by

- animal products such as milk, eggs, meat
- contact with infected persons/animals (pigs, cows, pets, reptiles...) or
- other feces- or urine-contaminated products such as sprouts, vegetables, fruits....

Farm animals take salmonellas from their fellows, contaminated feed or water, rodents, or pests.

Symptoms of Salmonellosis can be severe

In the case of typhoid or paratyphoid Salmonellosis, the onset of illness is gradual. People can suffer from sustained high fever, unwellness, severe headache, and decreased appetite, but also from an enlarged spleen irritating the abdomen and dry cough.

A study conducted in Thailand with children suffering from enteric fever caused by the typhoid serovars S. Typhi and Paratyphi showed a sudden onset of fever and gastrointestinal issues (diarrhea), rose spots, bronchitis, and pneumonia (<u>Thisyakorn et al., 1987</u>)

The non-typhoid Salmonellosis is typically characterized by an acute onset of fever, nausea, abdominal pain with diarrhea, and sometimes vomiting (WHO, 2018). However, 5% of the persons – children with underlying conditions, e.g., babies, or people who have AIDS, malignancies, inflammatory bowel disease, gastrointestinal illness caused by non-typhoid serovars, and hemolytic anemia, or receiving an immunosuppressive therapy can be susceptible to bacteremia. Additionally, serovars like S. Cholerasuis or S. Dublin are apt to develop bacteremia by entering the bloodstream with little or no involvement of the gut (Chiu, 1999). In these cases, consequences can be septic arthritis, pneumonia, peritonitis, cutaneous abscess, mycotic aneurysm, and sometimes death (Chen et al., 2007; Chiu, 2004, Wang et al., 1996).

In pigs, S. Cholerasuis causes high fever, purple discolorations of the skin, and thereinafter diarrhea. The mortality rate in pigs suffering from this type of Salmonellosis is high. Barrows orally challenged with S. Typhimurium showed elevated rectal temperature by 12h, remaining elevated until the end of the study. Feed intake decreased with a peak at 48h after the challenge and remained up to 120h after the challenge. Daily gain reduced during the following two weeks after infection. A higher plasma cortisol level and a lower IGF-I level could also be noticed. All these effects indicate significant changes in the endocrine stress and the somatotropic axis, also without significant alterations in the systemic pro-inflammatory mediators (Balaji et al., 2000)

To protect humans, Salmonella in pork must be restraint

There are three main steps to keep the contamination of pork as low as possible:

- 1. Keeping Salmonella out of the pig farm
- 2. Minimizing spreading if Salmonella is already on the farm
- 3. Minimizing contamination in the slaughterhouse

1. How to keep Salmonella out of the pig farm?

To answer this question, we must look at how the pathogen can be transported to the farm. According to the Code of Practice for the Prevention and Control of Salmonella on Pig Farms (Ministry of Agriculture, Fisheries and Food and the Scottish Executive Rural Affairs Department), there are several possibilities to infiltrate the pathogen into the farm:

- Diseased pigs or pigs which are ill but don't show any symptoms
- Feeding stuff or bedding contaminated with dung
- Pets, rodents, wild birds, or animals
- Farm personnel or visitors
- Equipment or vehicles

Caution with purchased animals!

To minimize/prevent the entry of Salmonella into the livestock, bought-in animals must come from reputable breeding farms with a salmonella monitoring system in place. As possible carrier animals are more likely to excrete Salmonella when stressed; they should be kept in isolation after purchasing. Additionally, the animals must go through a disinfectant foot bath before entering the farm.

Keep rodents, wild animals, and vermin in check!

Generally, the production site must be kept clean and as unattractive as possible for all these animals. Rests of feed must be removed, and dead animals and afterbirths must be promptly and carefully disposed of. A well-planned baiting and trapping policy should be in place to effectively control rodents.

Only selected people should enter the hog houses

In any case, the number of persons entering the hog house must be kept as low as possible. Farmworkers should be trained in the principles of hygiene. They should wear adequate clothing (waterproof boots and protective overalls) that can be easily cleaned/laundered and disinfected. The clothes/shoes should always be used only at this site. Thorough hand washing and the disinfection of the boots when entering and leaving the pig unit are a must.

If visits are necessary, the visitors should take the same measures as the farm workers. And, of course, they should not have had contact with another pig farm during the last 48 hours.

Keep pens, farm equipment, and vehicles clean!

Farm equipment should not be shared with other farms. If this cannot be avoided, it must be cleaned and disinfected before re-entering the farm. Also, the vehicles for the transport of the animals must be cleaned and disinfected as soon as possible after usage, as contaminated transporters always pose the risk of infection.

Feed should be Salmonella-free!

To get high feed quality, the feed should be purchased from feed mills/sources with a well-functioning bacterial control to guarantee the absence of Salmonella. It is essential that birds, domestic and wild animals cannot enter the feed stores.

It is also advised to keep dry feed dry as possibly contaminating Salmonella can multiply in such humid conditions. Additionally, all feed bins and delivery pipes for dry and wet feed must be consciously cleaned, and the damp feed pipes also disinfected.

The change from pellets to mash could be helpful as the pellets facilitate Salmonella colonization by stimulating the secretion of mucins (<u>Hedemann et al., 2005</u>).

For sanitation of the feed, we offer organic acids (<u>Acidomix product range</u>) or mixtures of organic acids and formaldehyde in countries where formaldehyde products are allowed (<u>Formycine</u>) to decrease the pathogenic load of the feed materials. In vitro trials show the effectiveness of the products:

For the in vitro trial with Formycine, autoclaved feed samples were inoculated with Salmonella enteritidis serovar Typhimurium DSM 19587 strain to reach a Salmonella contamination of 10^6 CFU/g of feed. After incubating at room temperature for three hours, Formycine Liquido was added to the contaminated feed samples at 0, 500, 1000, and 2000 ppm. The control and inoculated feed samples were further incubated at room temperature, and Salmonella counts (CFU/g) were carried out at 24, 48, 72 hours and on day 15.

The limit of Salmonella detection was set at 100 CFU/g (10^2) . Results are shown in figure 2.

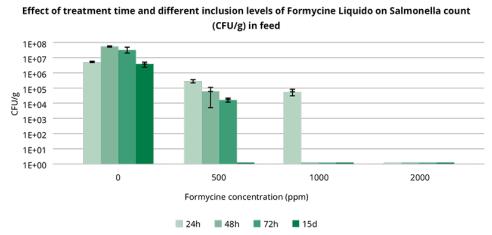


Fig. 2: Effect of treatment time and different inclusion levels of Formycine Liquido on the Salmonella count in feed

As important as uncontaminated feed is clean water for drinking. It can be achieved by taking the water from a main or a bacteriologically controlled water borehole. Regular cleaning/disinfection of the tanks, pipes, and drinkers is essential.

Bedding should be Salmonella-free

Straw material containing feces of other animals (rodents, pets) always carries the risk of Salmonella contamination. Also, wet or moldy bedding is not recommended because it is an additional challenge for the animal. To optimize the quality of bedding, the straw should be bought from reliable and as few as possible sources. The material must be stored dry and as far as practicable from the pig buildings (Ministry of Agriculture, Fisheries and Food & Scottish Executive Rural Affairs Department, 2000).

Vaccination is a beneficial measure

For the control of Salmonella in swine herds, vaccination is an effective tool. <u>De Ridder et al. (2013)</u> showed that an attenuated vaccine reduced the transmission of Salmonella Typhimurium in pigs. The vaccination with an attenuated S. Typhimurium strain, followed by a booster vaccination with inactivated S. Cholerasuis, showed better effects than an inactivated S. Cholerasuis vaccine alone (<u>Alborali et al., 2017</u>). <u>Bearson et al. (2017)</u> could delimitate transmission through less shedding and protect the animals against systemic disease.

To achieve the best effects, the producer must understand the diversity of Salmonella serovars to choose the most promising vaccination strategy (<u>FSIS</u>, <u>2023</u>).

2. How to minimize the spreading of Salmonella on the farm?

If there are already cases of Salmonella on the farm, infected animals must be separated from the rest of the herd. Small batch sizes are beneficial, as well as not mixing different litters after weaning. If feasible, separate units for different production phases with an all-in/all-out system could break the reinfection cycle and help reduce Salmonella contamination on the farm. And also in this case, vaccination is helpful.

Salmonella doesn't like acid conditions

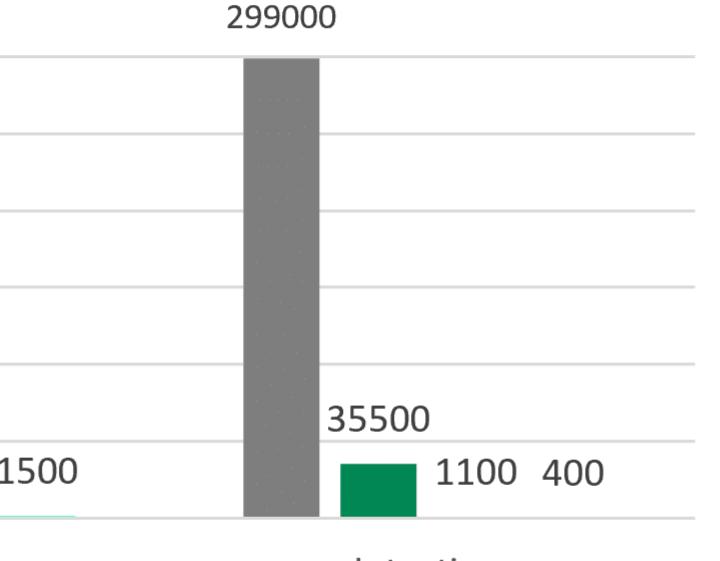
An effective tool is acidifying the feed with organic acids, as Salmonella doesn't like acid conditions. A trial was conducted with Acidomix AFG and Acidomix AFL to show their effects against Salmonella. For the test,

10⁵ CFU/g of Salmonella enterica ser. Typhimurium was added to feed containing 1000 ppm, 2000 ppm, and 3000 ppm of Acidomix AFG or AFL. The stomach and intestine were simulated in vitro by adjusting the pH with HCl and NaHCO3 as follows:

Stomach 2.8 Intestine 6.8-7.0

After the respective incubation, the microorganisms were recovered from feed and plated on an appropriate medium for CFU counting. The results are shown in figures 3 and 4.

lomix AFL



Intestine

0 ppm **2**000 ppm 3000 ppm

Phytomolecules can support pigs against Salmonella

Plant compounds or phytomolecules can also be used against Salmonella in pigs. Some examples of phytomolecules to be used are Piperine, Allicin, Eugenol, and Carvacrol. Eugenol, e.g., increases the permeability of the Salmonella membrane, disrupts the cytoplasmic membrane, and inhibits the production of bacterial virulence factors (Keita et al., 2022; Mak et al., 2019). Thymol and Carvacrol interact with the cell membrane by H bonding, also resulting in a higher permeability.

An already published in vitro trial conducted with our product Ventar D also showed excellent effects against Salmonella while sparing the beneficial gut flora. A further trial once more demonstrated the susceptibility of Salmonella to Ventar D. It showed that Ventar D controls Salmonella by suppressing their motility and, at higher concentrations, inactivating the cells (see figures 5 + 6):

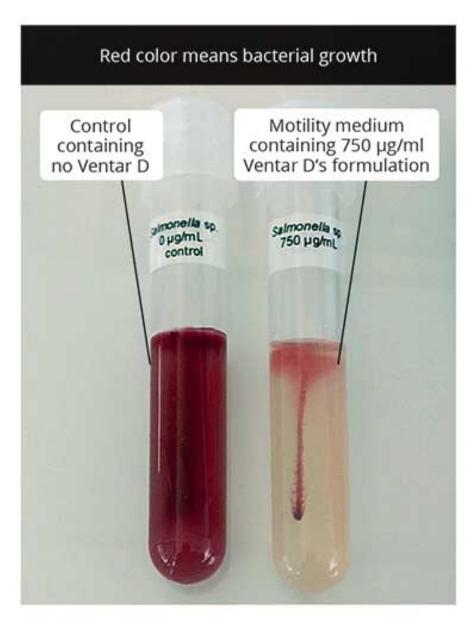


Figure 5: S. enterica motility test: on the left side – control; on the right side – motility medium containing.750 µg/mL of Ventar

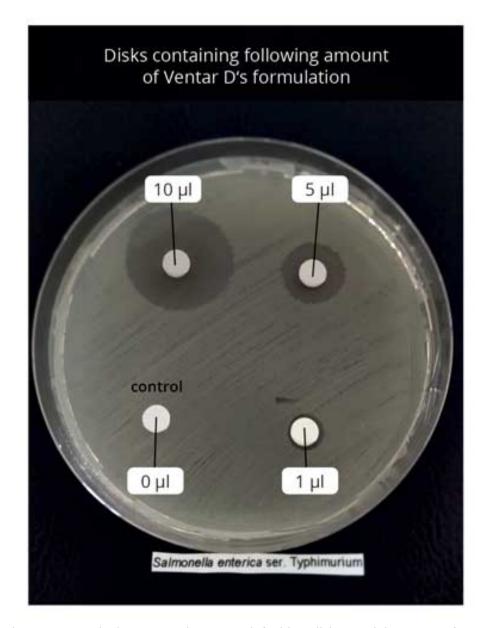


Fig 6 . Disk diffusion assay employing S. enterica. upper left side – disk containing 10 μ L of Ventar; upper right – 5μ L; lower left – control; lower right – 1μ L.

In addition to the direct Salmonella-reducing effect, essential oils / secondary plant compounds / phytomolecules improve digestive enzyme activity and digestion, leading to increased nutrient absorption and better feed conversion (Windisch et al., 2008).

3. How can the farmer keep Salmonella contamination low in the slaughterhouse?

In general, the slaughterhouse personnel is responsible for adequate hygiene management to prevent contamination of carcasses and meat. However, also the farmer can make his contribution to maintain the risk of contamination in the slaughterhouse as low as possible. A study by <u>Vieira-Pinto (2006</u>) revealed that one Salmonella-positive pig can contaminate several other carcasses.

According to a trial conducted by <u>Hurd et al. (2002)</u>, infection and, therefore, "contamination" of other pigs can rapidly occur, meaning that cross-contamination is a topic during transport to the slaughterhouse and in the lairages when the pigs come together with animals from other farms. The stress to which the pigs are exposed influences physiological and biochemical processes. The microbiome and animal's immunity are affected, leading to higher excretion of Salmonella during transport and in the lairages. So, the animals should not be stressed during loading and unloading or transportation. The trailer poses a further risk of

infection if it was not cleaned and disinfected before. So, reliable people who treat the animals well and keep their trailers clean should be chosen for transportation.

Pig producers are obliged to keep Salmonella in check - phytomolecules can help

At least in the EU, pig producers have the big duty to keep Salmonella low in their herds; otherwise, they will have financial losses. They are not only responsible for their farm, but also the slaughterhouses count on them. Besides the standard strict hygiene management and vaccination, farmers can use products provided by the industry to sanitize feed but also to support their animals directly with phytomolecules acting against pathogens and supporting gut health.

All these measures together should be a solution to the immense challenge of Salmonella, to protect people and prevent economic losses.

References:

Alborali, Giovanni Loris, Jessica Ruggeri, Michele Pesciaroli, Nicola Martinelli, Barbara Chirullo, Serena Ammendola, Andrea Battistoni, Maria Cristina Ossiprandi, Attilio Corradi, and Paolo Pasquali. "Prime-Boost Vaccination with Attenuated Salmonella Typhimurium Δznuabc and Inactivated Salmonella Choleraesuis Is Protective against Salmonella Choleraesuis Challenge Infection in Piglets." *BMC Veterinary Research* 13, no. 1 (2017): 284. https://doi.org/10.1186/s12917-017-1202-5.

Balaji, R, K J Wright, C M Hill, S S Dritz, E L Knoppel, and J E Minton. "Acute Phase Responses of Pigs Challenged Orally with Salmonella Typhimurium." *Journal of Animal Science* 78, no. 7 (2000): 1885. https://doi.org/10.2527/2000.7871885x.

Bearson, Bradley L, Shawn M. Bearson, Brian W Brunelle, Darrell O Bayles, In Soo Lee, and Jalusa D Kich. "Salmonella Diva Vaccine Reduces Disease, Colonization, and Shedding Due to Virulent S. Typhimurium Infection in Swine." *Journal of Medical Microbiology* 66, no. 5 (2017): 651–61. https://doi.org/10.1099/jmm.0.000482.

Brenner Michael, G, M Cardoso, and S Schwarz. "Molecular Analysis of Salmonella Enterica Subsp. Enterica Serovar Agona Isolated from Slaughter Pigs." *Veterinary Microbiology* 112, no. 1 (2006): 43–52. https://doi.org/10.1016/j.vetmic.2005.10.011.

Chen, P.-L., C.-M. Chang, C.-J. Wu, N.-Y. Ko, N.-Y. Lee, H.-C. Lee, H.-I. Shih, C.-C. Lee, R.-R. Wang, and W.-C. Ko. "Extraintestinal Focal Infections in Adults with Non-typhoid Salmonella Bacteraemia: Predisposing Factors and Clinical Outcome." *Journal of Internal Medicine* 261, no. 1 (2007): 91–100. https://doi.org/10.1111/j.1365-2796.2006.01748.x.

Chiu, Cheng-Hsun, Lin-Hui Su, and Chishih Chu. "Salmonella EntericaSerotype Choleraesuis: Epidemiology, Pathogenesis, Clinical Disease, and Treatment." Clinical Microbiology Reviews 17, no. 2 (2004): 311–22. https://doi.org/10.1128/cmr.17.2.311-322.2004.

De Ridder, L., D. Maes, J. Dewulf, F. Pasmans, F. Boyen, F. Haesebrouck, E. Méroc, P. Butaye, and Y. Van der Stede. "Evaluation of Three Intervention Strategies to Reduce the Transmission of Salmonella Typhimurium in Pigs." *The Veterinary Journal* 197, no. 3 (2013): 613–18. https://doi.org/10.1016/j.tvjl.2013.03.026.

Deane, Annette, Declan Murphy, Finola C. Leonard, William Byrne, Tracey Clegg, Gillian Madigan, Margaret Griffin, John Egan, and Deirdre M. Prendergast. "Prevalence of Salmonella spp. in Slaughter Pigs and Carcasses in Irish Abattoirs and Their Antimicrobial Resistance." *Irish Veterinary Journal* 75, no. 1 (2022). https://doi.org/10.1186/s13620-022-00211-y.

Edel, W., M. Schothorst, P. A. Guinée, and E. H. Kampelmacher. "Effect of Feeding Pellets on the Prevention and Sanitation of Salmonella Infections in Fattening Pigs1." *Zentralblatt für Veterinärmedizin Reihe B* 17, no. 7 (2010): 730–38. https://doi.org/10.1111/j.1439-0450.1970.tb01571.x.

EFSA. "Salmonella." European Food Safety Authority. Accessed August 7, 2023. https://www.efsa.europa.eu/en/topics/topic/salmonella.

Elbediwi, Mohammed, Daiwei Shi, Silpak Biswas, Xuebin Xu, and Min Yue. "Changing Patterns of Salmonella Enterica Serovar Rissen from Humans, Food Animals, and Animal-Derived Foods in China, 1995–2019." *Frontiers in Microbiology* 12 (2021). https://doi.org/10.3389/fmicb.2021.702909.

Elnekave, Ehud, Samuel Hong, Alison E Mather, Dave Boxrud, Angela J Taylor, Victoria Lappi, Timothy J Johnson, et al. "Salmonella Enterica Serotype 4,[5],12:I:- In Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade." Clinical Infectious Diseases 66, no. 6 (2018): 877–85. https://doi.org/10.1093/cid/cix909.

FCC Consortium. "Final Report - Food Safety." European Commission, 2010. https://food.ec.europa.eu/system/files/2016-10/biosafety_food-borne-disease_salmonella_fattening-pigs_slaught house-analysis-costs.pdf.

Ferrari, Rafaela G., Denes K. Rosario, Adelino Cunha-Neto, Sérgio B. Mano, Eduardo E. Figueiredo, and Carlos A. Conte-Junior. "Worldwide Epidemiology of *Salmonella s*erovars in Animal-Based Foods: A Meta-Analysis." *Applied and Environmental Microbiology* 85, no. 14 (2019). https://doi.org/10.1128/aem.00591-19.

"FSIS Guideline to Control Salmonella in Swine Slaughter and Pork Processing Establishments." FSIS Guideline to Control Salmonella in Swine Slaughter and Pork Processing Establishments | Food Safety and Inspection Service. Accessed August 14, 2023. https://www.fsis.usda.gov/guidelines/2023-0003.

Gal-Mor, Ohad, Erin C. Boyle, and Guntram A. Grassl. "Same Species, Different Diseases: How and Why Typhoidal and Non-Typhoidal Salmonella Enterica Serovars Differ." *Frontiers in Microbiology* 5 (2014). https://doi.org/10.3389/fmicb.2014.00391.

González-Santamarina, Belén, Silvia García-Soto, Helmut Hotzel, Diana Meemken, Reinhard Fries, and Herbert Tomaso. "Salmonella Derby: A Comparative Genomic Analysis of Strains from Germany." *Frontiers in Microbiology* 12 (2021). https://doi.org/10.3389/fmicb.2021.591929.

Government of South Australia. Typhoid and paratyphoid – including symptoms, treatment, and prevention, April 3, 2022.

https://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/conditions/infectious+diseases/typhoid+and+paratyphoid/typhoid+and+paratyphoid+-+including+symptoms+treatment+and+prevention.

Hauser, Elisabeth, Erhard Tietze, Reiner Helmuth, Ernst Junker, Kathrin Blank, Rita Prager, Wolfgang Rabsch, Bernd Appel, Angelika Fruth, and Burkhard Malorny. "Pork Contaminated with Salmonella Enterica Serovar 4,[5],12:I:—, an Emerging Health Risk for Humans." Applied and Environmental Microbiology 76, no. 14 (2010): 4601–10. https://doi.org/10.1128/aem.02991-09.

Health and Wellbeing; address=11 Hindmarsh Square, Adelaide scheme=AGLSTERMS.AglsAgent; corporateName=Department for. "Sa Health." Typhoid and paratyphoid - including symptoms, treatment, and prevention, April 3, 2022.

https://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/conditions/infectious+diseases/typhoid+and+paratyphoid/typhoid+and+paratyphoid+-+including+symptoms+treatment+and+prevention.

Hedemann, M. S., L. L. Mikkelsen, P. J. Naughton, and B. B. Jensen. "Effect of Feed Particle Size and Feed Processing on Morphological Characteristics in the Small and Large Intestine of Pigs and on Adhesion of Salmonella Enterica Serovar Typhimurium DT12 in the Ileum in Vitro1." *Journal of Animal Science* 83, no. 7 (2005): 1554–62. https://doi.org/10.2527/2005.8371554x.

Hendriksen, Susan W.M., Karin Orsel, Jaap A. Wagenaar, Angelika Miko, and Engeline van Duijkeren. "Animal-to-Human Transmission of *Salmonella* Typhimurium DT104A Variant." *Emerging Infectious Diseases* 10, no. 12 (2004): 2225–27. https://doi.org/10.3201/eid1012.040286.

Keita, Kadiatou, Charles Darkoh, and Florence Okafor. "Secondary Plant Metabolites as Potent Drug Candidates against Antimicrobial-Resistant Pathogens." *SN Applied Sciences* 4, no. 8 (2022). https://doi.org/10.1007/s42452-022-05084-y.

Ministry of Agriculture, Fisheries and Food, and Scottish Executive Rural Affairs Department. "Salmonella on Pig Farms - Code of Practice for the Prevention and Control Of." ReadkonG.com, 2000.

https://www.readkong.com/page/code-of-practice-for-the-prevention-and-control-of-5160969.

Morrow, W.E. Morgan, and Julie Funk. Ms. Salmonella as a Foodborne Pathogen in Pork. North Carolina State University Animal Science, n.d.

Soumet, C., A. Kerouanton, A. Bridier, N. Rose, M. Denis, I. Attig, N. Haddache, and C. Fablet. Report, Salmonella excretion level in pig farms and impact of quaternary ammonium compounds based disinfectants on Escherichia coli antibiotic resistance § (2022).

Thisyakorn, Usa. "Typhoid and Paratyphoid Fever in 192 Hospitalized Children in Thailand." *Archives of Pediatrics & Adolescent Medicine* 141, no. 8 (1987): 862. https://doi.org/10.1001/archpedi.1987.04460080048025.

Ung, Aymeric, Amrish Y. Baidjoe, Dieter Van Cauteren, Nizar Fawal, Laetitia Fabre, Caroline Guerrisi, Kostas Danis, et al. "Disentangling a Complex Nationwide Salmonella Dublin Outbreak Associated with Raw-Milk Cheese Consumption, France, 2015 to 2016." *Eurosurveillance* 24, no. 3 (2019). https://doi.org/10.2807/1560-7917.es.2019.24.3.1700703.

Vieira-Pinto, M, R Tenreiro, and C Martins. "Unveiling Contamination Sources and Dissemination Routes of Salmonella Sp. in Pigs at a Portuguese Slaughterhouse through Macrorestriction Profiling by Pulsed-Field Gel Electrophoresis." *International Journal of Food Microbiology* 110, no. 1 (2006): 77–84. https://doi.org/10.1016/j.ijfoodmicro.2006.01.046.

Waldron, P. "Keeping Cows and Humans Safe from Salmonella Dublin." Cornell University College of Veterinary Medicine, December 25, 2018.

https://www.vet.cornell.edu/news/20181218/keeping-cows-and-humans-safe-salmonella-dublin.

Wang, J.-H., Y.-C. Liu, M.-Y. Yen, J.-H. Wang, Y.-S. Chen, S.-R. Wann, and D.-L. Cheng. "Mycotic Aneurysm Due to Non-Typhi Salmonella: Report of 16 Cases." *Clinical Infectious Diseases* 23, no. 4 (1996): 743–47. https://doi.org/10.1093/clinids/23.4.743.

WHO. "Salmonella (Non-Typhoidal)." World Health Organization, February 20, 2018. https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal).

Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. "Use of Phytogenic Products as Feed Additives for Swine and Poultry1." *Journal of Animal Science* 86, no. suppl 14 (2008). https://doi.org/10.2527/jas.2007-0459.

Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. "Use of Phytogenic Products as Feed Additives for Swine and Poultry1." *Journal of Animal Science* 86, no. suppl_14 (2008). https://doi.org/10.2527/jas.2007-0459.

The future of coccidiosis control



By Madalina Diaconu, Product Manager Pretect D, EW Nutrition and

With costs of over 14 billion USD per year (Blake, 2020), coccidiosis is one of the most devastating enteric challenges in the poultry industry. With regard to costs, subclinical forms of coccidiosis account for the majority of production losses, as damage to intestinal cells results in lower body weight, higher feed conversion rates, lack of flock uniformity, and failures in skin pigmentation. This challenge can only be tackled, if we understand the basics of coccidiosis control in poultry and what options producers have to manage coccidiosis risks.

Current strategies show weak points

Good farm management, litter management, and coccidiosis control programs such as shuttle and rotation programs form the basis for preventing clinical coccidiosis. More successful strategies include disease monitoring, strategic use of coccidiostats, and increasingly coccidiosis vaccines. However, the intrinsic properties of coccidio make these parasites often frustrating to control. Acquired resistance to available coccidiostats is the most difficult and challenging factor to overcome.

Optimally, coccidiosis control programs are developed based on the farm history and the severity of infection. The coccidiostats traditionally used were chemicals and ionophores, with ionophores being polyether antibiotics. To prevent the development of resistance, the coccidiostats were used in shuttle or rotation programs, at which in the rotation program, the anticoccidial changes from flock to flock, and in the shuttle program within one production cycle (Chapman, 1997).

The control strategies, however, are not 100% effective. The reason for that is a lack of diversity in available drug molecules and the overuse of some molecules within programs. An additional lack of sufficient coccidiosis monitoring and rigorous financial optimization often leads to cost-saving but only marginally effective solutions. At first glance, they seem effective, but in reality, they promote resistance, the development of subclinical coccidiosis, expressed in a worsened feed conversion rate, and possibly also clinical coccidiosis.

Market requests and regulations drive coccidiosis control strategies

Changing coccidiosis control strategies has two main drivers: the global interest in mitigating antimicrobial resistance and the consumer's demand for antibiotic-free meat production.

Authorities have left ionophores untouched

Already in the late 1990s, due to the fear of growing antimicrobial resistance, the EU withdrew the authorization for Avoparcin, Bacitracin zinc, Spiramycin, Virginiamycin, and Tylosin phosphate, typical growth promoters, to "help decrease resistance to antibiotics used in medical therapy". However, ionophores, being also antibiotics, were left untouched: The regulation (EC) No 1831/2003 [13]of the European Parliament and the Council of 22 September 2003 clearly distinguished between coccidiostats and antibiotic growth promoters. Unlike the antibiotic growth promoters, whose primary action site is the gut microflora, coccidiostats only have a secondary and residual activity against the gut microflora. Furthermore, the Commission declared in 2022 that the use of coccidiostats would not presently be ruled out "even if of antibiotic origin" (MEMO/02/66, 2022) as "hygienic precautions and adaptive husbandry measures are not sufficient to keep poultry free of coccidiosis" and that "modern poultry husbandry is currently only practicable if coccidiosis can be prevented by inhibiting or killing parasites during their development". In other words, the Commission acknowledged that ionophores were only still authorized because it believed there were no other means of controlling coccidiosis in profitable poultry production.

Consumer trends drove research on natural solutions

Due to consumers' demand for antibiotic-reduced or, even better, antibiotic-free meat production, intensified industrial research to fight coccidiosis with natural solutions has shown success. Knowledge, research, and technological developments are now at the stage of offering solutions that can be an effective part of the coccidia control program and open up opportunities to make poultry production even more sustainable by reducing drug dependency.

Producers from other countries have already reacted. Different from the handling of ionophores regime in the EU, where they are allowed as feed additives, in the United States, coccidiostats belonging to the polyether-ionophore class are not permitted in NAE (No Antibiotics Ever) and RWE (Raised Without Antibiotics) programs. Instead of using ionophores, coccidiosis is controlled with a veterinary-led combination of live vaccines, synthetic compounds, phytomolecules, and farm management. This approach can be successful, as demonstrated by the fact that over 50% of broiler meat production in the US is NAE. Another example is Australia, where the two leading retail store chains also exclude chemical coccidiostats from broiler production. In certain European countries, e.g., Norway, the focus is increasingly on banning ionophores.

The transition to natural solutions needs knowledge and finesse

In the beginning, the transition from conventional to NAE production can be difficult. There is the possibility to leave out the ionophores and manage the control program only with chemicals of different modes of action. More effective, however, is a combination of vaccination and chemicals (bio-shuttle program) or the combination of phytomolecules with vaccination and/or chemicals (Gaydos, 2022).

Coccidiosis vaccination essentials

When it is decided that natural solutions shall be used to control coccidiosis, some things about vaccination must be known:

- 1. There are different strains of vaccines, natural ones selected from the field and attenuated strains. The formers show medium pathogenicity and enable a controlled infection of the flock. The latter, being early mature lower pathogenicity strains, usually cause only low or no post-vaccinal reactions.
- 2. A coccidiosis program that includes vaccination should cover the period from the hatchery till the end of the production cycle. Perfect application of the vaccines and effective recirculation of vaccine strains amongst the broilers are only two examples of preconditions that must be fulfilled for striking success and, therefore, early and homogenous immunity of the flock.
- 3. Perfect handling of the vaccines is of vital importance. For that purpose, the personnel conducting the vaccinations in the hatchery or on the farms must be trained. In some situations, consistent high-quality application at the farm has shown to be challenging. As a result, interest in vaccine application at the hatchery is growing.

Phytochemicals are a perfect tool to complement coccidiosis control programs

As the availability of vaccines is limited and the application costs are relatively high, the industry has been researching supportive measures or products and discovered phytochemicals as the best choice. Effective phytochemical substances have antimicrobial and antiparasitic properties and enhance protective immunity in poultry infected by coccidiosis. They can be used in rotation with vaccination, to curtail vaccination reactions of (non-attenuated) wild strain vaccines, or in combination with chemical coccidiostats in a shuttle program.

In a recent review paper (El-Shall et al., 2022), natural herbal products and their extracts have been described to effectively reduce oocyst output by inhibiting Eimeria species' invasion, replication, and development in chicken gut tissues. Phenolic compounds in herbal extracts cause coccidia cell death and lower oocyst counts. Additionally, herbal additives offer benefits such as reducing intestinal lipid peroxidation, facilitating epithelial repair, and decreasing Eimeria-induced intestinal permeability.

Various phytochemical remedies are shown in this simplified adaptation of a table from El-Shall et al. (2022), indicating the effects exerted on poultry in connection to coccidia infection.

Bioactive compound	Effect
Saponins	Inhibition of coccidia: By binding to membrane cholesterol, the saponins disturb the lipids in the parasite cell membrane. The impact on the enzymatic activity and metabolism leads to cell death, which then induces a toxic effect in mature enterocytes in the intestinal mucosa. As a result, sporozoite-infected cells are released before the protozoa reach the merozoite phase. Support for the chicken: Saponins enhance non-specific immunity and increase productive performance (higher daily gain and improved FCR, lower mortality rate). They decrease fecal oocyst shedding and reduce ammonia production.
Tannins	Inhibition of coccidia: Tannins penetrate the coccidia oocyst wall and inactivate the endogenous enzymes responsible for sporulation. Support for the chicken: Additionally, they enhance anticoccidial antibodies' activity by increasing cellular and humoral immunity.

Flavonoids and terpenoids	Inhibition of coccidia: They inhibit the invasion and replication of different species of coccidia. Support for the chicken: They bind to the mannose receptor on macrophages and stimulate them to produce inflammatory cytokines such as IL-1 through IL-6 and TNF. Higher weight gain and lower fecal oocyst output are an indication of suppression of coccidiosis.
Artemisinin	Inhibition of coccidia: Its impact on calcium homeostasis compromises the oocyst wall formation and leads to a defective cell wall and, in the end, to the death of the oocyst. Enhancing the production of ROS directly inhibits sporulation and also wall formation and, therefore, affects the Eimeria life cycle. Support for the chicken: Reduction of oocyst shedding
Leaf powder of Artemisia annua	Support for the chicken: Protection from pathological symptoms and mortality associated with Eimeria tenella infection. Reduced lesion score and fecal oocyst output. The leaf powder was more efficient than the essential oil, which could be due to a lack of Artemisinin in the oil, and to the greater antioxidant ability of A. annua leaves than the oil.
Phenols	Inhibition of coccidia: Phenols change the cytoplasmic membrane's permeability for cations (H+ and K+), impairing essential processes in the cell. The resulting leakage of cellular constituents leads to water unbalance, collapse of the membrane potential, inhibition of ATP synthesis, and, finally, cell death. Due to their toxic effect on the upper layer of mature enterocytes of the intestinal mucosa, they accelerate the natural renewal process, and, therefore, sporozoite-infected cells are shed before the coccidia reaches the merozoite phase.

Table 1: Bioactive compounds and their anticoccidial effect exerted in poultry

Consumers vote for natural - phytochemicals are the solution

Due to still rising antimicrobial resistance, consumers push for meat production without antimicrobial usage. Phytomolecules, as a natural solution, create opportunities to make poultry production more sustainable by reducing dependency on harmful drugs. With their advent, there is hope that antibiotic resistance can be held in check without affecting the profitability of poultry farming.

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.

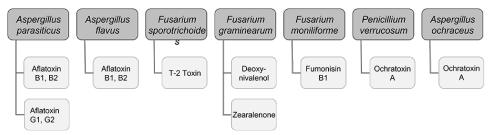


Figure 1: Fungi species and their mycotoxins of worldwide importance for poultry production (adapted from Bryden, 2012).

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

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 (<u>Devegowda and Murthy, 2005</u>). As a result of improper absorption of nutrients, egg production, eggshell formation, fertility, and hatchability are also negatively influenced.

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

Mycotoxins have specific targets

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

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

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

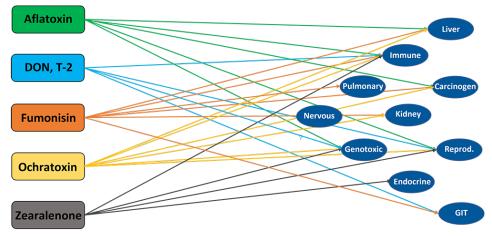


Figure 2: Main target organs of important mycotoxins

How to reduce mycotoxicosis?

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

Preventing mycotoxin production means preventing mold growth

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

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

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

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

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

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

- Dry properly: grains should be stored at <13% of moisture</p>
- 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 (<u>Raju and Devegowda 2000</u>).

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

Which characteristics are crucial for an effective toxin-mitigating solution

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

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

Depending on

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

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

Solis - the cost-effective solution

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

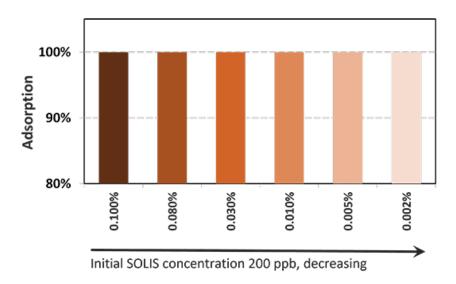


Figure 3: Binding capacity of Solis for Aflatoxin

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

<u>Solis Max</u> 2.0 has a synergistic combination of ingredients that acts by chemi- and physisorption to prevent toxic fungal metabolites from damaging the animal's gastrointestinal tract and entering the bloodstream.

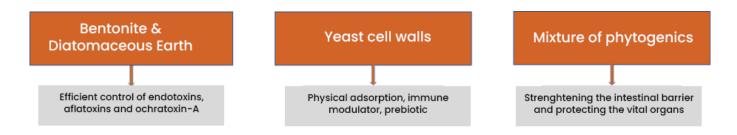


Figure 4: Composition and effects of Solis Max 2.0

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

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

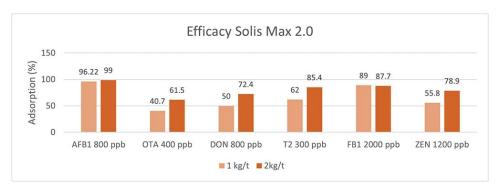


Figure 5: Efficacy of Solis Max 2.0 against different mycotoxins relevant in poultry production

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

Broiler trial shows improved performance in broilers

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

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

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

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

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

- 6% higher body weight than the negative control and 18.5% higher body weight than the challenged group
- 12 points and 49 points better FCR than the negative control and the challenged group, respectively
- Lower levels of AST and ALT compared to the challenged group, showing a better liver health

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

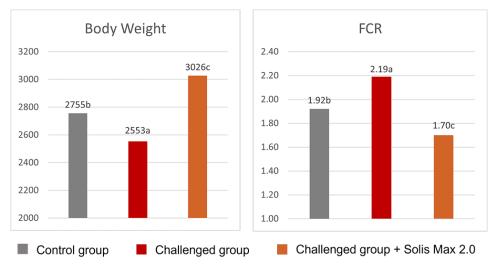


Figure 6: Better performance data due to the addition of Solis Max 2.0

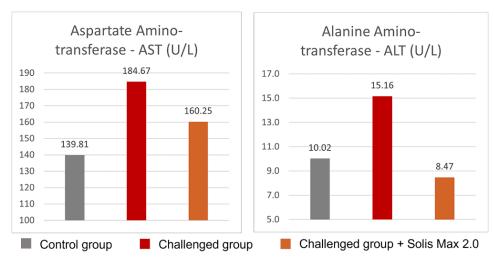


Figure 7: Healthier liver shown by lower values of AST and ALT

Effective toxin risk management: staying power is required

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

Toxin Risk Management



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