

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 [regain equilibrium](#). We can distinguish four major types of stressors in the poultry industry:

- Technological: related with management events and conditions
- Nutritional: involving nutritional imbalances, 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 an 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 [disrupting the intestinal barrier function](#), allowing endotoxins (as well as other toxins and pathogens) to cross the gut barrier and pass into the bloodstream
- By [alterations in the immune response](#), low doses of mycotoxins, such as trichothecenes, induce the upregulation of pro-inflammatory cytokines. A [possible synergy](#) 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 200ppb 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.

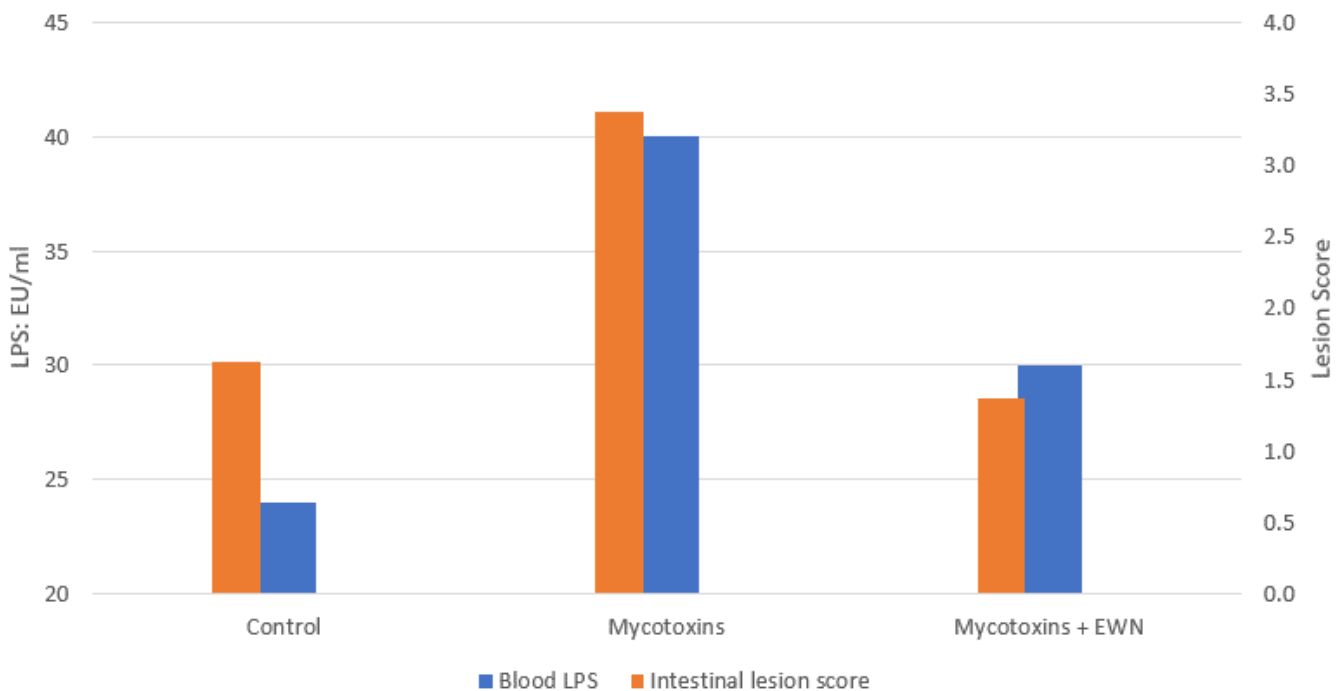


Figure 1 Blood LPS and intestinal lesion score of broilers challenged with 200ppb AFB1 + 350 ppb OTA from 1 to 21 days of age without and with an anti-toxin product from EW Nutrition GmbH (adapted from Caballero et al., 2021)

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, [including Salmonella and E.coli](#) also increases. The lesions associated with the pathogen compromise the epithelial permeability and the intestinal barrier function, resulting in [translocation of bacteria and LPS](#) (Figure 5) into the bloodstream and internal organs.

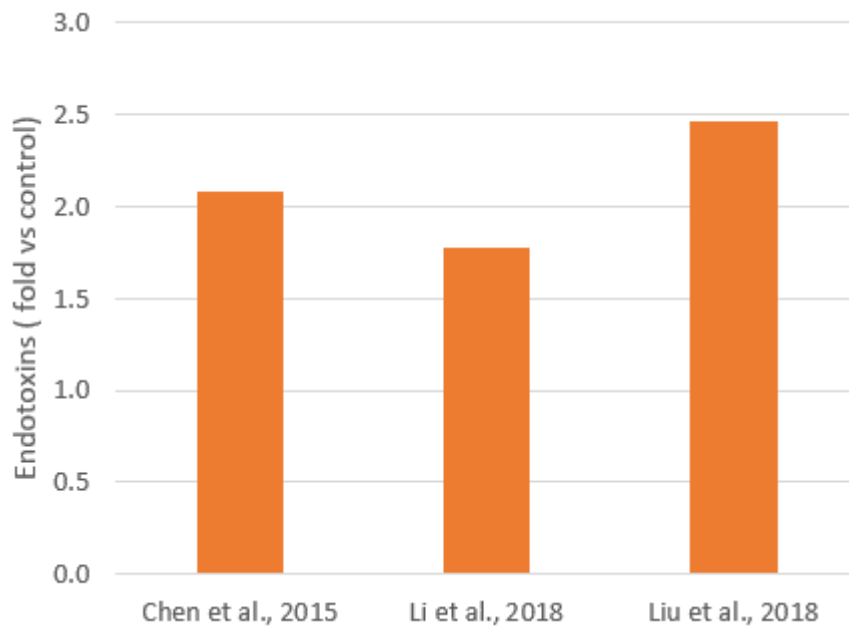


Figure 2 Increase in systemic LPS (vs a healthy control) after a NE challenge (adapted from Chen et al., 2015, Li et al., 2018 & Liu et al., 2018)

Environmental stress

Acute and chronic heat and cold stress increases gut permeability, by [increasing intestinal oxidative stress](#) and [disrupting the expression of tight junction proteins](#). 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.

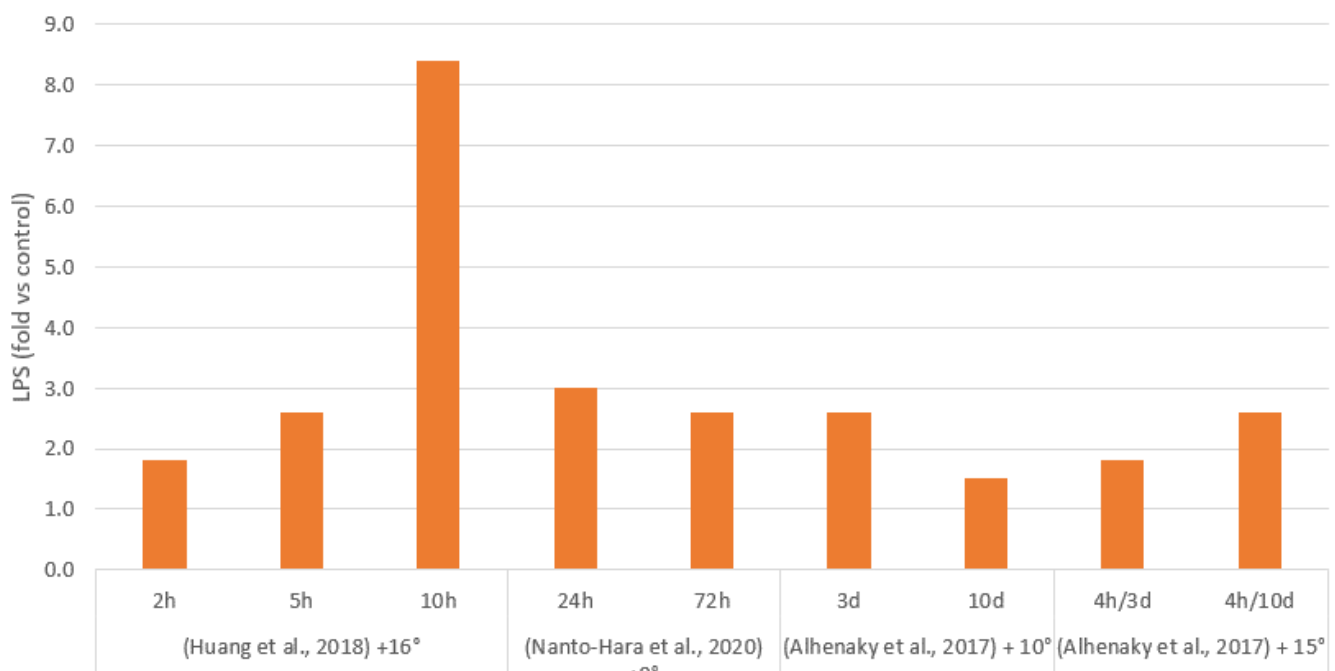
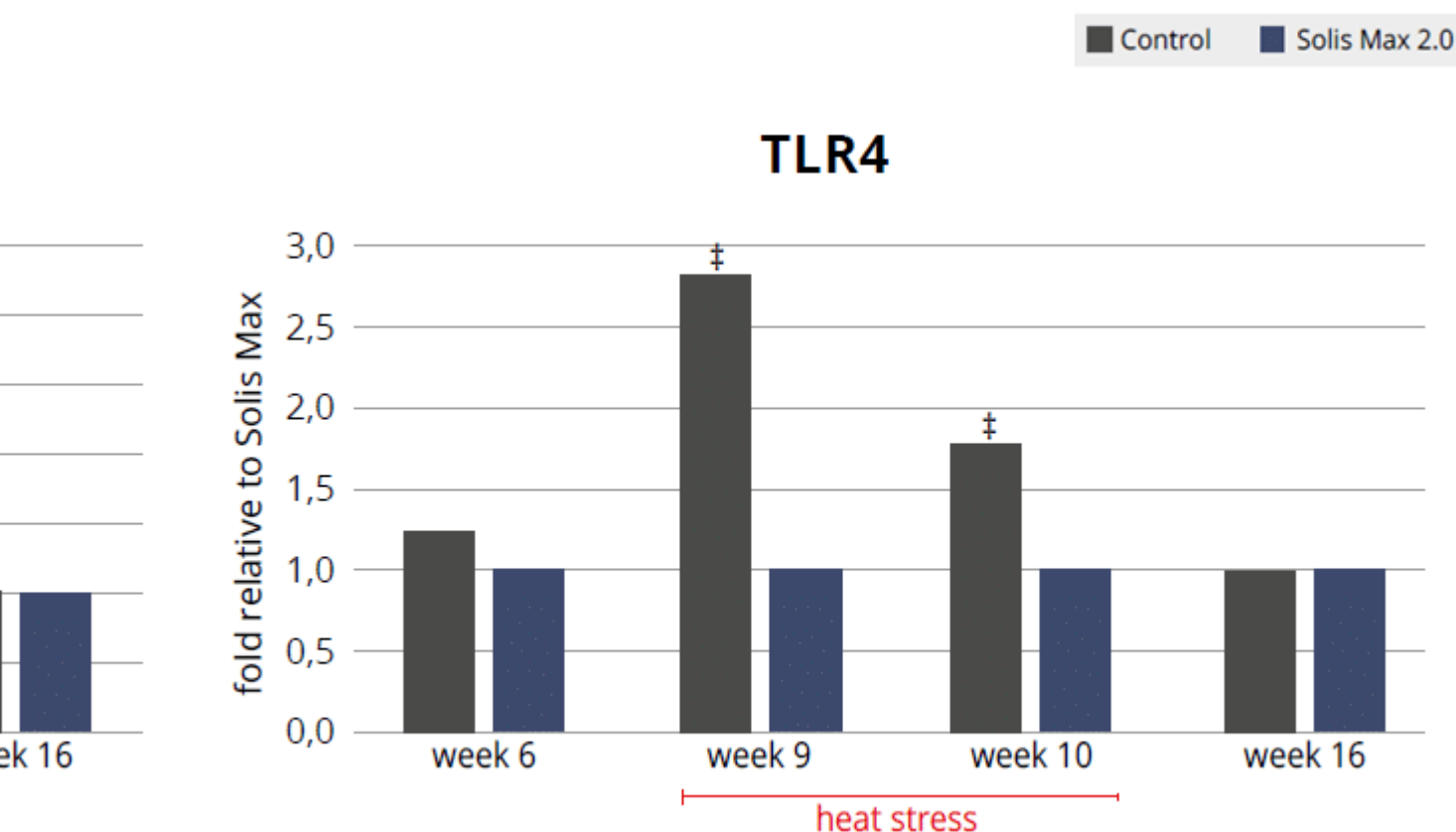


Figure 3 Systemic LPS increase (in comparison with a non-stressed control) after different heat stress challenges in broilers: 16°C increased for 2, 5 and 10 hours (Huang et al., 2018); 9°C increased for 24 and 72 hours (Nanto-Hara et al., 2020); 10°C continuously for 3 and 10 days, and 15°C 4 hours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

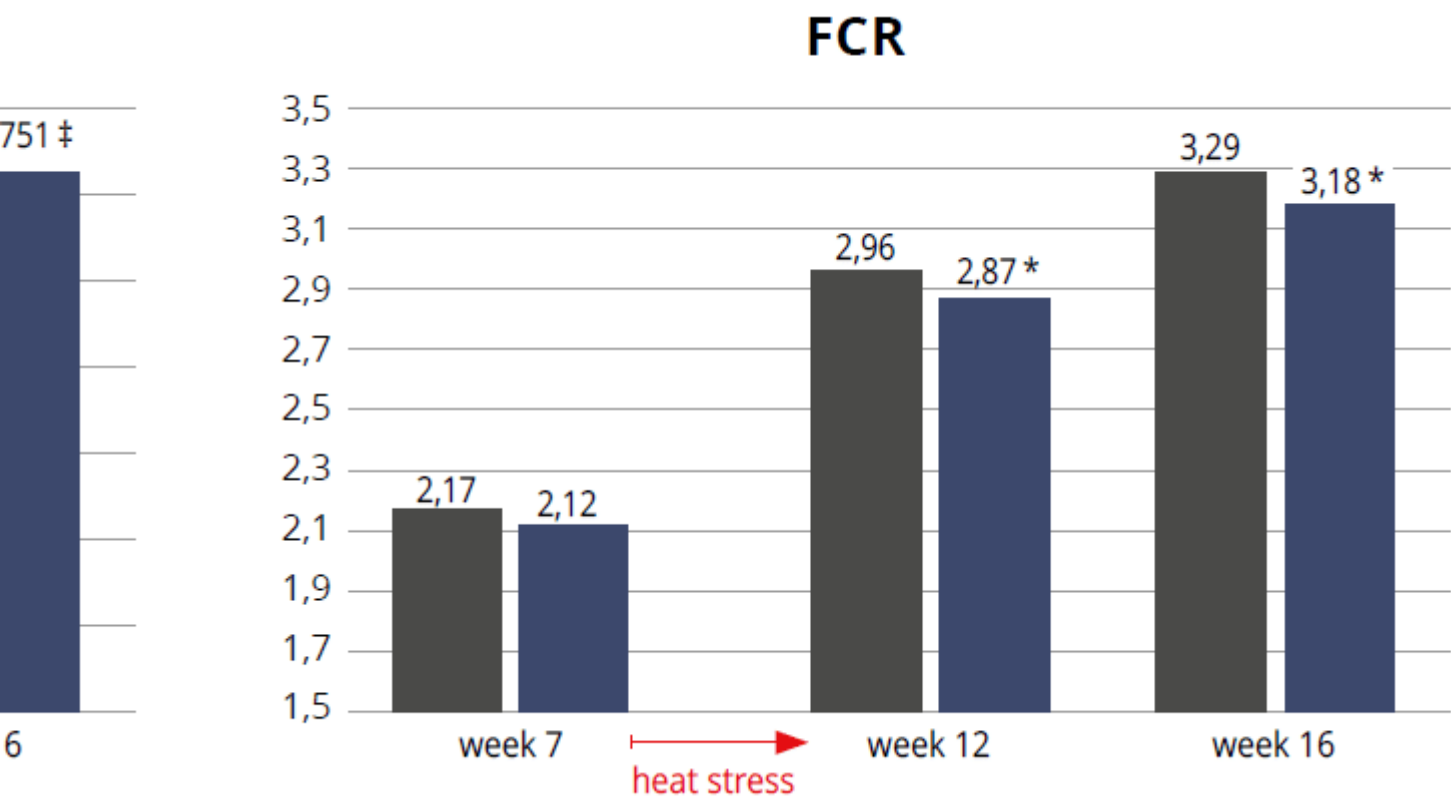
Throughout the heat stress period, blood LPS (Fig 4) was lower in the pullets receiving the product, which allowed lower inflammation, as evidenced by the lower expression of TLR4 (Fig. 5). Oxidative stress was also mitigated with the help of the combination of phytomolecules in the product, obtaining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

Broiler production with reduced antibiotics. The essentials



By **Dr. Inge Heinzl** and **Dr. Ajay Bhoyar** - EW Nutrition

Concerns about antibiotic resistance in humans and production animals have prompted a push across the board to reduce antibiotic use, including in livestock rearing. To meet these demands, the industry must keep the pathogenic pressure in production units as low as possible, enabling production with no antibiotics or minimum use of antibiotics.



The 3 essential steps for reducing

antibiotics in broiler production

In the following, we discuss experience-based insights and practical advice concerning best practices for broiler meat production with reduced antibiotic use, focusing on the following points:

- Farm biosecurity

- Management of the broiler house, including cleaning & disinfection, and environment & litter management

- Management of the flock, including DOC quality, disease prevention, and nutrition

1. General farm biosecurity

Biosecurity is the foundation for all disease prevention programs ([Dewulf et al., 2018](#)). Thus, it is essential in [antibiotic reduction](#) scenarios. It includes all measures taken to reduce the risk of introducing and spreading diseases, preventing diseases, and protecting against infectious agents. Its fundament is the knowledge of disease transmission processes.

The application of consistently high biosecurity standards substantially [reduces antimicrobial resistance](#) by preventing the introduction of resistance genes into the farm and lowering the need to use antimicrobials ([Davies & DWales, 2019](#)).

First of all: everyone must act in concert!

Biosecurity is one of the preconditions for the success of an ABR program, and it is crucial to bring all workers/staff on track through regular training on the best practices and their subsequent rigorous implementation. The biosecurity plan can only be effective if everyone on the operation follows it – all the time. Farm managers, poultry workers, and other persons entering the facility should adhere to the farm biosecurity measures, 24/24h – 7/7d.

Separation helps to prevent the spread of pathogens

One essential component for biosecurity is implementing a “line of separation” for the farm and each house. It is vital to have a good separation between high and low-risk animals and between areas on the farm that are dirty (general traffic) and clean (internal movements). In this way, it is not only possible to avoid the entrance but also the spread of disease, as potential sources of infection (e.g., wild birds) cannot reach the farm population.

The farm must be well isolated, not allowing the entry or passage of persons who do not work there and animals, including pets.

Inside the farm, the walls of the poultry house form the first line of separation, and the “Two-zone Danish Entry Protocol” constitutes a second line. This system utilizes a bench to divide the anteroom of a poultry house into two sides (outdoor / ‘dirty area’ and indoor / ‘clean area’). At a minimum, footwear should be changed, and hands washed or disinfected when passing over the bench; it is even better when workers have house-specific clothing and hairnets when entering the poultry area.

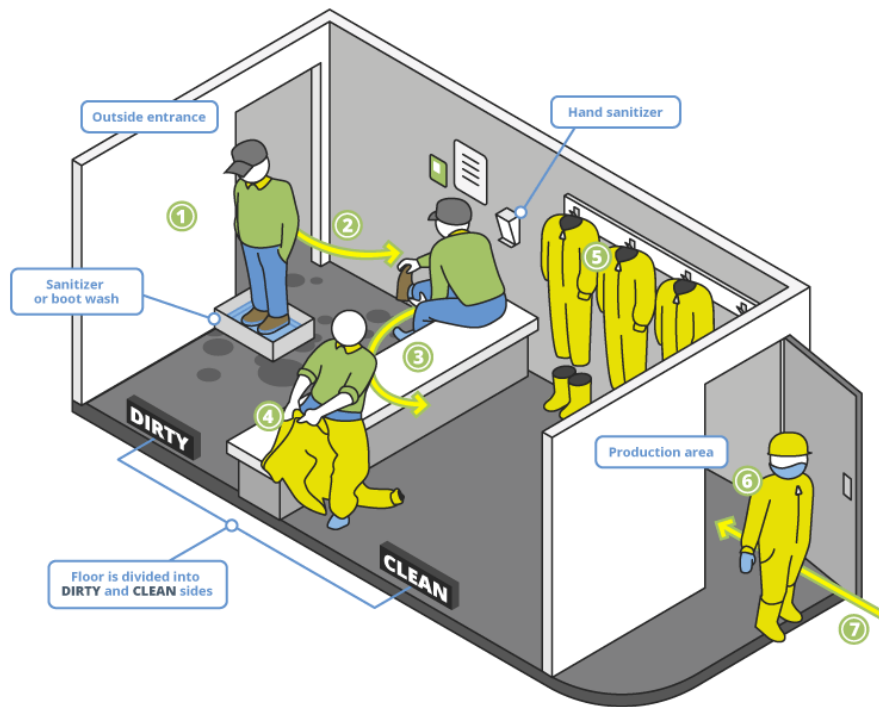


Figure 1: Safety procedures on the poultry farm – the Danish entry method

The room is divided into “dirty” and “clean” zones.

1. After the entrance from outside, workers/visitors step into a disinfectant boot tray.
2. They take off their street shoes and leave them on the dirty side of the entrance zone.
3. Then, they turn from the dirty to the clean side by swinging their legs without touching the floor.
4. They wash their hands and disinfect them by using the hand.
5. They must put on an overall, cap, mask, and boots of the poultry house.
6. Completely clothed, they can enter the poultry house.
7. When they leave the house, a reversed process must be followed.

Still more needs to be done to prevent the entrance and spread of disease.

Separate materials for each house

For each house, separate materials must be used, keeping a dedicated set of tools and equipment necessary for daily work.

Very important: no materials should be moved from one house to another unless thoroughly disinfected. Crates for bird transport in the case of thinning (partial depopulation of a broiler flock) are an important example.

Practice clean disposal of mortality

First, dead birds’ removal must be frequent (minimum twice a day) as carcasses are a source of infection. The next point is to make sure the route of birds’ disposal is strictly unidirectional, and the buckets or wheelbarrows for the transport of the dead birds do not reenter the poultry house. Finally, the carcasses should remain outside the farm or as far from the buildings as possible until collection, incineration, or composting.

2. Broiler house management

After the general organization on the farm, let's move on to the poultry houses.

Cleaning and disinfection of the house are the first steps - and check their efficacy!

Cleaning and disinfection are essential components in preventing the persistence and spread of pathogens. Both together aim to decrease microbial numbers on surfaces (and in the air) to a level that will ensure that most -if not all- pathogens and zoonotic agents are eliminated.

Cleaning refers to the physical removal of organic matter and biofilms, so the microorganisms and pathogens are afterward exposed to the disinfectant.

For effective cleaning and disinfection, the all-out/all-in system has proven of value. When birds are collected, all organic material, including feed residues and litter/feces, is removed.

Effective detergents and hot water are used to remove any grease or organic material. Pay special attention to the floors! Also, all surfaces and equipment should be sufficiently cleaned and given final disinfection.

Cleaning is crucial

A study by [Luyckx and collaborators \(2015\)](#) revealed that the mean total aerobic bacterial count on swab samples taken in broiler houses decreases significantly already after cleaning (figure 2). Good cleaning not only strongly reduces microbiological contamination and organic material but also ensures that the subsequent disinfection has a stronger impact on the remaining microorganisms. Consider that all disinfectants, even in high concentrations, are barely effective in the presence of organic material.

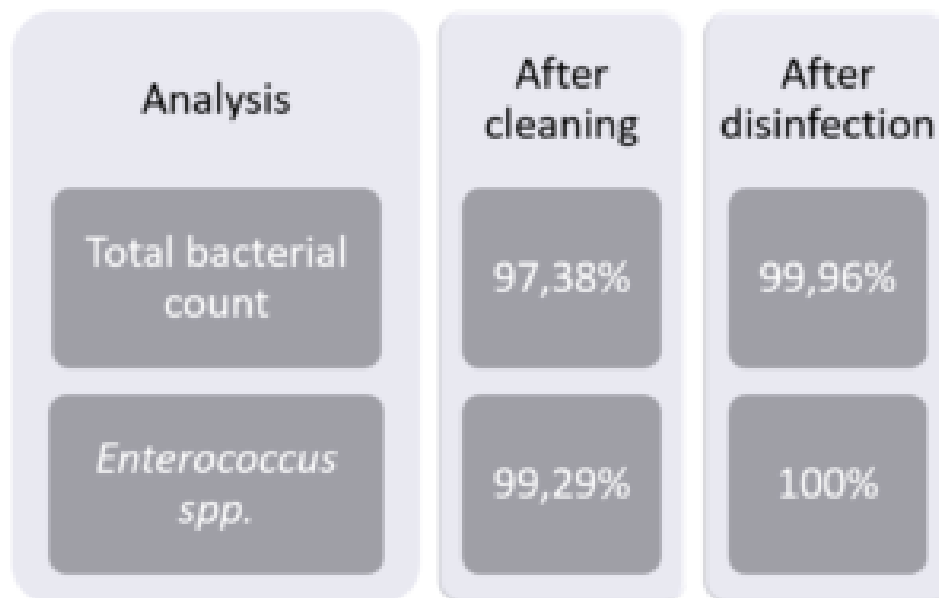


Figure 2: % of reduction of bacteria on surfaces after cleaning and after cleaning and disinfection (adapted from Luyckx et al., 2015)

Keep an eye on cleaning & disinfection efficacy

After cleaning and disinfection are complete, it is good practice to check the floors for Total Viable Count (TVC), Salmonella, and *E. coli* to test the efficacy of the cleaning and disinfection process. Recommended

levels of TVC should be less than ten colony forming units per square centimeter (CFU/cm²), and *E. coli* and *Salmonella* levels should be undetectable.

When high TVC are found, the cleaning and disinfection procedure must be evaluated, including the products (a rotation is recommended) and their application (e.g., dosage, dilution, water temperature, and exposure time). Also, possible reinfection by vermin or personnel during the downtime must be controlled.

Downtime:

After cleaning and disinfection, a down-time time of 10 days allows disease-causing pathogens to die ([UC Davis, 2019](#)).

Cleaning and disinfection of the waterline against biofilm

In the waterlines, the build-up of biofilms can be an issue. Biofilm is a sticky film that can be found inside water lines, regulators, and nipple drinkers. It starts when bacteria attach to a surface and produce a matrix of extracellular polymeric substances (EPS), including proteins and sugars, giving the biofilm the stickiness that traps other bacteria and organic matter. It provides the bacteria with protection from the external environment, and thus they multiply and thrive.

Biofilms not only block the water flow, but they can also include pathogenic bacteria. Thus, the waterline must be regularly cleaned and disinfected, not only between flocks but also within each flock.



Between flocks, an effective waterline cleaning should include:

- Application of hydrogen peroxide at high concentration, leaving it in the system for 24-48 hours to remove the biofilm from the pipelines)

- Flush the line to remove the detached biofilm, also activate the nipples with a broom or stick to flush them

- Immediately before the placement of the new chicks, the water lines should be flushed to have fresh drinking water available to the chicks

- The water pressure must be adjusted so that a droplet of water is visible at the end of each nipple, and the drinkers are put to the correct height to stimulate water intake and avoid spilling

During the life of the birds, a water disinfectant should be used to prevent biofilm formation, e.g., hydrogen peroxide in weekly applications or the continuous use of chlorine. Also, flushing is a good practice during the whole cycle to make sure that biofilm is removed and the birds count with fresh drinking water.

To a certain extent, biofilm build-up can be prevented by using organic acidifiers in the water, which improves the sanitizers' effectiveness and reduces bacterial growth in water lines.

Correct ventilation helps to prevent respiratory diseases

To keep broilers healthy, providing optimal ventilation in the poultry house is crucial. CO₂ and temperature are the most critical parameters. CO₂ should never exceed 2500 ppm and should be monitored continuously, most notably in the early morning before birds increase activity (e.g., eating). Ventilation rates should be adjusted to keep CO₂ below this limit. Draught or cold spots resulting in uneven distribution of birds in the house should be avoided, and causes should be investigated and repaired immediately.

Incorrect ventilation often is the reason for respiratory diseases and the need for antibiotic treatment. No matter if natural or power ventilation is used, proper monitoring of the system is indispensable to ensure the well-functioning of the equipment and, therefore, appropriate air quality ([Neetzon et al., 2017](#)).

Litter management to keep diseases in check

Effective litter management is another step on the road to keeping the birds healthy. Dryness of litter and ammonia level at bird's level are two significant key success factors in raising broilers. Dry litter preserves the footpads, so litter material should have a good moisture-absorbing capacity (e.g., chopped straw, wood shaving, rice husks, sunflower husks). When using build-up litter, litter sanitation and treatments need more attention.

Litter treatment (with acidifying or binding substances) and adequate ventilation are the most practical measures to control ammonia and improve litter quality ([Malone, 2005](#)). Keep litter temperature at 28 - 30°C (82.4 - 86°F), and use only litter tested or certified to have a TVC <10 CFU/g.

3. Flock management

The basis: healthy, high-quality day-old chicks

To produce good-quality day-old chicks, the parent flocks (PS) must be of good health status. PS should be free from vertically transmitted diseases such as Mycoplasma and Salmonella and be vaccinated/protected against important diseases:

Salmonella pullorum/*Salmonella Gallinari* should be assessed in PS by RPA serology in week 25-30, at least 60 samples per flock.

Mycoplasma gallisepticum should be checked by RPA/ELISA serology on a regular basis, preferably at least monthly, with a minimum of 30 samples per flock.

Parent flock vaccination leads to the production of maternal antibodies that help prevent horizontal infection (from the broiler farm environment) in chicks at an early age. This type of prevention is the primary function of some vaccinations, such as against Gumboro disease.

An essential part of the broilers' life occurs already in the hatchery. Single-stage incubation is recommended, and all floor eggs and dirty nest eggs should be excluded to assure the best day-old chick quality.

Comfortable conditions bring chicks to eat

The brooding phase needs special attention; it is about welcoming the chicks and making them comfortable in the house environment. For this, enough litter needs to be provided, the environment must be managed, and feed and water must be supplied.

At least 24 hours before chick placement, the house and floor temperature are increased to a minimum of 34°C and 28°C, respectively. Proper ventilation and lighting are also essential. These conditions need to be monitored and adjusted after the placement so the chicks feel comfortable and start feed and water consumption. Checking chick behavior is crucial during the first hours after placement.

Upon the placement of the chicks, it is recommended to have pre-starter crumble feed available on top of brooder paper underneath the drinking line. To stimulate early feed and water consumption, gently place the chicks onto that paper. The target is to have 100 % of chicks with crop filled within 48 hours of chick placement.

Reduce the stocking density

In general, high stocking density may restrict bird movement, interfere with airflow, and increase litter moisture and microbial growth, including pathogens, which potentially impairs broiler health, welfare, and performance.



When reducing antibiotics, increase the space per bird by 0.05 ft²/46 cm² per bird compared to your current conventional program. A lower stocking density helps keep litter moisture at a minimum, which reduces the shedding of cocci oocyst and pathogenic bacteria over the population.

Feed and water access must be granted to all animals at every moment. The number of chickens per feeder or drinker depends on the type of equipment used.

Consistent observation of the flock

To recognize emerging health issues, producers should critically observe the behavior of birds every day. On which points should they focus?

First, when entering the house, birds' behavior and response to the poultry worker should be observed with attention. Note the spread of birds throughout the house.
Note birds' drinking and eating behavior. Feed and water intake should be recorded daily, always at the same hour.
The quality of the fresh fecal droppings should be judged. Any changes in the fecal droppings (loss of consistency) can help notice emerging disease and take measures against it.

Especially during and after feed change, attention to changes in the usual feces consistency is necessary.

Vaccination and judicious antibiotic use are crucial

Carefully consider vaccination programs for broilers. Unnecessary vaccinations impact the immune system, possibly resulting in reduced performance and, in some circumstances, make the birds more susceptible to other diseases. Hence, the vaccination program must be diligently attuned ([Neetzon et al.](#)).

[2017](#)).



The disease background of the parent farm as well as the broiler farm where the chicks will be placed are essential factors for the vaccination program
If possible, vaccine strains that are the least immunosuppressive should be chosen
If coccidiostats are not permitted, an effective vaccination against coccidiosis is required and must be done as early as possible
All vaccinations must be given using a standard operating procedure that minimizes bird discomfort and optimizes the vaccine, and always administer vaccines following the advice from the manufacturer

After the vaccination, it is essential to monitor the effects of vaccination stress and take preventive measures to avoid any issues with broiler performance in terms of weight gain and mortality.

Use antibiotics with discernment

As we aim to reduce antibiotics, they should be limited to pure therapeutic use, only if other disease-prevention measures have not been successful, and mortality or disease symptoms make the treatment necessary. Before the treatment, the disease must be diagnosed by a qualified veterinarian. The diagnosis should be preferably followed up by isolation of the disease-causing bacteria, classification, and susceptibility testing before the antibiotics are applied.

Small-spectrum antibiotics that are less likely to cause antimicrobial resistance (AMR) should be preferred. Broad-spectrum antibiotics or antibiotics that are likely to cause AMR can only be used after susceptibility testing has demonstrated resistance to a first-choice antibiotic. The treatment effect must be evaluated by daily monitoring of disease symptoms, mortality, water, feed intake, and body weight gain.

Thinning - things to consider

If thinning (partial depopulation) is practiced, it should be done with the highest bio-security measures. Producers must ensure that the equipment used in the catching process is thoroughly cleaned before entering the house, and bird-catching personnel takes the same measures as farm personnel when entering the farm and the house. These policies will help to minimize the introduction of infectious agents.

Keep the feed withdrawal period for this process as short as possible to avoid flightiness, which can induce skin lesions (some regions catch birds in low light intensities to avoid flightiness). A short feed withdrawal period also prevents over-consumption of feed in a short amount of time, possibly disrupting feed passage in the gut and leading to bacterial imbalance and dysbacteriosis in the remaining birds. After thinning, feed and temperature must be adapted to the lower number of animals.

Provide your birds with high-quality water for drinking



Water is the most important nutrient for broilers. It plays an essential role in digestion and metabolism, thermoregulation, and waste elimination.

Several factors affect water quality: temperature, pH, bacteria, hardness, minerals, and total dissolved solids. These parameters should be analyzed at least twice per year. If necessary, corrective actions should be taken, e.g., a filtration to remove minerals, the addition of chlorine for disinfection, or the addition of organic acids to drop the pH.

Before each cycle, the water must be tested for total aerobic + enterobacteria, compared to reference values: Total plate count (TPC) should be < 1000 CFU/ml, and *E.coli*, Enterobacteriaceae, yeast, and molds at undetectable levels. The section about cleaning and disinfection of the waterline provides insights and practical advice about water sanitation and microbiological analysis.

Nutrition & feeding - a pillar for antibiotic reduction

Nutrition and feeding in ABR broiler production are not only about providing nutrients for growth but also about the effects of the feed on gut health. Gut health is essential for animals' overall health, welfare, and productivity, even more so in antibiotic reduction scenarios.

Feed should be of the highest quality - in all respects

High feed quality is necessary to provide the animal with the required nutrients and achieve their optimal utilization. Also important is the absence, limitation, or management of harmful substances and pathogens. High quality, therefore, includes:

- Form and composition of the final feed
- Nutritional value of the raw materials
- Management of harmful substances.

From reception and storage of the raw materials to the dispatch of the finished feed, the feed mill management emphasizes their quality assurance system, which is decisive in this connection.

First measure: quality assurance at the feed mill level

The feed mills producing for operations with no or reduced use of antibiotics must have a quality assurance (QA) and/or a good manufacturing program (GMP) in place that guarantees the production of consistently good quality feeds.

Proper raw material management and processing of feeds are necessary to achieve the lowest possible microbial-pathogen load, including:

An effective rodent and wild birds control
Disinfection of all the vehicles entering the feed mill
Proper storage and utilization of raw materials (e.g., first in-first out use, silo management)
Periodic thorough cleaning of milling equipment, premises and storage areas, and the monitoring of these activities
Standard operating procedure and quality assurance systems that guarantee [feed safety](#) and quality

Check the quality of the raw materials and the final feed

Digestion, absorption, and gut health depend on the quality of the feed ingredients. To provide the best preconditions for healthy growth, producers should avoid raw materials of a reduced and/or inconsistent quality. For this purpose, each raw material batch should be analyzed for its specific quality parameters. Quality parameters to consider are:

Physical ones, such as color, odor, particle size, and general appearance
Chemical ones, such as nutritional composition and specific parameters. For example, grains should be analyzed for mycotoxins and antinutritional factors; fats and oils need to be analyzed for free fatty acids (FFA), unsaturated/saturated (US) ratio, iodine value (IV), but also the peroxide value (PV) as oxidized fats have a lower energy value, and their intake is related to enteric diseases
Biological ones, including yeasts, molds, and enterobacteria

Also, the finished feed should be monitored by analyzing every batch concerning composition compared to values in the feed formulation, as well as physical, chemical, and microbiological quality parameters.

Clean storage on the farm prevents feed spoilage

As in the feed mill, keeping the farm facilities clean is of the highest importance. Warehouses, silos, bins, feeders, etc., should be emptied, cleaned, and disinfected after each flock; this avoids the formation of feed aggregates that can lead to mold growth and mycotoxin contamination; also, insects, bacteria, and parasites can remain in those residues.



Adapt feed formulation and feeding to the feeding phase

The value of phase feeding

Having the correct number of dietary phases to meet animal demands and avoid excess nutrients provides better intestinal health and thus aids production animals in ABR scenarios. The feeding phases should be designed to prevent abrupt changes in nutrition and raw material inclusions, possibly leading to dysbacteriosis.

Feeding for gut health

When feeding broilers in antibiotic reduction scenarios, extra care should be taken when formulating diets. The challenge is to achieve the same performance as conventional management at an optimum cost.

Don't waste nutrients: Improve feed digestibility, and at the same time, reduce the dangers of antinutritional factors coming from different ingredients by using suitable exogenous enzymes.

Keep an eye on fiber: Moderate levels of insoluble fibers with adequate structure and composition can be included to promote gizzard development and function. This measure leads to a better modulation of gut motility and feeds passage into the intestine. Additionally, it promotes gut health, resulting in higher nutrient digestibility.

Be careful with protein: Excess of undigested protein in the hindgut may lead to the proliferation of *Clostridium perfringens*; then, subclinical challenges of necrotic enteritis may occur. Moreover, the excess of nitrogen may increase feces moisture content, leading to wet litter. The optimization of the diets based on digestible amino-acid profiles and the use of synthetic amino acids decrease or eliminate the minimum requirements of crude protein, avoiding its excess.

Which feed form?

The feed form depends on the age or feeding phase: starter feeds can be offered as coarse mash, but preferably as crumble or mini-pellets (< 2 mm diameter) and grower and finisher diets as 3 - 4 mm pellets.

When using pelleted diets, quality is also the most crucial criterion. Poor pellet quality and thus the excess of fine particles increase feed passage rate, resulting in poor gizzard development and compromised gut health.

A high-quality pelleted feed can withstand - without much breakage - the handling that occurs after processing, such as transportation, storage, and farm management. Pellet quality can be measured by the Pellet Durability Index (PDI) obtained by simulating the impact and shear forces in a known quantity of feed for a determined amount of time. After this time, the sample is sieved, and the fines are separated, weighed, and compared with the initial sample

The PDI should be measured in the feed mill and compared to a standard. Later, it is also recommended to measure the PDI on the farm, and the producer should take corrective actions if the pellets cannot maintain their quality.

Additionally, it should be known that coarse ground grains stimulate gizzard development and function. So, about 30 % of the feed should consist of particles between 1-1.5mm (post pelleting) in all feeding phases.

Broilers' selection criteria for feed are form, color, size, and consistency



Broilers' selection criteria for feed are form, color, size, and consistency. They prefer feed that is easy to pick, such as crumbles or pellets.

Feed additives can support antibiotic reduction

The feed additive industry provides broiler farms and integrations with various solutions to make production more manageable and efficient.

A healthy start is half the battle

Let's start with the chicks. The early introduction of beneficial bacteria into the intestinal tract has proven helpful for gut health optimization. This colonization can be achieved with the administration of suitable probiotics preparation at the hatchery. Multi-strain probiotic preparations effectively initiate healthy microbiome development for optimum gut health. For these challenges, support is offered through EW Nutrition's [VENTAR D](#) and [ACTIVO LIQUID](#), phytomolecule-based products for the feed and the waterline, respectively.

Maintain gut health

Gut health is one of the essential preconditions for efficient growth. Only a healthy gut guarantees efficient digestion and absorption of nutrients. Several approaches are recommended to maintain gut health:

Promotion of beneficial and reduction of pathogenic gut flora: here, solutions can come in the shape of products based on phytomolecules that can be applied with the feed ([VENTAR D](#)) or the water ([ACTIVO LIQUID](#))

Management of bacterial toxins and mycotoxins: for this topic, products mitigating the toxins' negative impact on the birds (Product range of [MASTERSORB](#) and [SOLIS](#)) are offered

Protect your feed

When feed is stored, there is always the risk of bacteria, mold, or yeast overgrowth. Oxidation of feed ingredients, such as fats and oils, reduces their nutritional value. These issues can be prevented by applying:

- Acidifiers that have antimicrobial effects due to their pH-decreasing effect, which, later on, improves the feed digestibility and stabilizes the GIT flora ([ACIDOMIX](#), [FORMYCINE](#), and [PRO-STABIL](#))
- Antioxidants preserving ingredients susceptible to oxidation, such as fats and oils ([AGRADO](#), [SANTOQUIN](#), and [STABILON](#))

Improve pellet quality

Moisture retention during the conditioning process influences pellet quality: higher moisture retention entails a higher starch gelatinization resulting in higher digestibility, pellet binding, fewer fines, and a higher PDI. Surfactants (for example, [SURF•ACE](#)) are compounds that can reduce the surface tension between the water and the feed, improving moisture absorption during the conditioning process.

Besides that, moist steam in the pelleting process penetrates better and has a higher antimicrobial effect leading to lower production of bacterial and mycotoxins. The possible reduction of the pelleting temperature protects the nutrients.

ABR in broiler production is practicable - by observing some rules

As shown above, antibiotic-reduced broiler production needs many aspects to be considered and a lot of measures to be taken. All of these measures seek to keep animals healthy and avoid antibiotic use. Maintaining gut health is crucial, as only a healthy gut performs well, achieves the optimal utilization of nutrients, and increases growth performance.

Maintaining a successful production unit with no or reduced antibiotic use requires a holistic approach in which best practices must be assured at all levels of the production chain. The feed additive industry provides a broad range of solutions to support animal production through this challenging task. The objective could not be more critical: lowering antibiotic resistance to assure the future of animal and [human health](#).

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4 ways to cope with increased feed prices



by **Inge Heinzl**, Editor, **Marisabel Caballero**, Global Technical Manager Poultry, and **Ajay Bhoyar**, Global Technical Manager Poultry, EW Nutrition

In the last few months, the prices for feed grains and oilseeds such as soybeans have been climbing to multi-year heights. In part, this can be explained by high corn purchases by China and increasing export duties in Russia. The most significant cause, however, are weather events in producing countries: Just in the last year, droughts in the USA, Canada, and France raised the price of wheat by 40 %, the worst *La Niña* climate event in 91 years and the drought in China's biggest corn-growing area made corn about 100 % more expensive, and soybeans carry a 40 % higher price tag because of dry conditions in Argentina.



These events are a stark reminder that for global agriculture climate change impacts are already a reality.

High feed costs are an enormous challenge for the whole agricultural sector and sustainable strategies need to be adopted to enable a more efficient use of resources, both in the short and long term. This article explores possibilities to cope with the current situation. Through understanding the positions of farmers, integrators and feed millers and using targeted feed additive solutions, we can achieve a responsible use of resources that makes animal production more resilient to feed price increases.

Feed cost issues? Always start with this

The first question producers need to ask themselves is always if there is any step in the production process that could be done more effectively. Similar to [biosecurity programs](#), the basic steps seem self-evident, but to consistently implement them in the complex on-farm reality requires regular checks.

Feeding as “exactly” as possible

In case of high prices, the feed raw materials should be used as responsibly as possible:

Protein and energy content (but also other components, such as minerals and vitamins) must meet the requirements of the animals – age and production phase are decisive for the calculations.

Given variations in raw material quality, it is important to exactly determine nutrient contents to avoid over- and under-supply. For this purpose, technologies like the near-infrared spectroscopy (NIR) can be used.

Using locally available sources

In the initial stages of price hikes, it is often possible to resort to locally available sources, e.g., using sunflower or flaxseed meal to replace soybeans. Unfortunately, with increasing demand, these feed materials will usually become more expensive as well, and might not be suitable alternatives anymore. In general, however, it is worth using a maximum of local ingredients: they are often cheaper and less susceptible to transport and trade difficulties.

Feed additive solutions: use what is available in the best possible way

Once these first measures are exhausted, it is time to draw on industry solutions to derive maximum value out of the available feed ingredients. Let us consider four approaches that improve feed conversion and feed quality, adjust feed composition, and optimize feed production processes.

1. A critical goal: improving the feed conversion rate

The most direct way to better utilize feed is to improve the animals' feed conversion rate, with the help of the right supplements. Different product groups contribute to this aim in different ways.

1.1 Phytomolecules fight on different fronts

Phytomolecules are well-known for their antimicrobial effects against pathogenic bacteria ([Zhai et al., 2018](#)). Phytomolecules shift the balance of the microbiome towards the beneficial side (eubiosis instead of dysbiosis) and promote gut health. [A healthy gut](#) is able to digest the feed and absorb the nutrients in an efficient way.

Another value of phytomolecules is their digestive effect. They stimulate the secretion of saliva, gastric juice and digestive enzymes, and favor an adequate gastrointestinal motility, which leads to improved nutrient utilization (Jones, 2001; [Mendel et al., 2017](#)).

In trials testing the phytogenic [Activo product range](#), supplemented animals showed the following FCR improvements compared to non-supplemented control groups (Figure 1):

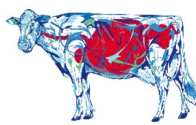
 Layers (feed / egg mass) 11 points	 Broilers 3 to 8 points
 Finishing pigs 17 points	 Dairy cows (milk / DMI) 10 points

Figure 1: FCR improvements for animals receiving Activo

1. 2 Enzymes improve nutrient availability

Even a corn-soybean meal diet is not fully digestible for monogastric animals. However, when feed prices increases, producers likely need to include more alternative ingredients in the diet that are much less digestible. Typically, these ingredients are rich in antinutritional factors such as non-starch polysaccharides (NSPs), which can cause detrimental effects on gut health.

Another disadvantage of NSPs is their “cage effect”. The water-insoluble NSPs cellulose and hemicellulose trap nutrients such as proteins and digestible carbohydrates. Consequently, digestive enzymes cannot reach them and they are not available to the organism.

Here is the point of attack for enzymes that enable a complete nutrient utilization: Making these substances available for the animals increases the energy content of the diet and, in the end, improves FCR. An example for laying hens receiving wheat-based diets can be found in Figure 2: [Axxess XY](#), a xylanase, significantly improved feed utilization by the hens.

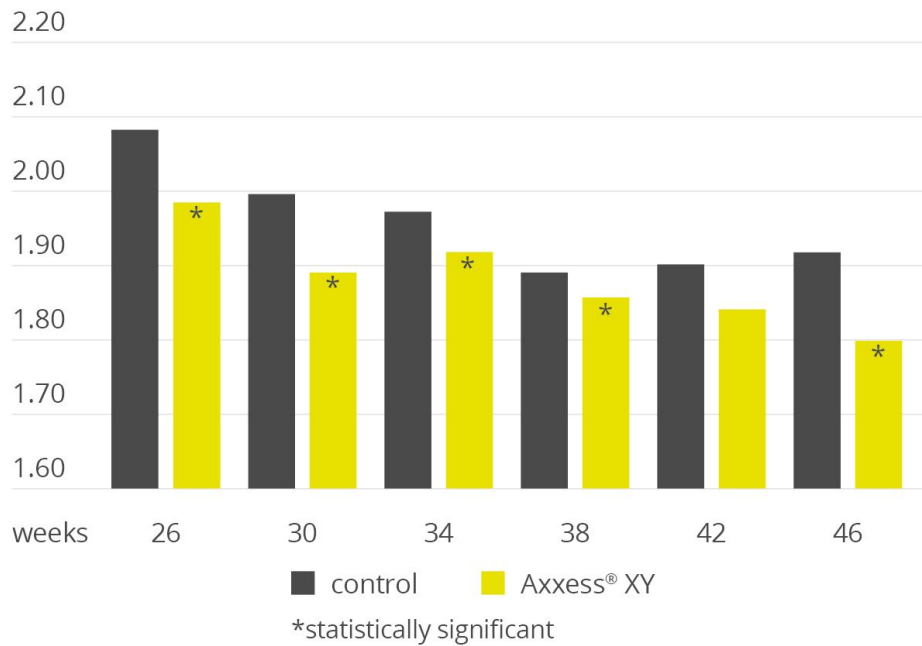


Figure 2: FCR in layers receiving Axxess XY, compared to control group (kg feed / kg egg mass)

1.3 Antioxidants maintain energy content of the diet

Corn Distiller's Dried Grains with Solubles (DDGS), a by-product of corn distillation processes, are used as an alternative to corn. In DDGS, the starch content is removed, but fat is concentrated, reaching about three times the fat level of corn. This is the reason why the energy content in DDGS and corn is similar. This makes DDGS an attractive ingredient for monogastric diets; however, fat, especially at hot temperatures in the summer, can be oxidized. The resulting rancidity and the accompanying destruction of vitamins, pigments, and amino acid leads to a decrease in the diet's bioavailability and energy content and to poor feed conversion.

The use of antioxidants can stabilize DDGS and other fatty ingredients in the feed, maintaining nutrient integrity and availability. Figure 3 shows the performance benefits of using antioxidant product [Santoquin](#) in pork finisher diets in the USA containing 30% of DDGS.

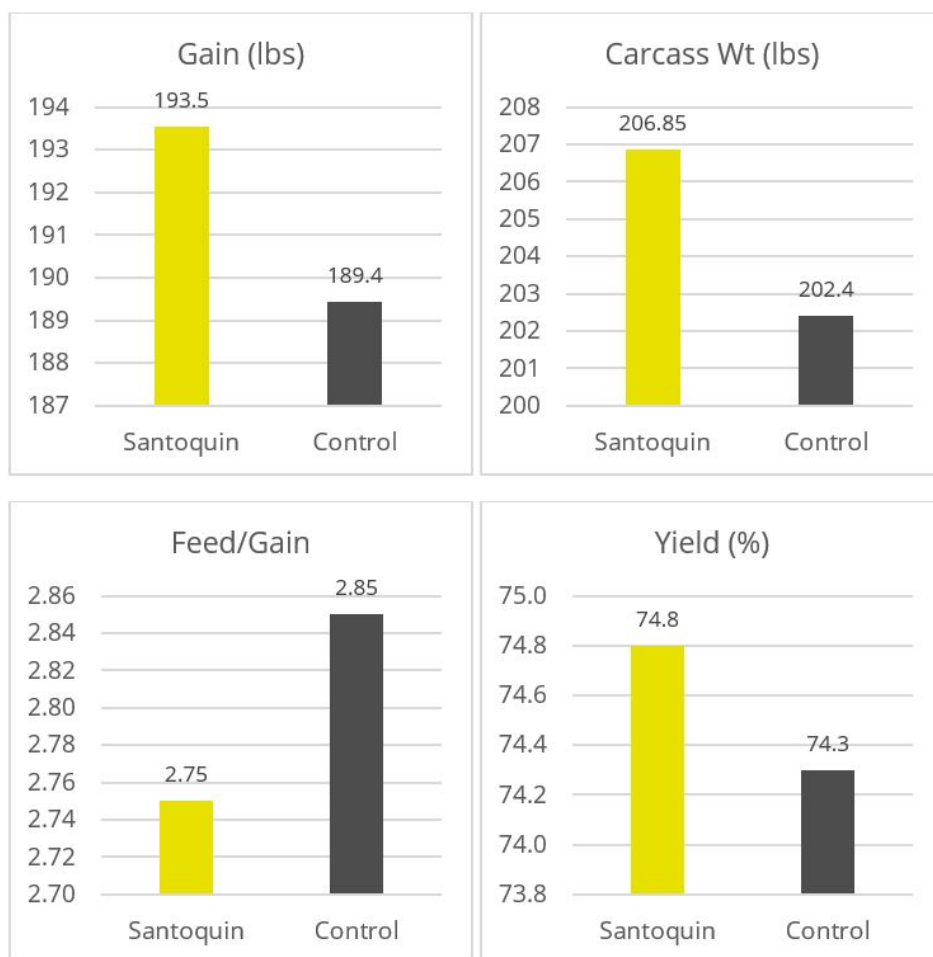


Figure 3: Performance results for pigs receiving Santoquin (trial with Midwest pork producer)

In poultry production, the use of DDGS is not as common as in swine. Antioxidants, however, can still help to protect the nutrients, maintain the energy content and improving FCR. The results from an extensive 2015 field study for broilers fed a diet without DDGS (shown in Figure 4) showed a net ROI of 6.7 to 1.

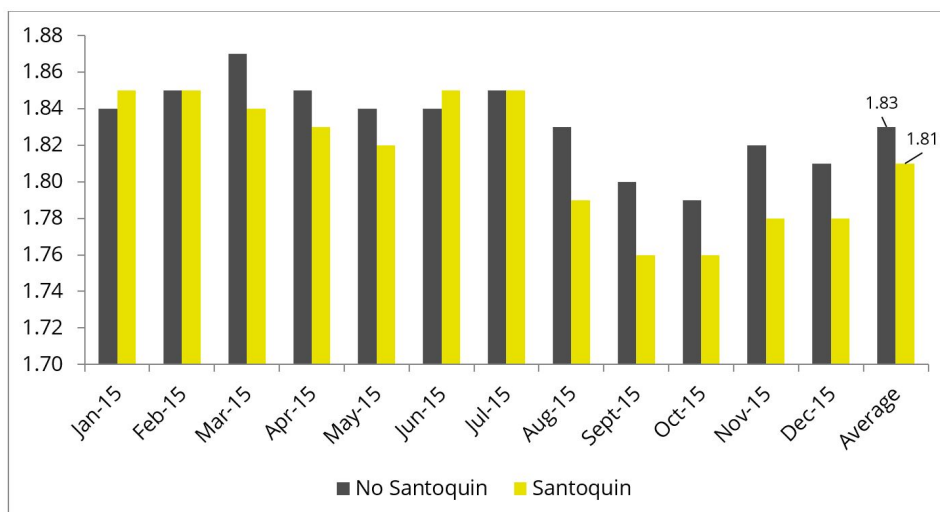


Figure 4: FCR in broilers receiving Santoquin, compared to non-supplemented control group

1.4 Organic acids improve intestinal processes

Organic acids, or acidifiers, can improve the gut microbiome, feed utilization, and gut health in production animals. The gut microbiome balance is aided by lowering the population of pathogenic bacteria susceptible to low pH, such as *E. coli*, *Salmonella*, and *Clostridium*.

Organic acids also directly attack pathogens by entering bacterial cells and changing the internal pH. Commensal bacteria such as *Lactobacilli* and *Bifidobacteria* survive as they can tolerate lower pH conditions. As pathogens constitute nutrient competitors, eliminating them improves gut health, which, is the most important precondition for optimal nutrient utilization.

The acidifying effect of organic acids furthermore favors digestion and nutrient utilization: for example, for weaned piglets that not able to produce enough HCl in the stomach, a low stomach pH is important for the activation of the proteolytic enzyme pepsin. Besides a non-optimal use of nutrients, undigested protein arriving in the intestine leads to the proliferation of undesired pathogens, decreasing health and performance.

[Organic acids](#), therefore, improve FCR directly, by promoting nutrient utilization through the stimulation of enzymes, and indirectly, by enhancing gut health.

2. Improving feed quality

Feed quality is not only a question of raw material quality. Feed additives play an important role in ensuring [feed safety](#) and enabling optimal utilization by the animal.

2.1 Mold inhibitors preserve the feed's value

Molds reduce the nutrient and energy content of the feed (table 1) and have a negative impact on animals' growth performance (table 2). Active water is the crucial point for mold growth. Compared to bacteria, which need about 0.90 – 0.97 Aw (active water), most molds require only 0.86 Aw.

Mold inhibitors contain different ingredients. Surfactants bind the free water, so that the moisture of the feed persists, but the active water important for molds is reduced. Organic acids, as already mentioned before, have antifungal properties. Together, they reduce molds and prevent the degradation of energy in the feed.

Table 1: Nutrient loss in corn infested with molds

	Crude protein %	Metabolizable energy Kcal/kg	Fat %
Non-infested corn	8.34 ^a	8.9 ^a	4.0 ^a
Molded corn	7.32 ^b	8.3 ^a	1.5 ^b
Loss in nutrients	1.02	0.6	2.5
% loss in nutrients	12.2	6.7	62.5

Table 2: Comparison of 28-day-old chicks performance fed not-infested and molded corn

	Weight gain (g)	FCR
Non-infested corn	767 ^a	1.79 ^a
Molded corn	713 ^b	1.96 ^b

2.2 Mitigating the negative impact of mycotoxins

Mycotoxins contamination of grains can occur in the field, during raw material harvesting, transportation,

storage, handling, and even during feed processing and storage. By mitigating the negative effects of mycotoxins – such as gut and liver inflammation, kidney degeneration or reproductive disorders – the animals’ health and performance can be maintained. In today’s contamination scenarios, it is absolutely necessary to use products that adsorb mycotoxins and contain their harmful impact on animals.

The effectivity of such products in animals is crucial. Table 3 shows an optimal experimental design and Figure 5 shows the results of its application: a total recovery of the performance pays off.

Table 3: Trial design, impact of Mastersorb Gold on broilers challenged with zearalenone and DON-contaminated feed

	Control	Mastersorb Gold	Challenge	Challenge + Mastersorb Gold
Challenge	---	---	300ppb zearalenone and 6000ppb DON	300ppb zearalenone and 6000ppb DON
Additive	---	MSG (2 kg / MT of feed)	---	MSG (1 kg / MT of feed)

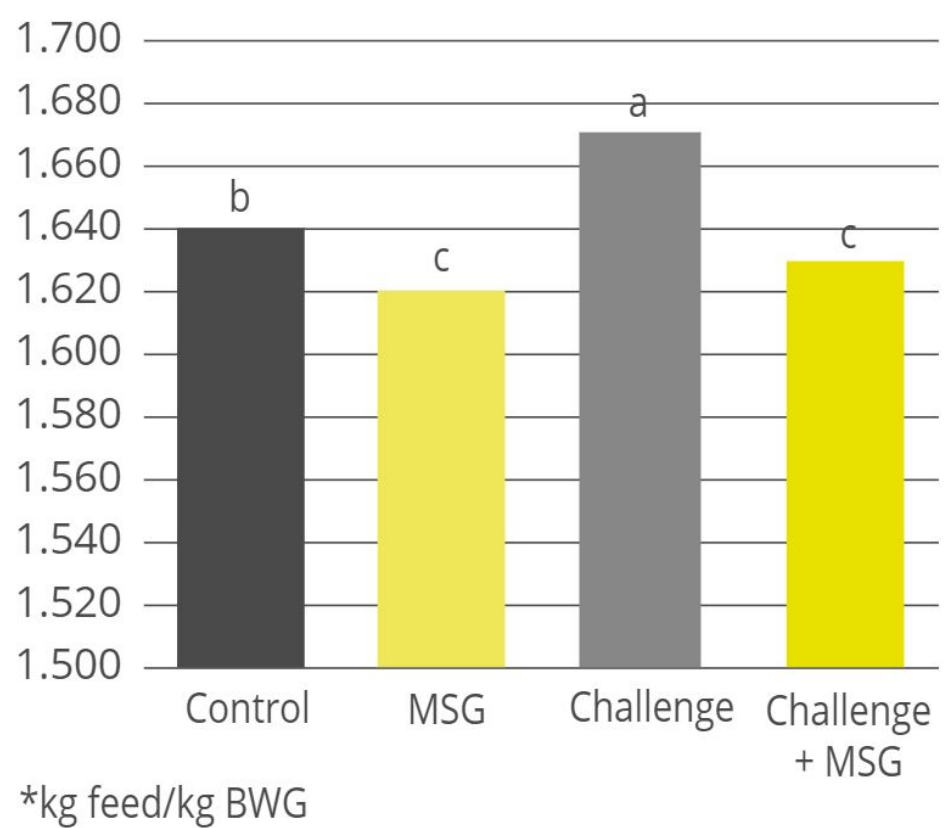


Figure 5: Average FCR for broilers, with or without zearalenone and DON challenge, with or without Mastersorb Gold supplementation

2.3 Surfactants for microbiological control and high pellet quality in the feed mill

Moisture is important. Too dry feed results in poor palatability and digestibility, and lower pellet quality. Also moisture loss has a direct impact on production and profitability.

The use of surfactants, makes it possible to bind the moisture to the feed, reaching a larger contact surface between water and feed particles, and improving starch gelatinization and pelleting efficiency. The improvement in starch gelatinization leads to a higher pellet quality, a lower proportion of fines and a higher content of metabolizable energy.

Moreover, moist steam has a better antimicrobial effect than dry steam, leading to lower fungal and

bacterial growth and preventing the production of toxins. The pelleting temperature can also be lower, protecting the nutrients.

Figure 6 shows how the use of [SURF•ACE](#), a synergistic blend of organic acids and surfactants, improves pellet durability, moisture content, and mold occurrence for beef and poultry pellet feed.

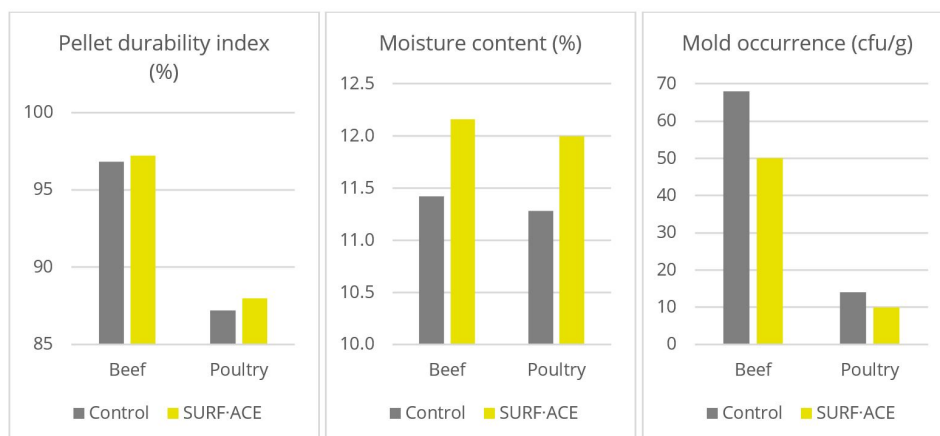


Figure 6: Improvements in pellet durability, moisture content and mold through using SURF•ACE

3. Using feed alternatives in ruminants – partial replacement of protein feed by urea

Ruminal bacteria are able to synthesize amino acids and, subsequently, generate a high-quality protein out of acid amides, a group of non-proteins occurring during the synthesis and degradation of proteins. What they require to do this is enough energy, minerals, and trace elements available in the feed (Weiß et al., 2011). When the bacteria arrive in the abomasum and in the small intestine they, or rather their proteins, are degraded by enzymes together with the undegradable rumen protein into useful amino acids.

With the aid of ruminal microbes, ruminants therefore partly cover their protein requirement through non-protein nitrogen. The most well-known is urea. It is critical that the urea given to animals has a degradation rate similar to other energy sources the animal consumes. Otherwise, there will be an imbalance between the quantity of usable nitrogen and the energy required for microbial protein synthesis: The urea accumulates in the rumen, becoming toxic for the microbiota and creating metabolic disorders.

[Special coating technology](#) allows for nitrogen to be released at a rate close to that of protein degradation of the main vegetable protein sources (e.g., soybean meal). This leads to a more constant nitrogen supply for the microorganisms and results in maximal synthesis of microbial protein.

4. Save costs in the production process

Besides high pellet quality, feed millers seek to maximize production efficiency. Factors contributing to this target are the amount of fines to be reprocessed, the utilization of steam, the pellet throughput and the energy demand. Once more, the moisture of the feed is of decisive importance. Substances can be added to the feed to achieve an optimal moisture content. These substances bind free water by generating an emulsion of dietary fat and the added water.

Besides the positive effects on pellet durability, moisture content and mold growth shown above, this leads to a better general lubrication of the machinery: The addition of feed mill processing aid SURF•ACE leads to a 10-15 % lower energy demand or a higher production output without increasing energy consumption

(Figure 7), depending on the mill's requirements. Good machinery lubrication additionally reduces wear and tear, another important dimension of production efficiency

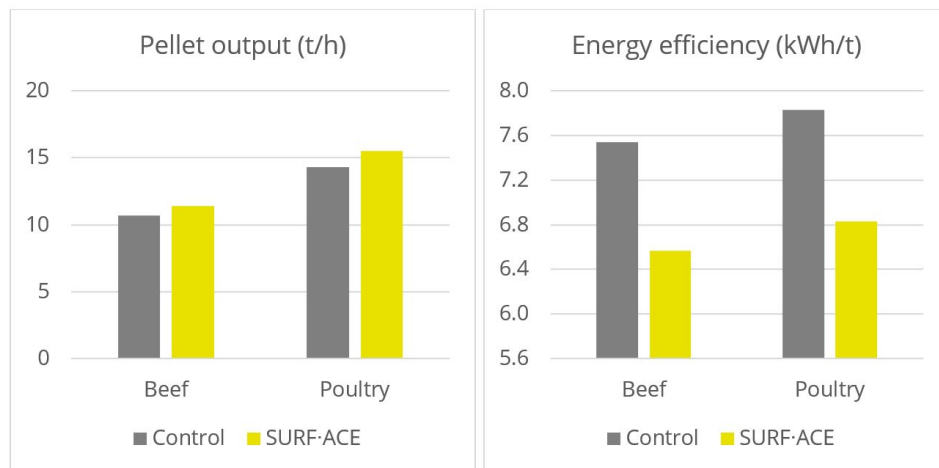


Figure 7: Improvements in pellet output and energy efficiency through using SURF•ACE

Producers can rise to the challenge of rising feed prices

Rising feed costs pose a significant challenge to everyone in animal production. We are all compelled to look for alternatives to optimize the utilization of resources. This firstly involves a critical look at the efficiency of every step in our operations, but also includes utilizing targeted feed additives. Various measures are available for animal producers to optimize feed conversion, improve feed quality, and resort to alternative ingredients. In feed production, tools are on hand to optimize the manufacturing processes, improve feed quality, and make a positive impact on animal performance. Feed price fluctuations will continue to challenge our industry. Still, while tackling short- and medium-term difficulties, we can also strategically build resilience – and take the measures today that will contribute to our long-term ambitions for sustainable and profitable production.

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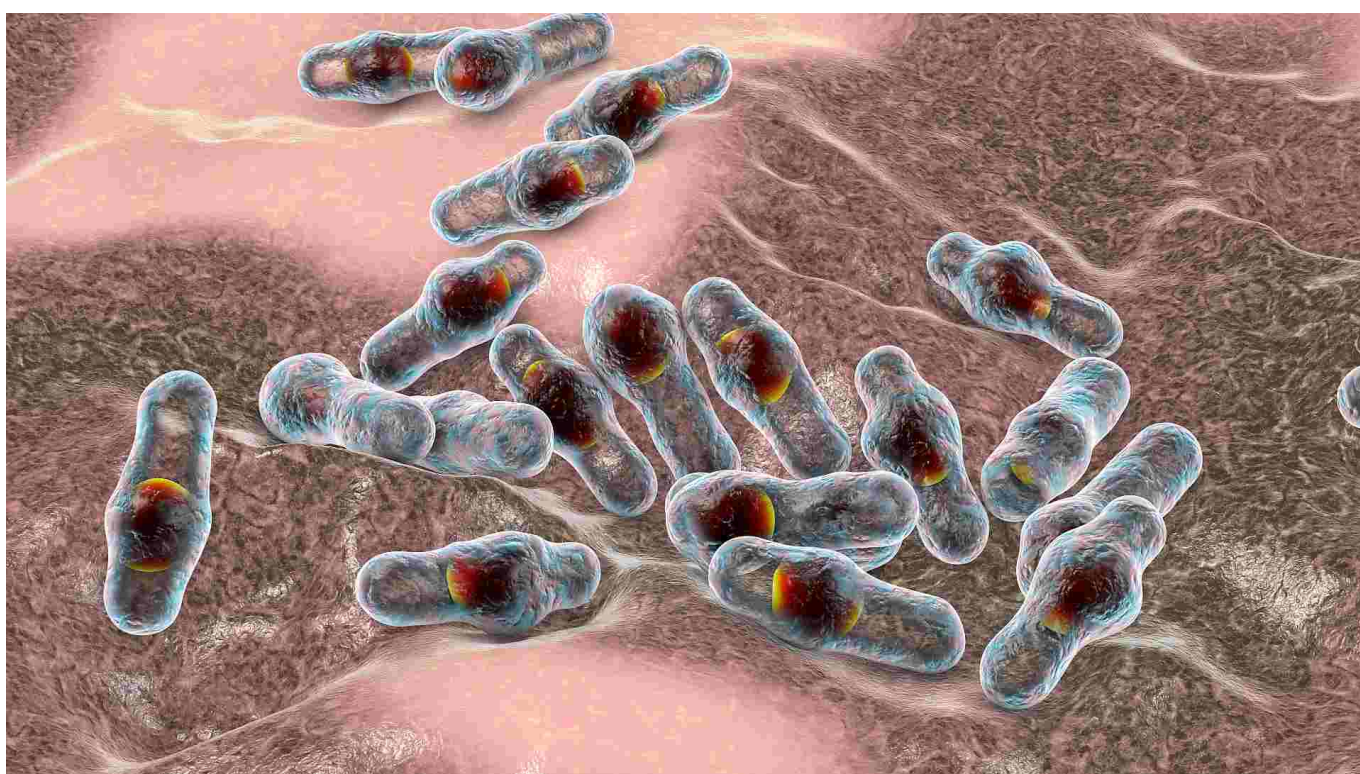
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Mitigating Necrotic Enteritis through Natural Alternatives in Antibiotic-Free Production Systems



by EW Nutrition USA, Inc.

In the poultry industry, Necrotic Enteritis is of great interest due to the potential detrimental growth effects it may have in a flock, even at subclinical levels⁵⁰. Coccidiostats and antibiotics have been used for a long time to get the disease-causing bacterium *Clostridium perfringens* under control, but with increasing antimicrobial resistance, alternative approaches are required. This article aims to give an overview of the disease and the measures against it.



***Clostridium perfringens* - a ubiquitous, highly resilient bacterium**

Clostridium perfringens is a Gram-positive, spore-forming, anaerobic, rod-shaped bacterium⁵⁰. This encapsulated, non-motile microorganism is fastidious in growth requirements⁵⁹. Most often, complex media like cooked meat or thioglycolate broth are used as enrichment³⁰.

It was Welch and Nuttall who first identified *C. perfringens* in 1892 as *Bacillus aerogenes capsulatus*¹⁸. In Great Britain, the bacterium was commonly known as *C. welchii* and sometimes called Frankel's bacillus in Germany until designated *C. perfringens* by Bergey¹³.

Clostridium perfringens is the causal microorganism for Necrotic Enteritis (NE)¹⁴. In humans, it is one of the most common causes of foodborne illness²⁰. The Centers for Disease Control and Prevention (CDC, 2012) estimates that nearly one million people are affected every year, making *C. perfringens* the third most frequent source of domestically acquired foodborne illness after Norovirus and *Salmonella*.

***Clostridium perfringens* can be found everywhere**

Clostridium perfringens is found in soil, water, and other organic materials. As far as poultry facilities, *C. perfringens* has been isolated from litter, dust, walls, floors, fans, transportation coops, feeders, and feed⁸⁹.

Additionally, *C. perfringens* is found in the GI tract of broiler chickens, humans, and other mammals⁴⁷. When intestinal samples of broiler chickens were analyzed for *C. perfringens*, 75-95 % tested positive²⁴. Drew and co-workers¹⁰ determined that *C. perfringens* is usually found at $\sim 10^4$ colony-forming units (CFU)/g of broiler digesta. These results agree with Jia et al.²⁶, who stated that *C. perfringens* is present at low levels in healthy poultry. In humans, investigations in different parts of the world showed a prevalence of *Clostridium perfringens* between 57-94%³².

Different types of *Clostridium perfringens* with different toxins

There are five types (A-E) of *C. perfringens*, which can be identified through their toxin production (see table 1). All strains produce alpha-toxin. Furthermore, *Clostridium perfringens* has been described to produce eight other toxins, three (delta, theta, kappa) can be lethal, but these are seldom involved in disease origin³⁷.

Table 1. Different types of *Clostridium perfringens*

		<i>C. perfringens</i> Type				
		A	B	C	D	E
Toxins	Alpha	x	x	x	x	x
	Beta		x	x		
	Epsilon		x		x	
	Iota					x
	Enterotoxin	x				
	Diseases/animals ¹⁸	Food-born disease/humans NE/fowl	Dysentery/lambs enterotoxaemia/ sheep, goats, guinea pigs	Food-born disease/humans NE/fowl	Enterotoxaemia/ sheep Pulpy kidney disease/lambs	Enterotoxaemia/ calves Dysentery/sheep, guinea pigs, rabbits

High resilience gives an advantage against competitors

Since *Clostridium perfringens* is a spore-forming bacterium, it is very resilient to high temperatures, slight pH variations, and toxic chemicals^{43, 7}.

Labbe et al.³⁰ established that *C. perfringens* can reproduce at temperatures between 15-50 °C. Hence, proper refrigeration temperatures (below 10 °C) can be an effective means of control. The optimum range is between 37-47 °C, and at these temperatures, the mean generation time – the time required for the bacterial count to double – is approximately 10-12 minutes⁴¹. These short generation times allow the bacteria to outcompete other microorganisms that may need similar resources in a certain environment.

The optimum pH range of *Clostridium perfringens* is between 5.5-7.0²². However, it can grow at a pH as low as 5 and as high as 9. In live broiler chickens, the pH in the small intestine has been determined to be between 6.00-7.78.

Necrotic enteritis in poultry

The disease necrotic enteritis was first described by Parish^{45, 46} in cockerels in England. Some of the symptoms include depression, reluctance to move, ruffled feathers, somnolence, diarrhea, loss of appetite, and anorexia²¹. Mortality ranges from 0-50% ⁶ have been reported in infected flocks. Since then, virtually every area that raises poultry has reported signs of necrotic enteritis.

Clostridium perfringens - How NE unravels

As already mentioned, 10^4 colony-forming units (CFU)/g of broiler digesta¹⁰ are normal and can be found in healthy birds. *C. perfringens* becomes problematic when counts reach 10^7 - 10^8 CFU/g⁶.

Necrotic enteritis is caused by types A and C of *Clostridium perfringens*, but normally, predisposing factors “set the stage”^{24, 48}. This could be seen in an investigation where they wanted to create a model to reproduce NE in a laboratory setting. Researchers realized that inoculation of *C. perfringens* alone did not cause the disease found in the field⁴⁸. Therefore, it was assessed that certain cofactors must play a significant role in the pathogenicity of *C. perfringens*. Williams⁵⁷ reviewed concurrent infections of coccidiosis and necrotic enteritis in chickens (Figure 1). The copious interactions of these diseases with predisposing factors, control methods, sources of infection, and disease form is a testament to the complexity of this poultry industry matter.

Coccidiosis creates access

Shane *et al.*⁵³ noted that several authors had considered coccidiosis to be a predisposing factor for NE. They proceeded to describe the pathogenesis of *Eimeria acervulina*, one of the protozoa responsible for coccidiosis in poultry. When the oocysts are ingested, they quickly attach to the intestinal wall causing lesions where the protozoa reproduce numerous times. These are the lesions to which *C. perfringens* attaches.

What happens in the animal?

Long *et al.*³³ proposed the pathogenesis for NE: First, epithelial cells are vacuolated, and the epithelium lifts off the lamina propria, which is congested and edematous. These lesions can be caused by a combination of factors like toxin production and/or, as just mentioned, coccidiosis. *Clostridium perfringens* cells attach to the lamina propria, where they thrive. The tissue becomes necrotic as large numbers of heterophils, a type of phagocyte, flood the foci (sites of lesions).

A combination of disease-inducing factors such as bacteria proliferation, heterophil lysis, and villus' necrosis seem to develop quickly. The inflammation zone then becomes riddled with mononuclear cells, cells containing lymphocytes, antigen-presenting cells, and eosinophilic-staining (proteinaceous) amorphous material. This necrotizing process moves from the tip of the villi to the crypt.

Chronic version

In chronic cases, villi may be found to have multiple cysts from recurrent necrosis. In birds that overcome the disease, injured epithelial cells are replaced by newly formed reticular structures. These new cells

travel from the crypt to the tip of the villi and replace the old, damaged cells. The result is a short, flat villus with a reduced surface area for nutrient absorption^{44, 45, 34}. These morphologically altered villi are the necrotic lesions found in the field and some *C. perfringens* challenge trials (Figure 2).

Acute form

The acute form of NE results in enlarged lesions along the gut wall, and the epithelium becomes eroded and detached; consequently, a diphtheritic membrane is formed. This yellow, green, or brownish pseudo-membrane is called the “Turkish towel,” which describes the appearance of the friable, gas-filled, foul-smelling GI tract⁵⁷.

Subclinical form

Poultry producers are not only concerned with the acute form of NE. Recent studies have shown that the disease’s subclinical form can be as detrimental as the acute illness¹⁹. Lovland and co-workers³⁵ stated that this symptomless disease is often overlooked at the farm, and the effects are only noticed at the processing facility.

Subclinical NE (SNE) can cause cholangiohepatitis, a condition where the liver is enlarged with pale reticular patterns and sometimes small, pale foci. In the United Kingdom, it was estimated that 4% of broiler carcasses and 12% of livers are condemned at processing plants due to clostridial infection; thereby, reducing profit³⁶. Moreover, sparse lesions that may be found in a case of SNE may be enough to hinder growth performance; thus, resulting in an underproductive flock³⁹.

Feeding Against Necrotic Enteritis

It has been reported that diet formulation has the greatest impact on the prevalence of *C. perfringens* in chicken GI tracts⁶¹. The poultry industry formulates diets on a least-cost basis, which may become problematic if nutritionists do not take into consideration the pathological consequences that some ingredients may have in the GI tracts of chickens. Every feed ingredient has a specific purpose in the diet. For instance, cereal grains are fed for their energy concentration as well as fiber. Also, some grain and animal/plant meals are used for their protein content. Since these ingredients are obtained from different sources, they are highly variable in macro and micronutrients¹.

The diet provides the conditions for proliferation

There are multiple elements that affect the proliferation of *C. perfringens* in chicken intestines, one of the most critical factors being diet formulation^{5, 36}. Some feed ingredients have been found to exacerbate the numbers of *C. perfringens* in chickens’ gastrointestinal tract. Diets formulated with wheat increased NE intestinal lesion scores compared to broiler chickens fed a corn-based diet⁴. In another study, Drew *et al.*¹⁰ investigated the effects of different protein sources on the intestinal populations of *C. perfringens* in broiler chickens. Diets were formulated to contain 230, 315, and 400 g/kg of fishmeal or soy protein concentrate (SPC). The numbers of *C. perfringens* in the ileum and ceca increased when the amount of protein increased from 230 to 400 g/kg.

Type of grain influences the occurrence of *Clostridium perfringens*

Authors have studied the effects of grain inclusion on gut microbiota, and it is well established that small cereal grains such as barley, rye, and wheat tend to increase the prevalence of *C. perfringens* in the GI tract. Shakouri et al.⁵² investigated the influence of barley, sorghum, wheat, and corn on counts of *C. perfringens* in the different intestinal segments. Corn and wheat had the lowest *C. perfringens* counts, followed by sorghum, while barley yielded the highest counts. These findings agree with Riddell and Kong⁵¹.

Other researchers have concluded that the increase in gut viscosity and increased chyme transit time elicit the overgrowth of *C. perfringens* in the intestines²⁸. Grains like wheat and barley contain high amounts of non-starch polysaccharides (NSP), which increase viscosity²⁶. Furthermore, it has been alleged that, since these grains are high in NSP, the bird cannot absorb nutrients as efficiently, thereby leaving them for microbes like *C. perfringens* to consume³¹.

Enzymes improve nutrient availability in the presence of *C. perfringens*

Shakori et al.⁵² and Jia et al.²⁶ also studied the impact of several diets with the inclusion of a blend of carbohydrases such as glucanase and xylanase. Their findings suggested that enzyme addition did not affect counts of *C. perfringens* in the different intestinal sections. However, they did find an improvement in growth performance. They stated that enzymes improved chyme viscosity by degrading the encapsulation of nutrients in diets.

For this reason, researchers have investigated the use of enzymes in wheat and barley-based diets on the incidence of *C. perfringens* in chicken intestines. Jackson et al.²⁵ studied the effect of beta-mannanase addition on flocks infected with *Eimeria* spp. and *C. perfringens*. They found that feeding this enzyme significantly reduced the impact of *C. perfringens* on the performance of infected flocks as well as intestinal lesion scores. Moreover, the authors explained that this might be due to beta-mannanase crossing the intestinal wall to provoke an immune response. They determined that this enzyme tended to ameliorate the symptoms of necrotic enteritis, but not significantly.

MOS may have a positive impact on immunity

Hofacre et al.²³ found similar results when birds were fed mannan-oligosaccharides. A marked effect was only found when mannan-oligosaccharides were included along with lactic acid-producing, competitive exclusion products (probiotics).

The feed form is decisive

Feed form has also been investigated on the incidence of *C. perfringens*. When birds were fed whole wheat compared to ground, researchers found reduced counts of *C. perfringens* in the gut². These results can be extrapolated to the findings of Engberg et al.¹¹. They found that when birds were fed coarse versus fine

mash or pellets, *C. perfringens* counts were consistently higher in flocks fed mash diets. These authors concluded that feeding pellets or whole grains increases gizzard activity, which consequently triggers hydrochloric acid production and decreases pH in the GI tract. This drop in pH of approximately 0.5 units may be responsible for decreased *C. perfringens* counts.

Mind the protein source

Another well-established fact is that the *C. perfringens* population can be affected by the type of the protein source and the inclusion rates.

Potato is worse than fish

Palliyeguru *et al.*⁴² studied the inclusion of protein concentrates (potato, fish, and soy) on subclinical NE. They determined that the potato-containing diet resulted in the highest incidence of *C. perfringens* in the gut, followed by fish and soy. Also, the potato-containing diet had the highest activity of trypsin inhibitors and lowest lipid content. Increased trypsin inhibition does not allow for the inactivation of alpha and beta toxins produced by *C. perfringens*, resulting in increased intestinal wall lesions.

Fish is worse than soy due to the amino acid composition

Drew *et al.*¹⁰ formulated diets containing fishmeal or a soy protein concentrate at different levels. Feeding dietary fishmeal resulted in a higher incidence of *C. perfringens* as compared to the soy protein diet. Furthermore, with increasing levels of soy and fishmeal diets, counts of *C. perfringens* increased as well. A notable difference in fishmeal protein concentrate compared to the soy protein concentrate was the amino acid ratio in this experiment; the methionine and glycine ratios were 1.3 times greater in fishmeal diets.

Muhammed *et al.*⁴⁰ determined that methionine was required for *C. perfringens* sporulation. This may be of interest to nutritionists since some authors have estimated that 10-20 % of synthetic amino acids are not absorbed and reach the lower intestinal tract, i.e., ceca; thereby, aiding in the proliferation of *C. perfringens*.

Fat source - animal fat is critical

The effects of fat sources on *C. perfringens* population remain largely unknown. Knarreborg *et al.*²⁹ studied the bacterial microflora in chicken intestines after feeding different dietary fats (soy oil and a tallow and lard mix) in rations containing antibiotic growth promoters (AGP). When soy oil was fed, *C. perfringens* counts were significantly lower than diets containing animal fats. The authors stated that, since plant oils contain higher amounts of unsaturated fatty acids, the chyme in birds fed oil diets would have decreased viscosity, decreasing transit time. Furthermore, an additive effect was found when soy oil was provided along with AGP, which may be due to facilitated antibiotic dispersion caused by the oil's lipophilic properties. Knarreborg *et al.* (2002) investigated the effects of fat sources on *C. perfringens*. They found that total anaerobic counts increased with animal fat addition. However, zinc bacitracin was included in their diets, specifically targeting Gram-positive microorganisms like *C. perfringens*; thus, potentially biasing their results.

Antibiotics and coccidiostats in the diet -

helpful, but finite

Antibiotics and coccidiostats have been commonly included in poultry diets since the mid-1940s and 1950s^{61, 58}.

Prescott *et al.*⁴⁹ studied the inclusion of zinc bacitracin to prevent necrotic enteritis and concluded that it successfully controlled the *C. perfringens* challenge. Flocks in the antibiotic treatments were able to overcome disease and perform similarly to unchallenged birds. Multiple authors have replicated these results using different antibiotics such as virginiamycin and salinomycin^{17, 3, 11}.

Improvements in flock performance with the inclusion of antibiotics and coccidiostats are well understood and omnipresent in the literature. However, the potential loss of subtherapeutic antibiotic usage in livestock in the United States due to increasing concerns over [antimicrobial resistance](#) and consumer demands makes research of viable alternatives to these compounds paramount.

So, what are your alternatives?

A lot of different approaches are possible. In general, these measures should act against *Clostridium perfringens* while supporting gut health.

Tested substances without the desired effects

Lastly, multiple options have been studied to control *C. perfringens* in poultry. Some researchers have studied the inclusion of complex carbohydrates and fibers like pine shavings, guar gum, and pectin with limited success^{4, 31}. Another popular alternative is the use of competitive exclusion-based products such as prebiotics and probiotics^{27, 16}. Still, these products failed to yield consistent results.

Other options that have been investigated are the addition of lactose and organic acids^{54, 38}. Potassium diformate did not produce lowered counts of *C. perfringens*. Lactose reduced *C. perfringens* counts but resulted in undesirable ceca characteristics including, enlargement and increased fermentation⁵⁴.

Essential oils alone or in combination may be a solution

Mitsch and coworkers³⁹ investigated the efficacy of two blends of essential oils with positive effects on the reduction of *C. perfringens* from the gut and feces of broilers. Gaucher and coworkers¹⁵ compared growth performance and gut health of broilers fed a conventional (anticoccidials and AGPs) vs. ABF (Coccidiosis vaccine and essential oil blends) diet. They established that livability, age at slaughter, and percentage of condemnation did not change with diet type. However, average daily weight gain and FCR were negatively affected. Furthermore, NE was more prevalent in ABF flocks. Still, many authors agree that a multifactorial approach is necessary if antibiotics should be completely replaced by these strategies³⁶.

A contemporary study by Wati *et al.*⁵⁶ aimed to compare AGPs to a commercial blend of essential oils fed to broilers. Authors found that chickens fed essential oils had body weights and FCRs that were statistically similar to the AGP treatment. Moreover, both AGP and essential oil treatments had statistically lower counts of *Salmonella* and *E. coli* after an oral challenge than the control group.

Conclusion

***C. perfringens* is a potential pathogen found in every place poultry is raised. Therefore, we must continue to identify strategies to control the development of Necrotic Enteritis. Since antibiotics alone may not always successfully control *C. perfringens* and have the potential for subtherapeutic use loss in the US, a multifactorial approach must be considered and investigated. Grain size, enzymes, feed form, animal protein source, fats, and feed supplements such as essential oils can affect the proliferation of *C. perfringens*. Nutritionists, veterinarians, and live production personnel must come together to develop the best approach for their specific complex circumstances.**

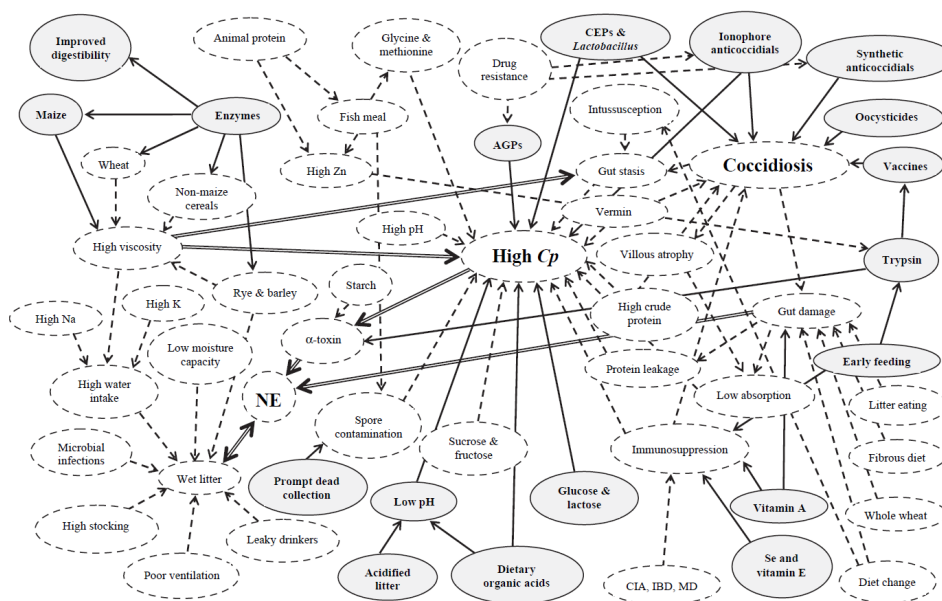


Figure 1. Interaction between coccidiosis and NE with environmental factors

Solid-line arrows are beneficial in controlling disease. Dashed-line arrows impart high disease risk factors. Double-line arrows depict major disease-risk factors. AGP, antibiotic growth promoter; CIA, chick infectious anemia; CEP, competitive exclusion product; Cp, *Clostridium perfringens*; IBD, infectious bursal disease; MD, Marek's disease; NE, necrotic enteritis. (Williams, R.B. 2005)

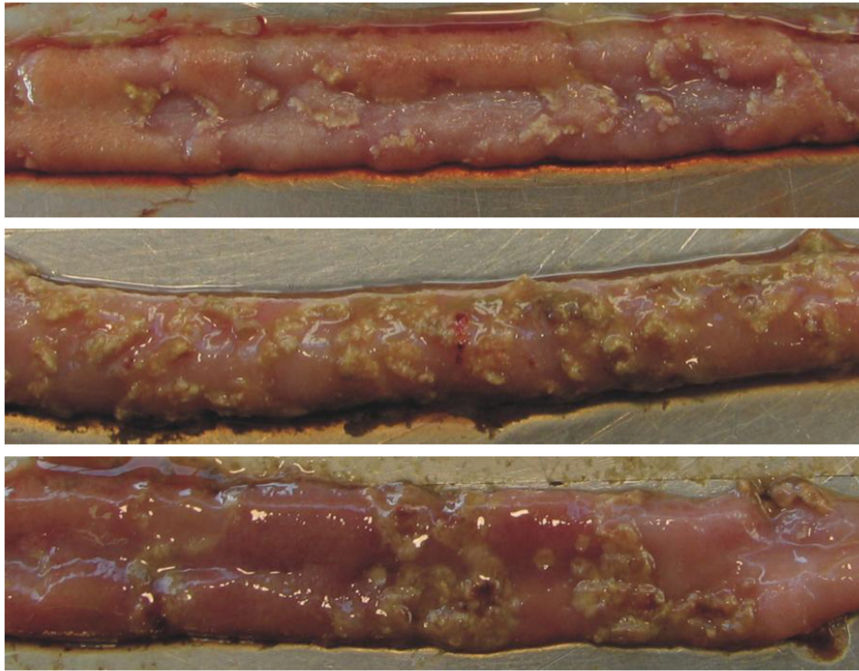


Figure 2. Necrotic Enteritis lesions in chicken intestines

Yellowish necrotic lesions in three intestinal samples. Intestines A and C show a few marked lesions. Intestine B shows clusters of lesions typical of the “Turkish towel” syndrome. (Source: <http://www.mdpi.com/2072-6651/2/7/1913/htm>. Accessed: January 14, 2021).

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Dysbacteriosis and gut health management in poultry



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The growing restrictions on the use of antibiotics growth promoters (AGPs), as well as the

development of resistance to some routinely used antimicrobials in the recent past, have increased the incidence of dysbacteriosis within intensive poultry farming. What is the solution to maintaining gut health and animal performance in these circumstances?



What is dysbacteriosis?

Dysbacteriosis has been defined as the presence of a qualitatively and/or quantitatively abnormal microbiota in the proximal parts of the small intestine. This abnormal microbiota produces a cascade of reactions in the [gastrointestinal tract](#), including reduced nutrient digestibility and impaired intestinal barrier function, increasing the risk of bacterial translocation and inflammatory responses (Panneman, 2000; Van der Klis, 2000 and Lensing, 2007). Dysbacteriosis is not a specific disease but a secondary syndrome. Along the entire GI tract, there is a diverse microbial community comprised of bacteria, yeasts, archaea, ciliate protozoa, anaerobic fungi, and bacteriophages, commonly referred to as the intestinal microbiota.

Dysbacteriosis is an imbalance in the gut microbiota as a consequence of an intestinal disruption. The impact of dysbacteriosis can be separated into economic and welfare issues (Bailey, 2010). Dysbacteriosis can lead to very wet litter and caking issues. The prolonged contact of broilers with the caked litter can result in painful ulceration of the feet and hocks (pododermatitis and hock-burn), leading to a serious welfare issue and degradation of the carcass.

Apart from these issues, a major economic impact comes from reduced growth rates, FCR, and increased veterinary treatment costs (Kizerwetter-Świda and Binek, 2008).

Causes of dysbacteriosis

It is believed that both non-infectious and infectious factors can play a role in dysbacteriosis (DeGussem, 2007).

Non-infectious causes are:

- Diet
- Brooding
- Biosecurity
- Risk periods
- Environmental conditions

Diet

Intestinal bacteria derive most of their energy from dietary compounds. Thus, diet has a major influence over the bacterial populations (Apajalahti et al., 2004). Any change in feed and feed raw materials, as well as the physical quality of feed, influence the balance of the gut microbiota. Processing significantly affects the characteristics of the feed as a substrate for the bacterial community. Perhaps the temperature and pressure of the conditioning process give its characteristic signature to the bacterial community structure.

Inappropriate brooding conditions

The provision of optimal brooding conditions is essential for ensuring optimal gut microbiota development. Birds receiving appropriate brooding develop a gut that performs well and has a greater capacity to cope with the challenges of the broiler shed. Early access to feed and water is crucial. One of the most critical factors for the occurrence of dysbacteriosis is the lack of digesta. The microbiota can change in a period of hours when nutrients are not present. The quality of water is also essential to maintain normal intestinal function and digesta pH.

Faulty biosecurity

If clean-out and disinfection procedures are improperly conducted, pathogens will be introduced into the poultry shed, and exposure to these pathogens will influence gut health and development. It has been proven that litter management regimes affect chicken gastrointestinal tract (GIT) and microbiota (Wang et al., 2016)

Risk periods

There are times during poultry production when the bird will be challenged, for example, during feed changeovers, vaccination handling and transportation, overcrowding, or placement in new housing. During these periods, the gut microbiota can fluctuate and, in some cases, if management is sub-optimal, dysbacteriosis can occur.

Environmental conditions

Achieving optimal environmental conditions will promote good gut health. Any perturbation in gastroenteric physiology or immunity of the bird, caused by temperature stress or other environmental discomforts, can cause dysbacteriosis and/or enteritis. These are associated with lower absorption of

nutrients by the host. Suzuki et al. 1983 demonstrated that overcrowding and heat stress, very commonly seen in intensive poultry farming, has a significant impact on the microbiota of chickens.

Infectious agents that potentially play a role in dysbacteriosis

- Mycotoxins
- Eimeria spp.
- Clostridium perfringens
- Other bacteria producing toxic metabolites

Mycotoxins

Many mycotoxins can stimulate the secretion of several antimicrobial molecules, which have positive effects on the maintenance of intestinal homeostasis. Fumonisin inhibit the growth of fungi, Fusarium toxins exhibit different antimicrobial defensive mechanisms, and aflatoxins exhibit a moderate antimicrobial activity against Escherichia coli, Bacillus subtilis, and Enterobacter aerogenes [Bevins et al. 1999 and Wan et al.2013]. Mycotoxins such as aflatoxins, trichothecenes, zearalenone, fumonisin, and ochratoxin can alter the normal intestinal functions, such as the barrier function and nutrient absorption. Some mycotoxins, like trichothecenes and ochratoxin, affect the histomorphology of the intestine (Winnie et al., 2018). Mycotoxicosis changes the population equilibrium, which can lead to dysbacteriosis.

Eimeria spp.

Coccidiosis caused by Eimeria spp. in chickens appears to be one of the principal destabilizing agents, causing the destruction of enterocytes and affecting the integrity of the intestinal mucosa and wall. The lesions that it causes, the inflammatory process, the reduced absorption and consequent excess of nutrients in the lumen all contribute to the proliferation of certain groups of bacteria. This situation clearly predisposes birds to intestinal dysbacteriosis and/or bacterial enteritis, and in particular to necrotic enteritis.

Clostridium perfringens

Clostridium perfringens is a natural part of the habitat in the hindgut that is not dangerous under normal circumstances. If it multiplies, the bacterium produces toxic substances that damage the intestinal mucosa and cause a condition called necrotic enteritis. The disease is characterized by necrosis and inflammation of the GIT. Without treatment, this can escalate to perforation of the intestines, hemorrhages, and eventual death from septic shock.

Signs and consequences of dysbacteriosis

Dysbacteriosis can have profound effects on the host. Dysbacteriosis alters the GIT environment and favors the growth of pathogenic bacteria. Pathogenic bacteria produce toxins that increase intestinal motility or cause alterations in the amounts of mucus produced or in its composition. They also result in modifications of gastric acidity, reduction in the production of bacteriostatic peptides in the pancreas, and

reduced immunoglobulin (IgA) secretion.

Toxins released by entero-pathogens damage intestinal villi, resulting in focal ulcerations of the mucosa, tissue necrosis, and shifts in gut microorganism numbers and metabolism. The costliest condition for animal production is the chronic inflammatory response of the animal to constant minor dysbacteriosis. These chronic responses can reduce weight gain and cause low feed conversion efficiency. Coccidiosis infections and any other enteric disease can be aggravated when dysbacteriosis is prevalent. Generally, animals with dysbacteriosis have high concentrations of *Clostridium* that generate more toxins, leading to necrotic enteritis.

In broilers, the syndrome is generally seen between 20 and 30 days of age (Wilson et al., 2005). Clinically, the main signs are:

- pale, glistening or orange droppings with undigested food particles
- wet and greasy droppings and hence dirty feathers
- sometimes foamy caecal droppings
- reduced physical activity
- increased water intake
- decrease in feed intake with a check in weight or reduced gain rates
- increased feed conversion

(Wilson et al., 2005; De Gussem, 2007)

Wet litter is also a general outcome of dysbacteriosis that may affect the air quality of the house, leading to a higher incidence of respiratory problems.

Additionally, foodborne pathogens such as *Salmonella* spp. and *E.coli* proliferate more in the dysbiotic intestine and can become persistent residents of the hindgut.

At necropsy, the main observations are

- a thin, fragile, often translucent intestinal wall
- watery or foamy intestinal contents
- frequent orange mucus and undigested particles in the intestines
- ballooning of the gut
- intestinal inflammation

(Pattison, 2002; De Gussem, 2007)

Prevention of dysbacteriosis

The most important factors to prevent dysbacteriosis are

- Minimizing environmental stress
- Maintaining good water quality
- Improving feed digestibility
- Avoiding antinutritional factors, mycotoxins, and rancidity
- Feed additives that could modulate microbial component and avoid dysbacteriosis

Growth-promoting antibiotics are well known for the inhibition of undesired microbiota and the negative effects of their metabolites, and selection for beneficial bacteria. However, the adverse result is that they diminish the natural diversity of the gut microbiota. Antibiotics can also result in animals developing bacterial resistance.

Other products have been proposed as alternatives to growth promotion, taking into consideration the increasing bacterial resistance to some antibiotic categories.

Alternate feed additive technologies that have a promising role in controlling dysbacteriosis are:

- Probiotics
- Prebiotics
- Enzymes
- Organic acids
- Essential oils and phytomolecules

Probiotics

The post-hatch period is very critical for the chicks' intestine development. Exposure to the environment in hatchery and farm affects microbial colonization in the intestine tract. The use of selective probiotics in day-old chicks at the hatchery and on the farm immediately after placement in broiler house reduces the risk of dysbacteriosis. Probiotics work by competitive exclusion, thereby prevent the colonization of potentially pathogenic bacteria. Probiotics prevent enteric diseases, improves intestine development and digestion process.

The benefits include enhanced growth and laying performance, improved gut histomorphology, immunity, and an increase in beneficial microbiota (Rajesh Jha et al., 2020)

Prebiotics: Mannan Oligosaccharide

(MOS) mimics the properties of the cells on the gut wall to attract and bind with harmful bacteria. Rather than allowing the bad bacteria to attach to the gut wall, the MOS acts as a sticky sponge, clearing up the harmful bacteria and removing them from the digestive system. MOS play an important role in gut functionality and health, through enhanced nutrient digestibility and improved gut barrier function and local defenses. MOS is also related to long villi and shallow crypts in the intestine, so a larger surface area helped with the absorption of nutrients and improved animal performance (Chand et al., 2016b)

Enzymes

Careful choice of feed enzymes will reduce nutrients available for pathogenic bacterial growth and improve gut health. Bacterial Xylanase is showing promise by digesting both soluble and insoluble arabinoxylans and reducing the viscosity of intestinal content. It maintains gut motility, improves nutrients digestibility, and impairs the growth of pathogenic bacteria in the hindgut.

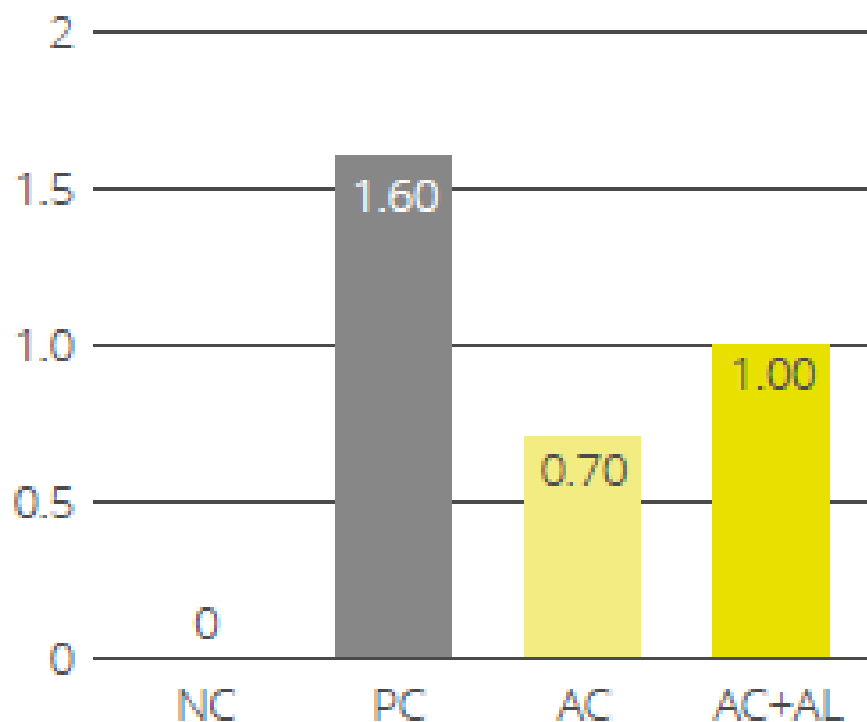
Organic acids

Organic acids ameliorate the conditions of the GIT through the reduction of GIT pH, promoting proteolytic enzyme activity, intensifying pancreatic secretions. They encourage digestive enzyme activity and nutrient digestibility. Organic acids are creating stability of the microbial population by stimulating the growth of beneficial bacteria (Papatisiros et al., 2013).

Phytomolecules

Multiple scientific studies have proven the positive effects of phytomolecules (also known as phytochemicals or secondary plant compounds) on the gut health of livestock animals. These substances support digestion and improve the utilization of nutrients. This results in higher daily weight gain and better feed conversion. In addition, phytomolecules have a proven antimicrobial effect, based on different biological modes of action.

EW Nutrition offers standardized phytomolecule-based solutions (Activo and Activo Liquid) that positively influence gut health and subsequent performance parameters in poultry. In scientific studies, the Activo product line has shown a positive effect on gut pathogenic bacteria, reducing necrotic enteritis (Fig 1) and improving production performance.



Conclusion

Dysbacteriosis can have profound effects on the host. Acute dysbacteriosis can result in the proliferation of pathogenic microorganisms that become enteropathogenic. Pathogenic bacteria can produce toxins and metabolites that increase gut motility, increase fermentation with gas production, change gut pH, irritate the mucosa, cause inflammation, and increase mucous secretion. This process reduces the digestibility and absorption of nutrients.

Maintaining the equilibrium of the gut ecosystem is key to avoiding dysbacteriosis. Improving feed digestibility and using feed additives that modulate gut microflora help to maintain more stable gut ecosystems, even during periods of intestinal stress preventing dysbacteriosis. Effective prevention and control of dysbacteriosis help increase poultry operations' economic profitability by way of improved performance, health, and welfare, and reduce foodborne pathogens and environmental impact of poultry production.

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All-rounder lutein supports animals and humans

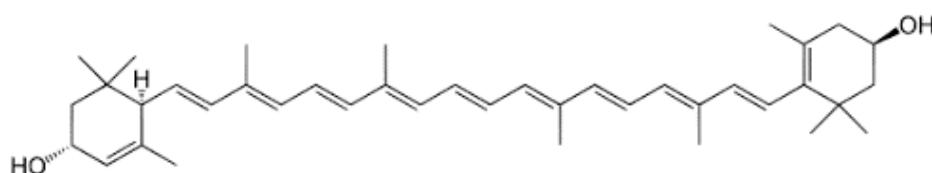


by *Inge Heinzl*, Editor, EW Nutrition

Lutein is a lipid-soluble pigment that can be found naturally in algae and plants. There, it is a component of the light-collecting complexes in the chloroplasts.

For example, kale contains a relatively high concentration of up to 0.25mg lutein per g wet weight. For industrial purposes, however, lutein is extracted from the petals of marigold; they contain up to 8.5mg/g wet weight.

In the animal organism, lutein occurs in the egg yolk, in milk, or the macula lutea ("yellow spot") of the animal/human eye. However, animals and humans cannot synthesize it.



Lutein belongs to the group of carotenoids, which is divided into carotenes and xanthophylls. Lutein, chemically expressed as "3,3'-dihydroxy- α -carotene", is a xanthophyll always accompanied by its isomer zeaxanthin. It is synthesized out of two α -carotenes through hydroxylation.

Lutein provides benefits for animals and humans

Due to its beneficial characteristics, lutein is an essential ingredient of plants and is used in animal nutrition as well as in human medicine.

Lutein has antioxidant protective properties

Under normal conditions, the cells in the animal and human organism control ROS (reactive oxygen species) levels. Usually, there is a balance between the generation of ROS and their elimination by scavenging systems. However, the high performance levels in modern animal production can easily lead to high ROS levels, translated into oxidative stress and leading to cell damage. Cell damage contributes to the generation of cancer and early aging in humans. In animals, the negative impact of oxidative stress can be responsible for lower performance and inferiority of meat and eggs.

Antioxidants stop ROS by taking up their energy

Through the uptake of energy, molecules can get into an excited state. One example is singlet excited oxygen, a highly reactive form of oxygen able to destroy proteins, lipids, and DNA. Carotenoids can intervene in this process: by exchanging electrons, the singlet excited oxygen gets neutralized, and the carotenoid gets into this excited state with higher energy. Once able to release this energy as heat into the environment, the carotenoid gets back to its normal state and can once again start acting as an antioxidant.

In this way, carotenoids, including lutein, 'quench' the energy of excited molecules and prevent the adverse effects of ROS (reactive oxidative substances).

Antioxidant properties profitably used

The antioxidant character of lutein plays an important role in the treatment or prophylaxis of macular degeneration in humans (Landrum & Bone, 2001). There is also evidence that lutein can be used to improve the visual and retinal function in dogs (Wang et al., 2016). In the eye, lutein and zeaxanthin, occurring in the retina and the macula, neutralize free radicals produced due to the ultraviolet light and thereby prevent damage to the macula.

Further possible applications are against cardiovascular diseases (Dwyer et al., 2001) and various types of cancer (e.g., breast cancer, Gong et al., 2018).

Lutein is important in infant nutrition

Lutein and its isomer zeaxanthin are the two primary carotenoids found in human milk (Giordano and Quadro, 2018). Stringham and co-workers (2019) postulate that lutein plays an important role in children's visual and cognitive development/optimization. They report that a lutein supplementation of the mother can lead to a higher concentration of this substance in the milk and, consequently, in the child's plasma (Sherry et al., 2014). In dairy cows, an increased level of lutein in the milk can also be observed (Xu et al., 2014), suggesting that lutein could also be essential in calf development.

Lutein stimulates the immune system

Another benefit of lutein is its positive influence on the immune system.

On the one hand, lutein stimulates the production of antibodies. In dogs, Guimarães Alarça et al. (2016) could show an increase of CD4+ and CD8+ T-lymphocyte subtypes. Kim et al. (2000) demonstrated the increase of lymphocytes and cells expressing CD5, CD4, CD8, and major histocompatibility complex class II (MHC II) molecules. Bédécarrats and Leeson (2006) provoked a higher secondary antibody response to infectious bronchitis vaccination in laying hens.

Besides, lutein acts as an anti-inflammatory agent, as shown in vitro by Chao et al. (2015) and in broiler chickens by Moraes and team (2016).

Lutein improves the attractivity of poultry products

In the marketing of poultry products, appearance and color are of central importance for evaluating quality. Egg yolk coloration is to a large extent a matter of regional preferences, however it is clear that an egg with a yolk that does not have the typical color is classified as inferior by the consumer. In areas with traditional corn growing, a white-skinned chicken is not commercially viable. Even when pullets are bought, the shanks and beaks should be yellow.

The use of xanthophylls like lutein and zeaxanthin enables producers to safely control the color of the egg yolk and of the broiler skin. It also leads to a healthy color of the shanks and beaks of the birds.

Lutein in a nutshell

Lutein is a true all-rounder: a substance that delivers benefits across the board. In plants, it helps fruits and petals become attractive for insects and other animals. It positively influences the animal, acting as an antioxidant, promoting infant development, and stimulating the immune system. As a pigment, it makes poultry and poultry products look more attractive to the consumer. Through its presence in eggs and milk, lutein provides clear and clean benefits to both animals and humans.

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Do we have the tools to reduce antibiotics in swine production?



The global swine industry is going through unprecedented challenges. On the one hand, the threat of the African Swine Fever virus is global, despite the fact it hasn't arrived in all markets. The virus is today alive among the wild boars in the Polish and Belgian forests. Every day it keeps gaining a few more meters to the border, threatening the German swine industry, one of the largest in the European Union.

If this happens, we might be seeing important changes to the pork supply chain on the meat market worldwide – in Europe in addition to current issues in the USA meat plants. The profitability of swine businesses depends in many ways on the export capacity of large corporations based in Germany, Spain, Denmark, etc.

On the other hand, the presence of COVID-19 in most countries is changing human behavior, meat consumption at home, and the way we look at the future. Perhaps a virus overload via the news, some

“fake news” conveying wrong messages on what’s coming, and suddenly we feel the future will never be the same.

The future of the swine industry

At least for the swine industry, the future will indeed never be exactly the same. We will be facing different challenges. Some of these will be structural, such as the issue of decreased manpower and how to substitute manpower by machines, through the implementation of Precision Livestock Farming, for instance.

We are also facing important health challenges to our animals: not just ASF, but also new and more aggressive PRRS strains, among other pathogens. Our sows’ production capacity is increasing annually, yet in some cases 25% of the new-born piglets are lost from birth to market. Increasingly, we may start to see increased levels of mortality not only in the nursery but in fattening pigs and sows as well.

It is becoming clearer all the time: the future of the global swine industry lies in producing more pigs with reduced antibiotics. To stay the course, we need to take further action and implement corrective measures.

Why we should remove antibiotics in pig production

Pressure from stakeholders and regulators

There is, and there will be, increasing pressure from many stakeholders worldwide to work toward pig production with reduced or no antibiotics. Meat suppliers, slaughterhouses and processors, governments at different levels, and, of course, the European Union – all are demanding reductions in the level of antibiotics in livestock production.

There is also an increasing awareness at the global societal level regarding [antimicrobial resistance](#) related to antibiotic usage in farming production. Consumer pressure will grow exponentially as the terrible COVID-19 experience will be “digested” by the global population.

Pressure to accede to the pork market

There is yet another important reason to start working in that direction: the global swine meat market. Today, China’s pork meat shortage is opening the market. Now any producer could potentially sell meat, either to China or to any other country. We are starting to see moves from companies in the USA or Brazil banning the use of Ractopamine in their operations because they want to get access to the ractopamine-free market (Europe & Asia, over 70% of the global population).

According to M. Pierdon (AASV 2020 Proceedings), there will be two types of markets: the “Niche ABFree” and the “Commodity ABFree”. Companies will have to analyse what their future is on the meat market. Not all the producers may be willing to enter this new phase, but for sure many will try.

Strategies for antibiotic reduction

In Europe, the time has arrived. Zinc oxide will be banned in June 2021 and there is now more than a trend in production with less or no antibiotic use. In some cases, there is a need; in others, this is simply profitable.

Challenges to antibiotic reduction

Producing pigs completely without antibiotics is not easy, and not affordable for all. Initially we may have to give up some performance parameters in order to achieve the balance between what we want and what we can achieve in animal performance. But the time will arrive when these two objectives will converge; there is no alternative.

To that end, we will have to include in our pig production strategy all the available tools and technologies: genetic selection, immunization against some key pathogens, environmental control (mandatory but often forgotten), early detection of diseases, etc.

In this new era we are entering, nutrition and feed additives will play a key role. It will be crucial to find solutions targeting the microbiome's stabilization and diversification, creating and maintaining healthy farms and achieving all the performance parameters.

Do we have the tools for antibiotic reduction?

Even today there are companies able to produce completely antibiotic-free pigs – proof that **yes, the tools are already in place**.

Nevertheless, for most producers, the answer to – **Can we produce without antibiotics?** is most likely “probably not”. This will require a holistic approach, given the specific case of piglets.

The microbiome of the piglet is strongly influenced by birth and the subsequent weeks. What, then, are the elements that will be part of this new game that comprises a new approach?

- The colostrum intake & the management of the piglets
- Antibiotic usage and its influence on the gut
- The piglets' microbiome and its evolution during the periweaning period
- The weaning process, appetite, and water intake
- Zinc oxide removal and its influence on the microbiome
- The immune system and the relationship with the GIT status
- Inflammation and its modulation at the gut level
- The health status and the effect on the concomitant infections: which ones are key and which ones are secondary pathogens
- The all-important biosecurity, management, and hygiene

To summarize: there is no one tool, but rather a **holistic approach** to face this new challenge that the swine industry is facing nowadays. The answer is not a silver bullet, but a journey that we all must undertake.

Available in Spanish [here](#).