Beyond AGPs: Controlling necrotic enteritis through gut health optimization

Antibiotic growth promoters (AGPs) have routinely been used in intensive poultry production for improving birds' performance. However, in recent years, reducing the use of antibiotics in animal production has become a top priority, due to concerns about the development of antibiotic-resistant bacteria and mounting consumer pressure. Multiple countries have introduced bans or severe restrictions on the non-

therapeutic use of antibiotics, including in the US, where the Food and Drug Administration has implemented measures to curb the use of antibiotics since 2017.

However, the removal of AGPs poses challenges for poultry performance, including reduced feed efficiency, decreased daily weight gain, as well as higher mortality. Moreover, the withdrawal of AGPs in feed is widely recognized as one of the predisposing factors for necrotic enteritis (NE). NE is one of the most common and economically important poultry diseases, with an estimated global impact of US$ 5 to 6 billion per year. As a result of withdrawing AGPs, the usage of therapeutic antibiotics to treat NE has increased. To break out of this vicious cycle and to secure the efficiency of poultry production, alternatives are needed that combat NE where it starts: in the gut.

Necrotic enteritis: a complex disease

NE is caused by pathogenic strains of *Clostridium perfringens* (CP): ubiquitous, gram-positive, spore-forming anaerobic bacteria. The spores of CP can be found in poultry litter, feces, soil, dust, and contaminated feed. Low levels of different CP strains are naturally present in the intestines of healthy birds, kept in check by a balanced microbiome. However, when gut health is compromised, pathogenic strains can proliferate at the expense of unproblematic strains, resulting in clinical or sub-clinical NE.

Animals suffering from the clinical form show symptoms such as general depression, reluctance to move, and diarrhea, with mortality rates of up to 50%. Infected birds suffer from degenerated mucosa lesions in the small intestines. Even in its “mild”, subclinical form, which often goes unnoticed, the damage to the animals’ intestinal mucosa can result in permanently reduced performance and consequent economic losses for the producer.

Certain predisposing factors have been found to enable the proliferation of pathogenic strains in the gastrointestinal tract. Diet is a key example: the composition of the gut flora is directly linked to feed composition. High inclusion rates of cereals (barley, rye, oats, and wheat) that contain high levels of non-starch polysaccharides (NSPs), high levels of indigestible protein, and inclusion of proteins of animal origin (e.g. fishmeal) have been shown to predispose birds to NE.

A range of diseases (e.g. chicken infectious anemia, Gumboro, and Marek’s disease), but also other factors that have immunosuppressive effects, such as heat or cold stress, mycotoxins, feed changes, or high stocking density, render birds more susceptible to intestinal infections. The single most prominent predisposing factor for the occurrence of NE is the mucosal damage caused by coccidiosis.

Gut health is key to combating necrotic enteritis

To control NE, a holistic approach to optimizing the intestinal health of poultry is needed. It should take into account not only parameters such as diet, hygiene, and stress, but should also make use of innovative tools.

Phytomolecules, also known as secondary plant compounds, are essentially plants’ defense mechanisms against pathogens such as moulds, yeasts, and bacteria. Studies have demonstrated the antimicrobial effects of certain phytomolecules, including against antibiotic-resistant pathogens. Phytomolecules have also been found to boost the production of digestive enzymes, to suppress pro-inflammatory prostaglandins and have antioxidant properties. These features make them a potent tool for optimizing gut health, potentially to the point of replacing AGPs.

Can phytomolecules mitigate the impact of necrotic enteritis?

To study the impact of phytomolecules on the performance of broilers challenged with a NE-causing CP strain, a trial was conducted at a US-based research facility. In this 42-day study, 1050 male day-old Cobb 500 broiler chicks were divided into 3 groups, with 7 replicates of 50 chicks each.

On the first day, all animals were vaccinated against coccidiosis through a live oocyst spray vaccination. The experimental diets met or exceeded the National Research Council requirements, and were fed as crumbles/pellets. On days 19, 20, and 21, all pens, except the negative control group, were challenged with a broth culture of *C. perfringens*. A field isolate of CP known to cause NE (originating from a commercial broiler operation) was utilized as the challenge organism. On day 21, three birds from each pen were selected, sacrificed, group weighed, and examined for the degree of present NE lesions.

The positive control group received no supplements. The trial group received a synergistic combination of two phytogenic products containing standardized amounts of selected, microencapsulated phytomolecules: an in-feed phytogenic premix (Activo®, EW Nutrition GmbH) and a liquid complementary feed supplied via the drinking water (Activo® Liquid, EW Nutrition GmbH). The products were given at inclusion rates corresponding to the manufacturer's baseline antibiotic reduction program recommendations (Figure 1):

Figure 1: Trial design

The trial results indicate that the addition of phytomolecules helps to mitigate the impact of NE on broilers' performance. The group receiving Activo® and Activo® Liquid showed a better feed conversion (Figure 2) compared to the positive control group (NE challenge, no supplement). Also, better lesion scores were noted for animals receiving phytomolecules (0.7 and 1) than for the positive control group (1.6).

The most significant effect was observed concerning mortality: the group receiving Activo® and Activo® Liquid showed a 50% lower mortality rate than the positive control group (Figure 3). These results clearly indicate that phytomolecules can play an important role in mitigating losses due to NE.

Figure 1: Adjusted FCR

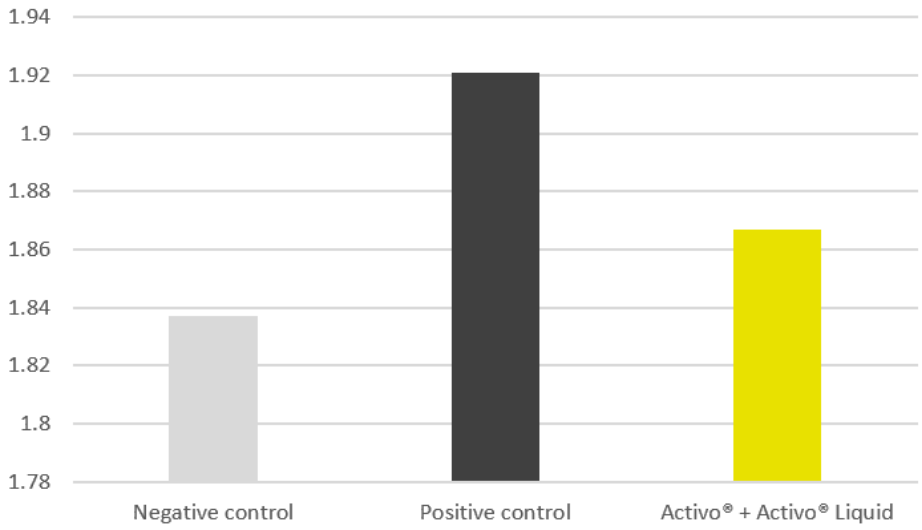
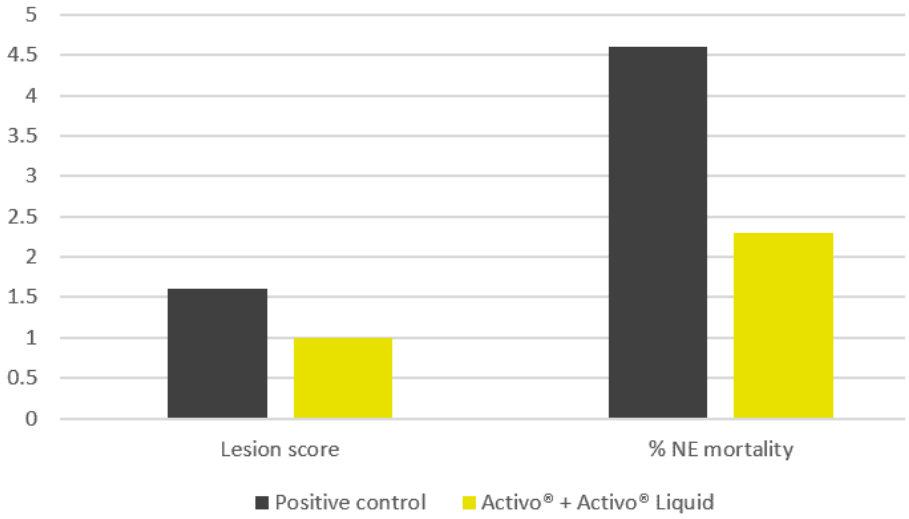


Figure 2: Lesion scores and mortality



Tackling necrotic enteritis in a sustainable way

In an age of AGP-free poultry production, a concerted focus on fostering animals' gut health is key to achieving optimal performance. This study strongly demonstrates that, thanks to their antimicrobial, digestive, anti-inflammatory and antioxidant properties, phytomolecules effectively support birds' intestinal health when challenged with NE. The inclusion of Activo® and Activo® Liquid, two phytogenic products designed to synergistically support birds during critical periods, resulted in improved feed conversion, better lesion scores, and 50% lower mortality.

In combination with good dietary, hygiene, and management practices, phytomolecules are therefore a potent tool for reducing the use of antibiotics: including Activo® and Activo® Liquid in their animals' diets allows poultry producers to reduce the incidence of NE, to mitigate its economic impact in case of outbreaks, and therefore to control NE in a sustainable way.

By A. Bhoyar, T. van Gerwe and S. Regragui Mazili

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Necrotic enteritis: The complete overview

by **Inge Heinzl, Marisabel Caballero, Ajay Bhoyar**, EW Nutrition

Necrotic enteritis is a profit killer in poultry production

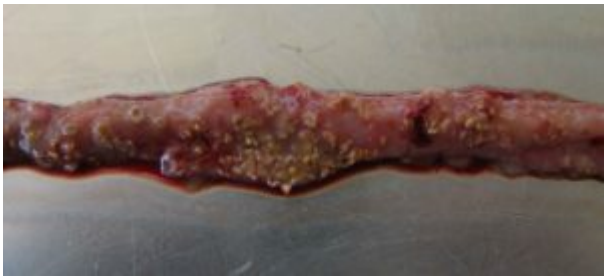
Necrotic enteritis is the cause of USD 6 billion losses every year in global poultry production, corresponding to USD 0.0625 per bird (Wade and Keyburn, 2015). This controllable disease is on the rise. One reason is the voluntary or legally required reduction of antibiotics in animal production due to the increasing occurrence of antimicrobial resistance but also consumer demand. Another reason is the administration of live Coccidiosis vaccines and partial reduction of ionophores, which also show efficacy against Gram-positive bacteria (Williams, 2005).

Necrotic enteritis and coccidiosis are the most significant health problem in broilers (Hofacre et al., 2018).

The disease generally occurs in broiler chickens of 2-6 weeks of age. It is caused by an overgrowth of *Clostridium perfringens* type A and, to a lesser extent, type C in the small intestine. The toxins produced by *C. perfringens* also damage the intestinal wall.

Clinical and subclinical forms of NE – which one causes more significant losses?

The clinical form is obvious



Intestine showing signs of NE

...is characterized by acute, dark diarrhea resulting in wet litter and suddenly increasing flock mortality of up to 1% per day after the first clinical signs appear (Ducatelle and Van Immerseel, 2010), sometimes summing up to mortality rates of 50% (Van der Sluis, 2013). The birds have ruffled feathers, lethargy, and inappetence.

Necropsy typically shows ballooned small intestines with a roughened appearing mucosal surface, lesions, and brownish (diphtheritic) pseudomembranes. There is a lot of watery brown, blood-tinged fluid and a foul odor during post-mortem examination. The liver is dark, swollen, and firm, and the gall bladder is distended (Hofacre et al., 2018).

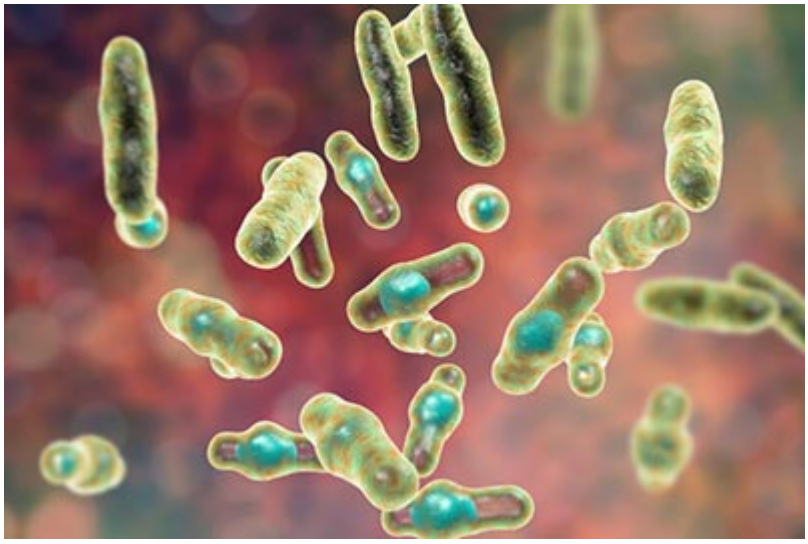
In the case of **peracute** Necrotic Enteritis, birds may die without showing any preliminary signs.

The subclinical form often only can be noticed at the end of the cycle

When birds suffer from the subclinical form, chronic damage to the intestinal mucosa and an increased quantity of mucus in the small intestine lead to impaired digestion and absorption of nutrients resulting in poor growth performance. The deteriorated feed conversion and the resulting decreased performance become noticeable around day 35 of age. As feed contributes approximately 65-75% of the input cost to produce a broiler chicken, poor feed conversion increases production costs and significantly influences profitability. Often, due to a lack of clear symptoms, this subclinical disease remains untreated and permanently impacts the efficiency of production.

The pathogen causing NE – a ubiquitous bacterium

Responsible for Necrotic Enteritis are Gram-positive, anaerobic bacteria, specific strains of *Clostridium perfringens* type A and, to a lesser extent, type C (Keyburn et al., 2008).



Clostridia primarily occur in the soil where organic substances are degraded, in sewage, and in the gastrointestinal tract of animals and humans. These bacteria produce spores, which are extremely resistant to environmental impact (heat, irradiation, exsiccation), some disinfectants, and can survive for several years. Under suitable conditions, *C. perfringens* spores can even proliferate in feed or litter.

Clostridium perfringens is a "natural inhabitant" of the intestine of chickens. In healthy birds, it occurs in a mixture of diverse strains at 10^2-10^4 CFU/g of digesta (McDevitt et al., 2006). The disease starts when *C. perfringens* proliferates in the small intestine, usually due to a combination of factors such as high amount protein, low immunity, and an imbalance in the gut flora. Then, the number rises to 10^7-10^9 CFU/g of digesta (Dahiya et al., 2005).

Highly important: NetB, a pore-forming toxin is a key virulence factor for NE

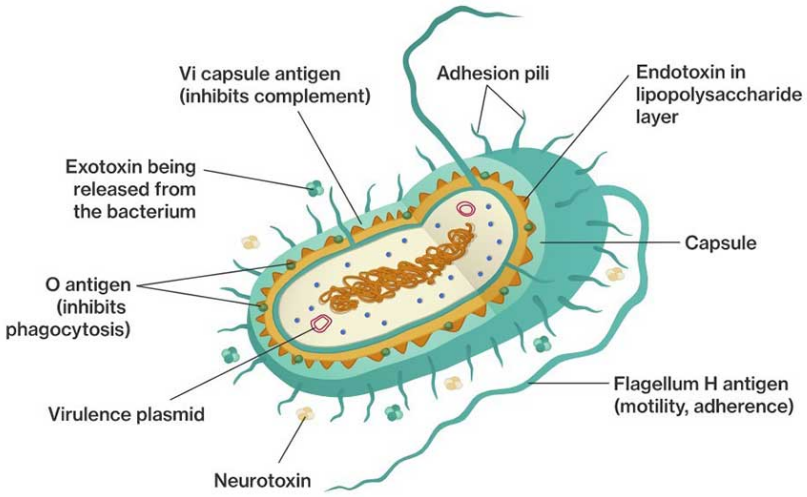
To establish in the host, *Clostridium* Spp. and other pathogens depend on virulence factors (see infobox). These virulence factors include for example "tools" for attachment, evasion or suppression of the host's immune system, "tools" for getting nutrients, and "tools" for entry into intestinal cells. Over the years, the α-toxin produced by *C. perfringens* was assumed to be involved in the development of the disease and a key virulence factor. In 2008, Keyburn and coworkers found another key virulence factor by using a *C. perfringens* mutant unable to produce α-toxin, while still causing Necrotic Enteritis.

Thus, another toxin was identified occurring only in chickens suffering from Necrotic Enteritis: *C. perfringens* necrotic enteritis B-like toxin (NetB). NetB is a pore-forming toxin. Pore-forming toxins are exotoxins usually produced by pathogenic bacteria but may also be produced by other microorganisms. These toxins destroy the integrity of gut wall cell membranes. The leaking cell contents serve as nutrients for the bacteria. If immune cells are destroyed, an immune reaction might be partially imparted.

Additionally, pathogenic strains of *C. perfringens* produce bacteriocins – the most important is Perfrin (Timbermont et al., 2014) – to inhibit the proliferation of harmless *Clostridium* Spp. strains and to replace the normal intestinal flora of chickens (Riaz et al., 2017).

Examples of virulence factors

1. **Adhesins**
   Enable the pathogen to adhere or attach within the target host site, e.g. via fimbria. Pili enable the exchange of RNA or DNA between pathogens.
2. **Invasion factors**
   Facilitate the penetration and the distribution of the pathogens in the host tissue (invasion and spreading enzymes). For example: hyaluronidase attacking the hyaluronic acid of the connective tissue or flagella enabling the pathogens to actively move.
3. **Toxins**
   Damage the function of the host cells or destroy them (e.g. endotoxins – lipopolysaccharides, exotoxins)
4. **Strategies of evasion**
   Enable the pathogen to undergo the strategies of defense of the immune system (e.g. antiphagocytosis factors provide protection against an attack by phagocytes; specific antibodies are inactivated by enzymes).

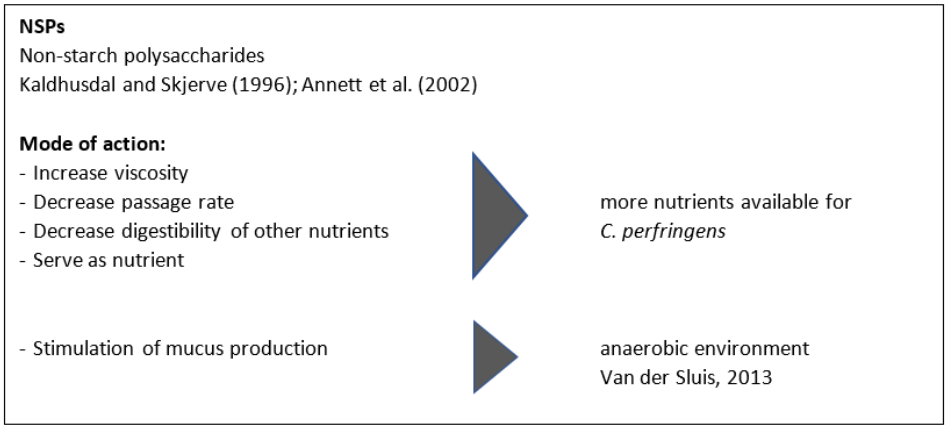


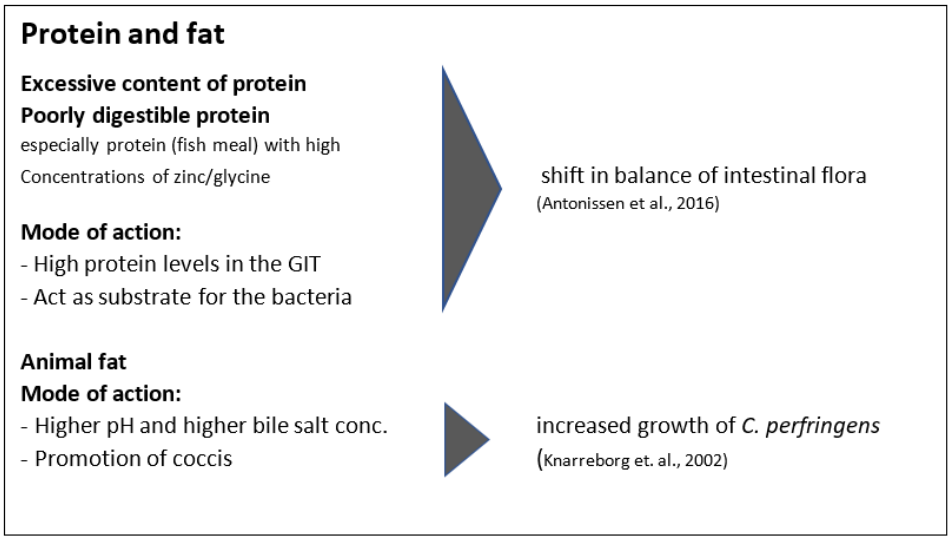
Predisposing factors favor the development of NE

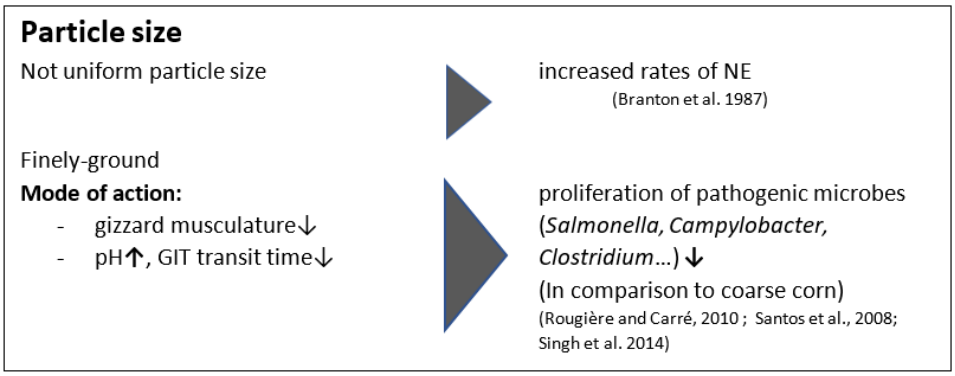
A chicken with **optimal gut health** may be less **susceptible to NE**. Additional **predisposing factors** are necessary to allocate nutrients and make the gut environment suitable for the proliferation of these pathogens, enabling them to cause disease (Van Immerseel et al., 2008; Williams, 2005).

1. FEED: composition and particle size are critical

Feed plays a role in the development of Necrotic Enteritis that should not be underestimated. Here, substances creating an intestinal environment favorable for *C. perfringens* must be mentioned.







2. Mycotoxins create ideal conditions for NE

Mycotoxins harm gut integrity and create ideal conditions for the proliferation of *Clostridium perfringens*:

Mycotoxins do not have a direct effect on *C. perfringens* proliferation, toxin production, or NetB transcription. However, mycotoxins disrupt gut health integrity, creating a favorable environment for the pathogen. For example:

- DON provides good conditions for proliferation of *perfringens* by disrupting the intestinal barrier and damaging the epithelium. The possibly resulting permeability of the epithelium and a decreased absorption of dietary proteins can lead to a higher amount of proteins in the small intestine. These proteins may serve as nutrients for the pathogen (Antonissen et al., 2014).
- DON and other mycotoxins decrease the number of lactic acid producing bacteria indicating a

shift in the microbial balance (Antonissen et al., 2016.)

3. Eimeria spp.: forming a perfect team with Clostridium perfringens

An intact intestinal epithelium is the best defense against potential pathogens such as *C. perfringens*. Here, coccidiosis comes into play. Moore (2016) showed that by damaging the gut epithelium, *Eimeria* species give *C. perfringens* access to the intestinal basal domains of the mucosal epithelium. Then, the first phase of the pathological process takes place and from there, *C. perfringens* invades the lamina propria. Damage to the epithelium follows (Olkowski et al., 2008). The plasma proteins leaking to the gut and the mucus produced are rich nutrient sources (Van Immerseel et al., 2004; Collier et al., 2008). A further impact of coccidiosis is shifting the microbial balance in the gut by decreasing the number of e.g., *Candidatus savagella* which activates the innate immune defense.

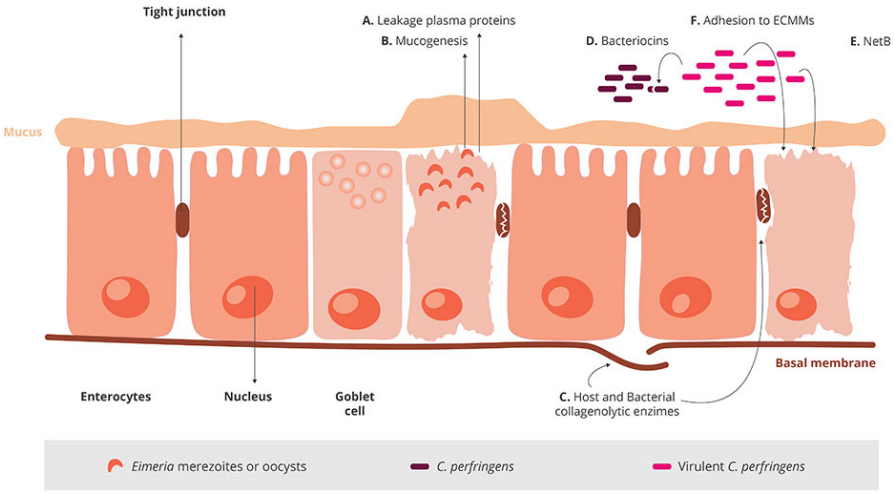


Figure 1

Figure 1:

A. *Eimeria* induce leakage of plasma proteins by killing epithelial cells
B. They enhance mucus production in the intestine

A+B lead to an increase in available nutrients and create an environment favorable for the proliferation of *perfringens*

Not only *Eimeria* Spp., also other pathogens (e.g. *Salmonella* Spp., Ascarid larvae, viruses) and agents, such as mycotoxins damaging the intestinal mucosa can pave the way for a *C. perfringens* infection. Predisposing factors like wet litter, the moisture of which is essential for the sporulation of *Eimeria* Spp. oocysts, must also be considered as promoting factors for Necrotic Enteritis (Williams, 2005).

4. Immunosuppressive Factors: Bacteria, viruses…, and stress

Any factor which induces stress in the animals disrupts the balance of the intestinal flora. The resulting suppression of the immune system contributes to the risk of Necrotic Enteritis (Tsiouris, 2016).

Bacteria

Shivaramaiah and coworkers (2011) investigated a neonatal *Salmonella typhimurium* infection as a predisposing factor for NE. The early infection causes significant damage to the gut (Porter et al., 1998) Additionally, Hassan et al. (1994) showed that the challenge with Salmonella typhimurium negatively impacted the development of lymphocytes which might also promote a colonization of *Clostridium perfringens*.

Viruses

Infectious Bursal Disease is known to increase the severity of infections with salmonella, staphylococci, but also clostridia. Another clostridia-promoting viral disease is Marek's Disease.

Stress:

The intestinal tract is particularly sensitive to any type of stress. This stress can be caused by e.g. too high temperatures, high stocking densities, an abrupt change of feed.

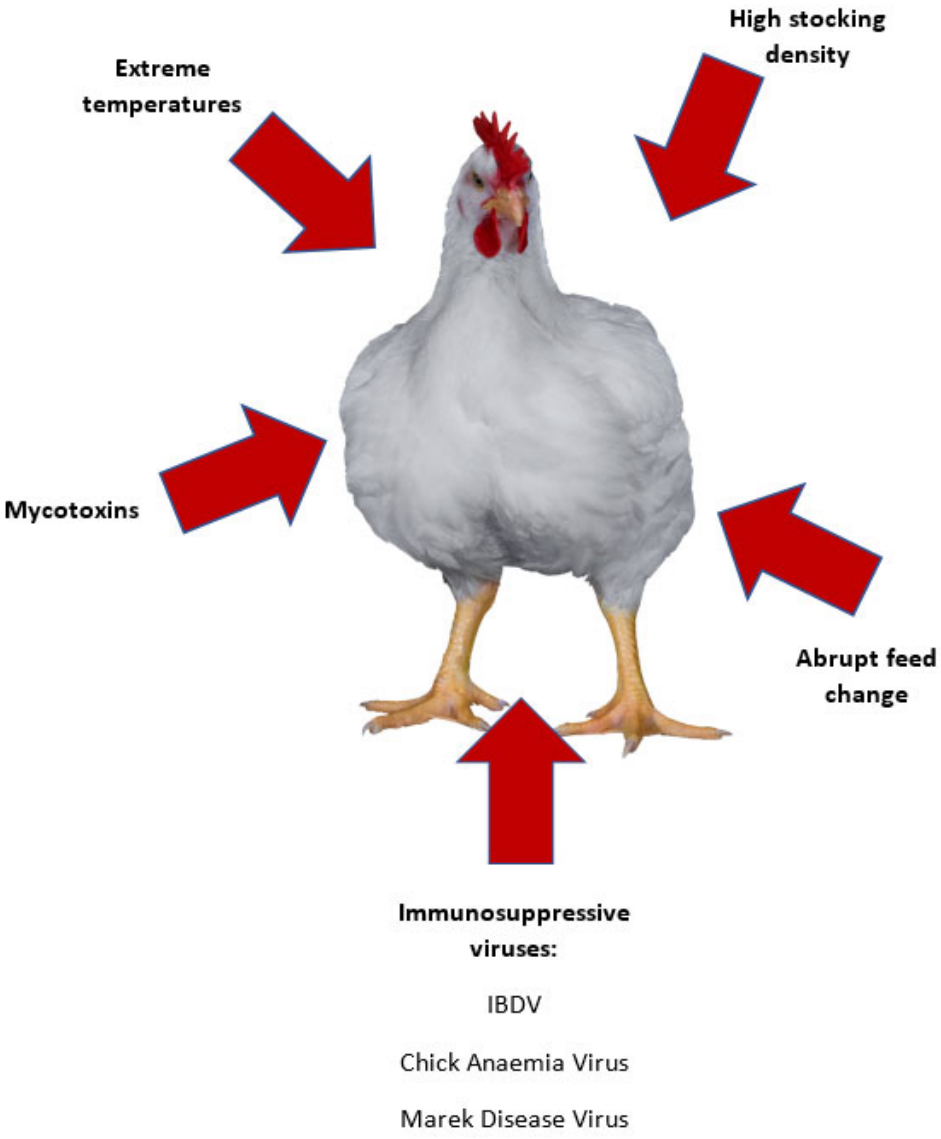


Figure 2: Predisposing factors weakening the birds and enabling Clostridium to attack

Treatment is necessary in the case of acute disease

In this instance, the farmer is obligated to consult a veterinarian and treat his birds.

It must be mentioned that, as the treatment takes place via feed or water, only birds which still consume water or feed may be treated.

Antibiotics are effective but also take a risk

Antibioitics targeting Gram-positive bacteria are commonly used for the treatment of acute NE. The antibiotic choice shall be addressed by a veterinarian, taking into account the mode of action and the presence of resistance genes in the farm/flock.

The profilactic use of antibiotics is not recommened and many countries have already banned it in order to reduce antimicrobial resistance (AMR).

Antimicrobial Resistance (AMR)

Some bacteria are less sensitive to certain antibiotics due to genetic mutations. They are able to:

- stimulate the production of enzymes, which break down or modify the antibiotics and inactivate them (1).
- eliminate entrances for antibiotics or promote the development of pumps, which discharge the antibiotic before taking effect (2).
- change or eliminate molecules to which the antibiotic would bind (targets for the antibiotics).

This means that, when the corresponding antibiotics are used, bacteria resistant against these antibiotics survive. Due to the fact that their competitors have been eliminated they are able to reproduce better.
Additionally, this resistance may be transferred by means of “resistance genes”

- to daughter cells
- via their intake from dead bacteria (3)
- through horizontal gene transfer (4)
- through viruses (5)

Every application of antibiotics promotes the development of resistance (Robert Koch Institute, 2019). A short-term use, better biosecurity, or an application at low dosage give the bacteria a better chance to adapt.

Bacteriophages would be possible but are still disputed

Experimental use of phage treatments has shown to be effective in reducing disease progression and symptoms of Necrotic Enteritis (Miller et al., 2010). By oral application of a bacteriophage cocktail, Miller and coworkers could reduce mortality by 92% in *C. perfringens*-challenged broilers compared to the untreated control.

Mode of action: the endolysins, highly evolved enzymes produced by bacteriophages, are able to digest the bacterial cell wall for phage progeny release (Fischetti, 2010). However, phages are still not approved by the EFSA.

Excurs:

Antimicrobial Resistance (AMR) Some bacteria are less sensitive to certain antibiotics due to genetic mutations. They are able to:

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- change or eliminate molecules to which the antibiotic would bind (targets for the antibiotics) (figure 3)

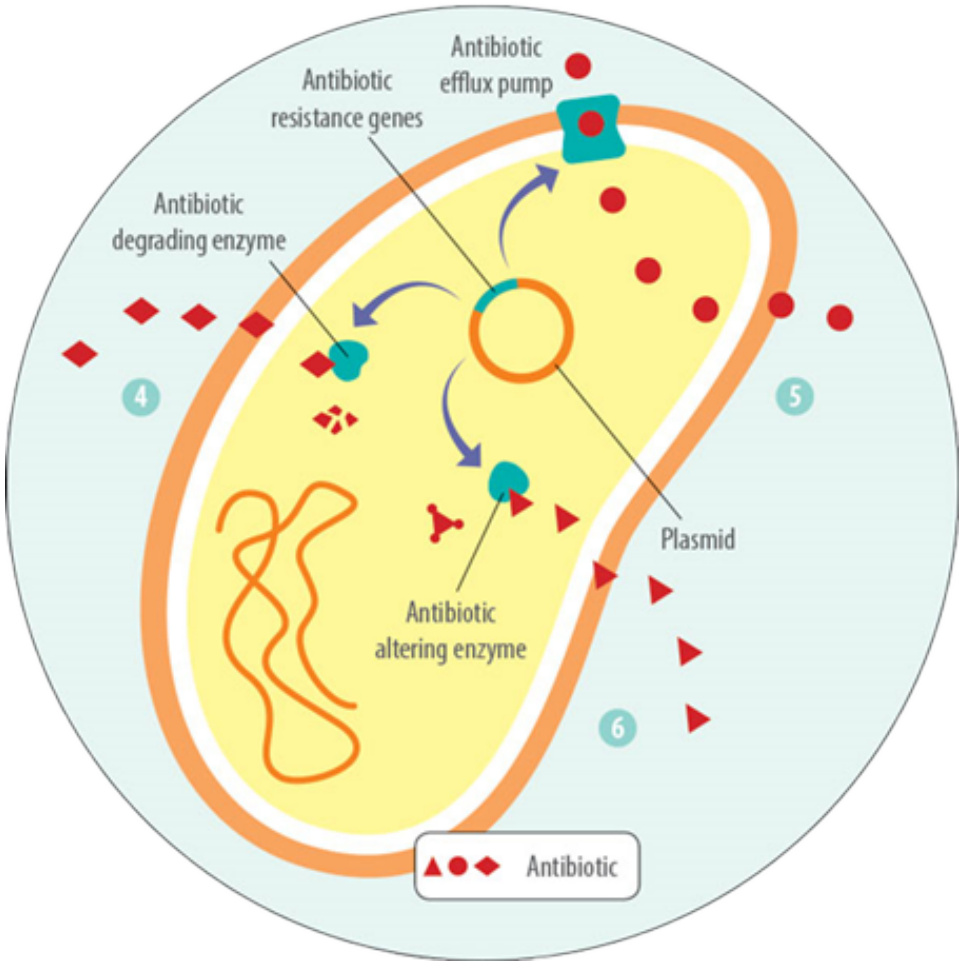

Figure 3: Possibilities of a bacterial cell to defend itself against antibiotics

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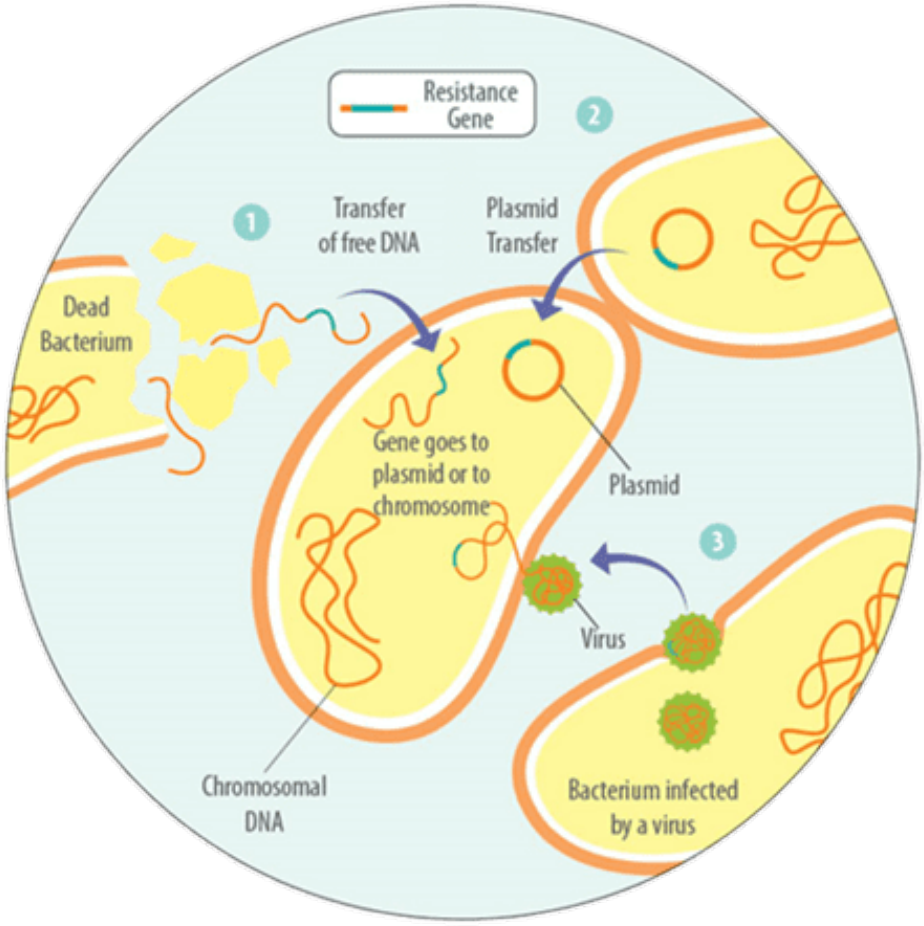


Figure 4: Possibilities to transfer resistance to other bacterial cells

Every application of antibiotics promotes the development of resistance (Robert Koch Institute, 2019). A short-term use or an application at low dosage give the bacteria a better chance to adapt.

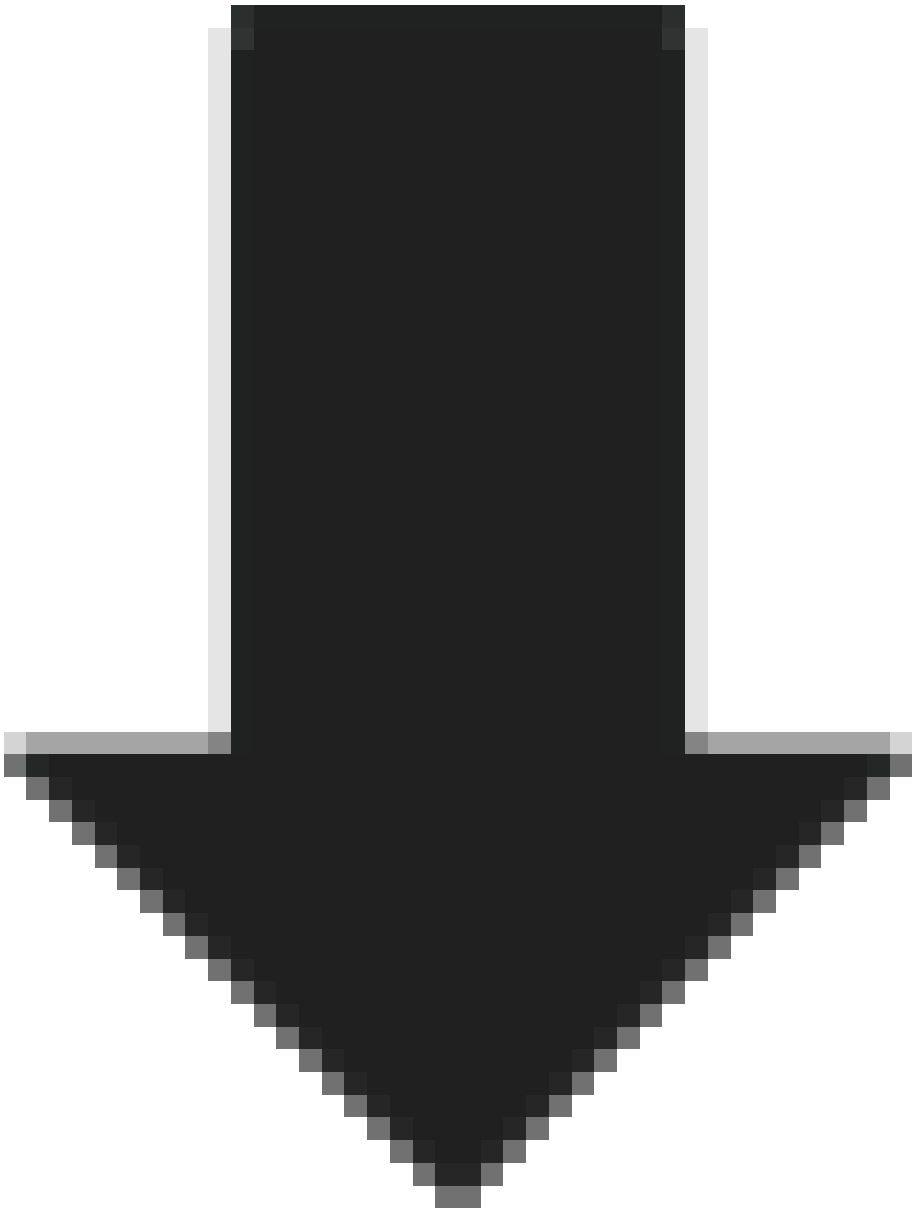
Preventing a disease is always better than its treatment!

But how to do it? Preventing the conditions that favor the proliferation of Clostridium perfringens and strengthening the host's immune response lowers the probability of disease. Besides eliminating the predisposing factors, the main targets are:

- **Balance of the gut flora**
- **Optimization of gut function and integrity**
- **Maintenance of immunity**

1. Biosecurity is of the highest importance!

There is evidence that most *Clostridium* strains isolated from birds suffering from Necrotic Enteritis could induce the disease experimentally, while strains isolated from healthy birds cannot. This confirms that only specific strains are problematic (Ducatelle and Van Immerseel, 2010).
So, it's of highest importance to avoid introducing these pathogenic strains to the farm.



Strict biosecurity measures!

Separate clothing, boots, and hand washing/disinfecting facilities in each poultry house
More than 14 days of down time between flocks

2. Specific measures against coccidiosis

1. **Vaccination**

According to parasitologists, 7 to 9 *Eimeria* species are found in chickens, and they do not cross-protect against each other. An effective vaccination must contain sporulated oocysts of the most critical pathogenic *Eimeria* species (*E. acervulina, E. maxima, E. tenella, E. necatrix, and E. brunetti*). The more species contained in the vaccine, the better. However, if not applied the correct way, vaccines can be ineffective or cause reactions in the birds that might lead to NE (Mitchell, 2017).

2. **Anticoccidials**

Alternate use of chemicals (synthetic compounds) and ionophores (polyether antibiotics) with different modes of action is important to avoid development of resistance.

Ionophores have a specific mode of action and kill oocysts before they are able to infect birds. Being very small, ionophore molecules can be taken up and diffused into the outer membrane of the sporozoite. There, it decreases the concentration gradient leading to an accumulation of water within the sporozoite causing its bursting.

3. Diet – favorable for the birds but not for clostridium!

Minimizing non-starch polysaccharides (NSPs) in cereals

To prevent a “feeding” of *Clostridium perfringens*, high content of water-soluble but indigestible NSPs such as wheat, wheat by-products, and barley should be avoided or at least minimized. Additionally, xylanases should be included in the feed formulation to reduce the deleterious effects of NSPs and improve feed energy utilization. Instead of these cereals, maize could be included in the diet. It is considered a perfect ingredient in broiler diets due to its high energy content and high nutrient availability.

Formulating low protein diets/diets with highly digestible amino acids

Feeding low-protein diets supplemented with crystalline amino acids might be beneficial to reduce the risk of Necrotic Enteritis (Dahiya et al., 2007). To improve protein digestibility and therefore reduce the proliferation of *C. perfringens*, proteases may be added to the feed.

Avoiding/Minimizing animal fats in the diet

Animal fats tend to increase the counts of *Clostridium perfringens*; thus, they should be replaced by vegetable fat sources.

Feed form is decisive

In terms of feed form, Engberg et al. (2002) found that birds fed pellets showed a reduced number of *Clostridium perfringens* in the caeca and the rectum than mash-fed birds. Branton and coworkers (1987) reported a lower mortality by feeding roller-milled (coarsely ground) than hammer-milled feed.

4. Additives

Additives can be used either to prevent the proliferation of *Clostridium perfringens* or to change the environmental conditions in a way that proliferation of *C. perfringens* is prevented.

1. Probiotics directly support the balance of the microbiome

These live microbial supplements can be used to help to establish, maintain or re-establish the intestinal microflora.

Mode of action:

They compete with pathogenic bacteria for substrates and attachment sites and produce antimicrobial substances inhibiting the growth of pathogenic bacteria (Gillor et al., 2008). They bind and neutralize enterotoxins (Mathipa and Thantsha, 2017) andpromote immune function of the host (Yang et al., 2012)

2. Prebiotics indirectly promote the microbiome

These feed ingredients serve as substrates to promote beneficial bacteria in the intestine.

Mode of action:

D-mannose or fructose, starches non-digestible by birds, selectively stimulate the growth and the activity of the “good” gut flora. Fructooligosaccharides decrease C. perfringens and E. coli in the gut and increase the diversity of Lactobacillus Spp. (Kim et al., 2011). Galactooligosaccharides, in combination with a B. lactis-based probiotic, have been reported to selectively promote the proliferation of Bifidobacterium Spp. (Jung et al., 2008).

3. Organic acids support gut health

Organic acids are often used in animal diets to improve intestinal health.

Mode of action:

A decreased pH promotes beneficial bacteria. Caprylic acid suppresses *C. perfringens* but also *Salmonella spp*. by inhibiting their utilization of glucose (Skrivanova et al., 2006). Lauric, citric, oleic, and linoleic acid, as well as medium-chain fatty acids (C8-C14), impede the growth of *C. perfringens.*

A trial with different organic acid products showed high efficacy for Acidomix AFG and Acidomix AFL against *Clostridium perfringens* as well as against *Salmonella enterica.* For the test, 50 µl solution containing different microorganisms (reference strains of S. enterica and C. perfringens; conc. 10^5 CFU/ml) together with 50 µl of increasing concentrations of various organic acids/organic acid products (Acidomix) were pipetted into microdilution plates. After the respective incubation, the MICs of every organic acid/organic acid product were calculated.

Figure 5 shows the minimum inhibiting concentrations (MIC). For Acidomix AFL and AFG, lower concentrations than for fumaric, lactic, and propionic acid were needed to inhibit the growth of *Salmonella en*terica and *Clostridium perfringens*.

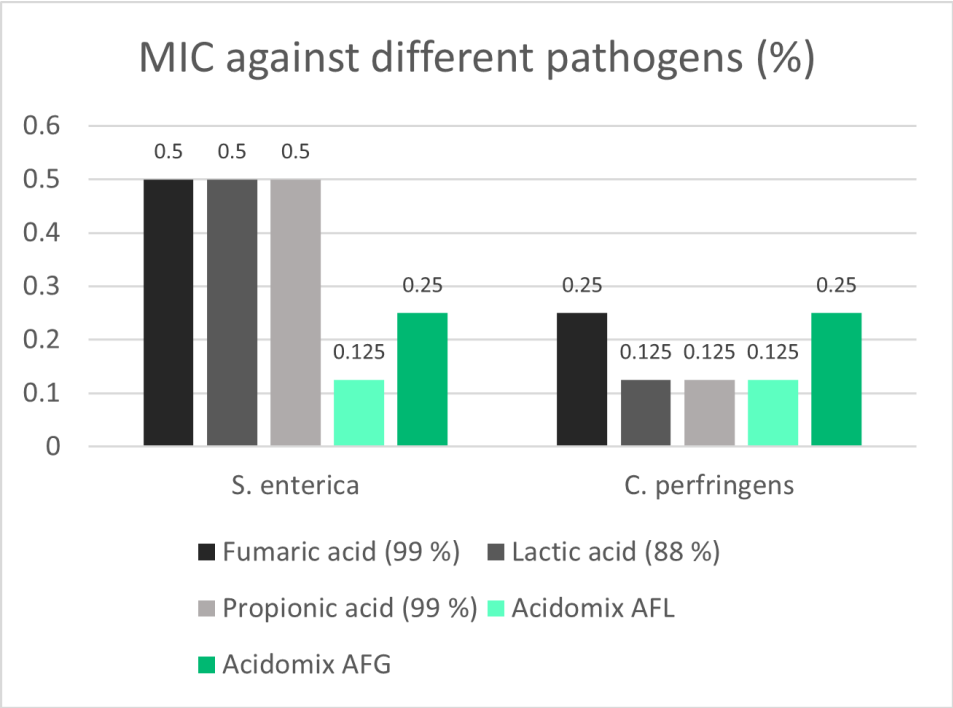


Figure 5: Minimal inhibiting concentrations of Acidomix AFL and Acidomix AFG against Salmonella enterica and Clostridium perfringens

Phytomolecules : different types are available against NE

Phytomolecules, also known as secondary plant compounds, have been used against pathogens for centuries. In general, two subgroups of these substances are known as effective against Clostridium perfringens: Tannins and Essential Oils.

Tannins

Many studies have shown the efficacy of tannins against different pathogens such as helminths, Eimeria spp., viruses, and bacteria. Extracts from the chestnut and quebracho trees are effective not only against *C. perfringens* but also its toxins (Elizando et al., 2010). Tannins act against Eimeria spp. (Cejas et al., 2011) and Salmonella Sp., two predisposing factors for NE.

A trial was conducted with Pretect D, a product based on tannins and saponins, to show its efficacy against coccidia, one of the predisposing factors of NE. For the 35-day study conducted at a commercial research facility in the US, 1800 one-day-old Cobb 500 broilers were divided into four groups of 450 birds each (with 9 replicates & 50 birds per replicate). They all received the standard feed of the farm (Starter D0-D21, Grower D22-D35).

The challenge was given in the form of a freshly prepared mixed inoculum with *E. acervulina* (100,000 oocysts/ bird), *E. maxima* (50,000 oocysts/ bird), and *E. tenella* (75,000 oocysts/ bird). The inoculum was mixed into the feed in the base of each pen's tube feeder.

The oocyst count per gram of feces (OPG) was done on D21, D27 & D35. The cocci lesion scoring (CLS) was done on D27 following Johnson and Reid (1970) with 0=normal; 4=most

Group	Challenge	Additive
Non-challenged Control (NC)	No	No
Challenged Control (CC)	Yes	No
CC + Ionophore	Yes	Ionophore@60ppm
CC + Pretect D	Yes	Pretect D@500ppm

The trial showed that, due to Pretect D, the lesion score showed a lower value indicating that lesions could be reduced or were less severe, which can be seen in figure 6:

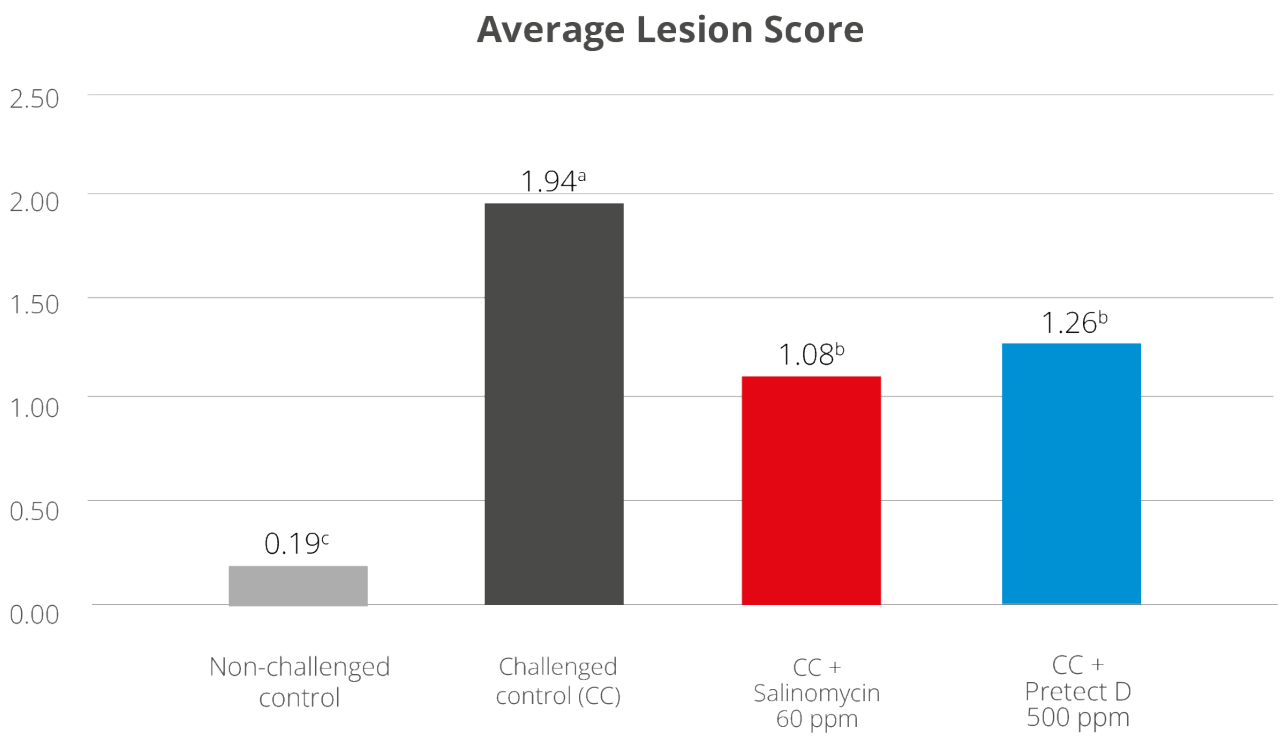


Figure 6: Average lesion score

Essential Oils

Their hydrophobic characteristic enables them to interact with the lipids of the membrane of C. perfringens. They can incorporate into the bacterial membrane and disrupt its integrity, increasing the permeability of the cell membrane for ions and other small molecules such as ATP and leading to the decrease of the electrochemical gradient above the cell membrane and the loss of the cell's energy equivalents. Besides their direct effect on *Clostridium spp*., many phytomolecules improve gut health and help prevent the proliferation of *Clostridium spp.* And, therefore, Necrotic Enteritis.

An In vitro-trial shows Ventar D reducing Clostridia and sparing the beneficial lactobacilli. In this trial, the bacteria (Clostridium perfringens, Lactobacillus agilis S73, and Lactobacillus plantarum) were cultured under favorable conditions (RCM, 37°C, anaerobe for Clostridium perfringens, and MRS, 37°C, 5 % CO_2 for Lactobacilli) and exposed to different concentrations of Ventar D (0 µg/ml – control, 500 µg/ml, 750 µg/ml, and 1000 µg/ml).

The results of the trial with Clostridium perfringens are shown in figure 7.

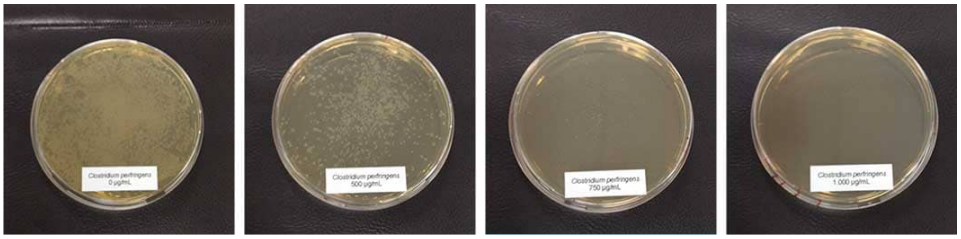


Figure 7: Different concentrations of Ventar D added to Clostridium perfringens cultures

Here, a significant reduction of colonies could already be observed at a concentration of 500 µg/ml of Ventar D. With 750 µg/ml, only a few colonies remained, and at a Ventar D concentration of 1000 µg/ml, Clostridium perfringens didn't grow anymore.

In contrast, the Lactobacilli showed a different picture: only at the higher concentration (1250 µg/ml of Ventar D) did Lactobacillus plantarum and Lactobacillus agilis S73 show a slight growth reduction (figure 8).

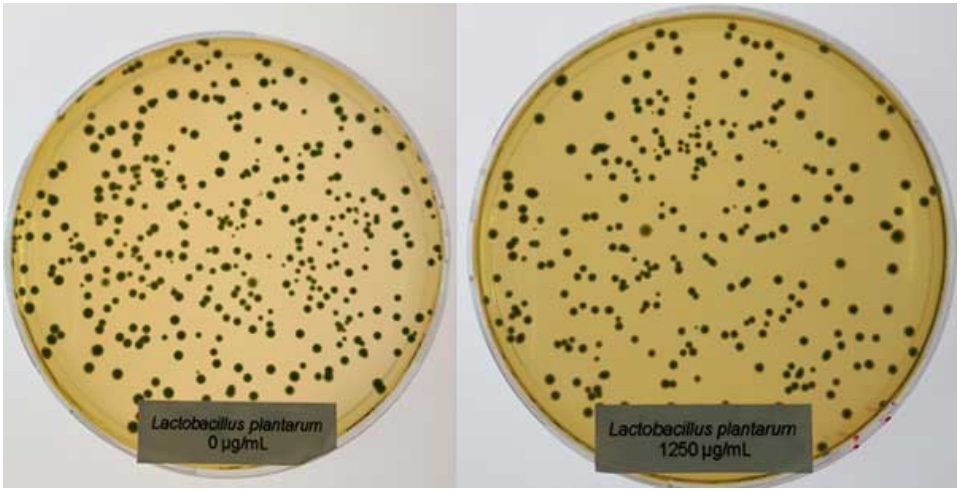


Figure 8: Lactobacillus plantarum exposed to 0 (left) and 1250 µg/ml (right) of Ventar D

1. In vivo-trial in poultry shows that phytomolecules reduce gut lesions

The study was conducted at Southern Poultry Feed & Research, Athens, GA (USA), over 42 days. It included in total 880 Cobb 500 broilers across 2 trial groups, with 11 repetitions per trial set-up and 40 animals per replicate floor pen. All animals received routine vaccinations at the hatchery and were healthy when starting the trial.

Control group	Built-up litter (no additive)
Ventar D group	Built-up litter + Ventar D, 100 g/MT of feed

All birds received standard feed, fed as crumbles/pellets ad libitum. Feed intake by pen was recorded per feeding phase for starter (D21), grower (D35), and finisher feed (D42). Bird weights were recorded at study initiation, on D21, D35, and D42. On D21 and D35, three birds per pen were sacrificed. The GIT was scored for necrotic enteritis lesions; figures 9 and 10 show the results.

Figures 9 and 10: Lesion score on days 21 and 35

Already on day 21, the birds of the Ventar D group showed a less impacted gut mucosa, indicated by a lower lesion score. Lesions were reduced in both groups until day 35; however, the value of the Ventar D group was still better.

A less impacted gut has a higher digestion and absorption capacity, which results in better performance (FCR and weight gain) and lower mortality (figures 11-14).

Figures 11-14: Performance data of a control group compared with birds supplemented with Ventar D

The two trials show that Ventar D allows the poultry producer to proactively strengthen broilers' gut health by controlling Clostridia perfringens and promoting/saving beneficial bacteria such as lactobacilli. The effects of the reduction of Clostridia can be seen in vivo in a lower lesion score and better performance.

Toxin binders adsorb bacterial and mycotoxins

These binders have two modes of action:

They bind mycotoxins and, therefore, reduce or prevent damage to the intestinal wall so that the preconditions for *Clostridium* spp. proliferation are not generated.

Additionally, binding toxins produced by *Clostridium perfringens* can reduce the occurrence or severity of lesions: Alpha-toxin, phospholipase C, hydrolyses membrane phospholipids and damages erythrocytes, leucocytes, myocytes, and endothelial cells and causes their lipolysis (Songer, 1996), leading to necrosis and tissue damage.

Binding NetB toxin, the key virulence factor, could reduce the severity of Necrotic Enteritis.

A trial was conducted in a laboratory in Valladolid/Spain to show the high binding capacity of Solis Plus 2.0. All tests were carried out as duplicates and using a standard liquid chromatography/mass spectrometry (LC/MS/MS) quantification. Interpretation and data analysis were carried out with the corresponding software. Toxin concentrations, anti-mycotoxin agent application rates, and pH levels were set as follows:

Mycotoxin	Challenge Level	Challenge (ppb)	Solis Plus 2.0 inclusion rate	Assay time
Aflatoxin	Low	150	0.2%	30 min.
	High	1500		
Fumonisin	Low	500		
	High	5000		
Ochratoxin	Low	150		
	High	1500		

The results are shown in figure 15:

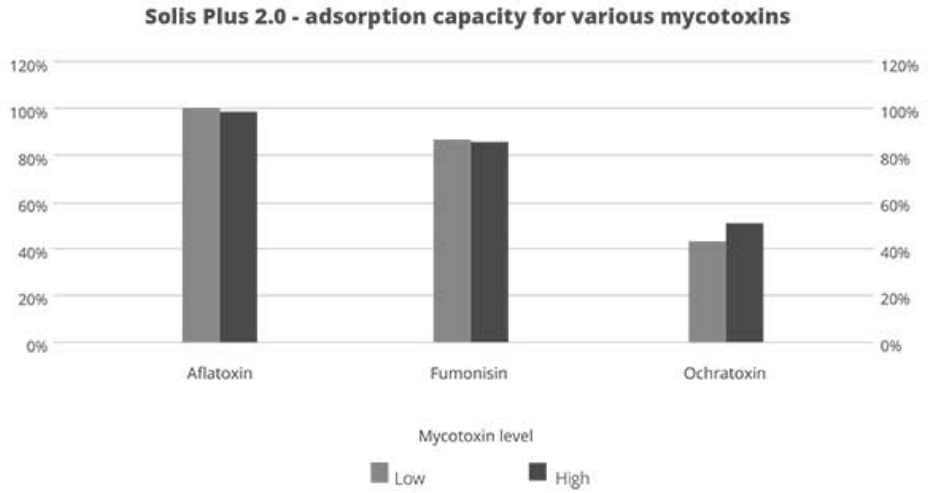


Figure 15: Adsorption capacity of Solis Plus for relevant mycotoxins

Under acidic conditions (pH 3), Solis Plus 2.0 effectively adsorbs the three tested mycotoxins at low and high contamination levels:

- Aflatoxin: 150 ppb -100 %; 1500 ppb – 98 %
- Fumonisin: 500 ppb – 87%; 5000 ppb – 86 %
- Ochratoxin: 150 ppb – more than 43 %; 1500 ppb – 52 %.

By binding harmful toxins and preventing their negative impact on the gut, toxin binders can also be a tool to reduce necrotic enteritis.

NE can be controlled – even in an antibiotic-free era

The ever-growing trend of reduced antibiotic and ionophore use increases the incidence of Necrotic Enteritis in poultry production. Especially the subclinical form, which generally goes unnoticed, results in poor feed efficiency and is a major cause of financial losses to poultry producers.

Maintaining optimum gut health is key to preventing the occurrence of Necrotic Enteritis. In the era of antibiotic-free poultry production, alternatives acting against the pathogenic bacterium and also against its predisposing factors must be considered to control this devastating disease. The industry already provides solutions like phytomolecules-based products or toxin binders to support the animals.

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Challenging times for broilers? Phytomolecules, not antibiotics, are the answer

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Anyone working with today's fast-growing broiler chicken knows that it is a sensitive creature – and so is its gut health. Thanks to continuous improvements in terms of genetics and breeding, nutrition and feeding, as well as general management strategies, broiler production has tremendously upped performance and efficiency over the past decades. It is estimated that, between 1957 and 2005, the broiler growth rate increased by over 400%, while the feed conversion ratio dropped by 50%.

These impressive improvements, however, have come at the cost of intense pressure on the birds' digestive system, which needs to process large quantities of feed in little time. To achieve optimal growth, a broiler's gastrointestinal tract (GIT) needs to be in perfect health, all the time. Unsurprisingly, enteric diseases such as necrotic enteritis, which severely damages the intestinal mucosa, hamper the intestines' capacity to absorb nutrients and induce an inflammatory immune response.

The modern broiler's gut – a high-performing,

but sensitive system

However, in a system as high performing as the modern broiler's GIT, much less can lead to problems. From when they are day-old chicks up to slaughter, broilers go through several challenging phases during which they are more likely to show impaired gut functionality, e.g. after vaccinations or feed changes. Good management practices go a long way towards eliminating unnecessary stressors for the animals, but some challenging periods are unavoidable.

The transition from starter to grower diets is a classic situation when nutrients are very likely to not be well digested and build up in the gut, fueling the proliferation of harmful microbes. Immunosuppressive stress in combination with an immature intestinal microflora results in disturbances to the bacterial microbiota. At "best", this entails temporarily reduce nutrient absorption, in the worst case the birds will suffer serious intestinal diseases.

Phytomolecules – the intelligent alternative to antibiotics

To safeguard performance during stressful periods, poultry producers need to anticipate them and proactively provide effective gut health support. For many years, this support came in the form of antibiotic growth promoters (AGP): administered prophylactically, they were effective at keeping harmful enteric bacteria in check. However, due to grave concerns about the development of antimicrobial resistance, non-therapeutic antibiotics use has been banned in many countries. Alternatives need to focus on improving feed digestibility and strengthening gut health, attacking the root causes of why the intestinal microflora would become unbalanced in the first place.

Phytomolecules are secondary metabolites active in the defense mechanisms of plants. Studies have found that certain phytomolecules stimulate digestive enzyme activities and stabilize the gut microflora, "leading to improved feed utilization and less exposure to growth-depressing disorders associated with digestion and metabolism" (Zhai et al., 2018). With other trials showing positive effects on broilers' growth performance and feed conversion, the research indicates that phytomolecules might also specifically support chickens during challenging phases.

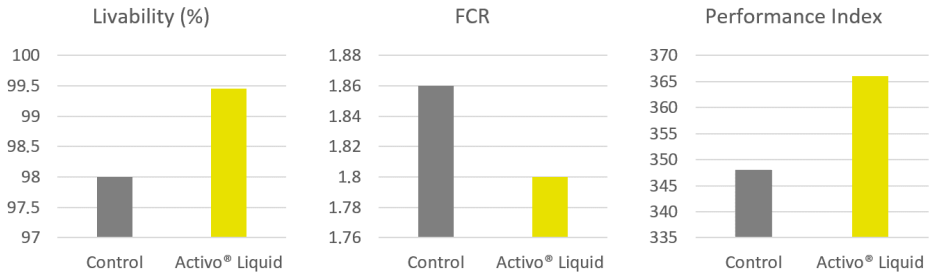
The effect of phytomolecules on broilers during a challenging phase

A study was conducted over a period of 49 days on a commercial broiler farm of an AGP-free integration operation in Japan. The farm reported gut health challenges in the second and third week of the fattening period due to vaccinations and changes to the animals' diets. The trial included 15504 Ross 308 broilers, divided into two groups. The negative control group included a total of 7242 birds, kept in another house.

All the birds were fed the standard feed of the farm. The trial group (8262 birds) received Activo® Liquid, which contains a synergistic combination of phytomolecules, administered directly through the drinking water. Activo® Liquid was given at an inclusion rate of 200ml per 1000L of water (3.3 US fl oz per gallon of stock solution, diluted at 1:128), from day 8 until day 25, for 8 hours a day.

The results are summarized in Figure 1:

Figure 1: Improved broiler performance for Activo® Liquid group (day 49)



The Activo® Liquid group clearly showed performance improvements compared to the control group. Livability augmented by 1.5%, while the feed conversion rate improved by 3.2%. This resulted in a more than 5% higher score in terms of the performance index.

Challenging times? Tackle them using phytomolecules

Poultry producers take great care to eliminate unnecessary sources of stress for their birds. Nonetheless, during their lifecycle, broiler chickens face challenging periods during which the balance of the intestinal microflora can easily become disturbed, with consequences ranging from decreased nutrient absorption to full-blown enteric disease.

The trial reviewed here showed that, after receiving Activo® Liquid, broilers raised without AGPs showed encouraging performance improvements during a challenging phase of feed changes and vaccinations. Likely thanks to the activation of digestive enzymes and a stabilization of the gut flora, the broilers showed improved livability and feed conversion, thus delivering a much more robust performance during a critical phase of their lives. In times where the non-therapeutic use of antibiotics is no longer an option, phytomolecules allow poultry farmers to effectively support their animals during challenging times.

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Photo Source: Aviagen

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