Necrotic enteritis: The complete overview

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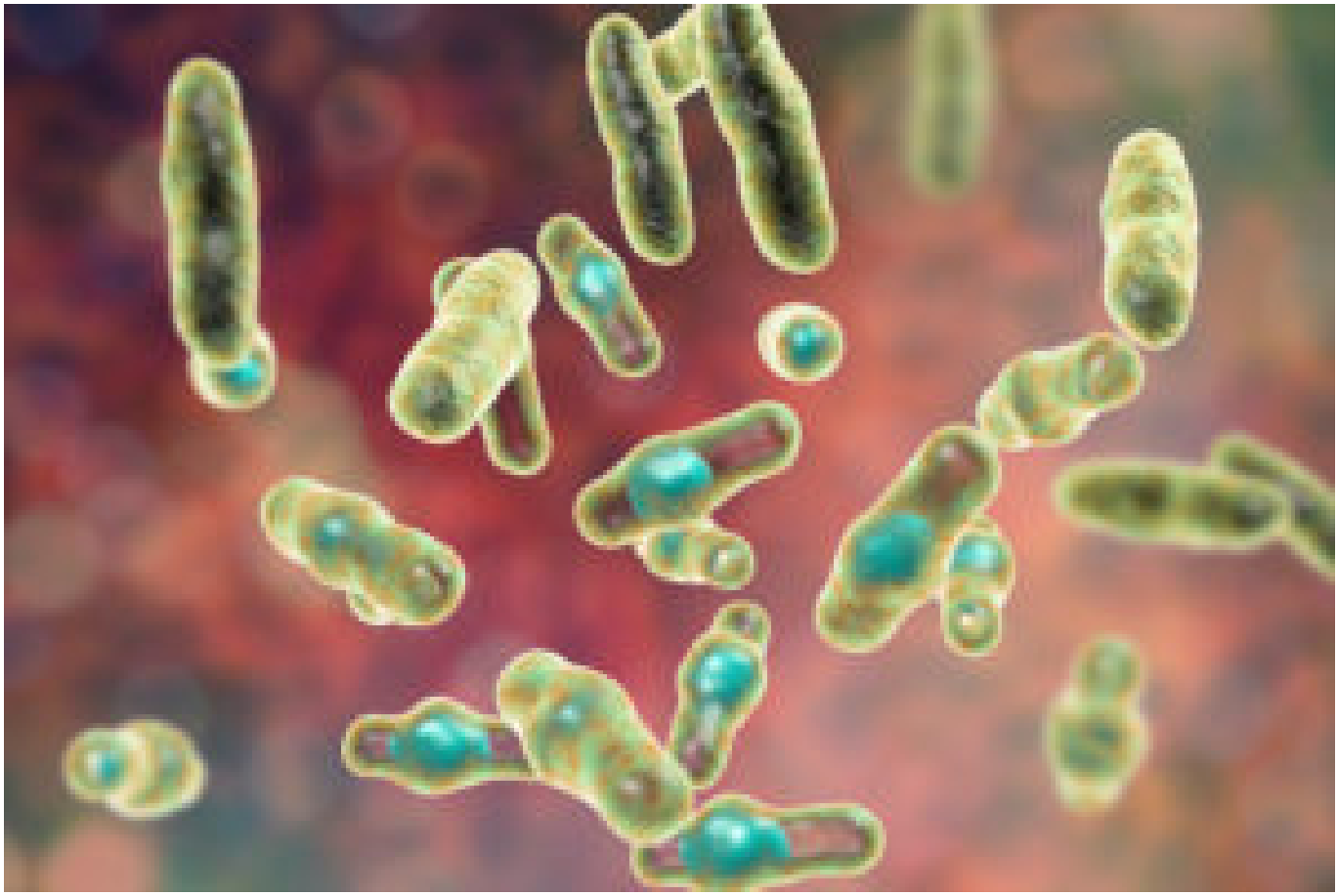
Eliminating necrotic enteritis from your operations starts from a good understanding of what it is, how to prevent it, and how to mitigate its effects on your poultry production.



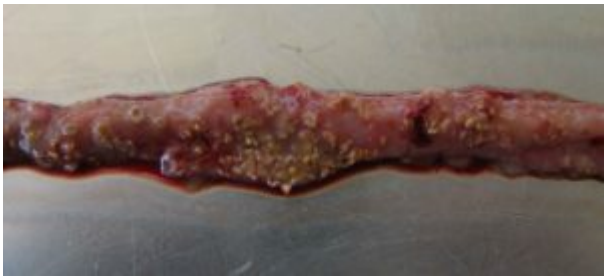
Necrotic enteritis is a poultry disease caused by an overgrowth of *Clostridium perfringens* type A, and to a lesser extent type C, in the small intestine. The toxins produced by *C. perfringens* also damage the intestinal wall. In general, it occurs in broiler chickens of 2-6 weeks of age. In subclinical forms, it is characterized by impaired digestion. Clinical forms lead to severe problems and increased flock mortality in a very short time.

Necrotic enteritis is the cause of USD 6 billion annual losses in global poultry production and this controllable disease is on the rise. One reason is the voluntary or legally required reduction of antibiotics in animal production. This trend is driven by the increasing occurrence of antimicrobial resistance, as well as by consumer demand. Another reason is the reduction of ionophores which, besides their activity against coccidia, also show efficacy against clostridia. When anticoccidial live vaccines are used, the application of these ionophores is not possible and clostridia / necrotic enteritis increase (Williams, 2005).

While this is a widespread problem in all poultry, for broilers in particular, necrotic enteritis and coccidiosis are the most significant health problem.

Clinical and subclinical forms of NE

The clinical form



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...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 mucosal surface, lesions, and brownish (diphtheritic) pseudo-membranes. 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

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.

Pathogens

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 the gastrointestinal tract of animals and humans. These bacteria produce spores, which are extremely

resistant to environmental impact (heat, irradiation, exsiccation) as well as 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).

NetB, 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, yet 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 impacted (Los et al., 2013).

Additionally, pathogenic strains of *C. perfringens* produce bacteriocins – the most important being 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)

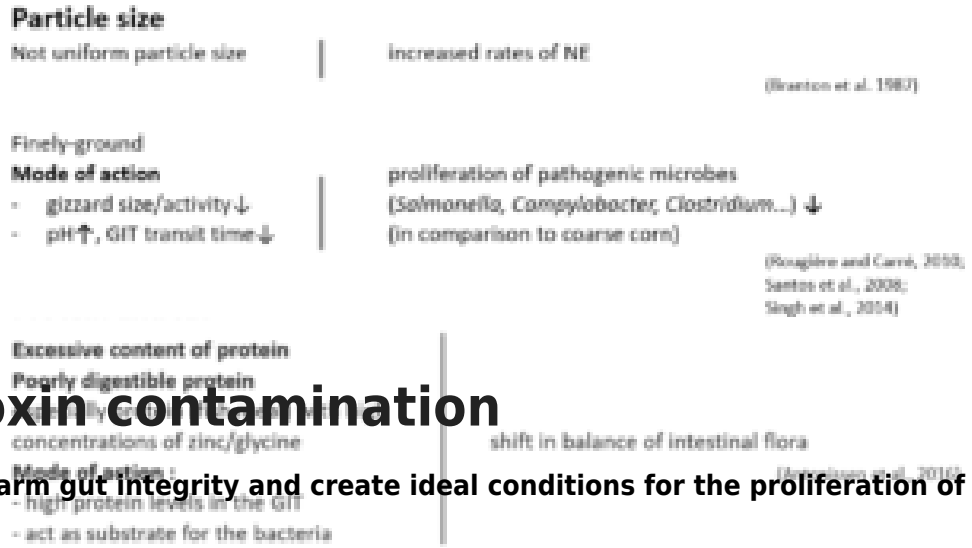
4. Strategies of evasion
Enable the pathogen to bypass the strategies of defense of the immune system (e.g. antiphagocytosis factors provide protection against an attack by phagocytes; specific antibodies are inactivated by enzymes).

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).

Predisposing factors

Feed: composition and particle size

The role of feed in the development of necrotic enteritis should not be underestimated. This is where substances creating an intestinal environment favorable for *C. perfringens* come into play.



Mycotoxin contamination

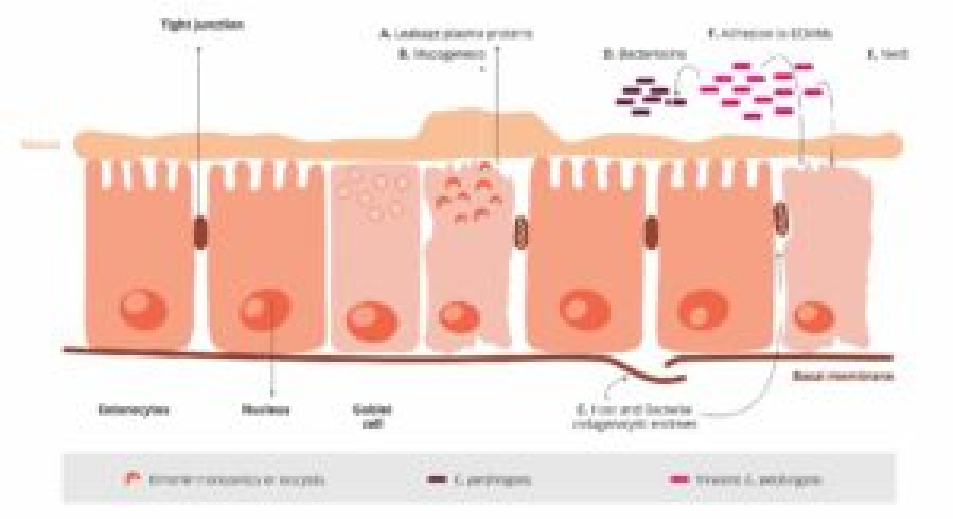
Mycotoxins harm gut integrity and create ideal conditions for the proliferation of *Clostridium perfringens*.

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:

1. DON provides good conditions for proliferation of *C. 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).
2. DON and other mycotoxins decrease the number of lactic acid producing bacteria indicating a shift in the microbial balance (Antonissen et al., 2016.).

Eimeria ssp.

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.



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

1+2 lead to an increase in available nutrients and create an environment favorable for the proliferation of *C. 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).

Immunosuppressive factors

Besides the already explained influencers feed, mycotoxins and coccidia, also other predisposing factors must be mentioned. In general, 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). These factors include:

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.

Treatment

In acute cases, the farmer should 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

Antibiotics 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 mode of action and the presence of resistance genes in the farm/flock.

The prophylactic 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

Experimental use of phage treatments have 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.

Prevention

Preventing a disease is always better – and more cost-effective – that its treatment.

How, then, should it be done?

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

Biosecurity

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).

It is therefore of the 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

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.

Diet

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 poor quality fats / animal fats in the diet

These fats tend to increase the count of *Clostridium perfringens*; thus, they should be replaced by higher quality and/or vegetable fats, respectively.

Feed form

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 co-workers (1987) reported a lower mortality by feeding roller-milled (coarsely ground) than hammer-milled feed.

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.

Probiotics

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

Mode of action:

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

Prebiotics

- 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* ssp. (Jung et al., 2008).

Organic acids

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

Mode of action:

- 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*

Phytomolecules

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

- Many studies have shown the efficacy of tannins against different pathogens such as helminths, *Eimeria*, 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)
- Activity of tannins against *Eimeria* (Cejas et al., 2011) and *Salmonella* Sp., two predisposing factors for NE.

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.
- This increases the permeability of the cell membrane for ions and other small molecules such as ATP, 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., a lot of phytomolecules improve gut health and help to prevent a proliferation of *Clostridium* ssp. and therefore necrotic enteritis.

Mycotoxin/bacterial toxin binders

These binders have two modes of action:

- Binding mycotoxins, damage of the intestinal epithelium can be reduced or even prevented, so that the preconditions for *Clostridium* proliferation are not generated.
- 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 lysis (Songer, 1996). This leads to necrosis and tissue damage.

- Binding NetB toxin, the key virulence factor, could reduce the severity of necrotic enteritis.

Conclusion

The ever-growing trend of reduced antibiotic and ionophore use is contributing to an increased incidence of necrotic enteritis in poultry production.

The subclinical form of necrotic enteritis generally goes unnoticed, resulting 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 this pathogenic bacterium and also against its predisposing factors must be considered to control this devastating disease.

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