Necrotic Enteritis control for ABF poultry production

By T.J. Gaydos

Control of Necrotic Enteritis (NE) can be one of the most difficult challenges in a system without the availability of antibiotics. In addition, NE is a costly disease because of mortality and loss of performance. Necrotic enteritis is a multifactorial disease that requires damage to the intestinal mucosa, disruption of the intestinal microflora, and a toxin-producing strain of *Clostridium perfringens*. If any one of these three items is removed or lessened, the severity or incidence of NE will be reduced.

The 3 must-haves for antibiotic-free necrotic enteritis control in poultry

1. Prevent mucosal damage

The most common cause of damage to intestinal mucosa in broilers is excessive cycling of *Eimeria maxima*. The ubiquitous nature of this parasite in poultry production makes it one of the most important contributors to NE. This species of coccidia is most relevant with respect to NE because its life cycle invades deeper into tissues than other species leading to more damage to the intestinal mucosa.

The life cycle of coccidiosis lasts roughly seven days, with each cycle producing exponentially higher numbers of the parasite. Three consecutive replication cycles are needed to produce immunity. The biology of *E. maxima* is a significant reason why NE commonly occurs around 18-21 days. However, many other things may damage the intestinal mucosa, including mycotoxins, worms, and rancid fat. Managing all sources of mucosal disruption are critical to preventing and controlling NE.

2. Support the microflora

The importance of the microbiome on health is well known; the ability to modify the microbiome to a more appropriate or healthy status is a more difficult challenge. There is a tremendous volume of research in all species about the impact and importance of intestinal microflora on immunity, health, and disease. The microflora is not static but rather a dynamic community of microorganisms that change with bird age, time of day, composition of the diet, and treatment with antibiotics or other additives. Management of intestinal microflora is a very difficult process because its development and manipulation are not fully understood.

Any significant feed formulation or feed form change is a stress event for intestinal microflora. Feed changes are thus high-risk periods for the development of NE. It is a best practice to avoid feed changes when birds are in the NE risk window. It is important to support the intestinal microflora with either in-feed or in-water products to improve intestinal health during feed changes.

It is important to avoid feed outages. After a feed outage, the disruption to the microflora and the increase in mucus production increases the likelihood of an NE outbreak in the following days. Preemptively adding a water additive to improve intestinal health directly after a feed outage can reduce the risk of NE in the flock.

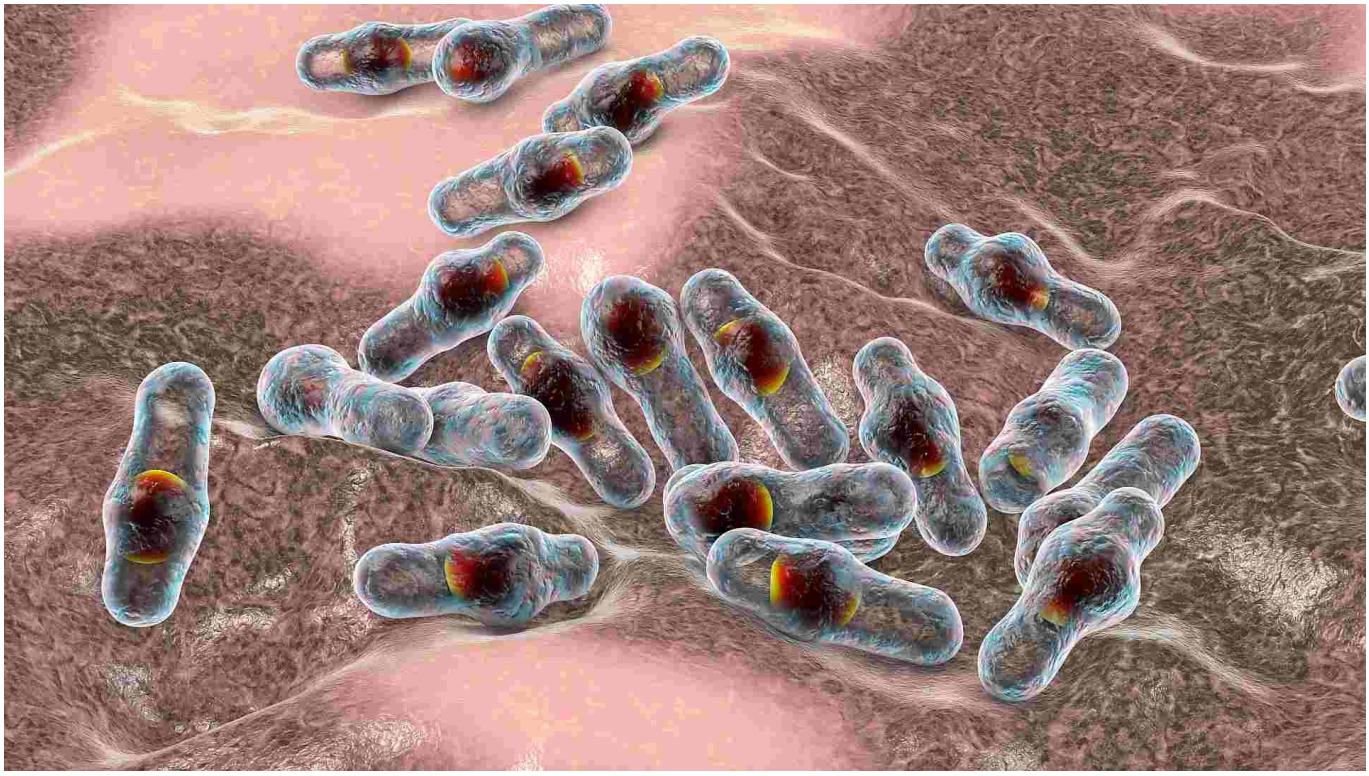
When managing intestinal microflora: probiotics, prebiotics, plant extracts, enzymes, and organic acids are the most commonly used tools. Each of these product classes interacts with the bird and its flora in a different way and selecting additives with complimentary modes of action is critical to the success of the program. Direct colonizing organisms like *Lactobacillus* spp. can help to directly change the microflora, providing a more mature and healthier microbiome.

Prebiotics such as mannan- and fructo-oligosaccharides provide a food source for beneficial microorganisms and can interact directly with the immune system of the bird. Plant extracts can have antimicrobial or anti-inflammatory properties that can also modulate the microflora by impacting the growth and metabolism of different species of microorganisms in the intestine.

3. Limit *Clostridium perfringens* growth

It is not possible to eliminate toxin-producing *C. perfringens* from the environment. Clostridia are spore-forming microorganisms that are very resistant to disinfectants. However, it is possible to manage the abundance of these microorganisms in a system through proper litter management, sanitation, and disposal of mortality.

A house that has a history of NE should have the litter completely removed and the environment cleaned and disinfected as much as the facility will allow. New clean shavings should be brought into the house at a sufficient depth to limit access to the floor. Several non-antimicrobial feed and water additives have shown promise in reducing numbers of *C. perfringens* in feces of infected birds. Feed and water additives are an essential tool to reduce the impact of NE.



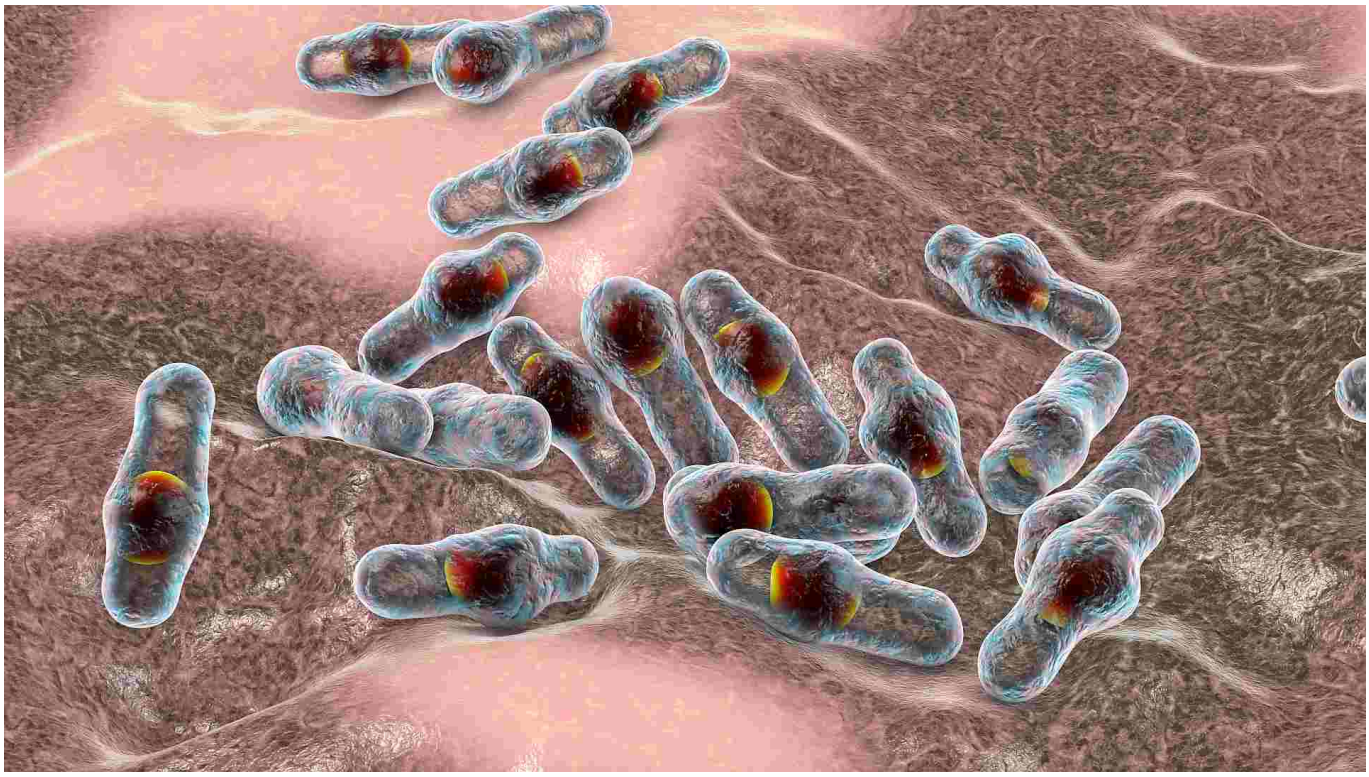
Conclusion: the more you prevent, the less you have to treat

Even with the best management practices, outbreaks of NE will happen. In order to successfully treat a flock with NE, it is critical to catch the mortality early. Once a flock is experiencing high mortality from NE, it is very difficult to treat because the sickest birds will not be drinking enough water to receive a significant amount of water additives. Treating or managing an outbreak is as much art as science, but it is a combination of reducing the inciting causes.

Manage microflora and clostridial growth with organic acids, copper sulfate, phytogenics, or probiotics. Reduce coccidiosis cycling with amprolium, saponins, or other phytogenics. With excellent husbandry, the impact of NE can be reduced drastically even without using antibiotics. Managing NE incidence in poultry is a mixture of animal husbandry, managing coccidiosis cycling, feed and water additive selection, and high-quality nutrition.

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 levels[50]. 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 bacterium[50]. This encapsulated, non-motile microorganism is fastidious in growth requirements[59]. Most often, complex media like cooked meat or thioglycolate broth are used as enrichment[30].

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 Bergey[13].

Clostridium perfringens is the causal microorganism for Necrotic Enteritis (NE)[14]. In humans, it is one of the most common causes of foodborne illness[20]. 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 feed[89].

Additionally, *C. perfringens* is found in the GI tract of broiler chickens, humans, and other mammals[47]. When intestinal samples of broiler chickens were analyzed for *C. perfringens*, 75-95 % tested positive[24]. Drew and co-workers[10] determined that *C. perfringens* is usually found at ~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 origin[37].

Table 1. Different types of *Clostridium perfringens*

		***C. perfringens* 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 minutes[41]. 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 5.5-7.0[22]. 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 anorexia[21]. 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 digesta[10] are normal and can be found in healthy birds. *C. perfringens* becomes problematic when counts reach 10^7-10^8 CFU/g[6].

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 field[48]. Therefore, it was assessed that certain cofactors must play a significant role in the pathogenicity of *C. perfringens*. Williams[57] 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 tract[57].

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 illness[19]. Lovland and co-workers[35] 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 profit[36]. Moreover, sparse lesions that may be found in a case of SNE may be enough to hinder growth performance; thus, resulting in an underproductive flock[39].

Feeding Against Necrotic Enteritis

It has been reported that diet formulation has the greatest impact on the prevalence of *C. perfringens* in chicken GI tracts[61]. 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 micronutrients[1].

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 diet[4]. 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 Kong[51].

Other researchers have concluded that the increase in gut viscosity and increased chyme transit time elicit the overgrowth of *C. perfringens* in the intestines[28]. Grains like wheat and barley contain high amounts of non-starch polysaccharides (NSP), which increase viscosity[26]. 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 consume[31].

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 gut[2]. 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 fermentation[54].

Essential oils alone or in combination may be a solution

Mitsch and coworkers[39] 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 coworkers[15] 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 strategies[36].

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.

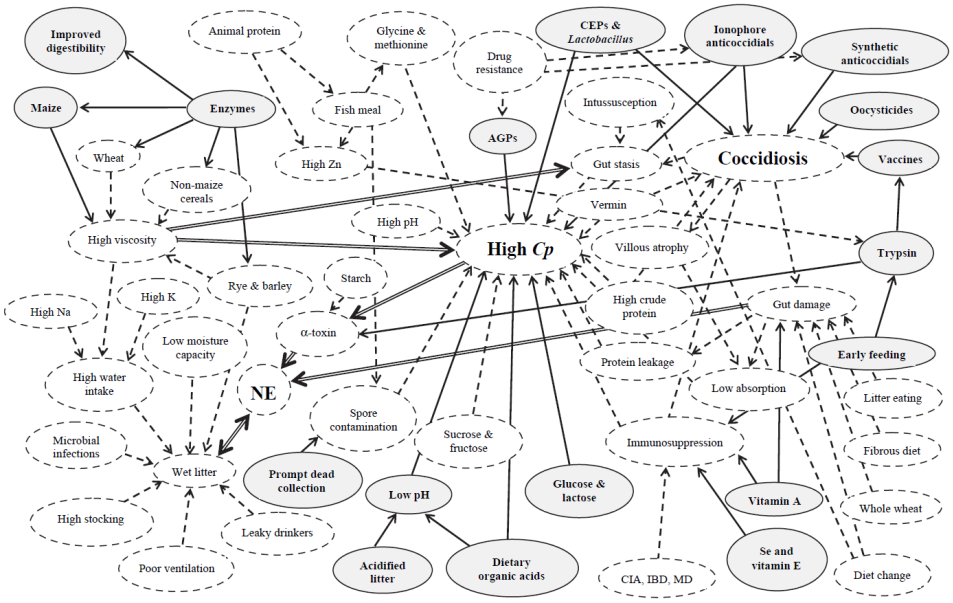


Figure 1. Interaction between coccidiosis and NE with environmental factors

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)

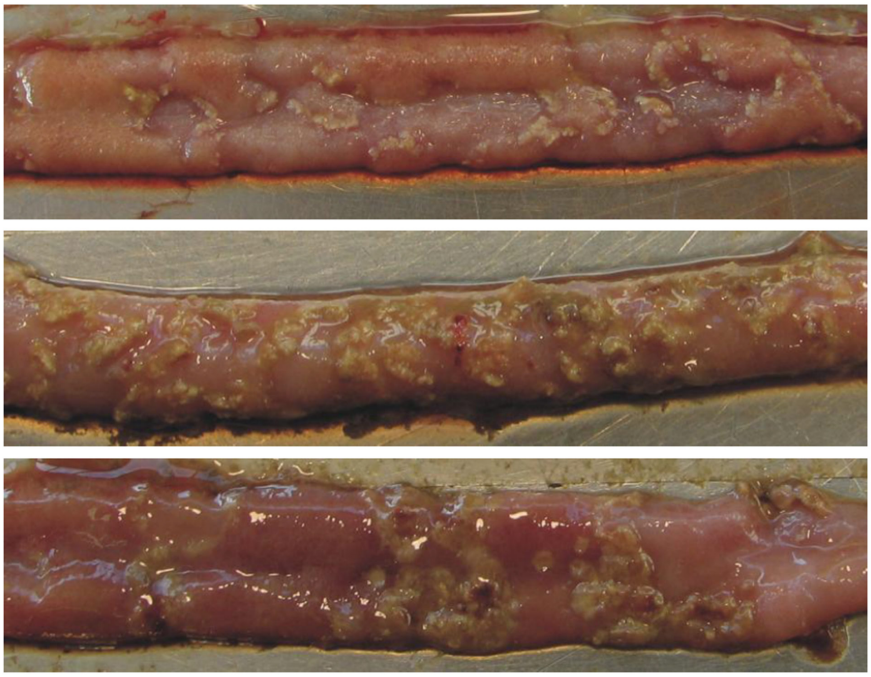


Figure 2. Necrotic Enteritis lesions in chicken intestines

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).

References

1. Bedford, M.R. 1996. Interaction Between Ingested Feed and the Digestive System in Poultry. Applied Poultry Science 5:86-95.
2. Bjerrum, L., K. Pedersen, and R. M. Engberg. 2005. The Influence of Whole Wheat Feeding on Salmonella Infection and Gut Flora Composition in Broilers. Avian Disease 49:9-15.
3. Bolder, N. M., J. A. Wagenaar, F. F. Putirulan, K. T. Veldman, and M. Sommer. 1999. The Effect of Flavophospholipol (Flavomycin^{R}) and Salinomycin Sodium (Sacox^{R}) on the Excretion of *Clostridium perfringens*, *Salmonella enteritidis*, and *Campylobacter jejuni* in Broilers After Experimental Infection. Poultry Science 78:1681-1689.
4. Branton, S. L., B. D. Lott, J. W. Deaton, W. R. Maslin, F. W. Austin, L. M. Pote, R. W. Keirs, M. A. Latour, and E. J. Day. 1997. The Effect of Added Complex Carbohydrates or Added Dietary Fiber on Necrotic Enteritis Lesions in Broiler Chickens. Poultry Science 76:24-28.
5. Choct, M. 2009. Managing Gut Health through Nutrition. British Poultry Science 50:9-15.
6. Cooper, K., and J. G. Songer. 2009. Necrotic Enteritis in Chickens: A Paradigm of Enteric Infection by *Clostridium perfringens* Type A. Veterinary anaerobes and diseases 15:55-60.
7. Craven, S. E., N. J. Stern, N. A. Cox, J. S. Bailey, and M. Berrang. 1999. Cecal Carriage of *Clostridium perfringens* in Broiler Chickens Given Mucosal Starter Culture^{TM}. Avian Diseases 43:484-490.
8. Craven, S. E., N. A. Cox, N. J. Stern, and J. M. Mauldin. 2001a. Prevalence of *Clostridium perfringens* in Commercial Broiler Hatcheries. Avian Diseases 45:1050-1053.
9. Craven, S. E., N. J. Stern, J. S. Bailey, and N. A. Cox. 2001b. Incidence of *Clostridium perfringens* in Broiler Chickens and Their Environment during Production and Processing. Avian Diseases 45:887-896.
10. Drew, M. D., N. A. Syed, B. G. Goldade, B. Laarveld, and A. G. Van Kessel. 2004. Effects of Dietary Protein Source and Level on Intestinal Populations of *Clostridium perfringens* in Broiler Chickens. Poultry Science 83:414-420.
11. Engberg, R. M., M. S. Hedemann, Leser, T.D., and Jensen, B.B. 2000. Effect of Bacitracin and

Salinomycin on Intestinal Microflora and Performance of Broilers. Poultry Science 79: 1311-1319.
12. Engberg, R. M., M. S. Hedemann, and Jensen, B.B. 2002. The Influence of Grinding and Pelleting of Feed on the Microbial Composition and Activity in the Digestive Tract of Broiler Chickens. British Poultry Science 44:569-579.
13. Freeman, B.A. 1979. *Burrows* Textbook of Microbiology. W.B. Saunders Company, Philadelphia, Pennsylvania, USA.
14. Fukata, T., Y. Hadate, E. Baba, T. Uemura, and A. Arakawa. 1988. Influence of *Clostridium perfringens* and its Toxin in Germ-free Chickens. Research in Veterinary Science 44:68-70.
15. Gaucher, M.L., Quessy, S., Letellier, A., Arsenault, J., and M. Boulianne. 2015. Impact of a drug-free program on broiler chicken growth performances, gut health, *Clostridium perfringens* and *Campylobacter jejuni* occurrences at the farm level. Poultry Science 94: 1791-1801.
16. Geier, M. S., L. L. Mikkelsen, V. A. Torok, G. E. Allison, G. C. Olnood, M. Boulianne, R. J. Hughes, and M. Choct. 2010. Comparison of Alternatives to In-feed Antimicrobials for the Prevention of Clinical Necrotic Enteritis. Journal of Applied Microbiology 109:1329-1338.
17. George, B. A., C. L. Quarles, and D. J. Fagerberg. 1982. Virginiamycin Effects on Controlling Necrotic Enteritis Infection in Chickens. Poultry Science 61:447-450.
18. Hatheway, C. L. 1990. Toxigenic Clostridia. Clinical Microbiology Reviews 3:66-98.
19. Heier, B. T., A. Lovland, K. B. Soleim, M. Kaldhusdal, and J. Jarp. 2001. A Field Study of Naturally Occurring Specific Antibodies against *Clostridium perfringens* Alpha Toxin in Norwegian Broiler Flocks. Avian Diseases 45:724-732.
20. Heikinheimo, A., M. Lindstrom, and H. Korkeala. 2004. Enumeration and Isolation of *cpe*-Positive *Clostridium perfringens* Spores from Feces. Journal of Clinical Microbiology 42:3992-3997.
21. Helmboldt, C. F., and E. S. Bryant. 1971. The Pathology of Necrotic Enteritis in Domestic Fowl. Avian Diseases 15:775-780.
22. Hickey, C.S., and Johnson, M.G. 1981. Effects of pH Shifts, Bile Salts, and Glucose on Sporulation of *Clostridium perfringens* NTCT 8798. Applied and Environmental Microbiology 41:124-129.
23. Hofacre, C. L., T. Beacorn, S. Collett, and G. Mathis. 2003. Using Competitive Exclusion, Mannan-Oligosaccharide and Other Intestinal Products to Control Necrotic Enteritis. Journal of Applied Poultry Research 12:60-64.
24. Immerseel, F. V., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in Poultry: an Emerging Threat for Animal and Public Health. Avian Pathology 33:537-549.
25. Jackson, M. E., D. M. Anderson, H. Y. Hsiao, G. Mathis, and D. W. Fodge. 2003. Beneficial Effect of B-Mannanase Feed Enzyme on Performance of Chicks Challenged with *Eimeria* and *Clostridium perfringens*. Avian Diseases 47:759-763.
26. Jia, W., B. A. Slominski, H. L. Bruce, G. Blank, G. Crow, and O. Jones. 2009. Effects of Diet Type and Enzyme Addition on Growth Performance and Gut Health of Broiler Chickens During Subclinical *Clostridium perfringens* Poultry Science 88:132-140.
27. Kaldhusdal, M., C. Schneitz, M. Hofshagen, and E. Skjerve. 2001. Reduced Incidence of *Clostridium perfringens*-Associated Lesions and Improved Performance in Broiler Chickens Treated with Normal Intestinal Bacteria from Adult Fowl. Avian Diseases 45:149-156.
28. Klasing, K. C. 1998. Nutritional Modulation of Resistance to Infectious Diseases. Poultry Science 77:1119-1125.
29. Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of Dietary Fat Source and Subtherapeutic Levels of Antibiotic on the Bacterial Community in the Ileum of Broiler Chickens at Various Ages. Applied and Environmental Microbiology 68:5918-5924.
30. Labbe, R. G. 1991. *Clostridium perfringens*. Journal of the Association of Official Analytical Chemists 74:711-714.
31. Langhout, D. J., J. B. Schutte, P. Van Leeuwen, J. Wiebenga, and S. Tamminga. 1999. Effect of Dietary High- and Low-methylated Citrus Pectin on the Activity of the Ileal Microflora and Morphology of the Small Intestinal Wall of Broiler Chicks. British Poultry Science 40:340-347.
32. Lindstrom, M., A. Heikinheimo, P. Lahti, and H. Korkeala. 2011. Novel Insights into the Epidemiology of *Clostridium perfringens* Type A Food Poisoning. Food Microbiology 28:192-198.
33. Long, J.R., Pettit, J.R., and Barnum, D.A. 1974. Necrotic Enteritis in Broiler Chickens II. Pathology and Proposed Pathogenesis. Canadian Journal of Comparative Medicine 38: 467-474.
34. Long, J. R., and R. B. Truscott. 1976. Necrotic Enteritis in Broiler Chickens III. Reproduction of the Disease. Canadian Journal of Comparative Medicine 40:53-59.
35. Lovland, A., and M. Kaldhusdal. 1999. Liver Lesions Seen at Slaughter as an Indicator of Necrotic Enteritis in Broiler Flocks. FEMS Immunology and Medical Microbiology 24:345-351.
36. McDevitt, R. M., J. D. Brooker, T. Acamovic, and N. H. C. Sparks. 2006. Necrotic Enteritis; A Continuing Challenge for the Poultry Industry. World's Poultry Science Journal 62:221-247.

37. McDonel, J. L. 1986. *Clostridium perfringens* Toxins (type A, B, C, D, E). Pharmacology and Therapeutics 10:617-655.
38. Mikkelsen, L. L., J. K. Vidanarachchi, G. C. Olnood, Y. M. Bao, P. H. Selle, and M. Choct. 2009. Effect of Potassium Diformate on Growth Performance and Gut Microbiota in Broiler Chickens Challenged with Necrotic Enteritis. British Poultry Science 50:66-75.
39. Mitsch, P., K. Zitterl-Eglseer, B. Kohler, C. Gabler, R. Losa, and I. Zimpernik. 2004. The Effect of Two Different Blends of Essential Oil Components on the Proliferation of *Clostridium perfringens* in the Intestines of Broiler Chickens. Poultry Science 83:669-675.
40. Muhammed, S. I., S. M. Morrison, and W. L. Boyd. 1975. Nutritional Requirements for Growth and Sporulation of *Clostridium perfringens*. Journal of Applied Bacteriology 38:245-253.
41. Murray, P. R., K. S. Rosenthal, and M. A. Pfaller. 2009. Medical Microbiology. 6th ed. Elsevier Health Sciences, Philadelphia, PA, USA.
42. Palliyeguru, M. W. C. D., S. P. Rose, and A. M. Mackenzie. 2010. Effect of Dietary Protein Concentrates on the Incidence of Subclinical Necrotic Enteritis and Growth Performance of Broiler Chickens. Poultry Science 89:34-43.
43. Paredes-Sabja, D., Torres, J.A., Setlow, P., and Sarker, M.R. 2008. *Clostridium perfringens* Spore Germination: Characterization of Germinants and their Receptors. Journal of Bacteriology 190:1190-1201.
44. Parish, W. E. 1961. Necrotic Enteritis in the Fowl (*Gallus Gallus Domesticus*). I. Histopathology of the Disease and Isolation of a Strain of *Clostridium welchii*. Journal of Comparative Pathology 71:377-393.
45. Parish, W. E. 1961. Necrotic Enteritis in the Fowl. II. Examination of the Causal *Clostridium welchii*. Journal of Comparative Pathology 71:394-404.
46. Parish, W. E. 1961. Necrotic Enteritis in the Fowl. III. The Experimental Disease. Journal of Comparative Pathology 71:405-414.
47. Pedersen, K., L. Bjerrum, B. Nauerby, and M. Madsen. 2003. Experimental Infections with Rifampicin-resistant *Clostridium perfringens* Strains in Broiler Chickens Using Isolator Facilities. Avian Pathology 32:403-411.
48. Pedersen, K., L. Bjerrum, O. Heuer, D. Wong, and B. Nauerby. 2007. Reproducible Infection Model for *Clostridium perfringens* in Broiler Chickens. Avian Diseases 52:34-39.
49. Prescott, J. F., R. Sivendra, and D. A. Barnum. 1978. The Use of Bacitracin in the Prevention and Treatment of Experimentally-induced Necrotic Enteritis in the Chicken. Canadian Veterinary Journal 19:181-183.
50. Rehman, H., W. A. Awad, I. Lindner, M. Hess, and J. Zentek. 2006. *Clostridium perfringens* Alpha Toxin Affects Electrophysiological Properties of Isolated Jejunal Mucosa of Laying Hens. Poultry Science 85:1298-1302.
51. Riddell, C., and X. Kong. 1992. The Influence of Diet on Necrotic Enteritis. Avian Diseases 36:499-503.
52. Shakouri, M. D., P. A. Iji, L. L. Mikkelsen, and A. J. Cowieson. 2008. Intestinal Function and Gut Microflora of Broiler Chickens as Influenced by Cereal Grains and Microbial Enzyme Supplementation. Journal of Animal Physiology and Animal Nutrition 93:647-658.
53. Shane, S. M., J. E. Gyimah, K. S. Harrington, and T. G. Snider. 1985. Etiology and Pathogenesis of Necrotic Enteritis. Veterinary Research Communications 9:269-287.
54. Takeda, T., T. Fukata, T. Miyamoto, K. Sasai, E. Baba, and A. Arakawa. 1995. The Effects of Dietary Lactose and Rye on Cecal Colonization of *Clostridium perfringens* in Chicks. Avian Diseases 39:375-381.
55. Tschirdewahn, B., S. Notermans, K. Wernars, and F. Untermann. 1991. The Presence of Enterotoxigenic *Clostridium perfringens* Strains in Faeces of Various Animals. International Journal of Food Microbiology 14:175-178.
56. Wati, T., Ghosh, T., Syed, B., and S. Haldar. 2015. Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. Animal Nutrition 1(2015): 213-219.
57. Williams, R.B. 2005. Intercurrent Coccidiosis and Necrotic Enteritis of Chickens: Rational, Integrated Disease Management by Maintenance of Gut Integrity. Avian pathology 34(3):159-180.
58. Williams, R. B., R. N. Marshall, R. M. La Regione, and J. Catchpole. 2003. A New Method for the Experimental Production of Necrotic Enteritis and its Use for Studies on the Relationships Between Necrotic Enteritis, Coccidiosis and Anticoccidial Vaccination of Chickens. Parasitology Research 90:19-26.
59. Wise, M. G., and G. R. Siragusa. 2005. Quantitative Detection of *Clostridium perfringens* in the Broiler Fowl Gastrointestinal Tract by Real-Time PCR. Applied and Environmental Microbiology

71:3911-3916.
60. Wiseman, R.W., Bushnell, O.A., and Rosengerg, M.M. 1956. Effects of Rations on the pH and Microflora in Selected Regions of the Intestinal Tract of Chickens. Poultry Science 35:126-132.
61. Yegani, M., and D. R. Korver. 2008. Factors Affecting Intestinal Health in Poultry. Poultry Science 87:2052-2063.

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. Fumonisins 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.

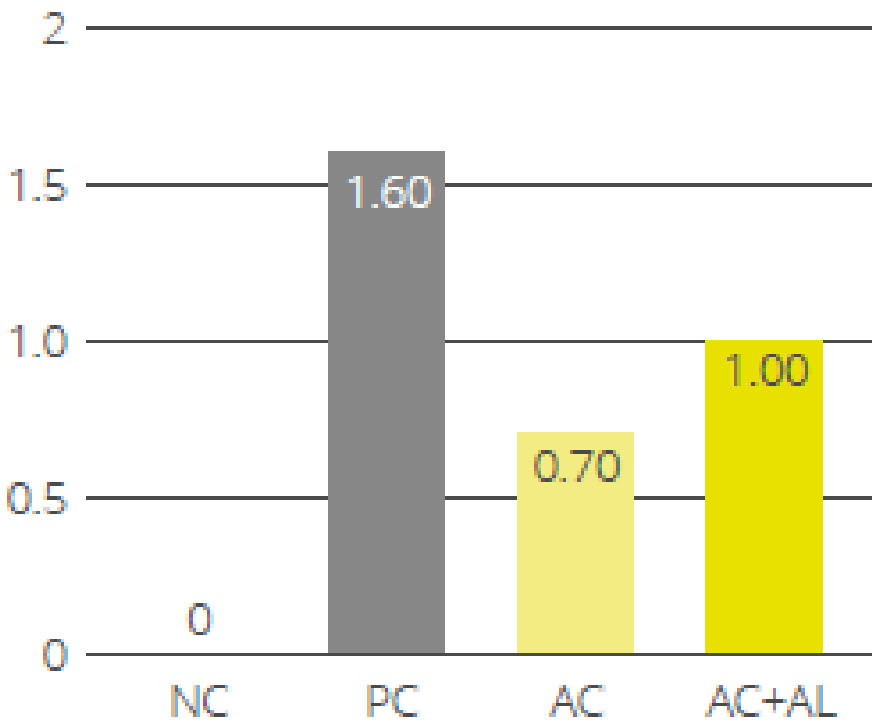
Organic acids

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 bacteriaPapatisiros et al., 2013).

Phytomolecules

Multiple scientific studies have proven the positive effects of phytomolecules (also known as phytogenics 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.

References

Apajalahti, J., Kettunen, A., and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World Poultry Sci J 60:223- 232.

Bailey, Richard A. 2010. Intestinal microbiota and the pathogenesis of dysbacteriosis in broiler chickens. PhD thesis submitted to the University of East Anglia. Institute of Food Research, United Kingdom.

Bevins, C. L.; Martin-Porter, E.; Ganz, T. Defensins and innate host defence of the gastrointestinal tract. Gut, 1999, 45, 911–915.

De Gussem , M. 2007. Coccidiosis in poultry: review on diagnosis, control, prevention and interaction with overall gut health . In Proceedings of the XVI European Symposium on Poultry Nutrition (pp. 160 169 . Strasbourg , France.

Gurrre, Philippe. 2020. Review Mycotoxin and Gut Microbiota Interactions. Toxins, 12, 769.

Jha, Rajesh, Razib Das, Sophia Oak, and Pravin Mishra, 2020. Probiotics (Direct-Fed Microbials) in Poultry Nutrition and Their Effects on Nutrient Utilization, Growth and Laying Performance, and Gut Health: A Systematic Review. Animals (Basel). 10(10): 1863.

Kizerwetter-Świda, M., and M. Binek. 2008. Bacterial microflora of the chicken embryos and newly hatched chicken. Journal of Animal and Feed Sciences 17:224-232

Panneman, H. 2000 . Clostridial enteritis/dysbacteriosis, fast diagnosis by T-RFLP, a novel diagnostic tool. In Proceedings of the Elanco Global Enteritis Symposium. Cork Ireland.

Papatisiros VG, Katsoulos PD, Koutoulis KC, Karatzia M, Dedousi A, Christodoulopoulos G. Alternatives to antibiotics for farm animals. CAB Rev Ag Vet Sci Nutr Res. (2013) 8:1–15. doi: 10.1079/PAVSNNR20138032.

Pui-Pui, Winnie, and Sabran Mohd-Redzwan. 2018. Mycotoxin: Its Impact on Gut Health and Microbiota. Frontiers in Cellular and Infection Microbiology, 8:60.

Rebel, J.M.J., Balk, F.R.M., Post, J., Van Hemert, S., Zekarias, B. and Stockhofe, N. 2006. Malabsorption syndrome in broilers. World's Poultry Science Journal, 62: 17–29.

Saeed, Mohammad, Fawwad Ahmad, Mohammad Asif Arain, Mohamed E Abd El-Hack, Mohamed Emam, Zohaib Ahmed Bhutto and Arman Moshaven, 2017. Use of Mannen – Oligosaccharides (MOS) As a Feed Additive in Poultry Nutrition. J. World Poult. Res. 7(3): 94-103.

Suzuki, K., R. Harasawa, Y. Yoshitake, and T. Mitsuoka. 1983. Effects of crowding and heat stress on intestinal flora, body weight gain, and feed efficiency of growing rats and chicks. Nippon Juigaku Zasshi 45:331-8.

Van der Klis, J.D. and Lensing, M. 2007. Wet litter problems relate to host–microbiota interactions. World Poultry, 23: 20–22.

Wan, M. L.; Woo, C. S.; Allen, K. J.; Turner, P. C.; El-Nezami, H. Modulation of porcine-defensins 1 and 2 upon individual and combined fusarium toxin exposure in a swine jejunal epithelial cell line. App. l. Environ. Microbiol., 2013, 79(7), 2225-2232

Wang L, Lilburn M, Zhongtang Y. 2016. Intestinal microbiota of broiler chickens as affected by litter management regimens Front. Microbiol (2016).

Wilson, J., Tice, G., Brash, M.L. and St Hilaire, S. 2005. Manifestations of Clostridium perfringens and related bacterial enteritides in broiler chickens. Worlds Poultry Science Journal, 61: 435–449.

Necrotic enteritis in poultry

Enteric diseases cause significant economic losses due to decreased weight gain, higher mortality, higher feed conversion, higher veterinary costs and medicine and a higher risk of contamination by poultry products in food production. The losses due to necrotic enteritis mainly occurring in broilers and fattening turkeys in intensive floor or free-range management are put at 2 billion US$ per year.

After the ban of antibiotic growth promoters, the relevance of this formerly well controllable disease reappeared and increased.

Necrotic enteritis is a disease of the gut
It is caused by specific gram-positive, anaerobic bacteria – *Clostridium perfringens,* mostly Type A.

Clostridia are found in litter, faeces, soil, dust and in healthy animals' guts. These spore forming bacteria are extremely resistant against environmental influences and can survive in soil, feed, and litter for several years and even reproduce.
Clostridium perfringens is a component of the normal gut flora. It occurs in a mixture of diverse strains in a concentration of up to 10^5 CFU / g intestinal content. In animals suffering from necrotic enteritis particularly one strain of *Clostridium perfringens* is found in a much more higher concentration of 10^6-10^8 CFU / g.
Necrotic enteritis affects chickens and turkeys at the age of 2-16 weeks, proliferating at the age of 3-6 weeks. There is an acute clinical, and a subclinical form.
Birds suffering from the clinical form clearly show symptoms like a poor general state of health and diarrhoea. Mortality rates up to 50 % can occur. Subclinical necrotic enteritis cannot be diagnosed easily, as there are no clear symptoms. This form, however, stays within the flock and causes losses due to decreased growth.

Factors promoting an infection with necrotic enteritis should be avoided!
In general, factors have to be cited that create an intestinal environment favourable for the facultative anaerobic *Clostridium perfringens* or weaken the immune status of the host:

1. **Feed:**
   Here **NSP's** have to be mentioned. Undigested NSP's serve as substrate and some of them cause higher production of mucus also serving as substrate and providing ideal anaerobic conditions. Undigested **proteins** due to high contents in the diet also serve as substrates. **Animal protein** and **fat** are worse than vegetable variants and a homogeneous size of particles in the diet is better than an **inhomogeneous mixture**.
2. **Stress**
   Stresses such as feed change or high stocking density favour NE
3. **Diseases**
   Immunosuppressive diseases such as infective chicken anaemia, Gumboro or Marek's decrease resistance against intestinal infections and facilitate their colonisation. Some pathogens exert pressure on the gut and prepare the way for clostridia. Here Cryptosporidia and salmonella have to be mentioned.

New approaches
Secondary plant compounds show good results against the two microorganisms just mentioned. In a trial conducted with free range broilers in France, a combination of a vaccination against coccidia and a mixture of secondary plant compounds (Activo liquid) resulted in a reduced occurrence of necrotic enteritis in the trial group compared to the control. Additionally due to an improved feed conversion, the margin per animal in the trial group was 5 Cent higher than in the control (1,44 € vs 1,39 €).
In an *in vitro* test, Activo liquid also showed bactericidal efficacy against field isolated *Salmonella pulmorum* and *Salmonella gallinarum* at a 2 % concentration.
The trials show that combined with a good feeding and stress management, secondary plant compounds, could be a good tool to eliminate predisposing factors for necrotic enteritis and could therefore help control this economically important disease.