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Antibiotic-Free and Reduced Antibiotic Use in Broiler Production: *Predisposing Factors and Control Strategies for Necrotic Enteritis*

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Introduction

Necrotic enteritis (NE) is a bacterial disease caused by *Clostridium perfringens* (CP) that affects broiler chickens between 2-5 weeks of age. It is an enterotoxemia (bacterial infection with production of intestinal toxins) with a very short clinical illness period followed by sudden increases in flock mortality. Some CP strains are capable of producing potent toxins that cause damage to the intestinal lining and liver. NE has been diagnosed worldwide, causing clinical and subclinical disease, leading to substantial economic losses due to mortality, added treatment costs and reduced live bird performance. For many years, the global cost of the disease was estimated to be $2 billion USD a year. However, the economic impact of NE has steadily increased since the 2000s due to a growing market trend demanding antibiotic-free (ABF) broilers, revised production costs and further research on its actual impact. Recently, losses attributed to NE have been estimated to be close to $6 billion USD a year worldwide.

During past decades, NE has been controlled by the addition of antibiotic growth promoters (AGP) such as bacitracin, lincomycin, avoparcin, virginiamycin and tylosin to the feed. More recently, avilamycin became available as another antibiotic (AB). However, voluntary or regulatory pressure to remove AGP and ionophore type anticoccidial medications have resulted in a greater frequency of clinical and subclinical NE problems and a growing challenge for the broiler industry. Also, it is widely recognized that NE is a complex, multifactorial disease that occurs following disruptions in the normal intestinal microflora, field challenges that cause damage to the intestinal lining and the use of feed ingredients that slow feed passage and can promote CP overgrowth and toxin production. The purpose of this article is to summarize the current knowledge on factors that contribute to the onset of the disease, and the prevention and control strategies being used in ABF and reduced AB use production systems globally for disease prevention, protect intestinal health and ensure optimal bird welfare and performance.

Necrotic Enteritis in Broilers

Causitive Agent: Clostridium perfringens

CP is a *Gram-positive*, anaerobic, spore-forming bacterium found in soil, dust, poultry litter and feces. The spores are extremely resistant to destruction by desiccation, chemicals and extremes in temperature. Spores allow CP to survive in poultry houses for extended periods and be readily transmitted to subsequent flocks. The CP strains not associated with NE are part of the normal flora of the intestinal tract of healthy chickens (commensals) and can be found in the crop, duodenum, jejunum, ileum and ceca. They are found in the intestines within a few hours after hatching, and their numbers increase gradually, eventually inhabiting the lower gut and ceca. In healthy birds, the intestine contains large numbers of CP (up to 10^5 colony-forming units/gram of content) comprising a mixed population including a low percentage of pathogenic or toxin-producing strains. However, evidence suggests that when outbreaks occur, a single CP pathogenic clone proliferates following a cascade of events (that continue to be investigated) and migrates along with its toxins (by reverse wave-like contractions or retroperistalsis) into the upper regions of the intestine, where the major damage occurs.

CP is an opportunistic pathogen that can also cause food poisoning in humans and enterotoxemia in other animal species. However, these CP strains are not the same types that cause disease in chickens. NE in chickens is caused by CP type G, previously identified as type A or C strains that proliferate in the small intestine and secrete one or more exotoxins. According to numerous reports, CP strains vary in virulence and ability to cause the disease. Initially, an α-toxin was considered the most important toxin involved, but recently another toxin known as NetB has been found. Both toxins have shown the ability to damage the intestinal epithelial surface, causing inflammation, ulceration and necrosis (death of tissue). Studies have suggested pathogenic CP strains may possess other virulence factors such as other toxins, bacteriocins and

hydrolytic enzymes that enable them to adhere, form biofilms, colonize, thrive and evade the birds' immune system. Infections in the liver and resulting toxins can also cause acute or chronic hepatitis leading to death or subclinical disease. Mild cases have been associated with subsequent condemnation of carcasses in the processing plant due to liver lesions.

Clinical Signs

NE occurs in both clinical and subclinical forms. The disease can occur in broilers between 2-5 weeks of age, but the onset is most commonly seen between 11-18 days of age. Predictably, it follows feed changes from starter to grower diets. The clinical form is usually short, with sick birds showing depression, immobility, ruffled feathers and diarrhea. Birds displaying clinical signs die within a few hours. Acute forms are followed by a sudden increase in flock losses (exceeding 0.2 % per day). In severe cases, loss can reach up to 1% per day without treatment. During suspected outbreaks, sick and dead birds must be removed from the house promptly to reduce the spread of the disease through direct contact.

Subclinical NE is probably the most economically important manifestation as it may persist in the flock undetected and untreated. Subclinical infections negatively impact key performance indicators such as growth, feed conversion ratio (FCR) and flock uniformity. Feed cost is approximately 65-75% of broiler production cost, and therefore, by reducing feed intake and body weight gain and increasing FCR, the disease can have a severe impact on the profitability of a broiler production system.

Lesions, Diagnosis and Treatment

It is important to examine euthanized or fresh dead birds for lesions that tend to be less obvious. They become more difficult to identify once the intestines start to decompose. Gross lesions are primarily found in the small intestine (descending loop of the duodenum into the jejunum and occasionally the ileum) and liver. The intestine can look congested, swollen (ballooned), friable, and contain gas and copious watery, brown, blood-tinged fluid with a foul odor (**Figure 1**). The intestinal lining can appear thickened with a granular surface (**Figure 2**) or covered by rough, brownish diphtheric pseudo-membranes (**Figure 3**). Sporadically, affected segments of the small intestine have a firm texture. Also, multifocal areas of pale or yellowish discoloration (**Figure 4**) may be seen in the liver along with a distended gall bladder. Both liver lobes can be enlarged, discolored and firm to the touch due to extensive damage. The kidneys are generally pale and often enlarged with a lobular pattern.

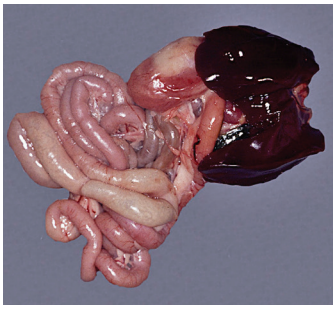

Figure 1. Lesions in the intestine are confined to the jejunum and ileum, which appear swollen (ballooned), friable and thin-walled, and contain gas and copious watery fluid.

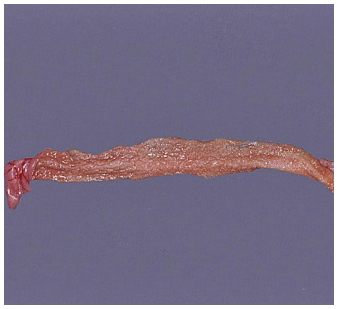

Figure 2. The intestinal lining can appear thickened with a finely roughened granular surface.

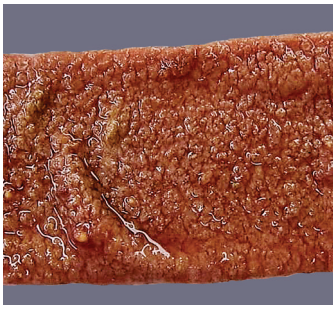

Figure 3. Thickened intestinal lining with a granular surface covered by rough brownish diphtheric pseudo-membrane.

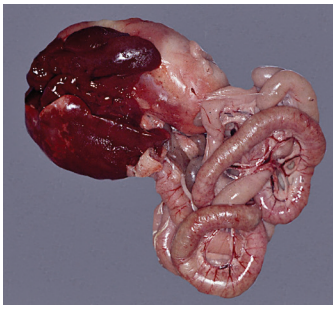

Figure 4. Intestine looks congested, swollen, and occasionally multifocal areas of pale or yellowish discoloration are seen in the liver.

Mucoid enteritis is characteristic of subclinical infections in the absence of significant mortality. Occasionally, a condition known as cholangiohepatitis, characterized by the presence of enlarged and firm yellowish livers, is found at the processing plant. These lesions result from CP colonizing the hepatic tissue and gall bladder, and are commonly found in subclinical infections.

The presumptive diagnosis of NE is based on gross lesions and smears of intestinal scrapings revealing large masses of *Gram-positive* rods. Confirmatory diagnostic methods include histopathological examination of intestinal lesions and isolation of CP in anaerobic conditions using blood agar and differential culture media incubated at 37°C (98°F) followed by PCR typing.

Occasionally, NE is diagnosed in broiler breeder pullets in rear. It commonly occurs following feed outages, uneven feed distribution problems and coccidiosis breaks. Discrete areas of swelling and necrosis in the lining of the lower third of the intestine are occasionally seen following or combined with infections of *Eimeria brunetti*.

Penicillin, tetracycline, lincomycin, tylosin and bacitracin are the AB most commonly used in drinking water to treat flocks with clinical signs and mortality due to NE. Flocks typically respond favorably to AB treatment within 24-48 hours unless the dose is incorrect or there is another concurrent disease such as coccidiosis. AB-treated flocks will not be suitable for ABF products. Today, and as presented herein, efforts to prevent the disease are focused on management practices and non-antibiotic (alternative) strategies. However, it is important to highlight that when severe outbreaks occur, veterinarians must prescribe an AB treatment to protect the birds' health and welfare.

Predisposing Factors

CP is the primary cause of NE in broilers, and recent studies have shown that pathogenic strains can alter the normal intestinal microflora by displacing commensal (non-pathogenic) clostridia bacteria. The exact mechanisms and molecular properties that allow CP to selectively multiply and produce damage have and continue to be investigated. However, it is broadly accepted that initiation and development of the disease is a complex process requiring a range of predisposing factors (**Figure 5**). These factors appear to play a role by disrupting the gut microflora (causing dysbacteriosis) and generating favorable conditions for pathogenic CP strains to multiply (bloom), exert their virulence factors (exotoxins and enzymes), elude the immune system and cause intestinal and liver damage. The most well-known predisposing factors are summarized in **Table 1** and discussed in the sections below.

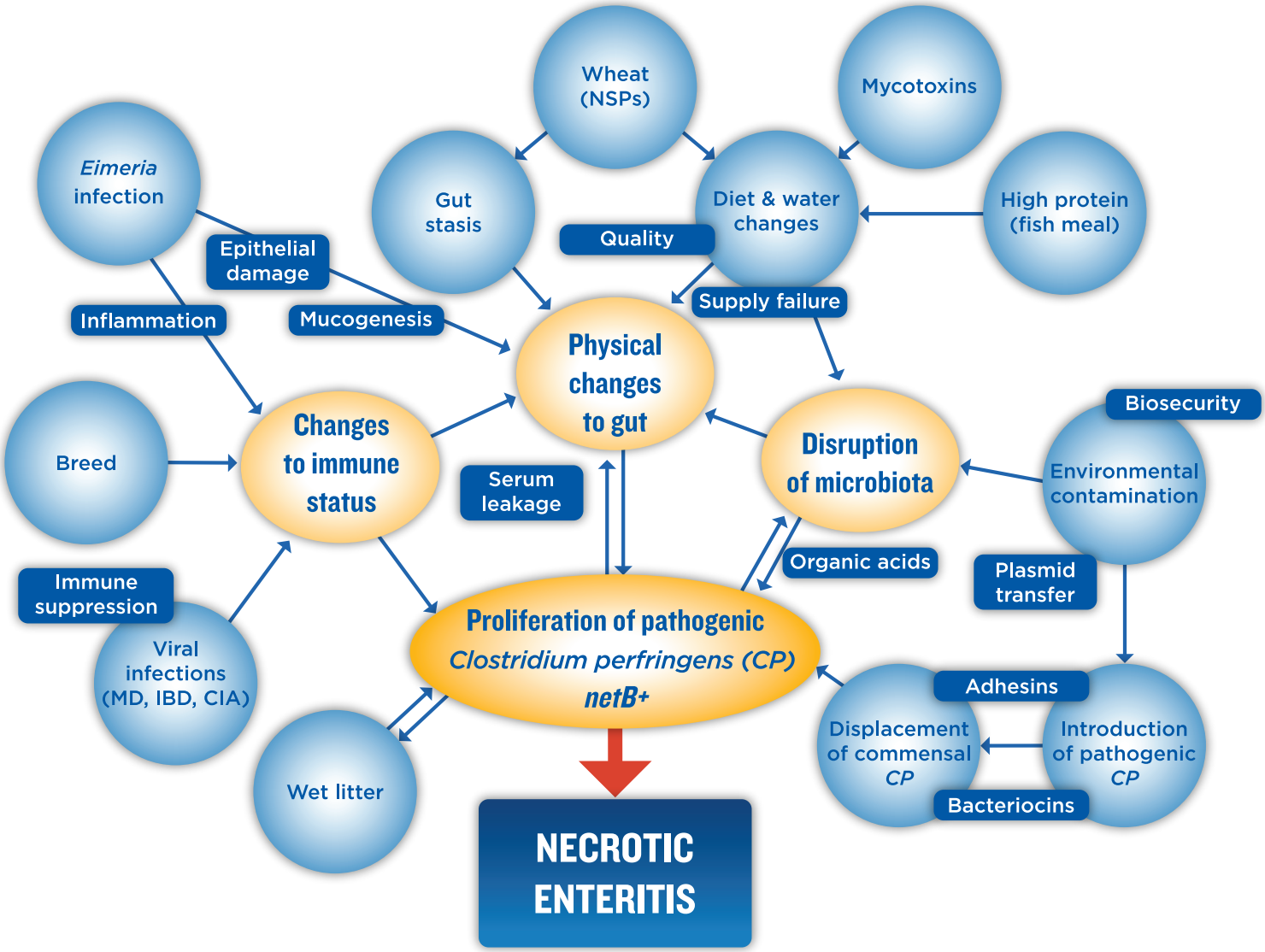

Figure 5. Summary of predisposing factors for the development of NE in chickens. Predisposing factors are shown in circles, and the major effects of these are shown in ovals. Important factors that may drive the influence of the predisposing factors are shown in the small rectangular boxes. MD= Marek's disease, IBD=Infectious bursal diseases, CIA=Chicken infectious anemia, NSP=Non-starch polysaccharides.

Source: Moore R. J. (2016). Necrotic enteritis predisposing factors in broiler chickens. Avian Pathology 45:3, pages 275-281. Reprinted by permission of Taylor & Francis Ltd.

Table 1. Main predisposing factors associated with NE episodes in ABF broiler flocks.

FACTOR	MAIN EFFECTS	COMMENTS
COCCIDIOSIS	Damage to the intestinal epithelium, leakage of plasma proteins, increased mucus production and disruption of normal gut microflora	Infections caused by *Eimeria maxima* and *Eimeria acervulina* are the most common
• **Ban of ionophore anticoccidials**	Subclinical and clinical coccidiosis results in intestinal damage	Control by chemically synthesized anticoccidial medications requires careful selection and rotation of available products
• **Resistance to anticoccidial medications**	Subclinical and clinical coccidiosis results in intestinal damage	Live performance data and regular monitoring of intestinal health by lesion scoring and/or oocyst counts help determine the effectiveness and/or need to rotate anticoccidial products
• **Live coccidiosis vaccines**	Uneven recycling and exposure to a high dose of oocysts cause intestinal damage	Vaccine administration and brooding management during the first three weeks are critical to avoid adverse effects
IMMUNOSUPPRESSION AND STRESS	Impaired development and function of the immune and digestive systems. Alterations in normal gut microflora	Increased risk of NE due to increased susceptibility to bacterial diseases
• **Viruses that cause immunosuppression and/ or mycotoxins**	Damage to the immune system and intestinal barrier at an early age increases the susceptibility and severity of NE lesions	Must have adequate maternal immunity and prevent early exposure to immunosuppressive viruses and mycotoxins
• **Cold brooding, wet litter, high stocking density, sudden changes in diet, feed shortages**	Harsh management conditions have detrimental effects on early growth, gut development and establishment of a normal microflora	Individual farms with poor management are associated with increased risk and/ or repeated NE episodes and during the transition from starter to grower diet
DIET COMPOSITION	Nature and specific components can stimulate the growth of CP and influence the incidence of NE	Ingredients create favorable conditions for pathogenic CP to proliferate and produce toxins
• **Small grains such as wheat, rye, barley and oats**	High levels of water-soluble non-starch polysaccharides (NSP) increase the viscosity of the digesta	Decreased digestibility and slower transit time through the intestine
• **High protein diets, animal proteins**	Imbalanced amino acid profiles can impair digestibility, provide additional undigested protein in the lower gut	Negatively affects the gut microflora and create conditions for pathogenic CP to bloom
• **Fish, and meat and bone meals**	High concentrations of CP, biogenic amines and fat rancidity can disrupt gut microflora	The compromised quality of these ingredients is associated with NE episodes
• **Finely ground diet**	Reduced feed retention time	Allow CP to grow faster

Coccidiosis

Coccidiosis continues to be the most common cause of intestinal damage in broilers worldwide, followed by NE. Although there are several factors capable of damaging the lining of the intestine (such as enteric viruses, helminth parasites, mycotoxins, biogenic amines, rancid fat, or stress), the leading predisposing cause is coccidiosis in the duodenum and jejunum by *Eimeria maxima* and *Eimeria acervulina*. Damage from coccidiosis infections may occur due to increased resistance to ionophore or chemically synthesized anticoccidial medications, or following vaccination with live coccidiosis vaccines.

Several commercially available vaccines (live sporulated oocysts) are composed of non- or slightly attenuated *Eimeria* species that must invade and multiply in the intestinal lining (3 consecutive infection cycles are required as a minimum to develop solid immunity), which causes some degree of intestinal epithelial damage. This effect could be aggravated by uneven vaccine administration or uptake that can result in a significant number of birds being missed (non-immunized), and subsequently exposed to a higher and more damaging dose of oocysts as live vaccine coccidias spread from vaccinated to non-vaccinated birds. Damage to the intestinal epithelium caused by wild or vaccine coccidias results in leakage of nutrients (plasma proteins) and increased mucus production (due to a T-cell induced inflammatory response) that become a rich substrate for CP to proliferate, produce toxins, further disrupt the normal microflora and prompt the development of NE (**Figure 6**). For these reasons, coccidia species such as *Eimeria maxima*, in conjunction with pathogenic CP strains, are used in experimental models to induce NE and/or evaluate the efficacy of AB and alternative products to control the disease.

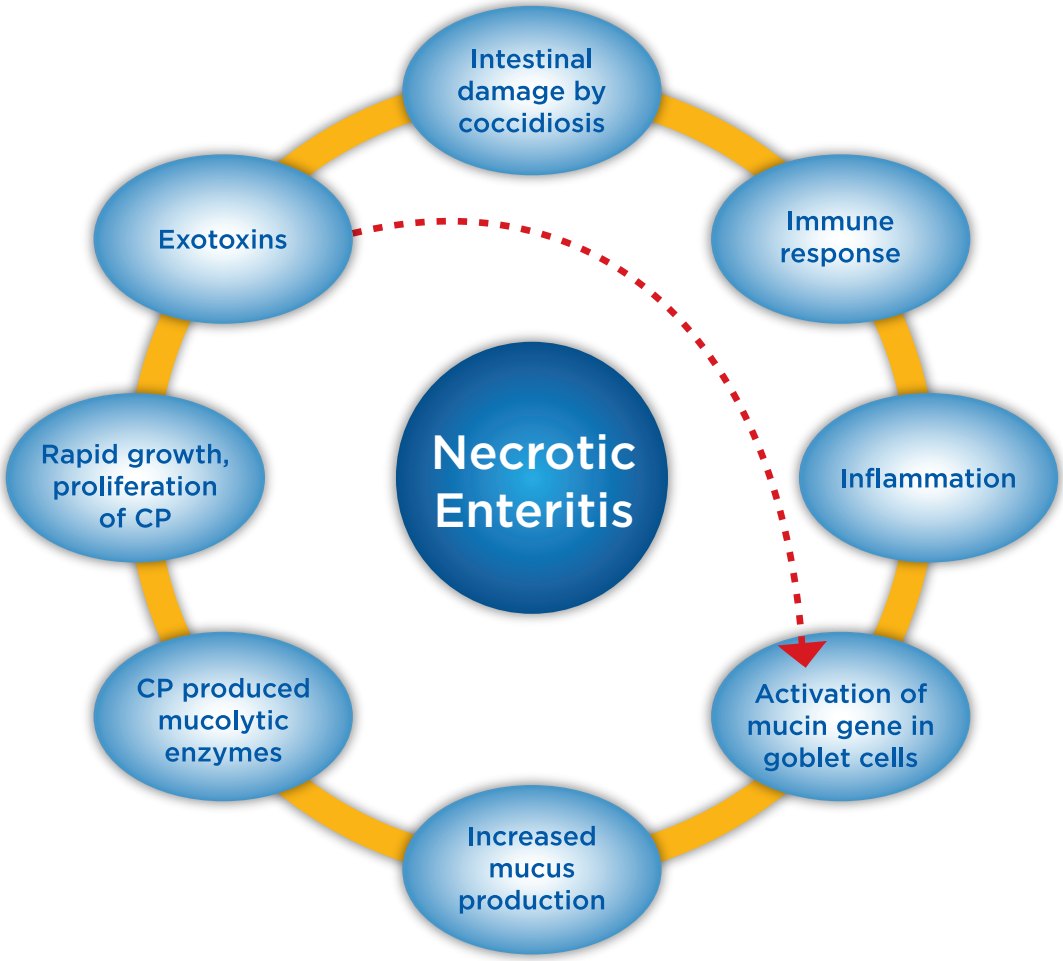

Figure 6. Hypothetical illustration of how a coccidiosis infection induces a mucogenic response providing a growth advantage to CP and leading to NE.

Adapted from: Collier, C.T., C.L. Hofacre, A.M. Payne, D.B Anderson, P. Kaiser, R.I. Mackie, and H.R. Gaskins (2008). Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth. Veterinary Immunology and Immunopathology, 122: 1-2, pages 104-115.

Following coarse spray or gel bead coccidiosis vaccination at the hatchery, there is a peak in oocyst counts in the litter 18 days post-vaccination. Predictably, field experiences have shown that broiler flocks vaccinated with live coccidiosis vaccine and not fed any anticoccidial medications are at greater risk of suffering NE just before day 18.

In-feed polyether ionophore type anticoccidials (monensin, salinomycin, lasalocid, narasin, maduramycin and semduramicin) have been, and continue to be very valuable tools to control coccidiosis and reduce the risk of NE thanks to their added anticlostridial activity. Either by mandate or voluntarily, AGP have been withdrawn from broiler production in the EU. However, ionophore anticoccidials are still being used. Although ionophores are not used in humans and have a different mode of action than therapeutic AB, they are considered AB in the US, and therefore, have been banned from ABF production systems. The commonly used terms in the US for broiler systems with no AB include "raised without antibiotics" (RWA), "no antibiotics ever" (NAE) and ABF. In general, field reports demonstrate that operations without ionophore medications are more likely to experience NE breaks than those that use ionophores as long as there are no coccidia drug-resistance problems.

Immunosuppression and Stress

Immunosuppression and stress caused by management and infectious factors can alter the birds' intestinal microflora and increase the risk of developing NE. Sudden changes in feed composition or transition from starter to grower diets are frequently associated with NE. Cold brooding conditions (more common during winter months) combined with reduced ventilation and increased litter moisture have been associated with an increased risk and repeated episodes of NE on known problem farms. Field experiences have demonstrated that high stocking density is a significant NE predisposing factor due to its detrimental effects on welfare and gut health. Infection with viruses such as Marek's disease virus, chicken anemia virus and infectious bursal disease virus, which impairs the birds' immune system and reduces the resistance to bacterial infections in the gut, could increase the severity of NE.

Studies have also shown aflatoxins, the most toxic mycotoxins known to decrease immune function, and NE can interact in decreasing broiler performance. Dietary aflatoxins in flocks exposed to NE environments increase the severity of intestinal lesion scores and further decrease feed intake, weight gains and feed efficiency. Fusarium mycotoxins (deoxynivalenol or fumonisins) alone or combined can cause disruptions of the intestinal barrier, resulting in leaked proteins from the plasma to the gut, reduced nutrient absorption and increased levels of nutrients available for CP growth.

Optimal chick quality and handling with care at hatch, processing and transport are essential to ensure chick health and the optimal immune and digestive system development. Overheating, dehydration or chilling during these steps can have detrimental effects on the maturation of chicks' intestinal tract and, in turn, reduce feed intake and nutrient absorption. Chick quality has been identified as an important predisposing factor and a key opportunity to minimize the risk of NE in all types of broiler production.

Diet Composition

The nature and specific components of the diet are important factors that can stimulate the growth of CP and influence the incidence of NE. Diets with small grains such as wheat, rye, oats and barley with high levels of indigestible, water-soluble non-starch polysaccharides (NSP) can pose a higher risk of NE than corn-based diets. These effects are possibly related to differences in the viscosity of the digesta, decreased digestibility and slower transit time through the intestine. NSP are hydrophilic compounds that encourage birds to increase their water consumption. The increased water intake also leads to greater water excretion resulting in additional moisture and deterioration of litter conditions. Furthermore, NSP can also interact with some proteins in the intestinal lining to increase the production and secretion of mucus, allowing pathogenic CP strains to adhere and proliferate.

High protein diets with imbalanced amino acid profiles result in excessive amino acids in the lower gut. Fermentation of this excess protein or amino acids can produce phenols, thiols, amines, ammonia, indoles and increase the pH of the lower gut, which impacts the microflora profile and creates favorable conditions for the proliferation of enteric pathogens such as CP. Animal origin proteins, in particular, can contain relatively high concentrations of poorly digestible proteins, which can serve as a substrate for CP. Fish, meat and bone meals have been related, particularly when quality is compromised (due to the presence of biogenic amines and fat rancidity), to outbreaks of the disease. Fish meal is known as a relatively rich source of zinc, glycine and methionine. Zinc increases the production of α-toxin and protects it against the effects of trypsin, whereas glycine appears to stimulate the growth of CP. Animal protein sources (fish meal and meat and bone meal) have also been associated with high concentrations of CP and NE episodes in broilers and breeders.

Animal fat sources of poor quality could also increase the incidence of NE, and this is a reason for preferring vegetable oil in current broiler diets. Rancid fats (high content of free radicals generated by oxidation) decrease palatability and reduce nutrient utilization by reacting with proteins, lipids and fat-soluble vitamins. In addition, free radicals can cause intestinal inflammation and lead to impaired absorption. Least cost formulation (use of low quality/priced ingredients), frequent changes in formulation, high variability in alternative products, biogenic amines, rancid fats and over-under-processed soybean meals may increase the risk of NE breaks.

Studies have revealed that high levels (15%) of distillers dried grains with solubles (DDGS) can increase the severity of NE incidents. Excess dietary calcium has also been recognized as important to promote NE, but the mechanism is unknown. Other less significant factors associated with the incidence of NE include lectins, tannins, trypsin inhibitors (present in soybean meal) and mycotoxins, which alone or in combination can impact digestibility, tissue irritation, gut's microflora and absorption of nutrients.

The physical form of the feed could also play a role as finely ground diets have been identified as a risk factor, particularly when using small grains. Coarsely ground feed stimulates gastric function (secretion of hydrochloric acid) and feed retention time in both the proventriculus and the gizzard. Contrarily, finely ground feed reduces feed retention time and allows CP to grow faster than coarsely ground feed.

Integrated Control Strategy

Based on current knowledge, it stands to reason that preventing clinical and subclinical NE requires recognition that any potential damage to the intestinal lining must be avoided, the proliferation of pathogenic CP prevented and the establishment of a healthy intestinal microflora promoted and preserved. These goals are likely to be accomplished by an integrated management strategy that considers the most significant risk factors known to avoid creating the conditions that lead to the propagation of pathogenic CP strains and the inception of the disease.

Coccidiosis Control

Coccidiosis prevention and control is the foremost factor in reducing the risk of NE. Rotation of anticoccidial medications (to preserve their effectiveness) or rotation between medications and vaccination strategies based on challenge levels and seasonal incidence must be considered to maximize coccidiosis control and reduce the incidence of NE. Consistent live coccidiosis vaccine administration (to ensure optimum vaccine dosing and coverage), followed by proper brooding management (to promote adequate vaccine cycling), has been recognized as critical to avoid possible adverse effects and predisposing flocks to NE. Coccidiosis vaccination is used by some ABF companies all year-round, while others vaccinate during summer and

autumn and use non-ionophore anticoccidials (i.e., decoquinate, nicarbazine, zoalene, amprolium) during the winter months (higher moisture and colder temperatures). Some operations have achieved benefits using live coccidiosis vaccination followed by in-feed administration of a non-ionophore anticoccidial such as decoquinate, robenidine or zoalene from 14-30 days of age. This approach is referred to as "bio-shuttle" and may result in fewer NE breaks following vaccination. The success of this method depends on allowing immunity against coccidiosis to develop, and therefore, it is important not to administer an anticoccidial too early (allow one and a half to two cycles of the vaccinal coccidia oocysts). Again, this strategy could be part of a rotation program.

When coccidiosis vaccination is part of a preventive strategy, it is critically important to ensure vaccine viability, proper handling, adequate administration and sufficient litter moisture to achieve maximum coverage and uniform cycling and immunization of the flock. If the litter is too dry and the sporulation rate of the vaccine oocysts is low, it can delay or alter the cycling of viable oocyst in the house. A delay in cycling could cause some birds to remain naive (not exposed) while others start shedding oocysts from a second life cycle. As a result, naïve birds could ingest and get exposed to a high dose of live vaccine oocysts or even oocysts from wild strains leading to intestinal damage and clinical coccidiosis. Bird stocking density is another important factor to ensure a balanced housing environment that will allow adequate live vaccine oocyst cycling. In general, live non-attenuated vaccines require 0.5 square feet/bird or 21 birds/square meter for the first seven days. After the first week, birds should have more space (at least 0.65 square feet/bird or 16.5 birds per square meter). To ensure proper oocyst shedding, sporulation and recycling occurs, it is important that birds are not provided the entire whole house space earlier than 10-12 days. In many operations this is accomplished using a half-house brooding method and keeping birds in the half-house (brooding chamber) for at least 10 days while maintaining adequate house temperature, relative humidity and ventilation ***(please see Editorial Note below).***

Feed Adjustments, Management and Alternative Products

Balancing the composition of broiler diets, the careful selection of feed ingredients and feeder and feeding management are probably the next most important and cost-effective components of an integrated prevention strategy. The goals are to avoid irritation of the gut, mucus secretion, impaired digestion and absorption and excessive undigested nutrients in the lower section of the gut. Supplementation of enzymes (i.e., xylanase) to diets with wheat, barley, oat and rye helps reduce the amount of indigestible carbohydrates and the viscosity of the diet. Also, studies have shown that dietary supplementation of young broilers with organic selenium or zinc might be beneficial to reduce the negative consequence of NE. Optimal feed management (easy access, feeder space and distribution) must be considered; and avoid feed outages with proper feeding and lighting management practices. Flocks that experience prolonged periods of darkness and without feed appear to be at higher risk of NE breaks.

Alternative products, feed additives composed of beneficial microorganisms (probiotics) to colonize and/or balance the intestinal microflora and prevent disruptions that can predispose birds to NE, are becoming increasingly popular. Probiotics or direct-fed microbials (DFM) containing *Bacillus subtilis* and *Bacillus licheniformis* have been shown to reduce NE lesion scores and mortality comparable with virginiamycin-treated birds. Different species of *Lactobacillus spp.* have been able to reduce CP counts and NE lesion scores while modulating some immune responses.

EDITORIAL NOTE: There may be instances where management recommendations from the author(s) differ from published Aviagen advice. For more information on Aviagen recommended stocking densities and control of coccidiosis, please refer to the ***Aviagen Broiler Management Handbook*** and ***Coccidiosis Control in Broilers with Use of Vaccines***. Stocking density may need to be adjusted based on a particular housing and/or farm conditions. Continuous monitoring of flock performance, routine coccidia lesion scoring examinations and oocyst count evaluations may help evaluate the effectiveness of a control program.

In general, probiotics or DFM are intended to increase the population of beneficial gut bacteria (*Lactobacillus spp., Bacillus spp., Bifidobacterium spp.* and *Enterobacter spp*.) that will:

- Reduce the concentration of enteric pathogens by competitive exclusion or production of proteins (bacteriocins) that inhibit the growth of pathogenic bacteria.
- Improve the digestive process by enhanced enzymatic activity.
- Improve feed intake and nutrient absorption.
- Reduce ammonia production.
- Neutralize enterotoxins.
- Enhance immune functions.
- Reduce inflammatory responses.
- Produce beneficial fermentation products such as volatile fatty acids.

Probiotics have been used alone or in combination with organic acids (formic, propionic, sorbic and butyric) to promote an acidic gut environment which may help enhance their favorable effects. Growing evidence has been published on the benefits of in-feed administration of microencapsulated sodium butyrate to protect the intestinal epithelial layer and reduce damage caused by NE on known problem farms. However, research and field experiences with various alternative products have reported inconsistent results when used to control NE episodes. The variability of results may be due to different environmental conditions, the impact of specific predisposing factors and the virulence of the CP strains in a particular operation. Oral administration of undefined-normal gut flora from adult healthy chickens, known as competitive exclusion (CE) products, has been shown under experimental and field conditions to reduce CP counts in the intestine, NE lesion scores, mortality and performance losses.

Other alternative products, including yeast cell wall extraction products containing mannan-oligosaccharides (MOS) or β-glucans, have shown the potential to reduce the clinical effects and severity of lesions caused by NE due to their anti-inflammatory effects, promotion of immunoglobulin secretion and production of short-chain fatty acids (SCFA). Numerous plant extracts (phytochemicals) known to have various antibacterial ingredients, such as tannins and essential oils (EO), could also be promising alternatives to AGP. Tannin-rich plants such as muscadine, chestnut, grape pomace, grapeseed and quebracho contain products (phenolic and flavonoid compounds) that may have antibacterial activity against various enteric pathogens. Grape products, chestnut and quebracho, have *in vitro* antibacterial and antitoxin activities against CP, and in vivo studies have confirmed their ability to reduce CP counts and the severity of lesions in broilers challenged with coccidia and CP. The ability of some tannins to reduce bacterial loads in the litter after their fecal excretion could be a valuable tool to reduce the likelihood of CP re-infection. EO and their compounds (thymol, carvacrol, eugenol and citronellol) have demonstrated antibacterial activity against CP and some possible modulating effects in gut microflora and digestive functions. Although tannins and EO appear to be potential alternatives, further work is needed to understand their mode of action on the gut microflora and ensure proper dosing, stability and consistency.

There is a wealth of alternative and feed additive options that have demonstrated benefits, but it is still not possible to suggest a single product or an ideal strategy to safeguard intestinal health and prevent NE. However, the availability of products with promising potential may be used alone or with one another to develop synergistic strategies that could prevent the overgrowth of pathogenic CP while helping maintain a healthy gut microflora. Any combination of products (i.e., a CE or probiotic in combination with an organic acid and another feed additive such as yeast and/or plant extract) must demonstrate measurable benefits in NE prevention, performance and cost-effectiveness under commercial conditions. In addition, it will be important to consider that CP and other enteric pathogens are highly adaptable microorganisms. Therefore, it may be necessary to use these products judiciously as resistant strains may evolve.

Chick Quality, Brooding and House Management

Chick quality is a broad term encompassing optimum size, weight, liveliness, health, livability and performance during the first days of life. In general, the following factors are associated with chick quality:

- Breeder health and nutrition
- Egg shell quality and sanitation
- Egg storage, incubation and hatching practices
- Hatchery sanitation
- Chick handling, transport and delivery

Ensuring the production and consistent supply of quality chicks is of the utmost importance to support ABF programs and reduce the risk of NE in broilers. Furthermore, adequate levels of maternal immunity and preventing immunosuppression and management or environmental stresses are critical to maintain broiler health and promote the optimum development of the birds' digestive and immune systems.

Good brooding and growing conditions are critical to guarantee steady feed and water consumption, optimum growth, intestinal development and reduced incidence of NE during the first four weeks of life (the NE window). During the last few years, some practices that have gained popularity to ensure optimal brooding and growing are as follows:

- Infrared thermometers used to monitor uniform bedding temperatures (28-30°C/82-86°F, relative humidity at 60-70%) before chicks are placed, and digital ear thermometers to measure chick vent (rectal) temperatures (39.4-40.5°C or 103-105°F) to evaluate hatching, transport and brooding conditions.
- LED lights along the water and feed lines are being used to attract chicks to feed and water.
- Cleaning water lines and drinkers to remove biofilms (where CP can persist and be protected against most disinfectants and antimicrobials) during down-times.
- Brooding paper at chick placement with dispersed crumbled feed help get flocks off to a good start.
- Checking "crop fill" is a practical way to evaluate the quality and uniformity of brooding practices (ideally 95% by 24 hours after placement).
- The use of alternative products has proven to be effective in promoting normal intestinal flora development and reducing the incidence of NE in ABF production systems. Some companies have observed benefits by administering a probiotic or CE product at the hatchery and acidifying the drinking water (pH 4-6) during the first 7-10 days of life.

Adequate litter depth with good absorptive material is indispensable. Litter moisture exceeding 35% is correlated with high-risk factors associated with bird health and welfare. Drier litter is also achieved by the use of circulation fans and proper drinker management (leak prevention). In dirt floor houses, where previous outbreaks have been linked to high spore soil counts, thorough house cleaning and disinfection followed by the addition of NaCl (0.292-0.366 kg/m^2 or 60-75 lb/1000 ft^2) may be useful to prevent the reappearance of the disease. Ideally, operations using built-up litter must have a minimum down-time of 14-18 days between flocks. During down-time, litter management practices such as crusting off, litter windrowing (in-house composting) and the addition of litter amendments to reduce microbial loads and pH (promote an acidic environment) are often used to inhibit the growth and persistency of various enteric pathogens, including CP. Experiences in the US have shown that NE occurs less frequently in broiler flocks with re-used litter, suggesting that early exposure to small numbers of CP and beneficial gut microflora may help reduce the risk of NE. However, it is also known that on some farms, CP isolates are extremely pathogenic and can cause severe outbreaks even in the absence of coccidiosis as a predisposing factor. Therefore, following severe NE outbreaks, and because CP spores are highly resistant, it is recommended to remove the litter and conduct complete house cleaning and disinfection procedures.

Future Control Options

Future research can better explain the particular mechanisms that encourage pathogenic CP strains to proliferate, and further identify virulence factors that could be targeted by alternative products and/or combined strategies. In addition, further understanding of alternative products and their mode of action could develop targeted and preventive measures. This knowledge will help improve current prevention and intervention strategies. Finally, identifying specific biomarkers and developing diagnostic tools that could help monitor intestinal health, allow early detection of flocks at risk and even differentiate between coccidiosis and NE, would be very useful to determine the appropriate corrective actions and ensure flock health and welfare.

Research exploring vaccination continues to develop products capable of inducing early immunity against CP. Vaccines may be a potential tool if live vectorized (recombinant) products are designed and can be easily administered to protect the intestinal lining against critical virulence factors. Recently a live recombinant attenuated *Salmonella Typhimurium* vaccine (RASV) expressing CP genes coding for exotoxins a-toxin, and NetB has been developed and licensed in the US for mass administration in the hatchery. This *Salmonella* vaccine is designed to colonize lymphoid tissues, and after replicating a few times in the birds, undergo programmed cell lysis facilitating the release of antigens and preventing the spread of the vaccine strain in the environment. Experimentally, oral administration of this recombinant vaccine in broiler chicks resulted in a significant mucosal immune response (by producing immunoglobulin (Ig) A, Ig G and Ig M antibodies) that protects against clinical signs and adverse effects of CP challenge. At the time of this writing, field studies evaluating the protection and compatibility of this vaccine with other vaccines and alternative products are underway.

Summary

- NE is a complex and multifactorial enterotoxemia in broilers caused by CP strains that possess virulence factors and produce exotoxins that damage the lining of the small intestine and liver.
- NE is one of the costliest broiler diseases worldwide due to its subclinical and clinical effects on broiler health and performance.
- The incidence of NE has increased in the wake of the growing ABF broiler production trend.
- Several predisposing factors can help create conditions leading to the proliferation of pathogenic CP strains and the onset of NE breaks.
- An integrated prevention strategy is based on current information about predisposing factors and how to avoid their harmful effects on intestinal health, gut microflora and nutrient digestion and absorption.
- Coccidiosis control and avoiding other factors capable of causing intestinal damage, disruptions of the gut microflora and digestive and absorption processes are critical in preventing this disease.
- As of today, it is unlikely that a single alternative product or strategy will replace AGP and eliminate the risk of NE in an ABF production system.
- Alternative products used as feed additives are being increasingly used either alone or in combination to promote intestinal health, prevent disruptions in the gut microflora and reduce the establishment and proliferation of enteric pathogens such as CP.
- On-going research to determine the mechanisms and virulence factors by which CP causes NE, and a further understanding of the interactions and development of alternative products and recombinant vaccines, will generate the knowledge needed to improve current prevention strategies.

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