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Original article

Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide

Shawkat A. M'Sadeq [a], Shubiao Wu [a, *], Robert A. Swick [a], Mingan Choct [a, b]

[a] School of Environmental and Rural Science, Armidale 2351, Australia
[b] Poultry Cooperative Research Centre, Armidale 2351, Australia

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ABSTRACT

Poultry production has undergone a substantial increase compared to the livestock industries since 1970. However, the industry worldwide is now facing challenges with the removal of in-feed antibiotics completely or gradually, as the once well-controlled poultry diseases have re-emerged to cause tremendous loss of production. Necrotic enteritis (NE) is one of the most important diseases which costs the industry over two billion dollars annually. In this paper, we review the progress on the etiology of NE and its control through dietary modifications, pre- and probiotics, short chain fatty acids, and vaccination. The other likely measures resulted in the most advances in the toxin characterization are also discussed. Vaccine strategies may have greater potential for the control of NE mainly due to clearer etiology of NE having been elucidated in recent years with the identification of necrotic enteritis toxin B-like (NetB) toxin. Therefore, the use of alternatives to in-feed antibiotics with a better understanding of the relationship between nutrition and NE, and limiting exposure to infectious agents through biosecurity and vaccination, might be a tool to reduce the incidence of NE and to improve gut health in the absence of in-feed antibiotics. More importantly, the combinations of different measures may achieve greater protection of birds against the disease. Among all the alternatives investigated, prebiotics, organic acids and vaccination have shown improved gastrointestinal health and thus, have potential for the control of NE.

1. Introduction

Poultry production has undergone a substantial increase compared to other animal food-producing industries since 1970 (Yegani and Korver, 2008). Improvements in housing, genetic selection for growth rate, and advances in feed formulation achieved by matching nutrient requirements of the birds and nutrient contents of the feedstuffs, have resulted in higher meat yield, improved feed conversion and lower mortality rates (Choct et al., 1999; Cooper and Songer, 2009). As growth rate and feed conversion ratio improve, the birds' nutrition and health care are becoming more demanding (Choct et al., 1999). The nutritional and health status of poultry are interlinked with gut health which includes immune system, gut microbial balance and macro and micro-structural integrity of the gut. The health of the gastrointestinal tract (GIT) affects digestion, absorption and metabolism of nutrients, disease resistance and immune response (Kelly and Conway, 2001; Yegani and Korver, 2008). The disturbances of these processes can result in enteric diseases (Dekich, 1998). This makes it important to pay attention to gastrointestinal health; usually any slight change is mostly accompanied by disruption of gut health and thus overall performance.

Enteric diseases are one of the most important problems in the poultry industry because of high economic losses due to decreased weight gain, increased mortality rates, worse feed conversion ratio, greater medication costs, and increased risk of contamination of poultry products for human consumption (Timbermont et al.,

* Corresponding author.
E-mail address: shubiao.wu@une.edu.au (S. Wu).
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2011). Several pathogens including viruses, bacteria, macroparasites and other infectious and non-infectious agents are reported as possible causes of enteric diseases either alone or in synergy (Reynolds, 2003). Many conditions have been associated with gastrointestinal problems such as diarrhea, wet droppings, dysbacteriosis, intestinal colibacillosis, malabsorption syndrome, coccidiosis and necrotic enteritis (NE). Enteric disorders are frequently associated with an overgrowth of *Clostridium perfringens*. Infections with this bacterium in poultry can cause NE, necrotic dermatitis, cholangiohepatitis, as well as gizzard erosion (Hafez, 2011). NE is the most common clostridial enteric disease in poultry, which typically occurs in broiler chickens (Cooper et al., 2013). NE is characterized by necrosis and inflammation of the GIT with a significant decline in growth performance and, in clinical cases, a massive increase in flock mortality. The total cost of NE outbreaks globally is estimated to be over $2 billion annually (Van der Sluis, 2000).

Antibiotics have been used as an effective tool to improve animal performance, by selectively modifying the gut microflora, decreasing bacterial fermentation, reducing thickness of the intestinal wall and suppressing bacterial catabolism. All these are important to improve health, nutrient availability and growth performance (Carlson and Fangman, 2000). Hence, dietary antimicrobials not only improve poultry growth and feed conversion efficiency, but also control enteric disease outbreak (Kim et al., 2011). The use of antibiotics in feed and for treating animals is second only to the medical use (Dahiya et al., 2006). It has been estimated that 11.15 million kg of antibiotics are used in animal feed in the USA alone each year (Union of Concerned Scientists, 2001) and 4.7 million kg or 35% of all antibiotics administered in Europe in 1999 were used in animal feed (European Commission Directorate General XXIV Directorate B, 1999). Hence, antibiotics have come under increasing scrutiny by government regulators, scientists and consumers because of the emergence of antibiotic-resistant "superbugs". European countries have now prohibited the use of in-feed antibiotics in poultry feed (Van Immerseel et al., 2004). Without the use of in-feed antibiotics, the Animal Health Institute of America has estimated that the USA will require an additional 12 million pigs, 23 million cattle and 452 million chickens to reach the levels of production attained by the current practices (Dahiya et al., 2006).

With a ban of in-feed antibiotics in European countries, the incidence of NE has increased on the broiler farms of these countries (Casewell et al., 2003; Hofacre et al., 2003). At the same time, the focus on alternative strategies has increased to secure animal health and thus the efficiency of livestock production. These alternative strategies include modulation of gut microflora, augmentation of immune response and pathogen reduction through management, vaccination, nutritional strategies and feed additives.

2. Necrotic enteritis in broiler chickens

2.1. Epidemiology

NE has a high mortality rate with severe economic losses. The disease has been reported in many countries, including the United Kingdom (Parish, 1961), Australia (Nairn and Bamford, 1967), Canada (Helmboldt and Bryant, 1971) and France (Casewell et al., 2003). The primary causative agent of NE is *C. perfringens* and the source of *C. perfringens* is ultimately the chickens themselves (Cooper and Songer, 2009). Outbreaks of NE in poultry, past and present, have been associated with *C. perfringens* contamination of the chickens' feed (Eleazer and Harrell, 1976; Hofacre et al., 1986; Nairn and Bamford, 1967). Occurrences of NE are also affected by season (Kaldhusdal and Skjerve, 1996), dietary restriction (Olkowski et al., 2006), bedding on high fiber litter (Branton et al., 1997) and management-related stress (Craven, 2000).

NE usually occurs in broiler chicks at 2–6 weeks of age (Cooper and Songer, 2010). Generally, NE is not typically known as a seasonal disease, although the occurrences of NE between different latitudes appear to contradict this. Kaldhusdal and Skjerve (1996) suggested that univariate regression analysis in south-eastern Norway indicated that NE occurred more often during the months October–March than during the months April–September, whilst in Canada it mostly appeared in July–October (Long, 1973). In the United Kingdom, the peak incidence of NE is during winter with a lower incidence during the warmer seasons (Hermans and Morgan, 2007).

2.1.1. C. perfringens as a causative agent of NE

C. perfringens is a Gram-positive, spore forming anaerobic bacterium, able to produce several enzymes and toxins responsible for NE symptoms and lesions (Van Immerseel et al., 2004). *C. perfringens* can be found in poultry litter, feces, soil, dust and in healthy bird intestinal contents (Dahiya et al., 2005). It is expected that small numbers of *C. perfringens* are resident, transiently or permanently, in the GIT of most bird species (Cooper and Songer, 2009). When poultry meat is analyzed for *C. perfringens*, in some cases up to 84% of meat samples are positive (Craven et al., 2001b). It is reported that colonization or contamination of poultry by *C. perfringens* occurs during the early life of the animal, and can commence in the hatchery environment (Van Immerseel et al., 2004). *C. perfringens* is found in eggshell, paper pads and chicken dander in the hatchery (Craven et al., 2001a). Craven et al. (2003) indicated that the *C. perfringens* contamination found in broiler carcasses can begin in the breeder hen, and be transmitted through the hatchery and growing area. Free-living birds such as crows have high counts of *C. perfringens* in their intestinal droppings, which indicate that wild birds also suffer from NE (Asaoka et al., 2004). Craven et al. (2001b) found that swabs taken from poultry farms showed an incidence of *C. perfringens* from a variety of sources, including live flies, walls, dirt outside the entrance, fans, floor, nipple-drinker drip-cups, water pipes, litter material, chick delivery-tray liners and boots of farm staff before chicks were placed. Even feed samples taken after 2 weeks following bird placement had an incidence of *C. perfringens*. This indicates that different sources and strains of *C. perfringens* can colonize in the birds and produce NE. Additionally, NE has been reported in a variety of wild birds including ducks (Chakrabarty et al., 1983), wild geese (Wobeser and Rainnie, 1987), Laysan albatross (Work et al., 1998), mute swan (Duff et al., 2011), bobwhite quail (Shivaprasad et al., 2008) and wild crows (Asaoka et al., 2004; McOrist and Reece, 1992). With the gradual move from caged or housed chicken raising to free range worldwide, occurrence of NE in free range flocks becomes a major concern as the domestic chickens are prone to be infected through wild birds. This poses eminent challenge in the management and care of free range chickens which may be less than when they are raised in housed conditions.

2.1.2. Toxins to produce NE

C. perfringens is able to produce several types of toxins, while individual strains only produce a subset of these toxins (Van Immerseel et al., 2008). *C. perfringens* strains (A, B, C, D and E) are classified according to the production of four major extracellular toxins alpha (α), beta (β), epsilon (ε) and iota (ι), while various strains can also produce other toxins, including β 2 toxin, perfringolysin O [θ-toxin], collagenase [κ-toxin], enterotoxin, theta toxin etc. (Petit et al., 1999). Type A produces α-toxin, type B produces α, β and ε toxins, type C produces α and β toxins, type D

produces α and ε toxins and type E produces α and ι toxins (Brynestad and Granum, 2002). In poultry, NE has been reported being caused by type A and to a lesser extent by type C (Engström et al., 2003).

Alpha-toxin has been reported to be the main virulence factor in poultry NE (Van Immerseel et al., 2008) with phospholipase C and sphingomyelinase activity (Flores-Díaz and Alape-Girón, 2003). Although this toxin was believed to be the main virulent factor in the pathogenesis of NE for long time, the importance of α-toxin in the pathogenesis of NE has been questionable. Early studies on the factors inducing NE showed that intro-duodenal infusions of both large volumes of *C. perfringens* broth culture and crude toxins (Al-Sheikhly and Truscott, 1977) into chickens, resulted in typical NE lesions. From these studies the authors concluded that α-toxin was the main toxin produced by *C. perfringens*, and then it was considered the most virulent factor in the pathogenesis of NE. Fukata et al. (1988) found that 21 out of 56 germ-free chickens, inoculated with either purified α-toxin or a supernatant of broth cultures of *C. perfringens* died after inoculation, whereas no bird died after receiving a culture supernatant neutralized by anti-alpha-toxin serum. Later on, Hofshagen and Stenwig (1992) found a significantly higher amount of α-toxin in isolates from birds with NE compared with isolates from birds without NE. In a more recent study, Cooper and Songer (2009) suggested that immunization with α-toxin gave substantial protection against NE and Rehman et al. (2009) concluded that α-toxin can damage the intestinal mucosal barrier. Contradicting these studies, an *in vitro* study (Gholamiandekhordi et al., 2006) demonstrated no difference in α-toxin production between *C. perfringens* isolated from healthy flocks and those isolated from NE outbreaks flocks. The most convincing evidence that α-toxin of *C. perfringens* was not a major factor in producing NE in chickens came from a study using an α-toxin negative mutant of *C. perfringens* generated from a virulent strain isolated from NE infected birds. This study demonstrated that the constructed mutant negating α-toxin gene induced the same type of NE lesion as that of the wild type strain (Keyburn et al., 2006). Other studies also argue against the role of α-toxin in NE. Furthermore, histological analysis of tissue in early stages of NE lesion development is inconsistent with the α-toxin phospholipase C or sphingomyelinase activities (Olkowski et al., 2008).

Recently, a new pore-forming toxin named necrotic enteritis toxin B-like (NetB) was discovered by Keyburn et al. (2008) as a virulence factor for the development of NE. NetB toxin showed limited amino acid similarity with pore-forming β-toxin (38% identity) and the α-toxin of *Staphylococcus aureus* (31% identity). This toxin was identified from *C. perfringens* type A strain isolated from infected chickens. Both recombinant and native NetB showed cytotoxic activities against the chicken leghorn male hepatoma cell line and the mechanism of action appeared to include the formation of a hydrophilic pore in the cell membrane with a functional diameter of 1.6–1.8 nm. A complemented NetB mutant was able to cause necrotic lesions in the gut of experimentally infected broilers. Additional evidence for the role of NetB in disease comes from the observation that chickens suffering from NE carry the NetB gene and produce highly conserved NetB toxin (Keyburn et al., 2010). This has been confirmed by surveys in North America, where all *C. perfringens* isolates from birds infected with NE carried the NetB gene, while only a small percentage of isolates from healthy birds carry the gene (Chalmers et al., 2008; Martin and Smyth, 2009). Keyburn et al. (2010) reported that NetB positive *C. perfringens* strain isolates from diseased birds were able to produce NetB *in vitro* and therefore only strains producing NetB were able to induce disease. This was supported by the report of Smyth and Martin (2010) where all NetB positive isolates induced NE in challenged birds, whereas none of the NetB negative isolates produced disease. This suggests that the presence of NetB is a critical factor in the development of NE.

2.2. Symptoms of necrotic enteritis in poultry

2.2.1. Clinical signs

The clinical form of NE is associated with signs such as ruffled feathers, relative immobility, depression, anorexia, diarrhea and decreased appetite (Al-Sheikhly and Al-Saieg, 1980; Al-Sheikhly and Truscott, 1977; Helmboldt and Bryant, 1971). Wet litter is also sometimes an early indicator of the disease (Riddell and Kong, 1992). Birds displaying clinical signs generally die within a few hours, with mortality rates up to 1% per day (Helmboldt and Bryant, 1971). In the acute form of NE, characterized by a sudden increase in flock mortality, birds usually die without premonitory signs (Kocher and Choct, 2008).

Diarrhea and associated wet litter were noted in the wood shaving litter of birds suffering from NE (Kalshusdal and Hofshagen, 1992). Elwinger and Teglöf (1991) found a direct correlation between poor litter condition and sticky droppings. In field surveys conducted globally (Van der Sluis, 2000) and in the United Kingdom (Hermans and Morgan, 2003), researchers associated NE with diarrhea or wet litter. Williams (2005) noted that in the United Kingdom, any sudden increase in litter moisture of poultry farms is associated with NE and antibiotic therapy is initiated. Helmboldt and Bryant (1971) suggested that acute NE may be associated with diarrhea or wet litter (Nairn and Bamford, 1967).

2.2.2. Subclinical signs

In subclinical forms of NE there is no peak of mortality and no clinical signs are present (Timbermont et al., 2011). Subclinical forms of NE are usually associated with reduced feed intake and weight gain and increased feed conversion ratio (Kalshusdal and Hofshagen, 1992; Lovland and Kaldhusdal, 2001). They are also associated with hepatitis and cholangiohepatitis (Van Immerseel et al., 2004). During subclinical infection, bacteria can reach the portal blood stream and bile duct. Colonization of high numbers of *C. perfringens* in hepatic tissue result in cholangiohepatitis (Timbermont et al., 2011). Diseased livers are greatly enlarged and have a pale reticular pattern with white or red foci and histopathological lesions characterized by bile duct hyperplasia, cholangitis, fibrinoid necrosis and sometimes focal granulomatous inflammation (Løvland and Kaldhusdal, 1999). Onderka et al. (1990) reported that inoculation of birds with *C. perfringens* produced cholangiohepatitis, enlarged liver, tan-colored liver with red and white foci and edematous gall bladder. During meat inspection at a slaughter house, infected livers were found, without any clinical signs in the flock (Timbermont et al., 2011). Although clinical forms of NE may cause high levels of mortality, the subclinical form of NE is more economically important than the clinical form because it may persist in the flock without any clinical manifestation. Thus, untreated birds suffering from subclinical NE can cause huge economic loses in the poultry production industry (Dahiya et al., 2006).

Gross lesions are usually restricted to the duodenum, jejunum and ileum (Timbermont et al., 2011) and also can occur in the caeca (Van Immerseel et al., 2004). The small intestine of infected birds is friable, dilated, hyperemic, thin walled and filled with gas, and the mucosal surfaces are covered by tan-orange pseudo-membranes and occasional hemorrhages (Broussard et al., 1986; Olkowski et al., 2006). Microscopic examination shows a strong inflammatory reaction to *C. perfringens* in the early stages of NE. The lamina propria is infiltrated and hyperemic with several inflammatory cells, especially at the interface of the basal domain of enterocytes and lamina propria. These areas are edematous, permitting the extensive disorder of the structural integrity between the enterocytes

and the lamina propria (Olkowski et al., 2006). Histopathological examination of later stage NE lesions shows extensive villous necrosis (Broussard et al., 1986), coagulation of necrosis of the mucosa in all segments of the small and large intestine (Olkowski et al., 2006) and a clear line of demarcation between necrotic and viable tissue. An accumulation of heterophilic granulocytes at the junction is seen (Al-Sheikhly and Al-Saieg, 1980; Long, 1973). Hemorrhage or patchy congestion is present over all the lamina mucosa, particularly in the vicinity of the crypts. The crypts are usually misshapen and inflated by pink mucus and necrotic cellular debris (Olkowski et al., 2006).

2.3. Predisposing factors for necrotic enteritis

Although *C. perfringens* is the primary causative agent of NE in poultry, other contributory factors that alter the microenvironment of GIT and create a favorable environment for *C. perfringens* overgrowth are essential to produce both the clinical and subclinical types of NE. Factors that predispose the bird to NE include co-infection of other parasites most evidently coccidiosis, stress and immunosuppression, and nutrition.

2.3.1. Coccidiosis

Coccidiosis is an enteric disease caused in the fowl by numerous *Eimeria* species. Some species (*Eimeria praecox*, *Eimeria mitis* or *Eimeria acervulina*) produce less severe clinical coccidiosis than others (*Eimeria brunetti*, *Eimeria maxima*, *Eimeria necatrix*, and *Eimeria tenella*) (Williams, 2005). The intestinal damage caused by coccidia is an essential predisposing factor for NE (Al-Sheikhly and Al-Saieg, 1980; Rodgers et al., 2015; Williams, 2005), allowing *C. perfringens* overgrowth and production of toxins (Van Immerseel et al., 2008). Intestinal damage during *Eimeria* infection will result in leakage of plasma proteins into the lumen of the intestinal tract, which is a rich nutrient substrate and favorable for *C. perfringens* proliferation and toxin production (Van Immerseel et al., 2004). Collier et al. (2008) suggested that coccidial infection induces mucogenesis as a result of a host mucogenic response, providing a growth advantage for *C. perfringens*.

For these reasons, several studies have used *Eimeria* spp. in conjunction with *C. perfringens* to induce NE experimentally. *E. maxima*, *E. acervulina* and *E. necatrix* are known to be the most suitable species to induce NE (Al-Sheikhly and Al-Saieg, 1980; Hofacre et al., 1998; Van Immerseel et al., 2004; Williams, 2005). *Eimeria* vaccine has also been used to enhance *C. perfringens* to induce NE (Gholamiandehkordi et al., 2007; Timbermont et al., 2009). The time and dose of administration of the virulent *Eimeria* or coccidial vaccine are important for inducing experimental NE. The time should not be more than 4–5 days before *C. perfringens* challenge (Williams et al., 2003; Williams, 2005). The doses of *Eimeria* administration needed for experimentally inducing NE are different according to the models which have been used. For example, Wu et al. (2014) used a suspension of 5000 sporulated oocysts each of *E. maxima* and *E. acervulina*, and 2500 oocysts of *E. brunetti*; Williams et al. (2003) used 30,000 sporulated oocysts of *E. maxima* and Gholamiandehkordi et al. (2007) suggested using attenuated coccidial vaccines at 10-times the recommended vaccination doses.

2.3.2. Stress and immunosuppression

Any stressful condition in broiler chickens could predispose them to NE, because it could change the intestinal environment in such a way that the risk of induction of NE is raised (McDevitt et al., 2006). For example, alteration in feeding regime (moving from a starter diet to a grower diet) in young chicken causes such stress in the GIT. Increases in stocking density are also frequently associated with NE (McDevitt et al., 2006; Tsiouris et al., 2015). Immunosuppressive agents such as infectious bursal disease, Marek's disease, chicken anemia virus and Gumboro disease have been suggested as causing an increase in the severity of NE (Lee et al., 2011; McReynolds et al., 2007; Timbermont et al., 2011; Williams et al., 2003). Indeed, in several studies, infectious bursal disease vaccine has been used as a predisposing factor to experimentally induce NE (Gholamiandehkordi et al., 2007; McReynolds et al., 2004, 2007; Timbermont et al., 2009). This has been done by inoculation with usual doses of infectious bursal disease vaccine (intermediate class) (Gholamiandehkordi et al., 2007; Timbermont et al., 2009) or at 10-times the recommended vaccination dose rate with infectious bursal disease vaccine (intermediate plus) (Nikpiran et al., 2008).

2.3.3. Nutritional factors

The key risk factor for the development of NE is alteration of the GIT environment which creates a favorable environment for *C. perfringens* growth. Diet is now widely recognized as having a strong impact on the incidence of NE in broiler chickens (Annett et al., 2002; Drew et al., 2004). Evidence arising from several studies has shown that there is a relationship between cereal type used in the diet, dietary protein levels and anti-nutritional factors and the incidence of NE (Kalshusdal and Hofshagen, 1992; McDevitt et al., 2006; Riddell and Kong, 1992). It is widely believed that a diet with high levels of indigestible water soluble non-starch polysaccharides (NSPs) strongly influences the incidence of NE in broilers. Numerous studies have revealed that a diet comprising cereals such as barley, rye and wheat increase the digesta viscosity and enhance the development of NE as these grains contain high amounts of NSPs such as arabinoxylans or β glucans (Annett et al., 2002; Dahiya et al., 2006; Kalshusdal and Hofshagen, 1992; McDevitt et al., 2006). The NSPs and certain types of starch in the cereals are not digestible by the enzymes present in the bird's GIT (Iji and Tivey, 1998; Juśkiewicz et al., 2004). They act as substrates for the gut microflora and provide an opportunity for these organisms, including pathogenic bacteria, to proliferate (Apajalahti et al., 2004; Choct et al., 1996; Iji and Tivey, 1998; McDevitt et al., 2006). As higher NSPs in diets lead to increased digesta viscosity, prolonged transit time and decreased nutrient digestibility (Choct et al., 1999), this may lead to the increased proliferation of *C. perfringens*, predisposing birds to NE (Annett et al., 2002). The NSPs are also hydrophilic, which encourages birds to drink more water in order to maintain homeostasis. The increased water intake also increases water excretion, affecting litter quality and thus allowing the pathogenic bacteria to proliferate (McDevitt et al., 2006). Furthermore, some NSPs interact with epithelial protein and glycoproteins, increasing mucin secretion from the tissues (Kleessen et al., 2003). This provides an opportunity to pathogenic microorganisms to adhere to the mucin and proliferate (McDevitt et al., 2006).

The level of crude protein, protein source, and the amino acid content of a diet have been thought to be associated with the incidence of NE. Poultry diets with high protein content or those rich in animal protein such as meat and bone meal or fish meal predispose birds to NE (Kaldhusdal and Skjerve, 1996; Williams et al., 2003; Williams, 2005; Wu et al., 2010, 2014). Diets that have a high protein concentration or imbalanced amino acid profiles reduce the digestibility of these compounds in the upper part of digestive system (McDevitt et al., 2006). Thus the indigestible protein in the lower part of GIT acts as substrate for the gut microflora (Timbermont et al., 2011). The fermentation of protein produces unfavorable outcomes such as phenols, thiols, amines, ammonia, indoles and increases the pH of the lower part of the GIT, which encourages the proliferation of pathogenic bacteria such as *Clostridium* sp. (Juśkiewicz et al., 2004; Lan et al., 2005). Another

possible explanation of the association between fish or meat meal and NE could be related to their higher zinc, glycine and methionine concentrations. Fish meal is relatively high in zinc (NRC, 1984), glycine and methionine (Dahiya et al., 2007). In an *in vitro* study, Baba et al. (1992) proposed that dietary zinc increased the production of α-toxin and protected the α-toxin destruction by trypsin. Glycine is an amino acid that stimulates the growth of *C. perfringens* (Dahiya et al., 2007) and is positively correlated to the *C. perfringens* population in the intestine (Wilkie et al., 2005).

Other anti-nutritional factors other than NSPs such as lectins, trypsin inhibitors and tannins may also predispose birds to NE. Lectins from wheat and soybeans are proteins and glycoproteins, which interact vigorously with epithelial tissues (Pusztai and Bardocz, 1996) and cause damage, change in microflora population and immune response in animals (Lan et al., 2005; Pusztai and Bardocz, 1996). Lectins alter bacterial attachment to the GIT and change the extent and rates of bacterial growth (Giovannini et al., 1996; McDevitt et al., 2006). Trypsin inhibitors, found in soybean meal, reduce the digestibility of protein and thus increase nitrogen concentration in the lower GIT, which provides suitable conditions for the growth of proteolytic bacteria such as *C. perfringens* (Clarke and Wiseman, 2005; McDevitt et al., 2006). Tannins are present in many dietary ingredients such as rapeseed meals and beans, and can interact strongly with protein, leading to tissue damage that may predispose birds to NE (McDevitt et al., 2006; Robins and Brooker, 2005). There are also other compounds such as mycotoxins, glucosinolates, alkaloids and polyphenols which interact with bacteria, altering bacterial proliferation (McDevitt et al., 2006) and may play a role in predisposing birds to NE.

The dietary factors play important roles in predisposing the birds to NE infection. However, diets having higher NSPs or containing high protein level may lead to more frequent NE occurrence in broiler chickens. Whereas other nutritional factors such as lectins, trypsin inhibitors and tannins may cause birds to be infected with NE, they are less significant. Attention may need to be paid to reduce levels of NSPs and protein especially animal protein in the diets to minimize the possibility for the flocks to succumb to NE disease.

2.3.4. Physical form of diet

The physical form of poultry diets may affect the physiological and morphological characteristics of the GIT (Engberg et al., 2004). Finely ground feed allows *C. perfringens* to grow faster than coarsely ground feed, which can lead to occurrence of NE in the field (Engberg et al., 2002). Branton et al. (1987) observed that use of a coarsely ground wheat diet decreased mortality from NE to 18.1%, whereas a finely ground wheat diet resulted in mortality of 28.9%. This may be related to that coarsely ground mash stimulates gastric function, including secretion of hydrochloric acid, and simultaneously increases the retention time of feed in the proventriculus and gizzard (Engberg et al., 2002).

3. Measures to control necrotic enteritis post antibiotic era

Antibiotics have played a major role in the suppression of clinical NE (Williams, 2005) and extensive reviews have been performed in the past. However, public concern over the use of in-feed antibiotics and the emergence of antibiotic-resistant bacteria has led many countries to ban the use of dietary antimicrobials. To be able to cope with the removal of antibiotics from animal feed, many studies have been conducted to investigate the possible alternatives to be used to control the diseases in animal production. Herein, we will discuss the development of antibiotics alternatives and their utilization in broiler chicken production. As a result of consumer pressure to reduce in-feed antibiotics in animal feed, Sweden was the first country to ban the use of antimicrobials for growth-promoting purposes (Kocher and Choct, 2008). In 1995, Denmark banned the use of avoparcin and virginiamycin in animal feed (Casewell et al., 2003). In 1997, the European Union restricted the use of some antibiotics in animal feed and followed this by a general ban on the use of all antibiotics in all animal feed in 2006 (Kocher and Choct, 2008). After the banning of sub-therapeutic antibiotics in animal feed, the incidence of NE in poultry farms increased in many European Union countries (Van Immerseel et al., 2004). For example, the incidence of NE in France was 4% in 1995 and increased to 12.4% in 1999 (Drouin, 1999). Similarly, in the USA, who also stopped using in-feed antibiotics, the incidence of NE and other diseases like gangrenous dermatitis, botulism and cholangiohepatitis has increased (Shane, 2004). Thus, the question has now become 'how to control this disease in the absence of antibiotics'. To investigate a method for controlling NE, the factors that predispose birds to develop NE must be better understood and alternative dietary supplements and management strategies to control NE must be developed.

Based on the literature, there are three basic strategies used to control NE. These strategies are: amplification of immune response, pathogen reduction, and dietary modification and/or use of feed additives (Dahiya et al., 2006). This paper will review numerous strategies for controlling NE, including dietary modification, the use of feed enzymes, probiotics, prebiotics and organic acids, and vaccination strategy will be discussed in the following sections.

3.1. Dietary modifications and feed enzymes

Modification of diet and the addition of enzymes cannot provide total protection against NE, but may reduce the risk of NE by improving digestion. For example, dietary cereals such as barley, rye and wheat contain high amounts of arabinoxylans or β glucans, mannans, cellulose, lignin and ingredients, which cannot be digested by poultry. These non-digestible feed constituents increase the digesta viscosity and encourage the development of NE (Dahiya et al., 2006; McDevitt et al., 2006). Also, poultry diets with high protein content, or those rich in animal protein such as meat and bone meal or fish meal predispose birds to NE (Kaldhusdal and Skjerve, 1996; Williams et al., 2003; Williams, 2005; Wu et al., 2010, 2014). Thus, reducing indigestible carbohydrates and proteins that predispose birds to NE may decrease the risk of NE outbreak in poultry farms.

It has been demonstrated that inclusion of exogenous enzymes to wheat, barley, oat or rye based diets can significantly decrease digesta viscosity in the small intestine (Bedford and Classen, 1992; Choct et al., 1999). Choct et al. (1999) reported that the addition of xylanase to wheat base diets decreased digesta viscosity and fermentation, increased nutrient digestion and digesta passage, and reduced the amount of nutrients available to the microflora, which, in turn, may reduce the bacterial population in the small intestine. Sinlae and Choct (2000) also demonstrated that dietary supplementation of xylanase reduced numbers of *C. perfringens*. Jackson et al. (2003) proposed that β mannanase would reduce the severity of challenge by *Eimeria* spp. and *C. perfringens* in broiler chickens, and thus reduce NE lesion scores in the intestine. However, supplementation of pentosanase in a wheat based diet did not have any beneficial effect on the susceptibility to NE in broiler chickens. Despite the contradictory results from published studies on the effect of feed enzymes on various bacterial populations, including *C. perfringens* in the broiler intestine, the supplementation of exogenous enzymes alone cannot provide complete protection against NE (Elwinger and Teglöf, 1991; Riddell and Kong, 1992). Enzymes have no direct effect on *C. perfringens,* but only change the intestinal environment (Kocher and Choct, 2008).

Therefore, further research is needed to investigate the effect of feed enzymes on the incidence of NE, so as to clarify the roles of the enzymes in the possible control of the disease.

3.2. Probiotics

Probiotics have been defined as a "live microbial feed supplements, which beneficially affect the host animal by improving its microbial balance" (Fuller, 1990). The characteristic of ideal probiotics are that they must be from host origin, resist to gastric acids and bile, persist in the intestinal tract, adhere to epithelium or mucus, produce inhibitory compounds, alter immune response and modulate the microflora activity (Patterson and Burkholder, 2003). The modes of action of probiotics include stimulating the immune system, maintaining gut microflora by competitive exclusion, altering metabolism through increased digestive enzyme activity, decreasing bacterial enzyme activity and ammonia production, and neutralizing enterotoxins (Collins and Gibson, 1999; Patterson and Burkholder, 2003; Simmering and Blaut, 2001; Walker and Duffy, 1998). The mechanisms of competitive exclusion of pathogens include competitive use of nutrients and mucosal binding sites, or production of short-chain fatty acids (SCFAs), low pH and bacteriocins, which are bactericidal or bacteriostatic for pathogenic bacteria (Ohland and MacNaughton, 2010).

A number of studies have reported the potential of undefined (normal gut flora) or defined (characterized bacterial strain) probiotics on the colonization of pathogenic bacteria. Mead (2000) reported that normal gut flora preparations showed efficacy against food borne pathogens such as *Escherichia coli*, *Yersinia enterocolitica*, *Clostridium botulinum*, *C. perfringens*, *Salmonella*, and *Campylobacter jejuni*. Morishita et al. (1997) showed that *Lactobacillus acidophilus* and *Streptococcus faecium* reduced the colonization of *C. jejuni* in the jejunum by 27%. A number of studies have been conducted to evaluate the effects of probiotics on NE in chickens. It has been reported that commercial probiotics reduce gross intestinal lesions from NE and improve feed efficiency (Hofacre et al., 1998). Craven et al. (1999) proposed that feeding normal gut flora to broiler chickens would reduce the *C. perfringens* colonization and decrease the incidence of NE. Hofacre et al. (2003) showed that mortality due to NE was reduced from 60 to 30% when birds were treated with lactic acid bacteria. La Ragione and Woodward (2003) found that colonization and determination of *C. perfringens* was suppressed when 1 day old and 20 day old birds were inoculated with 10^9 spores of *Bacillus subtilis* strain, then challenged 24 h later with 10^5 CFU of *C. perfringens*, colonization and determination of *C. perfringens* was suppressed. Jayaraman et al. (2013) found that dietary supplementation of *B. subtilis* in experimentally challenged broiler chickens with *Eimeria* and *C. perfringens* reduced FCR, intestinal lesion scores, and *C. perfringens* counts, and increased villus height to crypt depth ratios compared with an infected control without such supplementation.

3.3. Prebiotics

Prebiotics are generally defined as indigestible feed ingredients that selectively stimulate the growth or activity of beneficial bacteria that are already resident in the GIT (Gibson and Roberfroid, 1995). The potential effects of prebiotics on animals were recognized in the 1980s (Hajati and Rezaei, 2010). For a feed ingredient to be classified as a prebiotic, it must be neither digested nor absorbed in the upper part of digestive system, and it must be a selective substrate for one or a limited number of useful bacteria in the GIT, stimulate the bacteria to grow, be metabolically activated, be able to alter the intestinal microflora toward a healthier composition, and be palatable as a food ingredient (Collins and Gibson, 1999; Hajati and Rezaei, 2010). Among those, carbohydrates are commonly seen in the literature as prebiotics. For example, lactose, a disaccharide, has been used as a prebiotic in chickens (Hajati and Rezaei, 2010). We list here several other predominantly used prebiotics, i.e., mannooligosaccharides (MOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides, glycol-oligosaccharides, galacto-oligosaccharides, gluco-oligosaccharides, lactitol, lactulose, malto-oligosaccharides, and trans-galacto-oligosaccharides (Collins and Gibson, 1999; Gibson and Roberfroid, 1995; Patterson et al., 1997). In general, prebiotics can prevent the colonization of bacterial pathogens in the GIT (Bengmark, 2001), lower the gut pH through SCFA production (Gibson and Wang, 1994), and stimulate the immune system (Monsan and Paul, 1995). The following sections will detail the effectiveness of some prebiotics on the control of NE.

MOS is extracted from the yeast cell wall of *Saccharomyces cerevisiae* yeast (Hofacre et al., 2003). The yeast cell wall consists of protein, glucans, and mannan (Klis et al., 2002). MOS consists of mannoproteins, chitin, β (1,3) glucan, and β (1,6) glucan (Kollár et al., 1997). The exact mode of action of MOS is unclear, but β-glucans and mannans are the units with primary functions on bacteria indirectly. The mannans act as a receptor for type-1 fimbriae which are used by some harmful bacteria such as *E. coli* and *Salmonella* to attach to the gastrointestinal wall (Oyofo et al., 1989). β-glucans act as microbial recognition receptors of the innate immune system (Gantner et al., 2003) and both mannans and β-glucans structures stimulate the immune system (Spring et al., 2000). The effects of MOS supplementation on the broiler immune system, gut microflora and gut morphology have been well documented. For instance, Shanmugasundaram and Selvaraj (2012) reported that dietary prebiotics (killed whole yeast cells) increased interleukin-10 mRNA by 9 fold in comparison to the control. Shanmugasundaram et al. (2013) also proposed that 0.2% of killed whole yeast cell prebiotics increased body weight gain, macrophage nitric oxide production and caecal tonsil interleukin-1 mRNA amounts in broilers challenged with coccidia. Ghosh et al. (2011) reported that antibody titer against Newcastle disease was significantly higher in birds fed a yeast cell wall preparation. In the same study, birds were orally infected with *Salmonella pullorum* at 45 d of age. After the oral infection, *Salmonella* counts were lower in digesta in birds fed the yeast cell wall preparation. This was supported by a number of studies, where dietary MOS were effective in reducing *Salmonella* infection (Fernandez et al., 2000; Spring et al., 2000). It has also been reported that the mannan oligosaccharides improved intestinal morphology, such as increasing villi height and altering mucosal architecture (Iji et al., 2001), and decreasing the crypt depth of the mucosa of the small intestine (Yang et al., 2009).

Some studies have been conducted on the effects of prebiotics on *C. perfringens* in reducing the severity of NE. Data from Sims et al. (2004) showed that 6 week old turkeys in a MOS treatment group had significantly fewer *C. perfringens* in their large intestines compared with control birds. This finding is supported by Kim et al. (2011) who reported that dietary supplementation with MOS decreased *C. perfringens* populations in broilers' small intestines. Dietary MOS also reduced the abundance of *C. perfringens* in the broiler caeca at 21 d of age (Yang et al., 2008). During NE infection, Mohamed and Hafez (2011) observed that dietary MOS reduced mortality by 12% compared with the positive control group. In the same study, dietary MOS supplementation showed a reduction in the severity of lesion scores to 0.57 in contrast to 1.8 in the control group. Also, the results obtained in our group showed that yeast cell wall extract was effective in reducing lesions and improving livability compared with the challenged control (M'Sadeq et al., 2015). In contrast, Hofacre et al. (2003) suggested neither addition of MOS nor FOS to the broilers diet had significant effect on

intestinal lesion scores and mortality caused by NE. Although some controversial results have been observed, prebiotics such as MOS did show its effectiveness to control diseases including NE. Thus, prebiotics may be potentially effective antibiotic alternatives and it is worthwhile to further explore its utilization in the control of NE in broilers.

3.4. Organic acids

Organic acids have been used for decades in feed, to protect feed from microbial and fungal damage. These acids are added to foods as preservative agents and can also be used to control microbial contamination (Kum et al., 2010). In fact, the organic acids that have antimicrobial activity are simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing a hydroxyl group on the alpha carbon such as lactic, malic, and tartaric acids. It has been noted that salts of some of these acids have performance benefits (Dibner and Buttin, 2002). Some short chain carboxylic organic acids, such as sorbic and fumaric acids containing double bonds, also have antifungal activity (Dibner and Buttin, 2002). Several of these organic acids can be produced in small quantities as a result of the fermentation activity of the GIT (Dorsa, 1997) where anaerobic microflora are predominant (Dixon and Hamilton, 1981).

The effects of organic acids on broiler chickens have been well documented. Hassan et al. (2010) proposed that the inclusion of organic acids to broiler diets enhanced growth and feed utilization and feed conversion ratio. Acidification with different organic acids has been shown to reduce pathogenic bacteria toxicity through controlling pathogenic colonization on the gastrointestinal wall, and by inhibiting damage to epithelial cells (Langhout, 2000). The basic principal mode of action of organic acids is that non-dissociated organic acids can diffuse through lipophilic bacteria and mold membranes, and can disrupt the enzymatic reaction and the transport system of the bacteria (Cherrington et al., 1991). As described by Lambert and Stratford (1999), after penetration of organic acids into bacterial cytoplasm the non-ionized organic acids decompose to H (H^+) ions and (A^-) ions. By the time of the decline of the pH inside the bacteria, a specific mechanism (H^+-ATPase pump) will act to return the intracellular levels to normal pH. This process requires energy, which will lead to reduced energy accessibility for the proliferation of cells and eventually stop the bacterial growth or even kill it. Izat et al. (1990) observed that dietary supplementation of buffered propionic acid significantly reduced the total number of *E. coli* in the intestinal tract. Thompson and Hinton (1997) found that organic acids are bactericidal for *Salmonella* serotype Enteritidis PT4. Chaveerach et al. (2004) demonstrated that organic acids in the drinking water of young chicks could have a potential effect on *Campylobacter* infection. Czerwiński et al. (2012) found a negative correlation between *Enterobacteriaceae* numbers and the concentration of undissociated propionate, acetate and butyrate in the caeca. Dietary addition of organic acids can also improve the digestibility of protein and amino acids (Afsharmanesh and Pourreza, 2005), calcium, phosphorus, magnesium and zinc (Garcia et al., 2007). Also, it has been reported that organic acid supplementation can improve gastrointestinal villi height and cell proliferation (Adil et al., 2010). Other activities of organic acids associated with acidification include the increase of pancreatic secretions, microbial phytase activity, and the improvements of digestive enzyme activity (Dibner and Buttin, 2002).

Recently, microencapsulated organic acids have been used in poultry diets. The rationale behind using coated organic acids is that non-protected organic acids are digested and absorbed in the upper part of digestive tract, while coating organic acids prevents dissociation in the upper part of the digestive tract and directs their bioactivity towards the lower GIT. The applications of microencapsulated organic acids on animals are limited. Smulikowska et al. (2009) suggested that there is no growth promoting response to coated sodium butyrate or its salt in birds raised under optimized conditions. This finding has been supported by a number of other researchers (Czerwiński et al., 2012; Smulikowska et al., 2009; Zhang et al., 2011). Jerzsele et al. (2011) suggested that protected sodium butyrate had no beneficial effects in birds challenged with *C. perfringens*, but a combination of sodium butyrate with essential oils protected with vegetable fat increased body weight gain, villus height and decreased gross lesion scores compared with a control. In conclusion, organic acids have not shown effective protection of birds from pathogenic *C. perfringens* infection to alleviate the NE symptoms. However, further studies are required to investigation why such additives are not so far effective against NE as they are protective against *E. coli* (Izat et al., 1990) or *Campylobacter* (Chaveerach et al., 2004) in broiler chickens.

3.5. Vaccination

Vaccination has been an effective way to prevent humans and animals from many infectious diseases. It can enhance specific immunity of the organisms to viral and bacterial diseases. Vaccines have also been successfully applied to control numerous clostridial diseases in livestock animals (Walker, 1992). Therefore, vaccination against NE disease is proposed to provide an alternative treatment for NE in poultry especially when more convincing evidences was revealed that the toxin NetB is responsible for the disease (Keyburn et al., 2008, 2013).

Vaccination strategies have been put forward for the control of NE mainly in broiler chickens. These strategies were extensively reviewed recently by Mot et al. (2014). Prior to the discovery of NetB, the earlier vaccination focused on toxins that may not be associated to NE by and large, for example, α-toxin. Thus, the vaccines developed only had limited success in controlling NE (Cooper et al., 2009; Hoang et al., 2008; Zekarias et al., 2008), while the partial protective effect of α-toxin based vaccine may be due to the association of α-toxin protein with cell membrane that can have immune interaction to perform such protection (Keyburn et al., 2013). On the other hand, vaccination against coccidiosis has been also used to protect birds from NE occurrence (Jackson et al., 2003; Tsiouris et al., 2013; Williams et al., 2003). As *Eimeria* infection is widely recognized as one of the most important NE predisposing factors in broilers, it seems reasonable that the reduced risk of coccidiosis in birds would alleviate NE outbreak in the flock.

The most important step forward to developing vaccines to immunize the birds against NE occurred following the discovery of NetB toxin (Keyburn et al., 2008). A recombinant NetB *C. perfringens* (rNetB) was constructed and attenuated as a vaccine by (Keyburn et al., 2013). The birds immunized with rNetB were significantly protected against NE challenged with a mild dose of virulent bacteria, while the effectiveness of the vaccination was not so when a more robust challenge was performed. Alternatively, when the birds were immunized with a combination of rNetB, bacterin and cell free toxoid, significant protection against moderate and severe challenge was observed. It was suggested that *in vitro* levels of NetB produced by virulent *C. perfringens* isolates were too low to produce strong immune response in the birds and thus the combined vaccination of birds with rNetB and other cellular or cell-free antigens may be necessary. Jang et al. (2012) compared four *C. perfringens* recombinant proteins as vaccine candidates using the Montanide™ ISA 71 VG adjuvant in an experimental model of NE. When the broilers were immunized with purified clostridial

recombinant NetB toxin, pyruvate: ferredoxin oxidoreductase (PFO), α-toxin, or elongation factor-Tu, significantly reduced gut lesions were observed. Furthermore, birds immunized with NetB toxin exhibited significantly increased body weight gains and greater NetB toxin antibody titers. The authors suggested that vaccination with NetB toxin or PFO, in combination with ISA 71 VG enhances protective immunity against NE in broiler chickens. Fernandes da Costa et al. (2013) employed similar strategy but used only a formaldehyde NetB toxoid or NetB genetic toxoid (W262A) without attenuated NetB containing *C. perfringens* strains or recombinants. The immunization of poultry with these toxoids resulted in the induction of antibody responses against NetB and provided partial protection against the disease. Furthermore, Mot et al. (2013) used crude supernatant containing active toxin or formaldehyde-inactivated supernatant (toxoid) of a NetB positive *C. perfringens* strain and administered vaccination on 1, 3 and 12 days post hatch. It was found that double vaccination at day 3 and day 12 resulted in a significant decrease in the number of chickens with NE lesions although single vaccination with crude supernatant at day 3 also produced significant protection. However, the efficacy of vaccination using toxoid was lower compared with crude supernatant, and vaccination of 1-day-old chickens with crude supernatant or toxoid did not induce protection, a way supposed to be practical in the broiler production. To be more practical for the industry, Keyburn et al. (2013) performed maternal immunization in hens injected subcutaneously with genetically toxoided rNetB(S254L) alone, *C. perfringens* type A toxoid and toxoid combined with rNetB (S254L). They observed strong serum immunoglobulin Y response to NetB immunized with rNetB (S254L) formulations with anti-NetB antibodies transferred to the eggs and then onto the hatched progeny. It was confirmed that birds derived from hens immunized with rNetB (S254L) combined with toxoid and challenged with a homologous strain (EHE-NE18) had significantly lower levels of disease in a subclinical form compared with birds from adjuvant only vaccinated hens. They demonstrated that maternal immunization with a NetB-enhanced toxoid vaccine is promising for the control of NE in broiler chickens.

Although the protection of birds against NE was achieved by vaccination using NetB or other antigen related vaccines or in combination, more practical protocols and effective vaccines have to be extensively examined to achieve full protection of birds against the disease. The vaccines targeting against NetB antigen and possibly other undiscovered toxins responsible for NE should be explored through a practical vaccination regime for industry application. Undoubtedly, other beneficial combinations of different vaccines, such as non-inactivated supernatants, formalin-inactivated crude toxoids, immunogenic proteins and modified toxins including those that indirectly protect against NE are also important aspects to be investigated so as to achieve desired immunization levels suitable in a commercial application.

3.6. Other potential measures in controlling NE

Recent progress on the characterization of NetB toxin has suggested other possibilities to control NE. NetB has been characterized as a pore-forming toxin with a heptameric structure (Savva et al., 2013; Yan et al., 2013) where the polymerization of the proteins leads to the toxicity of the toxin to the epithelial cells of chicken intestine. It has been recognized that cholesterol plays a key role in the ability of NetB to oligomerize and form functional pores (Savva et al., 2013). This may provide some hints on the development of measures to reduce toxicity of NetB and thus to control NE in birds. Furthermore, the detailed information on the residues important for binding and toxicity will also facilitate vaccine development against NE.

Apart from the toxin NetB which directly produce toxicity to the intestinal tissues, a bacteriocin from NetB positive *C. perfringens* characterized by Timbermont et al. (2014), perfrin, was considered important in the pathogenesis of NE in broilers. This bacteriocin inhibits growth of other *C. perfringens* strains and leads to extensive and selective growth of *C. perfringens* secreting toxins that cause gut lesions. Consequently, it is reasonable to speculate that mechanisms preventing the production of such bacteriocin in the gut would be able to protect birds from infection of NE at least to some extent.

Selenium regulates major physiologic pathways of humans and animals as an essential micronutrient. It can enhance the immune and antioxidant systems. Recently, selenium was used for the in ovo injection to broiler eggs at 18 d of embryo age and the birds were later challenged with NE, and the protection effect was observed to increase the levels of antibody against NetB and α-toxin (Lee et al., 2014b). Again, the same group injected selenium in ovo that was incorporated into hydrolyzed soybean protein (B-Taxim [BT]) and observed similar protection of challenged birds against NE (Lee et al., 2014a). Accordingly, it seems promising that selenium administered in single or incorporated in hydrolyzed soybean protein shows certain degree of protection thus worth to pursue further for industrial implementation.

4. Conclusions

Many factors associated with infectious agents and diet can negatively affect the chicken gut health which may affect the health status and production performance of birds. Phasing out of in-feed antibiotics from poultry feed in Europe and recent removal or reduction of these compounds in the other parts of the world, either by government regulation or voluntarily, in countries including Australia and USA, is a challenge to the poultry industry. Studies from Denmark and Sweden have confirmed that the key problem of in-feed antibiotic withdrawal from poultry diets is the control of NE. Currently, much extensive multidisciplinary research has been done to alleviate the problems associated with antibiotic withdrawal from poultry diets, but to date no single preventive therapy has been established to substitute in-feed antibiotics. Several nutraceutical supplements including probiotics, prebiotics, essential oils, organic acids, enzymes and vaccines have been used to reduce the incidence of NE. However, no product has been as effective as antibiotics in terms of controlling NE and registered for commercial applications. Recent investigations have shown that the use of some antibiotic alternatives in poultry diet, for instance, prebiotics, organic acids and vaccines have improved gastrointestinal health, integrity and reduced the intestinal colonization of pathogenic bacteria including NE. In particular, the vaccine strategies may have greater potential for the control of NE mainly due to clearer etiology having been elucidated in recent years with the identification of NetB toxin. Therefore, using alternatives to in-feed antibiotics with a better understanding of the relationship between nutrition and NE, and limiting exposure to infectious agents through biosecurity and vaccination, might be a tool to reduce the incidence of NE and improve gut health in the absence of in-feed antibiotics. More importantly, the combinations of different measures may achieve greater protection of birds against the disease.

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