

Nutritional manipulation of necrotic enteritis outbreak in broilers

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Summary

A significant threat to the broiler industry is the sporadic outbreak of necrotic enteritis (NE). Damage to the intestinal mucosa through coccidial infection (*Eimeria* spp.) or a change in the normal intestinal microflora predisposes birds to the proliferation of *Clostridium perfringens* (CP) resulting in NE. At present in Australia the occurrence of NE in poultry is still controlled by adding antibacterial growth promotants (AGP) to feed. However, the ban of AGP in many European countries and recent trends to reduce and ban throughout the world the inclusion of AGP in all animal feed has forced the poultry industry worldwide to look for different means by which to control the disease. The choice of feed ingredient can significantly alter the intestinal microflora and predispose or protect birds from the rapid proliferation of potential pathogens like CP. More recently, the focus has shifted towards the inclusion of alternative feed supplements such as feed enzymes, prebiotic, probiotic and organic acids, which not only improve broiler performance but also provide considerable protection against NE.

Keywords: necrotic enteritis, chicken, feed ingredients, intestinal microflora

Introduction

The most common and financially devastating bacterial disease in modern broiler flocks is necrotic enteritis (NE). Necrotic enteritis was first described by Parish (1961) and is caused by the α -toxin of *Clostridium perfringens* (CP) types A and C. *Clostridium perfringens* is a gram positive, anaerobic bacterium that can be found in soil, litter, dust, and at low levels in the intestine of healthy birds. Damage to the intestinal mucosa through coccidial infection (*Eimeria* spp.) or a change in the normal intestinal microflora as a result of a change in diet can predispose birds to the rapid proliferation of CP (Ficken and Wages 1997). The intestine of infected birds is friable and distended with

gas and gross lesions caused by the α -toxin are usually found in the small intestine. In its acute form, birds often die without clinical signs. However, in its subclinical form the disease is much more financially damaging for the producer. The commonly observed symptoms of the disease vary with age of the birds (van der Sluis 2000b) and early signs of an NE outbreak such as wet litter, diarrhoea and a small increase in mortality of less than 1% are often overlooked. However damage to the intestine and the subsequent reduction in digestion and absorption can reduce weight gain by more than 200 g (van der Sluis 2000a) and the feed conversion ratio at 35 days of age by up to 5% (Kaldhusdal and Løvland 2000). Furthermore, increased condemnations at processing due to liver lesions associated with subclinical NE can occur (Kaldhusdal and Løvland 2000). It has been estimated that the total cost of clinical and subclinical NE can be as high as US\$ 0.05 per bird (van der Sluis 2000b) or a staggering US\$ 220 million based on the global production of chicken meat in 2002 (FAOSTAT 2002).

This paper will discuss the factors associated with the occurrence of NE and possible prevention mechanisms. In particular, the paper will focus on nutritional manipulation of the intestinal flora and on the reduction in risk of occurrence of NE.

Commonly used strategies to prevent NE

Outbreaks of NE can be effectively prevented or treated with antibiotics such as virginiamycin, bacitracin, penicillin, tylosin, flavophospholipol or avoparcin (Watkins *et al.* 1997). When used as so-called antibacterial growth promotants (AGP) the same agents can be very effective in controlling and preventing NE. Included at sub-therapeutical dosage AGP selectively modify the gut flora, suppress bacterial catabolism and reduce bacterial fermentation. All these changes lead to increased nutrient availability for the animal and

increased growth performance (Corpet 1999). Mucosal damage caused by coccidial infection can predispose the birds to rapid proliferation of *CP*. The effective control of coccidia can therefore greatly reduce the risk of NE. The inclusion of some anti-coccidial medications of the ionophore type has been effective in reducing the level of *CP* in chickens (Elwinger *et al.* 1998).

Reports of the occurrence of vancomycin resistance enterococci (VRE) in hospitals which has been linked to the AGP avoparcin (Collignon 1999) and the general fears of the development of an antibiotic resistant 'super-bacteria' led to a reduction of AGP registered for use in animal feed. In 1999, the European Union (EU) placed a partial ban on the use of AGP which will be replaced in 2006 by the general ban of all AGP in animal feed. It was shown that the removal of AGP led to a massive increase in NE outbreaks in many European countries and the widespread occurrence of ill-defined intestinal dysbacteriosis (Kaldhusdal and Løvland 2000; Pattison 2002). Although in Australia growth promotants other than avoparcin are still used to control NE, it is widely anticipated that Australia will follow the European lead. Use of alternative methods to control NE will become inevitable.

Data from Europe showed that strict hygiene management, climate in the buildings and feed composition are possible alternatives to AGP and can help to maintain broiler performance and control the occurrence of NE (Inbarr 2000). Nutritional management such as lowering the inclusion rate of fishmeal, wheat or barley in the diet may prevent NE (Ficken and Wages 1997). It has been recognised that modulation of the natural bacterial population of the intestine in broilers through nutritional manipulation such as the selection of feed ingredients or the use of alternate feed supplements can be effective tools to control NE and other *CP* related diseases.

Intestinal flora in broiler chickens

The intestinal tract of a newly hatched chick is sterile but within a few hours after hatching the first organisms are found. The first organisms that are present include *Escherichia coli*, *C. perfringens* and *Streptococci* depending on the cleanliness of the environment (Woolcock 1979). Complete colonisation of the intestine can take up to 6 weeks and is a fine balance between all the organisms present (Barnes *et al.* 1972). Conditions in the intestine range from a relatively aerobic environment in the crop and duodenum to complete anaerobic condition in the caeca. Initially the small intestine is colonised by *E. coli*, *Lactobacillus*, *Streptococcus* and *Enterococcus* (Mead 2000). Although obligate anaerobes (anaerobic cocci, *Clostridium*) may also be present, in the first weeks the oxygen concentration for rapid proliferation is generally too high. This may explain the fact that the occurrence of NE in birds younger than two weeks is rare. In the presence of these facultative anaerobes conditions

change to a more anaerobe environment, which can make it possible for *CP* to proliferate. The bacteria population in the small intestine reaches a stable balance within two to three weeks after hatching. In contrast the development of the microflora in the caeca can take up to 30 days and changes can occur until six weeks after hatching (Barnes *et al.* 1972). The caeca is the main place of bacterial fermentation in a chicken. Dominant bacteria in caeca are obligate anaerobes and can reach up to 10^{11} organisms/g of caecal content (Barnes *et al.* 1972).

The microflora population depends very much on the balance between communities of organisms and the diet composition as the source of available substrates for microorganisms. Therefore the main factor influencing the intestinal microflora is the diet composition. Because only between 10 and 60% of the intestinal bacteria can be grown in culture it is impossible to link changes in the bacterial population with a specific dietary ingredient (Apajalahti 1999). Despite these limitations, it is possible to measure changes the products of the bacterial metabolism or changes in the number of culturable isolates. For example, Wagner and Thomas (1978) reported that the inclusion of rye significantly increased butyric acid concentration and gas production in the small intestine. However, when an antibiotic such as penicillin was added to the diet the butyric acid and gas-producing component were removed. The authors concluded that the penicillin sensitive, gas and butyric acid-producing bacterium is most likely a *Clostridium*.

Effects of dietary ingredients on the proliferation of *CP* in the intestine and the occurrence of Necrotic Enteritis

The use of different feed ingredients will affect the environment in the intestine and subsequently influence the gut microflora. In order to find successful nutritional strategies to reduce the risk of NE it is necessary to have a broad understanding of the composition of feed ingredients and their effect on the microbial population in particular the growth of *CP*.

Maize

Maize is considered an excellent ingredient in broiler diets due to its high energy content and high nutrient availability. Broiler diets high in maize may also help to reduce the incidence of NE in comparison to diets based on wheat or barley (Kaldhusdal 2000). In an observational study attempting to link the incidence of NE with the cereal content of broiler diets in Norway, (Kaldhusdal and Skjerve 1996) found a strict correlation between the ratio of wheat plus barley to maize and the outbreak of NE. These authors showed that in periods with higher inclusion of maize over wheat or barley,

the occurrence of NE was reduced. The inclusion of maize in broiler diets was also linked to a reduced susceptibility to coccidiosis (Williams 1992).

Wheat, barley, rye

Reports in the literature emphasise the fact that the incidence of NE is significantly higher in broilers fed diets based on wheat, barley, oats or rye compared to diets based on maize (Kaldhusdal and Hofshagen 1992; Riddell and Kong 1992). Data by Choct and Sinlae (2000) confirmed that in three-week old broilers one day after the inclusion of a wheat-based diet the numbers of total anaerobe bacteria and in particular, the number of *C. perfringens* significantly increased. It is well known that these cereal grains contain high levels of indigestible water-soluble non-starch polysaccharides (NSP) (Annison and Choct 1991). The ingestion of high levels of soluble NSP leads to increased digesta viscosity and decreased digesta passage rate and nutrient digestibility (Hesselman and Aman 1986; Choct *et al.* 1996). Larger amounts of undigested material in the small intestine together with a slower flow of digesta increases the chances of rapid bacterial colonisation. A highly viscous intestinal environment will increase the proliferation of facultative anaerobes like gram-positive cocci and enterobacteria (Vahjen *et al.* 1998) and in return create an environment that can support obligate anaerobes such as *CP*. The change in the microbial balance in the small intestine will lead to dysbacteriosis, which was defined by Tice (2000) as “the presence of a qualitatively and/or quantitatively abnormal flora in the intestine”. Birds affected with dysbacteriosis have substantial higher numbers of bacteria in the small intestine and in particular increased numbers of *Clostridium* spp. (Panneman 2000). Excessive proliferation of *Clostridium perfringens* will lead to an outbreak of subclinical or clinical NE.

It is well documented that the addition of feed enzymes to diets based on wheat, barley, oats or rye significantly decreases viscosity in the small intestine by partially depolymerising the soluble NSP (Bedford and Classen 1992; Annison and Choct 1993). It has been demonstrated that the inclusion of xylanase in wheat based diets significantly reduced bacterial population in the small intestine (Apajalahti 1999; Choct *et al.* 1999) and in particular the numbers of *CP* (Choct and Sinlae 2000). The addition of enzyme reduced digesta viscosity in the small intestine and increased nutrient digestion as well as increased digesta flow rate all of which effectively reduced the amount of available nutrients to the microflora (Choct *et al.* 1999). Apajalahti (1999) suggested that the depolymerisation of larger arabinoxylans in wheat with xylanase produced xylo-oligomers and xylose which could only be partially utilised by the microflora. Subsequently the total number of bacteria in the ileum was reduced by 60%. The same authors also found that the addition of xylanase significantly increased total volatile fatty acids (VFA)

concentration in the caeca and in particular the concentration of propionic acid. As a result of the increase in propionic acid the numbers of potential pathogenic bacteria like *Clostridium*, *Escherichia* and *Salmonella* were reduced. Despite the profound changes in the intestinal microflora and the apparent reduction in the numbers of *CP* the addition of feed enzyme alone cannot provide complete protection against NE (Elwinger and Teglöf 1991; Riddell and Kong 1992). Enzymes will only change the condition in the intestine but have no direct effect on the growth of *CP*.

Only a few studies investigated the effects of grinding or pelleting on the microbial composition of the intestine. The mortality attributes to NE was significantly higher in birds fed crumbles with hammer-milled wheat compared to roller-milled wheat (Branton *et al.* 1987). In contrast Engberg *et al.* (2002) found no differences in the numbers of *CP* in the small intestine between diets with hammer milled (fine) or roller milled (coarse) wheat. The same study however found that birds fed pellets had higher counts of *CP*, total anaerobic bacteria and lactobacilli compared to birds fed mash. These authors concluded that pelleting improved nutrient digestibility hence reducing the amount of available substrate for the microflora.

Animal protein ingredient

Animal protein ingredients such as fishmeal or meat and bone meal are often associated with increased risk of NE (Ficken and Wages 1997; Ross Breeders 1999). Smith (1965) showed that the increase in the numbers of *CP* in the intestinal tract is directly related to the proportion of meat and bone meal in wheat based diets. Most current models to reproduce NE under experimental conditions are based on diets containing in excess of 25% fish meal and/or meat and bone meal prior to a challenge with *CP* (Truscott and Al-Sheikhly 1977; Prescott 1979; Cowen *et al.* 1987). Challenge models without the inclusion of fishmeal are less successful in reproducing the disease. It can be speculated that the high nutrient density, in particular high levels of protein, alters the microflora and creates favourable condition in the intestine for the proliferation of *CP*. Kaldhusdal and Skjerve (1996) found in their survey that the level of animal protein modified the association between cereal type and incidence of NE. It is important to note that the inclusion of increased level of fishmeal alone is no guarantee for the proliferation of *CP*. The inclusion of 25% fishmeal in a wheat based diet compared to only 9% fishmeal had no effects on the numbers of *CP* in the period from 2 to 4½ weeks of age (Barnes *et al.* 1972). Unfortunately, these authors did not report the crude protein content of the feed. Considering that a marked increase in performance was observed when more fishmeal was added, it is likely that the protein level in the diet with only 9% fishmeal was below the requirement, hence the excess protein was in fact utilised by the chicken and did not change nutrient availability for the microflora.

Vegetable protein ingredient

The association of animal protein ingredients with the increased occurrence of NE together with the general ban of protein sources of animal origin in Europe (Adams 2000) has placed great emphasis on vegetable proteins in feedstuff. *In vitro* studies showed that in modified growth medium some isolated soy protein stimulated the growth of *CP* (Busta and Schroder 1971). However, there are no reports in the literature which link the occurrence of NE with the inclusion of soybean meal or any other vegetable proteins.

It is known that low molecular weight carbohydrates such as α -galactoside oligosaccharides in vegetable proteins cannot be digested in the small intestine of monogastric animals due to the absence of endogenous α -galactosidase and are subsequently broken down by microbial fermentation in the caeca (Carré *et al.* 1990). Bacterial degradation of α -galactosidase can lead to increased hydrogen production, impaired utilisation of nutrients and subsequently reduced performance (Saini 1989). Despite the apparent abundance of nutrients for the intestinal microflora, there appear to be no changes in the composition of the microflora (including *CP*) when feeding diets containing up to 80% peas compared to a corn/soy control diet (Brenes *et al.* 1989).

Feed supplements: Prebiotic, probiotic, organic acids

By definition, prebiotics are short chain carbohydrates that are neither hydrolysed nor absorbed by the host and therefore are available to the microflora in the intestine (Gibson and Roberfroid 1995). The inclusion of substrates like fructo-saccharides (FOS), transgalacto-oligosaccharides (TOS) or inulin can selectively stimulate the growth beneficial microorganisms (bifidobacteria, *Lactobacillus* spp.) in the intestine (Ziggers 2001; Bielecka *et al.* 2002). Pathogens like *E. coli* or *CP* are unable to use FOS as an energy source hence the number of FOS fermenters will increase. The increase in numbers of these bacteria not only reduces the amount of available substrate to potential pathogens but also decreases pH in the intestine due to increased fermentation and production of VFA. Alternatively, it is known that manno-oligosaccharides (MOS) can reduce unwanted enteric pathogens such as *Salmonella* or *Campylobacter* by blocking the type-1 fimbriae which enables the pathogens to attach to the intestinal lining (Dawson and Pirvulescu 1999). Although clostridia do not express type-1 fimbriae it was reported that the addition of manno-oligosaccharides to diets fed to birds challenged with NE had some effects in reducing mortality as well as reducing secondary effects of NE on feed conversion ratio (Hofacre 2001). These effects are believed to be indirectly associated with the shift in growth of beneficial bacteria that inhibited the proliferation of *CP*.

In contrast to prebiotics, which stimulate the growth of beneficial bacteria within the host, the concept of probiotics is based on the direct use of live cultures of these bacteria. Microorganisms used as probiotics in animal nutrition are *Enterococcus* spp., *Saccharomyces* yeast, spore-forming *Bacillus* spp. and to a lesser extent *Lactobacillus* spp. (Simon *et al.* 2001). The functional benefits of probiotic include modification of the microflora, influencing the mucosa permeability, preventing binding of potential pathogen to intestinal mucosa by blocking binding sites, modulation of the immune system and production of bacteriocins (reviewed by Simon *et al.* (2001)). *In vitro* assays showed that the adhesion of *CP* could be reduced by lacto acid bacteria (LAB) (Rinkinen *et al.* 2003). Most studies on the effects of probiotic are conducted in humans, and data on the effects of probiotics in poultry diets are limited. Growth studies without disease challenge showed that the inclusion of *Bacillus coagulans* or *Lactobacillus* cultures in broiler diets significantly improved growth performance (Cavazzoni *et al.* 1998; Jin *et al.* 1998), and with challenge studies using *CP* a commercially available probiotic significantly reduced the severity of NE (Hofacre *et al.* 1998).

Conclusions

Feed ingredients consist of numerous compounds and it is often impossible to connect a single compound with the overall changes in the microflora. Knowing that specific feed ingredients or feed supplements can influence the proliferation of *CP* is a powerful tool to formulate feed rations without AGP. Reports from Europe show that it is possible to control the occurrence of NE through nutritional manipulation and alternative supplements. However, there are still big gaps in the understanding of how and under what conditions these ingredients will alter the numbers of *CP*. NE is a complex multifactorial disease with many unknown factors and future research has to focus on the understanding of the disease itself and the development of reliable and repeatable disease models to investigate nutritional manipulation in details.

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