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Re-Emergence of Necrotic Enteritis in the Broiler Industry

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Abstract: Clostridial enterotoxemia occurs in the clinical forms of necrotic enteritis, cholangiohepatitis, and botulism. Broiler and turkey flocks which are fed diets devoid of antibiotic supplements with activity against Gram positive intestinal flora are susceptible to these conditions which detract from optimal productivity and yield. Factors which predispose to clostridial enterotoxemia include incorporation of more than 10% wheat or barley in diets, acute changes in the form or composition of diets, starvation or climatic and environmental stress including saturation of litter. Mild intestinal coccidiosis may frequently precipitate outbreaks of necrotic enteritis. Antibiotic dietary supplements including bacitracin, virginiamycin and lincomycin will effectively suppress proliferation of toxigenic clostridia when incorporated at sanctioned levels. Alternatives to antibiotics include commercial suspensions of "beneficial" bacteria including *Lactobacillus* spp. *Bifidobacterium* spp and *Pediococcus* spp. These are administered to chicks or poults in drinking water after placement or as a coarse spray after transfer to delivery boxes at the hatchery. Additional preventive measures include acidification of water and feed for newly placed flocks and incorporation of oligosaccharides and enzymes in diets. Supportive modalities comprise management of ventilation to maintain litter moisture between 20% and 25%, effective vaccination to prevent immunosuppressive infections (IBD, MD, CA) and control of coccidiosis.

Key words: Broiler industry, necrotic enteritis, toxigenic strains

Introduction

Necrotic enteritis is re-emerging as a clinical and economic problem in the broiler industry, affecting both broiler breeder replacements and commercial meat flocks. The causal organism, *Clostridium perfringens*, Type A or C, can proliferate in the jejunum and ileum producing alpha or beta toxins, resulting in necrotic enteritis (Wages and Opengart, 2003) or cholangiohepatitis (Loveland and Kaldhusdal, 2001). Necrotic enteritis and cholangiohepatitis (Onderka *et al*., 1990) (Sasaki *et al*., 2000) are usually associated with proliferation of toxigenic *Clostridium perfringens* in the intestinal tract. Botulism which may occur concurrently with necrotic enteritis in commercial broilers results from elaboration of Type C toxin by *Cl. botulinum* in the lumen of the intestine (Dohms, 2003).

The occurrence of necrotic enteritis as a clinical condition under commercial conditions is influenced by a broad range of factors including deficiencies in design, installation and operation of ventilation and watering systems, coccidiosis and immunosuppression. Removal of growth-promoting antibiotics which suppress proliferation of Gram positive anaerobes in the intestinal tract is in part responsible for the increase in incidence rate of necrotic enteritis and botulism in broilers during the past ten years.

Acute mortality, which can attain 5% in affected flocks, is a significant factor in a highly cost-sensitive industry. Depression in growth rate and feed conversion efficiency in survivors which undergo mild chronic enteritis is probably responsible for more significant losses, especially with reoccurrence in subsequent flocks on specific farms.

Etiology of Necrotic Enteritis: Necrotic enteritis is a specific enterotoxemia of chickens and turkeys caused by toxigenic strains of *Clostridium perfringens* (Shane *et al*., 1985). The first formal description of the condition was provided by Parish (1961). Subsequent reports confirmed necrotic enteritis in Australia (Nairn and Bamford, 1967) following outbreaks in farms in Western Australia from 1961 onwards. These authors reproduced necrotic enteritis under controlled conditions by administering oral suspensions of *Clostridium perfringens*. Their observations on field outbreaks documented up to 5% peracute mortality on 30 farms with a higher incidence in spring and summer. Based on experience with enterotoxemia in sheep caused by proliferation of enterotoxic *Cl. perfringens*, it was hypothesized that changes in the ingredient composition of diets fed to broilers favored both intestinal stasis and a change in the pH of ingesta which favored multiplication of *Cl. perfringens* with toxigenic potential. This sequence was probably mediated by changing the composition of microflora producing conditions favoring anaerobes. Outbreaks of necrotic enteritis in broilers in Queensland, Australia, were described by Baines (1968). Gross lesions characterized by pseudomembranous enteritis conformed to the descriptions provided by Parish (1961) and previous

authors. Necrotic enteritis emerged as a field problem in Canada during the late 1960's (Long, 1973) and was subjected to intensive study under controlled conditions. Outbreaks resulted in mortality ranging from 11% to 26%, when feed contained 10^7 *Cl. perfringens* per gram (Long and Truscott, 1976). *Clostridium* spp which are the predominant anaerobes in the intestinal tract of chicks (Shane *et al.*, 1984) are generally confined to the cecum (Barnes *et al.*, 1972). Occurrence of this organism in the jejunum is sporadic (Timms, 1968). Only a small proportion of "*perfringens*-like" colonies isolated from the intestinal tract of chickens using SPS selective agar medium were identified as *Cl. perfringens* (Shane *et al.*, 1984). Experimental subjects were housed on wire mesh, or on either new or used broiler litter. In contrast, no "*perfringens*-like" organisms were isolated from specific pathogen-free chicks maintained in isolation units.

Difficulty in reproducing necrotic enteritis under experimental conditions is exemplified by the series of trials conducted by Cowen *et al.* (1987). Administering a culture of *Cl. perfringens* in the diet from 15 to 19 days failed to reproduce necrotic enteritis and *Cl. perfringens* could not be re-isolated from either sacrificed or dead birds. Losses increased to 10% when the subjects were placed on litter derived from a flock which had demonstrated mortality attributed to necrotic enteritis. The authors emphasized difficulty in developing a suitable model to study preventive and therapeutic medication. Necrotic enteritis has been described in cage-reared commercial laying pullets (Broussard *et al.*, 1986). The condition has also been described in turkeys (Gadzinski and Julian, 1992). In a case report describing three outbreaks in turkey hens aged 7 to 12 weeks, lesions similar to those observed in broilers were documented in addition to duodenitis, which is not generally encountered in broilers.

Factors predisposing to necrotic enteritis

Diet: Inclusion of animal-source protein ingredients including fish meal in broiler diets may increase the prevalence and severity of necrotic enteritis (Truscott and Al-Sheikhly, 1977). Fish meal is often incorporated into experimental diets used to reproduce necrotic enteritis in laboratory model systems. The changes in intestinal pH and in the composition of intestinal flora are considered to be responsible for proliferation of *Cl. perfringens* as a result of abrupt inclusion of fish meal in diets fed to broilers at mid-cycle.

Wheat has been implicated as a dietary factor predisposing to necrotic enteritis. It is noted that countries in which the condition was initially described include Australia and Canada, which traditionally incorporate wheat as the principal grain. In a trial designed to investigate milling of wheat on performance, Branton *et al.* (1987) inadvertently reproduced necrotic enteritis by feeding wheat-based diets. Mortality of 2.9% was recorded when yellow corn was the principal grain in growing diets. Wheat processed with a hammer mill induced 29% mortality but roller-milling the ingredient reduced losses to 18%. A combination of wheat processed by roller-milling and corn in equal proportions in the diet was associated with 3% mortality in contrast to 13% when the wheat component was passed through a hammer mill. It is considered significant that in this trial, histological examination of intestines confirmed infection with *Eimeria maxima* and *E. acervulina*.

Wheat, rye, barley and oat groats, which contain complex non-starch polysaccharides, increase the incidence of necrotic enteritis (Riddell and Kong, 1992). Trials were conducted in battery brooders with experimental diets fed from 14 days of age onwards. The *Cl. perfringens* challenge model involved feeding a broth culture containing from 4×10^6 to 2×10^9 cfu over a five-day period. Mortality in the treatments receiving a wheat-based diet ranged from 30% to 80% depending on the duration of challenge. Approximately 25% of chicks died of necrotic enteritis when fed either rye or barley-based diets when challenged from 18 through 20 days. Inclusion of rye in broiler diets fed to cage-housed Leghorn chicks increased the quantum of *Cl. perfringens* in cecal contents (Takeda *et al.*, 1995). At a 50% inclusion rate of rye, cecal contents were incompletely digested and significantly higher levels of *Cl. perfringens* were enumerated. Inclusion of 65% wheat in diets fed to 20 or 30-day broilers in cages induced gross and histological enteritis, considered as a precursor to necrotic enteritis. No changes were observed in the intestines of controls receiving a corn-based diet (Branton *et al.*, 1997). Desquamation of mucosa was observed in 40% of subjects fed the wheat diet and then challenged with *Clostridium perfringens*. In contrast, chicks fed a corn-based diet which were challenged showed an incidence of 7%. No obvious changes were recorded in the negative control group. No mortality was recorded despite the significantly higher intestinal lesion score in chicks receiving the wheat-based diet compared to either corn or the controls. The authors attributed the deleterious effect of wheat to an increase in the viscosity of ingesta which promoted either proliferation of *Cl. perfringens* or the propensity of the organism to elaborate toxin.

The distinction between these mechanisms was addressed by Annett *et al.* (2002) in a trial comparing crude or digested barley, wheat and corn diets and *in vitro* model. Digestion was carried out at 40°C and involved sequential addition of pepsin and pancreatin. The culture of *Cl. perfringens* was obtained from a field case of necrotic enteritis. Proliferation of *Cl perfringens* was significantly higher in digested wheat and barley diets compared to a digested corn diet, although no

differences in proliferation were observed among the non-digested diets. The authors concluded that the rate of *in vitro* proliferation of *Cl. perfringens* was correlated with the quantum of the organism in the intestinal tract which in turn influences the incidence of necrotic enteritis.

Coccidiosis: Intestinal coccidiosis has been associated with necrotic enteritis since the initial field observations of Nairn and Bamford (1967). Subsequently, studies on the pathogenesis of necrotic enteritis have implicated coccidiosis, including the reports by Helmboldt and Bryant (1971) and Long *et al.* (1974). A structured experiment to demonstrate the role of *Eimeria acervulina* and *E. necatrix* in the onset of necrotic enteritis was performed in Canada by Al-Sheikhly and Al-Saieg (1980). This study demonstrated that feed contaminated with *Cl. perfringens* on the 15th day resulted in mortality of 16% of chicks housed in wire brooders. Administering *E. necatrix* or *E. acervulina* oocysts three days before initiating clostridial challenge increased mortality attributed to necrotic enteritis to 28% and 53% respectively. Challenge with oocysts produced intestinal lesions graded 1 to 2+ on the Johnson and Reid (1970) scale in the control groups infected with coccidiosis. The severity of lesions was exacerbated in chicks subjected to coccidial challenge. A field case of necrotic enteritis in replacement-egg-strain pullets housed in cages was associated with *Eimeria maxima* infection, confirmed by examination of intestinal scrapings and histopathology (Broussard *et al.*, 1986). Mortality was controlled by administration of amprolium to suppress coccidiosis and bacitracin to eliminate Gram positive intestinal flora. A combination of *Eimeria brunetti* and *Cl. perfringens* resulted in high intestinal lesion scores and mortality from necrotic enteritis in a trial conducted to evaluate the interaction of these pathogens in seven to twelve-day old broiler chicks (Baba *et al.*, 1992). The model combining both pathogens resulted in 20% mortality and a lesion score of 3.5 when diets were supplemented with 1,000 ppm zinc. Deleting zinc from the diet resulted in a lesion score of 2.5 but with no mortality. Concurrent *in vitro* studies showed that supplementation of bacterial growth media with zinc decreased the elaboration of clostridial alpha toxin (phospholypase-C) and that addition of trypsin protected alpha toxin in media inoculated with a toxigenic *Cl. perfringens*. The deleterious effect of adding zinc to diets consumed by birds co-infected with *E. brunetti* and *Cl. perfringens* was attributed to the inactivation of intestinal trypsin which degrades alpha toxin produced by *Cl. perfringens*. The relative sequence of mild intestinal coccidiosis followed by clostridial infection in a challenge model was investigated by Shane *et al.* (1985) in relation to the pathogenesis of necrotic enteritis. Sporulated oocysts of *E. acervulina* were administered to cage-housed broilers at a dose of 3.5×10^5 to confirm that this dose induced mild subclinical coccidiosis. The *Cl. perfringens* challenge model comprised starvation for 24 hours at 20 days of age followed by initiation of feeding a diet containing a thioglycollate culture of *Cl. perfringens* (2.5×10^8 organism/g) for five days. The experimental design comprised a negative control, an *Eimeria acervulina* control, a *Cl. perfringens* control and treatments involving administration of *Eimeria acervulina* at either two or five days respectively before commencing infection with *Cl. perfringens*. Additional treatments comprised simultaneous administration of *Eimeria acervulina* and *Clostridium perfringens* and administration of *Eimeria acervulina* on the third day after onset of the *Cl. perfringens* challenge model. Administration of *Cl. perfringens* alone resulted in 9% mortality and a 76% prevalence of intestinal lesions ranging in severity from hyperemia to pseudomembranous enteritis. Administration of *Eimeria acervulina* either before or simultaneously with initiation of *Cl. perfringens* resulted in 41% and 35% mortality and 88% and 79% prevalence of lesions respectively. Mortality due to necrotic enteritis was reduced to 10% and 19% respectively in the treatments in which *Eimeria acervulina* was administered either five days before or on the third day after initiation of infection with *Cl. perfringens*. This study showed that not only did *E. acervulina* exacerbate mortality due to necrotic enteritis initiated by infection with *Cl. perfringens*, but the relative sequence of infection with oocysts was an important factor in the severity of infection. No evidence of clostridial enteritis was detected in the negative control or in the treatment receiving only *E. acervulina*. Statistical analysis demonstrated a correlation coefficient of 0.87 between necrotic enteritis lesion score and mortality. Concurrent studies were performed to investigate the pathogenesis of necrotic enteritis resulting from co-infection with *E. acervulina* and *Cl. perfringens*. Serum protein was reduced from 0.8 g/dl to 1.67 g/dl on the fifth day after infection with *E. acervulina*. Reduction in the pH value of duodenal contents from 5.9 to 4.7 and ingesta in the jejunum from 6.2 to 5.2 was observed five days after administration of oocysts. Intestinal passage time was increased by 70 minutes from 178 minutes to 248 minutes on the fifth day after infection with *E. acervulina*. The experimental design involving phased infection with oocysts confirmed previous observations by Helmboldt and Bryant (1971) that intestinal coccidiosis retarded intestinal motility and with the observations of Al-Sheikhly and Truscott (1977a) that damage to the intestinal mucosa was significant in the pathogenesis of necrotic enteritis due to toxigenic strains of Type A *Cl. prefringens*. Damage to the intestinal mucosa by sexual stages of *E. acervulina*, confirmed by histological examination, leads to effusion of protein from mucosa

into the lumen of the intestine. Degradation of protein into amino acids by proteolytic enzymes reduces pH below 5.6, decreasing the activity of smooth muscle and retarding intestinal motility. It was hypothesized that intestinal stasis promoted proliferation of *Cl. perfringens* with subsequent elaboration of alpha toxin.

Concurrent coccidiosis and necrotic enteritis has been diagnosed in turkeys involving primary breeders aged five weeks of age (Droual *et al.*, 1994) and in commercial turkeys aged six to eleven weeks (Droual *et al.*, 1995). A retrospective study on cases submitted to the Turlock Branch Laboratory of the California Veterinary Diagnostic Laboratory system attributed outbreaks to movement of turkeys from brooder to grower houses, presumably involving changes in feed composition, litter texture and antibiotic additives. Proliferation of intestinal flora as a result of these influences could have precipitated proliferation of *Cl. perfringens*. Most cases of primary necrotic enteritis in turkeys submitted for diagnosis showed lesions consistent with intercurrent intestinal infection including coccidiosis, hemorrhagic enteritis and possibly, other protozoal infections including trichomoniasis, hexamitiasis, histomoniasis and cochlosomiasis which have emerged as clinical entities or have been recognized during the past five years. Since a significant number of the cases submitted for diagnosis showed *E. coli* septicemia in addition to necrotic enteritis, it is speculated that immunosuppression and environmental stress may be predisposing factors to necrotic enteritis in commercial turkey flocks.

Pathogenesis and pathology of necrotic enteritis: A study of clinical data and specimens derived from 75 cases of necrotic enteritis was undertaken by the University of Connecticut (Helmboldt and Bryant, 1971). The predominant lesion comprised fibronecrotic enteritis with cystic dilatation of mucosal glands. Following the earlier description by Parish (1961), it is possible to recognize mild and severe manifestations of the clinical condition.

The pathology of experimentally-induced necrotic enteritis was described by Al-Sheikhly and Truscott (1977c). These studies described changes induced by intra duodenal infusion of *Cl. perfringens* Type A. Necrosis of the tips of villi was noticed after three hours, with visualization of *Clostridia* spp in the affected intestinal tissue. Destruction of villi progressed through the subsequent eight hours terminating in coagulation necrosis involving the entire villus structure. Similar lesions were noted in four-week old chickens infused with bacteria-free toxins of *Cl. perfringens* (Al-Sheikhly and Truscott, 1977b).

A recent study on the pathogenesis of necrotic enteritis applied light microscopy, immunohistochemical and ultrastructural investigation (Kaldhusdal *et al.*, 1995). Immunoperoxidase staining confirmed the presence of *Cl. perfringens* in necrotic tissues. It was concluded that toxin elaborated by the pathogen was responsible for the degenerative changes observed. Electron microscopy showed vesiculation of cell membranes and degenerative changes of cytoplasmic organelles. Light microscopy confirmed the presence of *Eimeria* sp from field cases, consistent with observations of previous investigators.

The role of antibiotics in prevention and therapy of Necrotic Enteritis: Successful treatment of necrotic enteritis by administration of either zinc bacitracin or penicillin in feed at 200 g/ton for periods ranging from 24 hrs to four days was confirmed in Australia (Nairn and Bamford, 1967). Water soluble oxytetracycline and bacitracin were found to be effective in Queensland (Baines, 1968). Penicillin in drinking water was also shown to be protective under laboratory conditions following experimental reproduction of necrotic enteritis. Prevention of necrotic enteritis was investigated by Williams Smith (1972) who evaluated nitrovin, a furan chemotherapeutic which was then available to the industry. Efficacy of nitrovin, bacitracin, virginiamycin, tylosin, ampicillin and benzyl penicillin was demonstrated, based on acceptable inhibitory concentration values against *Cl. perfringens* (*welchii*) derived from field outbreaks of necrotic enteritis.

Bacitracin was shown to be effective to prevent and treat necrotic enteritis under field conditions (Wicker *et al.*, 1977). A replicate pen trial confirmed 7% mortality attributed to necrotic enteritis in non-medicated controls placed on litter derived from a flock which had experienced an outbreak of the disease. In contrast, broilers receiving diets supplemented with zinc bacitracin at the level of 10 g/ton showed 0.4% mortality due to necrotic enteritis. Significant improvement in weight gain and feed conversion efficiency was recorded in broilers receiving zinc bacitracin. Administering the compound in drinking water prior to initiation of a clostridial challenge confirmed the protective effect of levels greater than 25 mg/l drinking water. Non-medicated controls showed 12% mortality and the presence of lesions consistent with non-fatal necrotic enteritis in 67% of survivors which were sacrificed for infection.

Bacitracin was shown to be effective in suppressing *Cl. perfringens* in the intestinal tract of chicks fed a soybean protein and sucrose-based diet (Stutz *et al.*, 1983). A level of 5.5 ppm was required to reproduce a statistically significant reduction in *Cl. perfringens* in ileal ingesta. The antibacterial effect increased proportionally with level but no incremental reduction could be determined at levels above 17 ppm. In chicks receiving practical diets, the natural flora of the distal intestinal tract comprised *Lactobacilli, Streptococci, Staphylococci,*

coliforms and then *Clostridia* in descending order. Bacitracin had a specific inhibitory effect on *Cl. perfringens* in the ileum without affecting aerobes.

Virginiamycin was shown to be effective to prevent necrotic enteritis in male broiler chicks in a replicate pen trial (George *et al.*, 1982). The basal feed comprised 26% fish meal and the challenge model comprised oral gavage of a culture of *Cl. perfringens* derived from a field outbreak of necrotic enteritis. Incorporation of virginiamycin in feed at levels exceeding 10 g/ton effectively suppressed mortality and restored growth rate and feed conversion efficiency compared to negative and positive controls. The positive control demonstrated a mortality of 37% with a lesion score of 2.1 and a body weight of 858 g. In contrast, the treatment receiving 10 g/ton virginiamycin recorded 1% mortality with a lesion score of 1.1 and a weight of 1061 g.

Lincomycin in drinking water was shown to be an effective treatment in a replicate pen trial in which necrotic enteritis was reproduced by placing chicks on infected litter derived from a field case. Non-medicated treatments showed 14% mortality compared to the absence of necrotic enteritis mortality in subjects receiving 17 mg lincomycin/l in drinking water (Hamdy *et al.*, 1983). Dietary supplementation of lincomycin at 20 ppm was shown to be effective in preventing necrotic enteritis in an experimental model when diets were inoculated with *Cl. perfringens*. In the first of two experiments, mortality was reduced from 18% to 4% with a concurrent significant reduction in lesion score. In the second experiment, mortality was reduced from 26% to 14% (Truscott and Al-Sheikhly, 1977).

The use of antibiotic growth promoters was banned in Denmark in 1998, resulting in a reduction in usage of bacitracin from 3,945 kg active compound in this year to 63 kg in 1999. Consumption of virginiamycin declined from 5,055 kg active compound in 1996 to 892 kg in 1998 and to zero in 1999 (Danmap II, 2002). According to the 2003 report of the Danish Poultry Board (Danske Fjerkraeraad, 2003), 140 million broilers were placed and slaughtered at an average age of 39 days at a biodensity of 45.2 kg/m^2. An average mortality of 3.3% was documented from placement through to slaughter at live weights ranging from 1.7 kg at 35 days to 1.9 kg at 38 days. This performance was achieved in the absence of feed additive antibiotic growth promoters with activity against Gram positive intestinal flora. Withdrawal of growth-promoting antibiotic feed additives in Scandinavia resulted in a decline in feed conversion efficiency of up to 1% (Emborg *et al.*, 2001).

A field study was performed to investigate the financial and practical effect of withdrawing growth-promoting antibiotic additives in the US broiler industry. The trial comprised seven million broilers over a three-year period, involving 158 paired-house comparisons in North Carolina and the Delmarva Peninsula. Deterioration of 0.9% in feed conversion efficiency was recorded and livability declined by 0.2% for the Delmarva comparisons and 0.14% in North Carolina. Coupled with a decrease in live weight of 0.03 lbs per broiler in Delmarva and 0.04 lbs in the North Carolina, reduced performance represents a potential annual loss of 98 m.tons in liveweight to the US industry, valued at $54 million, assuming an ex-farm production cost of 55¢/kg (Engster *et al.*, 2002).

Based on the risk of outbreaks of necrotic enteritis, producers continue to rely on zinc bacitracin, virginiamycin and lincomycin feed additives to suppress clostridial proliferation and to prevent enterotoxemia manifested as necrotic enteritis, botulism, and cholangiohepatitis. In regions where the use of growth-stimulating antibiotics which have specific action against Gram positive organisms are banned, alternative products are either under evaluation or are in commercial use.

Prevention of necrotic enteritis by alternatives to antibiotics: The European Union prohibition on antibiotic feed additives in 1999 stimulated a review of alternative products. Competitive exclusion flora may compete with *Clostridium perfringens* and other anaerobes in the distal intestinal tract, modifying in immature chicks and poults. A commercial competitive exclusion culture (Broilact™) was evaluated in broilers raised in replicate floor pens through 42 days of age (Elwinger *et al.* 1992). Administration of this non-defined competitive exclusion culture reduced both total mortality and the incidence of necrotic enteritis in broilers and resulted in lower levels of *Cl. perfringens* in the intestinal tract. The beneficial effect of Broilact™ was evident in a diet containing 6% animal protein but there was no advantage when an all-vegetable diet was fed. An ionophore anticoccidial (narasin) was incorporated in diets and may have contributed to suppression of *Cl. perfringens*. There was no statistically significant interaction between supplementation of diets with Broilact™ and narasin anticoccidial.

A necrotic enteritis model was used to evaluate Aviguard™, a non-defined intestinal culture licensed for use in the EU. This product was compared with two alternative intestinal flora preparations and a probiotic (Hofacre *et al.*, 1998a). The necrotic enteritis model was responsible for 2% mortality compared with 0.7% in the non-challenged negative control. Aviguard™ and one of the two intestinal-flora preparations reduced mortality attributed to necrotic enteritis to the level of the negative control. A second intestinal flora preparation and the probiotic were associated with 2.0% and 6.0% mortality respectively. Intestinal lesion scores on a scale ranging from zero for no gross changes to 3, represented by pseudomembranous enteritis, were determined for a random sample of broilers in each treatment at 21 days

of age. The Aviguard™ treatment resulted in an average total score of 3.2 compared to 7.3 for the non-treated positive control. Other treatments were intermediate in their protective effect as determined by intestinal lesion score. In a related study, the efficacy of Aviguard™ was compared to bacitracin and virginiamycin, using the necrotic enteritis model (Hofacre *et al.*, 1998b). The challenged unmedicated controls demonstrated 4.2% mortality compared to 2% for virginiamycin and 0.7% for bacitracin. No mortality was recorded in either the negative controls or the treatment receiving Aviguard™ and subjected to *Cl. perfringens* challenge. The authors concluded that Aviguard™ was as effective as virginiamycin or bacitracin in suppressing necrotic enteritis, under controlled experimental conditions. Critical evaluation of the protective effect of Aviguard™ could have been facilitated if the challenge model had induced necrotic enteritis mortality of 15%.

A mucosal starter culture developed by the USDA-Agricultural Research Service, Richard Russell Agricultural Research Center, was evaluated for potential to suppress *Clostridium perfringens* (Craven *et al.*, 1999). The defined competitive exclusion culture, originally developed to inhibit colonization with *Salmonella*, suppressed elaboration of alpha toxin but had no effect on the quantum of *Cl. perfringens* in the intestinal tract in two trials. Mucosal starter culture reduced the number of bacitracin-resistant *Cl. perfringens* in the ceca in one of four trials in which subjects were fed a corn-based diet and in two trials in which rye comprised 50% of the cereal component. The model against which the mucosal starter culture was evaluated did not produce mortality nor were intestinal lesion scores determined.

A number of competitive exclusion cultures and dietary additives were evaluated against a necrotic enteritis model at the University of Georgia (Hofacre and Beacorn, 2003). The products evaluated included bacitracin, Bio-Mos®, a mannanoligosaccharide, All-Lac™, a monoculture of *Lactobacillus*, and a treatment comprising combination of Bio-Mos® and All-Lac™, a competitive exclusion culture, a fructooligosaccharide and an acidifier added to drinking water. Competitive exclusion cultures were administered at day-old by application of a coarse spray. The challenge model comprised infection with *Eimeria acervulina* and *E. maxima* oocysts at 15 days of age, followed by administration of *C. perfringens* by oral gavage on the 18th, 19th and 20th days. Broilers were housed in cages for the duration of the post-infection observation period. There were no significant differences in liveweight among treatments. The challenge model resulted in 64% mortality which is considered to be higher than the level obtained under commercial conditions. In this trial, bacitracin reduced mortality due to necrotic enteritis to 40% of the level of the positive control indicating the severity of the challenge compared to the commercial situation. All-Lac™ reduced necrotic enteritis mortality to 35%, and was equivalent in protective effect to a combination of Bio-Mos® and All-Lac™.

Dietary mannanoligosaccharide supplementation increases the level of *Bifidobacterium* spp and *Lactobacillus* spp in the intestinal tract, depressing *Enterobacteriaceae* (Fernandez *et al.*, 2002). Since *Lactobacillus* competes with *Clostridium* spp, it is reasonable to assume that mannanoligosaccharide, commercially available as Bio-Mos®, has the potential to suppress *Cl. perfringens* in the distal intestinal tract and, indirectly, reduce the incidence of necrotic enteritis and cholangiohepatitis in broiler flocks.

Future control of clostridial enterotoxemia: In countries where feed-additive antibiotics are allowed and where supplementation with prophylactic levels of specified compounds is acceptable to consumers, broiler producers will continue to add either zinc bacitracin or virginiamycin to diets, especially on farms with a history of outbreaks of necrotic enteritis.

In countries and regions where supplementation of diets with antibiotics for the purpose of stimulating growth is disallowed, producers will employ alternative programs to suppress intestinal proliferation of *Clostridium* spp. Approaches to prevention will include administration of defined microbial products including selected strains of *Lactobacillus* spp, *Bifidobacterium* spp, *Enterococcus* spp and *Pediococcus* spp. Specific commercial products will be administered either as a coarse spray at the hatchery or can be proportioned into non-chlorinated drinking water after placement of the flock. Acidifiers containing citric and ascorbic acids in combination with a defined culture of *Lactobacillus acidophilus* can be added to drinking water supplied to chicks to promote a flora which inhibits colonization with *Salmonella* and *Campylobacter* spp. Mannanoligosaccharides derived from the outer cell wall of *Saccharomyces cerevisiae* promote *Bifidobacterium* spp and *Lactobacillus* spp which in turn inhibit proliferation of *Clostridium perfringens* and other Gram positive anaerobes. These compounds are predicated on the principle that modification of the intestinal flora indirectly suppresses *Clostridium perfringens* with the potential to reduce clinical enterotoxemia.

In view of the fact that mild intestinal coccidiosis is an important precursor of clostridial enterotoxemia, broiler producers will be obliged to employ more efficient procedures to suppress *Eimeria acervulina*, *E. necatrix* and *E. maxima*. This will involve appropriate management of ventilation systems to maintain litter moisture below 25%, installation of nipple drinking systems incorporating filtration of particulate contaminants in the water supply, implementation of effective anticoccidial programs including the use of

chemical and ionophore shuttles which are interspersed with one cycle of a synthetic coccidiocide followed by a cycle when flocks receive an oocyst vaccine. Anticoccidial programs should be monitored for efficacy by examination of intestines from sacrificed birds at 21-28 days, oocyst counts of litter and feces and assay of anticoccidial levels in feed. Surveillance for coccidiosis is especially important on farms with a history of necrotic enteritis and all flocks should be subjected to careful clinical evaluation and post-mortem examination of mortality for a few days after transition from the starter to the grower diet.

Effective vaccination programs to suppress immunosuppressive diseases should be implemented. Vaccination against Marek's and infectious bursal disease is critical to suppress clostridial enterotoxemia, both at the parent level and in commercial broiler flocks. Future prevention of necrotic enteritis and associated clostridial enterotoxemia will require a coordinated approach to management, nutrition, and suppression of immunosuppressive infections. The experience of producers in Scandinavia confirms that acceptable production parameters can be attained without supplementing diets with antibiotic additives.

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