

## MANAGING GASTROINTESTINAL HEALTH AND CELLULITIS

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### Introduction

The poultry industry has experienced an increase in the incidence of anaerobic clostridial cellulitis over the last decade, which can be a significant source of mortality in otherwise healthy market-age turkeys (Carr *et al.*, 1996). The disease, also called gangrenous dermatitis (GD, Wages and Opengart, 2003a), occurs in chickens and turkeys and primarily affects the skin and muscle of the abdomen and thorax, although necrosis in the wings and tail head area is often seen. The lesion is necrotic and usually hemorrhagic, with darkened skin overlying a thick serous exudate containing a significant amount of gas. Frequently the underlying muscle is also affected and can be discolored and necrotic. Often the disease occurs without warning with increased mortality as the initial event (Ritter, 2006).

The causative agents include Clostridia, an anaerobic, spore-forming Gram-positive rod. The species most commonly isolated include *C. perfringens* type A and *C. septicum*, although other Clostridial species are often found (Ritter, 2006). The genetic diversity of *C. perfringens* isolates from GD lesions is quite substantial (Neumann *et al.*, 2007), suggesting that the organism is highly adaptable, perhaps in association with stationary phase evolution in the ceca of normal hosts (Finkel and Kolter, 1999; Zambrano *et al.*, 1993; Zinser and Kolter, 2004). Other bacterial species such as *E. coli* and *S. aureus* may also be isolated from lesions and may contribute to the severity of the disease (Wages and Opengart, 2003a). In a recent study, Rehgerber *et al.* (2006) reported isolation of *C. perfringens* or *C. septicum*, or both, from skin and muscle lesions of 19 out of 20 broilers exhibiting symptoms associated with GD. Experimentally, GD has been reproduced in turkeys by the intramuscular inoculation of *C. septicum* (Saunders and Bickford, 1965).

This is not a new disease, as it was originally reported in 1930 (Niemann, 1930); however, historical interventions based on disinfection, litter management, and prevention of immunosuppression seem to be less effective than previously observed (Ritter, 2006). Nevertheless, a recent report reveals that immunosuppression remains a significant risk factor for GD in broilers (Hoerr, 2007). Recent *C. perfringens* isolates from cases of anaerobic cellulitis in market-age turkeys indicate that they continue to remain sensitive to

penicillin, the treatment of choice to control a clinical outbreak (Neumann *et al.*, 2007). The same authors report that there is some indication that the organism is developing resistance to monensin.

### **Clostridial vs Coliform Cellulitis**

One distinction to be made is between Clostridial and Coliform cellulitis. Coliform cellulitis, also known as inflammatory process, is more often seen in broilers than in turkeys, with diagnosis typically taking place at processing (Vaillancourt and Barnes, 2003). This condition is rarely fatal, and is a problem due to condemnation more than to morbidity or mortality. The lesion is usually a fibrinous or caseous solid found between the muscle and the skin, rarely extending into the muscle, and is often associated with scratches or other skin lesions (Norton *et al.*, 1999; Olkowski *et al.*, 2005). The most common species isolated from lesions is *E. coli*, and a reproducible model for the disease involves subcutaneous injection of *E. coli* originally isolated from a cellulitis lesion (Norton *et al.*, 1997). The pathology of Coliform cellulitis is associated with chronic inflammation (Vaillancourt and Barnes, 2003).

In contrast, Clostridial cellulitis is most often diagnosed during the life of the bird, at 6-7 weeks of age in broilers, 6-20 weeks of age in layers and 12-20 weeks of age in turkeys, in association with an abrupt increase in mortality (Wages and Opengart, 2003a). The skin lesions are moist and dark and frequently extend into the underlying muscle, with gas and blood tinged gel underneath skin that is often intact and undamaged. The pathology is associated with necrotizing  $\alpha$ -toxin rather than inflammation (Rehberger *et al.*, 2006). *C. perfringens* and *C. septicum* both produce and secrete necrotizing  $\alpha$ -toxins that are essential for virulence but unrelated to each other on a molecular level (Grossman *et al.*, 1967; Kennedy *et al.*, 2005; Sakurai *et al.*, 2004). Conditions for production of  $\alpha$ -toxin by *C. perfringens* include rapid vegetative growth in the presence of peptides or amino acids, in particular arginine and glycine, at neutral pH (7.4-7.6) (Murata *et al.*, 1975; Nakamura *et al.*, 1978). Free Zn is essential for  $\alpha$ -toxin stability once it is secreted by the organism (Murata *et al.*, 1975).

The absence of skin damage in birds exhibiting GD has led to some uncertainty about the route of infection. As discussed by Ritter (2006), there is evidence to support both “outside-in” and “inside-out” routes of infection. In the former case, organisms gain entry to the body from the environment. Since Clostridia are normally found in poultry litter, they are a constant threat and can enter through any breach in the integument and cause disease locally. In the latter case, Clostridia in the gut microflora become systemic in association with a failure in gut epithelial integrity. Intravenous injections of a mix of *C. perfringens* and *C. septicum* cause the disease, confirming the hypothesis that GD can result from a release of these organisms into the systemic blood, i.e. the “inside-out” route of infection (Ritter, 2006). Once systemic, the organism may then cause disease in a number of locations, but is particularly prone to subcutaneous sites with nearby damaged muscle tissue. Efforts to generate a reproducible animal model for GD research are ongoing in several locations and include oral delivery as one route of infection. The possibility that Clostridia responsible for GD can originate from the gut suggests the

strategy of reducing risk by manipulation of the gut environment and the resident microflora, a subject recently reviewed by Collett (2007).

Gut *C. perfringens*, especially in association with enteritis, can also cause necrotic enteritis (NE), a rapidly fatal disease in poultry, with lesions usually confined to the jejunum and ileum (Wages and Opengart, 2003b). The mechanism responsible for the ability of the same organism to cause NE in some flocks and GD in other flocks has been discussed by Ritter (2006) and may include such factors as antibiotic growth promotant use and the coccidial control program. Like GD, the pathology of NE is associated with the bacterial toxin and its ability to hydrolyze cell membranes (Stevens and Rood, 2000). For this reason, model systems of NE can yield some information that likely applies to GD.

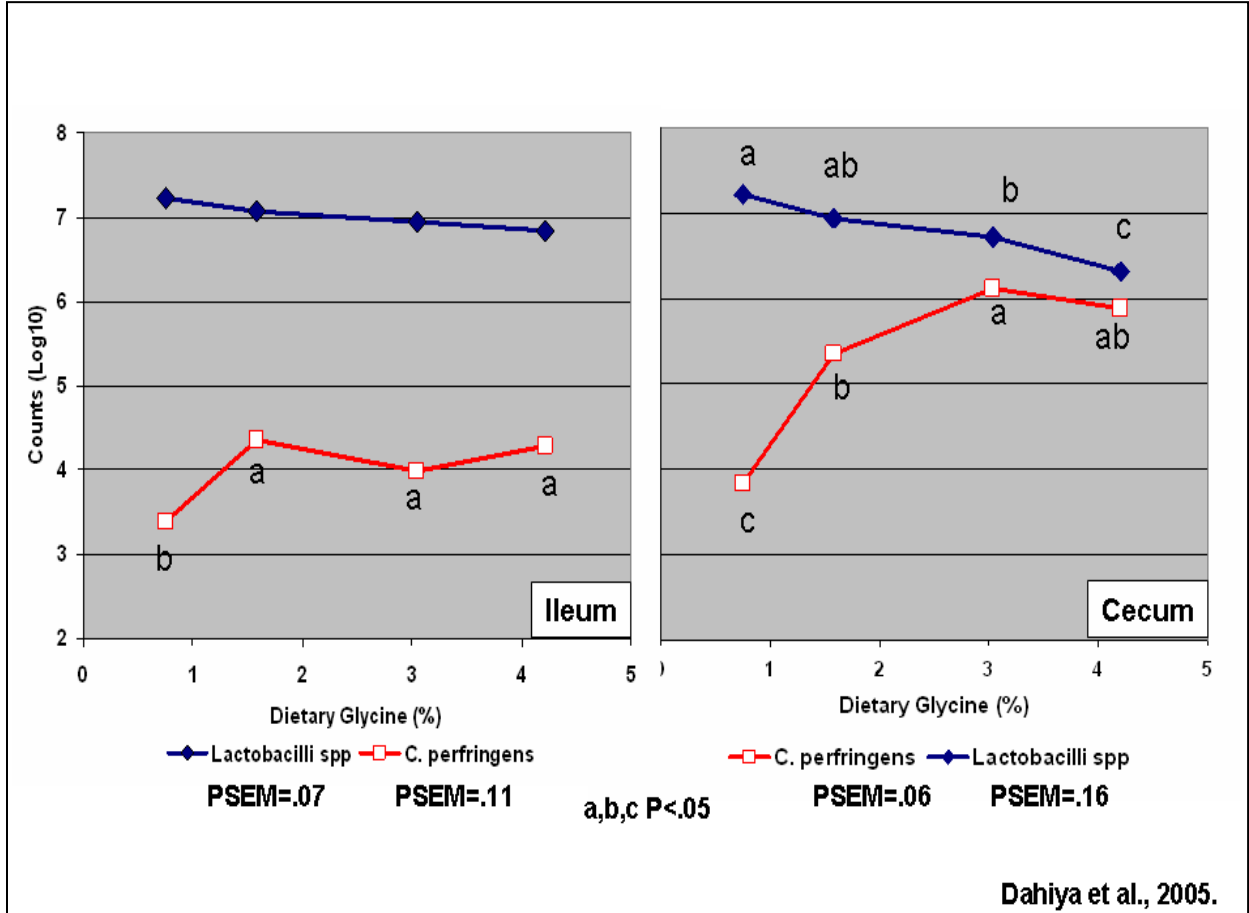
### **Role of Nutrition**

Perhaps the central risk factor for GD and NE is the presence in the animal of Clostridia in active proliferation. The production of  $\alpha$ -toxin takes place during vegetative growth of the organism in an anaerobic, amino acid-rich medium (Murata et al., 1975). Although Clostridia are normally found in the gut microflora of poultry, they are most common in the lower gut, particularly in the ceca, where low nutrient concentration controls their rate of growth (Bjornhag and Sperber, 1977; Stevens and Rood, 2000). However, the equilibrium of the microbiota is dynamic, responding to many environmental factors, including nutrition (Collett, 2005, 2007; Oviedo-Rondon *et al.*, 2006).

Nutrient availability in the lumen of the ileum can be increased in a number of ways. First, feeding poorly digestible feed ingredients can increase the presence of undigested protein in the lower gut. Second, the concentration of amino acids and other nutrients can be increased during periods of rapid feed passage due to disease, stress or other factors. In addition, water soluble non-starch polysaccharides may increase digesta viscosity, adversely affecting digestibility (Choct and Annison, 1992). Finally, the presence of free Zn can be increased by supplementation using inorganic Zn salts. A chelated form of Zn is more stable in the gut environment and less likely to dissociate and ionize in the lumen (Dibner and Richards, 2005). If the  $\alpha$ -toxin of *C. perfringens* is secreted in a Zn-free environment it is almost immediately degraded (Murata et al., 1975).

Since retrograde peristalsis is essentially continuous in poultry (Akester *et al.*, 1967; Duke, 1982; Sacranie *et al.*, 2007), Clostridia can be carried from the colon or cecum into the ileum at any time and enter a more rapid rate of growth in response to the nutrients available there. One factor that prevents this from occurring under normal circumstances is the presence of an acid-tolerant gut microflora in the ileum. This keeps the pH below neutral which reduces the likelihood of dominance by the acid intolerant species such as Clostridia. However, subclinical coccidiosis or enteritis can disrupt the normal microbiota, raising the pH and increasing the rate of mucin production by goblet cells, which would favor an overgrowth of Clostridia (Collett, 2005, 2007). Thus, the causative organism for GD can exist in a range of concentrations and locations within the animal's digestive system.

The association of dietary glycine supplementation with *C. perfringens* numbers and location,  $\alpha$ -toxin production and gut lesion scores has recently been reported (Dahiya *et al.*, 2005). In this research, dietary glycine ranged from 0.5 to 4.21% in a series of studies evaluating its effect on gut microbial populations, toxin production and NE lesions.

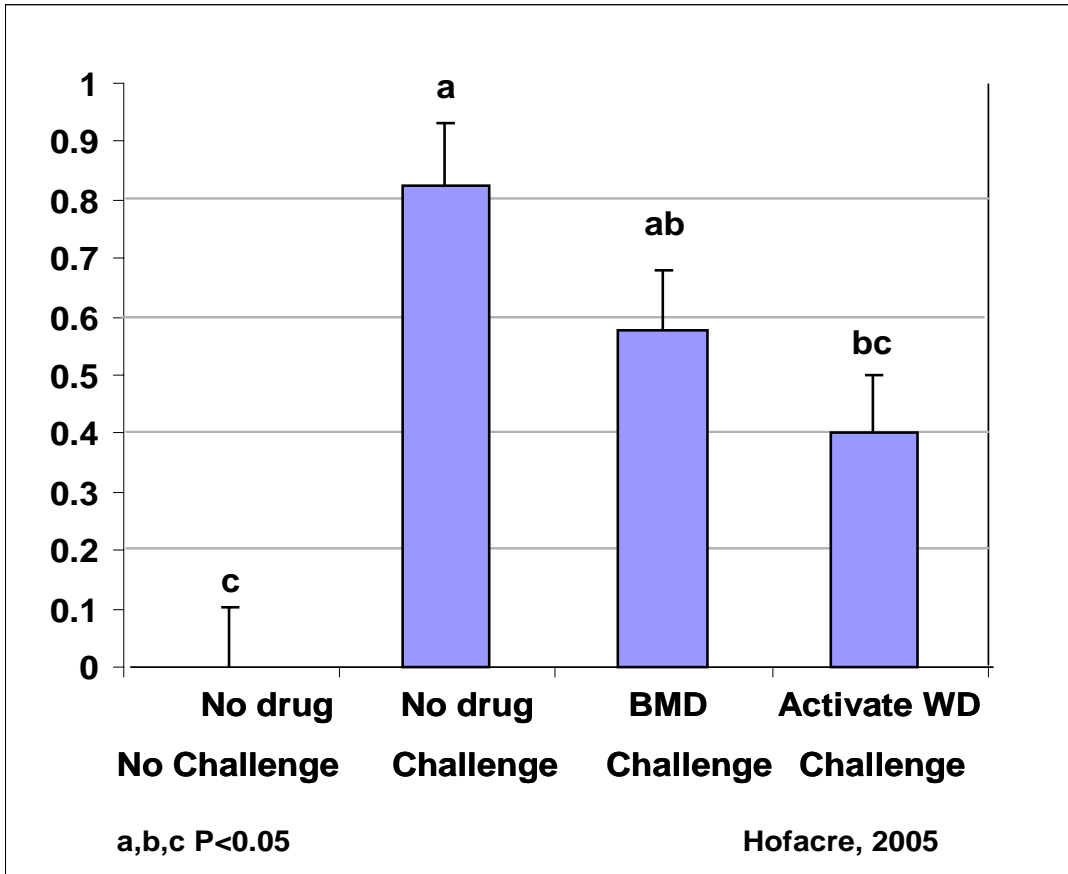


**Figure 1**

Figure 1 show the results of one of these studies (experiment 2), in which increasing dietary glycine supplementation was associated with a significant increase in *C. perfringens* in the ileum and cecum, and a decrease in Lactobacilli. In the same study, the microbial shift was accompanied by significant increases in  $\alpha$ -toxin in the ileal contents and in intestinal NE lesion scores (Dahiya *et al.*, 2005).

The gut environment and its resident microflora can also be modified using organic acids either in the feed or in the water. It is possible that providing an exogenous source of acid could be a factor discouraging overgrowth of Clostridia in the small intestine, but it may also simply favor the establishment and maintenance of the normal acid tolerant microbial flora present in the crop, gizzard, and upper small intestine. The effect of providing antimicrobial organic acids on necrotic enteritis incidence and severity has been reported by Hofacre (2005). The model system combined coccidial (day 14) and clostridial

(days 18, 19 and 20) challenge to generate NE in broilers. In this experiment (Figure 2), treatments consisted of unchallenged birds, challenged birds, birds challenged but treated with BMD, and birds challenged but treated with an organic acid blend (ACTIVATE® WD). As shown in Figure 2, post-challenge lesion scores for the control birds (treatment 1) were significantly better than for any of the challenge treatments.



**Figure 2**

Among the challenged treatments, the organic acid blend gave performance significantly better than the untreated birds and numerically superior to the antibiotic treatment. The possibility that this effect might be associated with a shift in gut environment that favors Lactobacilli over Clostridia is a subject for further research.

## Summary

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Gangrenous dermatitis, recently associated with high mortality in market-age turkeys, is a Clostridial infection that results in necrotizing lesions of skin and underlying muscle. The route of infection may include a breakdown in the barrier function of the gut, resulting in systemic distribution of *C. perfringens* and/or *C. septicum*. The organisms can then multiply and produce  $\alpha$ -toxin in an environment rich in free amino acids such as in damaged muscle or skin. One approach to proactively controlling GD risk is to modify the small intestinal environment and its microflora to be less susceptible to Clostridial overgrowth. Data presented demonstrate that Clostridia numbers can be manipulated through changes in the diet and that changing the gut microbial populations is associated with reduced toxin production and reduced pathology. Conversely, it is evident that a failure to manage the gut environment by ensuring highly digestible, good quality feed ingredients that are properly protected against degradation and microbial growth can favor Clostridial proliferation and the risk of high mortality in otherwise healthy market-age turkeys.

### References Cited

- Akester, A., R. Anderson, K. Hill and G. Osbaldiston. 1967. A radiographic study of urine flow in the domestic fowl. *Brit. J. Nutr.* 8:209-212.
- Bjornhag, D. and I. Sperber. 1977. Transport of various food components through the digestive tract of turkeys, geese and guinea fowl. *Swedish J Agric. Res.* 7:57-66.
- Carr, D., D. Shaw, D. Halvorson, B. Rings and D. Roepke. 1996. Excessive mortality in market-age turkeys associated with cellulitis. *Avian Diseases* 40:736-741.
- Choct, M. and G. Annison. 1992. The inhibition of nutrient digestion by wheat pentosans. *Brit. J. Nutr.* 67:123-132.
- Collett, S. 2005. Strategies for improving gut health in commercial operations. Pages 395-435. In: *Poultry Beyond 2010: 3rd International Poultry Broiler Nutritionist's Conference*. Auckland, New Zealand. April 3-8, 2005.
- Collett, S. 2007. Strategies to manage wet litter. 134-144. In: *Aust. Poult. Sci. Symp.*, Sydney, Australia, February 12-14, 2007.
- Dahiya, J., D. Hoehler, D. Wilkie, A. van Kessel and M. Drew. 2005. Dietary glycine concentration affects intestinal *Clostridium perfringens* and *Lactobacilli* populations in broiler chickens. *Poult. Sci.* 84:1875-1885.
- Dibner, J. and J. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci.* 84:634-643.
- Duke, G. 1982. Gastrointestinal motility and its regulation. *Poult. Sci.* 61:1245-1256.
- Finkel, S. and R. Kolter. 1999. Evolution of microbial diversity during prolonged starvation. *Proc. Natl. Acad. Sci. USA* 96:4023-4027.
- Grossman, I., D. Heitkamp and B. Sacktor. 1967. Morphologic and biochemical effects of *Clostridium perfringens* alpha-toxin on intact and isolated skeletal muscle mitochondria. *Am. J. Pathol.* 50:77-88.
- Hoerr, F. 2007. Case reports from Alabama. Pages 1-3. In: *56th Western Poultry Disease Conference*, Las Vegas, Nevada, March 26-29, 2007.
- Hofacre, C. 2005. Natural alternatives to prevent necrotic enteritis. *International Poultry Production* 13:7-9.

- Kennedy, C., E. Krejany, L. Young, J. O'Connor, M. Awad, R. Boyd, J. Emmins, D. Lyras and J. Rood. 2005. The alpha-toxin of *Clostridium septicum* is essential for virulence. *Mol. Microbiol.* 57:1357-1366.
- Murata, R., A. Yamamoto and H. Sato. 1975. Factors influencing the production of alpha-toxin (phospholipase C) by *Clostridium perfringens*. Pages 385-398. In: *Animal, Plant, and Microbial Toxins*. Ohsaka, A., K. Hayashi and Y. Sawai, (Eds.). Plenum Press, New York.
- Nakamura, M., M. Cook and W. Cross. 1978. Lecithinase production by *Clostridium perfringens* in chemically defined media. *Appl. Microbiol.* 16:1420-1421.
- Neumann, T., J. Skalecki, D. Karanadarun and T. Rehberger. 2007. Examining the diversity and antimicrobial susceptibility of *Clostridium perfringens* associated with anaerobic cellulitis in market-age turkeys. 136-139. In: 56th Western Poultry Disease Conference, Las Vegas, Nevada, March 26-29, 2007.
- Niemann, K. 1930. *Clostridium welchii* infection in the domesticated fowl. *J Am Vet Med Assoc* 77:604-606.
- Norton, R., S. Bilgili and B. McMurtrey. 1997. A reproducible model for the induction of avian cellulitis in broiler chickens. *Avian Diseases* 41:422-428.
- Norton, R., K. Macklin and B. McMurtrey. 1999. Evaluation of scratches as an essential element in the development of avian cellulitis in broiler chickens. *Avian Diseases* 43:320-325.
- Olkowski, A., C. Wojnarowicz, M. Chirino-Trejo, B. Wurtz and L. Kumor. 2005. The role of first line of defense mechanisms in the pathogenesis of cellulitis in broiler chickens: Skin structural, physiological and cellular response factors. *J Vet Med* 52:517-524.
- Oviedo-Rondon, E., P. Ferket and A. Santos Jr. 2006. The role of nutrition in the cause and prevention of gastrointestinal perturbation. 1-24. In: ACPV Workshop "Enteric diseases of poultry: The evolving challenges and new developments", Sacramento, CA, March 5, 2006.
- Rehberger, T., T. Neumann, K. Bos, G. Ritter and S. Dunham. 2006. Isolation and characterization of the *Clostridium* and gastrointestinal communities in broilers with gangrenous dermatitis. Pages 66-77. In: 41st National Meeting on Poultry Health and Processing, Ocean City, MD, Oct. 9-11, 2006.
- Ritter, G. 2006. Proposed pathogenesis of gangrenous dermatitis in chickens and attempts at experimental reproduction. 59-66. In: 41st National Meeting on Poultry Health and Processing, Ocean City, MD. Oct 9-11, 2006.
- Sacranie, A., P. Iji, L. Mikkelsen and M. Choct. 2007. Occurrence of reverse peristalsis in broiler chickens. 161-164. In: *Aust. Poult. Sci. Symp.*, Sydney, Australia.
- Sakurai, J., M. Nagahama and M. Oda. 2004. *Clostridium perfringens* alpha-toxin: Characterization and mode of action. *J. Biochem.* 136:569-574.
- Saunders, J. and A. Bickford. 1965. Clostridial infections of growing chickens. *Avian Diseases* 9:317-326.
- Stevens, D. and J. Rood. 2000. Histotoxic Clostridia. Pages 563-572. In: *Gram-Positive Pathogens*. Fischetti, V., R. Novick, J. Ferretti, D. Portnoy and J. Rood, (Eds.). ASM Press, Washington, DC.

- Vaillancourt, J.-P. and H. Barnes. 2003. Coliform cellulitis (inflammatory process). Pages 652-656. In. Diseases of Poultry. Saif, Y., (Ed.). Iowa State Press, Ames, Iowa.
- Wages, D. and K. Opengart. 2003a. Gangrenous dermatitis. Pages 791-795. In. Diseases of poultry. Saif, Y., (Ed.). Iowa State Press, Ames, Iowa.
- Wages, D. and K. Opengart. 2003b. Necrotic enteritis. Pages 781-784. In. Diseases of Poultry. Saif, Y., (Ed.). Iowa State Press, Ames, Iowa.
- Zambrano, M., D. Siegele, M. Almiron, A. Tormo and R. Kolter. 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. *Science* 259:1757-1760.
- Zinser, E. and R. Kolter. 2004. *Escherichia coli* evolution during stationary phase. *Res. Microbiol.* 155:328-336.