Using Competitive Exclusion, Mannan-Oligosaccharide and Other Intestinal Products to Control Necrotic Enteritis

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Primary Audience: Nutritionists, Producers, Veterinarians, Researchers

SUMMARY

Clinical and subclinical necrotic enteritis (NE) caused by the ubiquitous bacteria *Clostridium perfringens* can have a significant economic impact in broiler chickens. This impact is especially apparent when antibiotic feed additives are not used.

In this study, the effectiveness of five nonantibiotic feed additives and two competitive exclusion (CE) cultures were compared using an NE challenge model. Untreated male broiler chickens housed in an environmentally controlled facility were challenged with *C. perfringens* and had high mortality, gross intestinal lesions, poor feed utilization, and reduced body weights. Broilers treated with a defined lactic acid bacterial CE culture at 1 d and fed a diet containing 2 g/ton of mannan-oligosaccharide had the lowest mortality from necrotic enteritis. The feed efficiency of this combined treatment was similar to that of broilers fed a diet containing 50 g/ton of bacitracin methylene disalicylate.

The treatment in which broilers were given an undefined CE or the mannan-oligosaccharide alone, as well as three other feed additives, did not have any significant effect on feed efficiency, mortality, or body weight over the NE challenge control birds.

The findings of this study are very important in developing nutritional strategies to provide options for prevention of NE without continuously using antibiotics in feed.

Key words: competitive exclusion, mannan-oligosaccharide, bacitracin methylene disalicylate (BMD-50), chicken, *Clostridium perfringens*, necrotic enteritis

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DESCRIPTION OF PROBLEM

Necrotic enteritis (NE) is a common disease found in all poultry-growing areas of the world. It has been estimated that the cost of subclinical necrotic enteritis can be as much as $0.05 per bird [1]. The causative agent of necrotic enteritis is *Clostridium perfringens*, a nearly ubiquitous anaerobic bacteria that is found in soil, dust, feces, feed, poultry litter, and in intestinal contents [2]. The subclinical form of NE may be most economically important because it has been shown to impair feed conversion in broilers [3].

Traditionally, NE prevention strategies have taken the form of antibacterial feed additives, often referred to as growth promotants, such as baci-

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tracin, virginiamycin, avoparcin, lincomycin, tylosin, and penicillin [2, 3, 4, 5, 6, 7]. In 1999, the European Union (EU) prohibited use of antibacterial feed additives that have most successfully controlled the incidence of clinical and subclinical NE. Consequently, many EU countries have observed NE at near epidemic proportions (25 to 40%) in broiler flocks [8]. Therefore, new methods of prevention of NE must be investigated.

It has been documented that disturbances in the intestinal microflora can result in elevated levels of *C. perfringens* [9]. Fukata et al. [9] found that the pathogenic effects of *C. perfringens* could be reduced by feeding chicks a monoflora of *Lactobacillus acidophilus* or *Streptococcus faecalis*. Others have demonstrated that use of undefined competitive exclusion (CE) cultures of chicken intestinal flora, either fresh or freeze-dried, could reduce NE mortality and cecal colonization of *C. perfringens* and prevent subclinical negative effects on body weight and feed efficiency in broiler chickens [10, 11].

Feeding complex carbohydrates derived from the cell wall of the yeast *Saccharomyces cerevisiae* (mannan-oligosaccharide) or the yeast *Saccharomyces boulardii* has been found to decrease the colonization of *Salmonella* and *Campylobacter* [12]. Recent research has also shown these compounds may also be effective in reducing NE lesions in broiler chickens [13]. However, there has been no work to determine if including CE and complex carbohydrates will more effectively prevent clinical NE, subclinical NE, or both.

Given the ubiquitous nature of *C. perfringens* and the multiple factors, such as environmental stress, various feed ingredients (wheat, barley), or other disease insults (i.e., coccidia), that can trigger NE [2], a study was designed to evaluate the effectiveness of various nutritional strategies alone and in combination. These treatments were compared with a standard industry antibiotic.

MATERIALS AND METHODS

Treatments

The treatment groups were 1) nonchallenged control; 2) bacitracin methylene disalicylate, 50 g/ton [14]; 3) mannan-oligosaccharide, 2 g/ton [15]; 4) a freeze-dried lactic acid bacterial preparation (*L. acidophilus*, *Enterococcus faecium*, *Lactobacillus plantarum*, and *Pediococcus acidilactici* culture [16]; 5) an undefined freeze-dried CE culture [17]; 6) fructose oligosaccharide, 2 kg/ton [18]; 7) propionic acid, 8 kg/ton [19]; 8) a proprietary herbal supplement of oils and mannan-oligosaccharide [20]; 9) both lactic acid bacteria product (All-Lac XCL) and mannan-oligosaccharide (Bio-Mos); and 10) *C. perfringens* challenge control group. The undefined CE product and the lactic acid bacteria product were applied to the chicks at 1 d of age as a course spray (25 g/L of distilled water at 0.2 mL/chick). All of the other treatments were added to the feed during the entire study at inclusion rates indicated above.

Chicken Husbandry and Challenge Model

The study used 960 male broiler chickens [21] that were housed from d 0 to 28 in starting batteries and then in growing batteries. All groups were located within the same environmentally controlled room. Chicks in all groups were identified by cage number and were given water and a corn-soybean meal- and fishmeal-based feed ad libitum to favor the pathogenesis of the infection model.

The 10 treatments of two CE products, five dietary additives, bacitracin methylene disalicylate (BMD), and two control groups had eight replicates with 12 chicks per replicate. To prevent cross contamination of each of the CE cultures, cages were randomized within each treatment of the respective CE culture.

The NE challenge model used in this study has been described by George et al. [4]. The model consisted of a solitary oral inoculation with *Eimeria acervulina* and *Eimeria maxima* at 15 d of age followed by serial oral inoculation with *C. perfringens* [22] (approximately 10^8 cfu/bird each day) at 18, 19, and 20 d. The basal diet offered from 0 to 16 d contained 26% fishmeal. Feed consumption was measured from 0 to 15 d and 15 to 28 d. All birds were weighed by cage at 0, 15, and 28 d. Necropsies of all mortalities from 18 d were conducted to determine the cause of death. At 22 d, two birds from each cage were randomly selected, killed, weighed, and scored for the level of NE intestinal lesions (0 = none, 1 = mild, 2 = moderate, 3 = marked/severe). All remaining chicks were killed, weighed, and scored for lesions at 28 d.

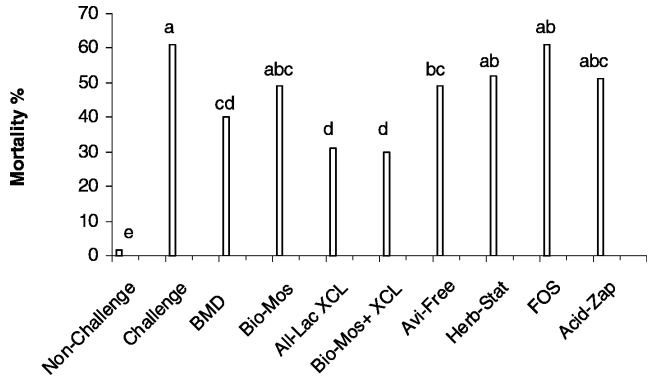

FIGURE 1. Necrotic enteritis mortality in broilers given an antibiotic, various feed additives, and competitive exclusion cultures. [a–d]Bars with different letters are significantly different ($P < 0.05$). BMD = bacitracin methylene disalicylate; Bio-Mos = mannan-oligosaccharide; All-Lac XCL = lactic acid bacterial preparation; Avi-Free = undefined competitive exclusion culture; FOS = fructose oligosaccharide; Herb-Stat = herbal supplement; Acid-Zap = propionic acid.

Biometrics

The treatments were analyzed using conventional ANOVA. The cause of significance was determined using the post hoc test of least significant differences (LSD with $P \leq 0.05$).

RESULTS AND DISCUSSION

The challenged control birds had the numerically highest mortality (60%) with 100% of the mortality due to NE (Figure 1), whereas broilers treated with the lactic-acid-producing bacterial culture alone (30%) or combined with the mannan-oligosaccharide (29%) in the feed had the lowest NE-associated mortality. These two novel treatments had mortalities similar to birds treated with the antibiotic BMD (39%). The birds treated with the undefined CE culture also had significantly less mortalities than the challenge controls.

There were no significant differences among treatments for weight gain (Figure 2). However, significant differences did occur among treatments for feed conversion (Figure 3). Birds receiving a combination of mannan-oligosaccharide and lactic acid had feed conversions similar to those fed BMD and the nonchallenged controls. Further,

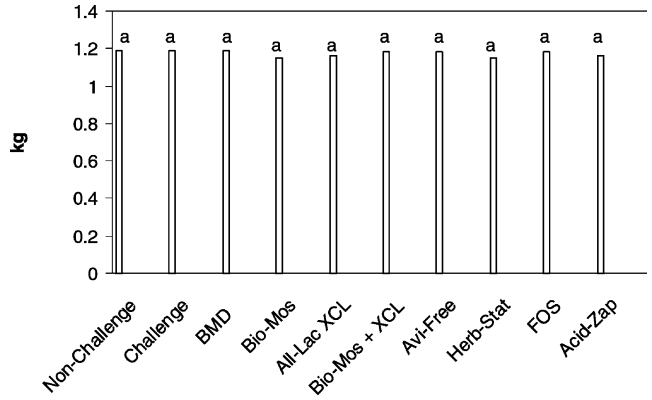

FIGURE 2. Weight gain for 0-to-28-d-old broilers given an antibiotic, various feed additives, and competitive exclusion cultures. [a]Bars with different letters are significantly different ($P < 0.05$). BMD = bacitracin methylene disalicylate; Bio-Mos = mannan-oligosaccharide; All-Lac XCL = lactic acid bacterial preparation; Avi-Free = undefined competitive exclusion culture; FOS = fructose oligosaccharide; Herb-Stat = herbal supplement; Acid-Zap = propionic acid.

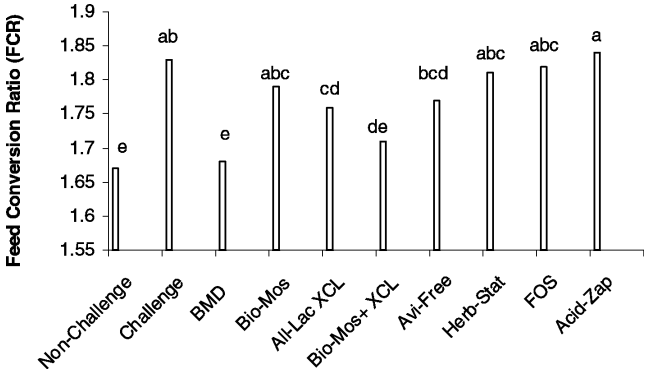

FIGURE 3. Feed conversion of 28-d-old broilers given an antibiotic, various feed additives, and competitive exclusion cultures. [a–e]Bars with different letters are significantly different ($P < 0.05$). BMD = bacitracin methylene disalicylate; Bio-Mos = mannan-oligosaccharide; All-Lac XCL = lactic acid bacterial preparation; Avi-Free = undefined competitive exclusion culture; FOS = fructose oligosaccharide; Herb-Stat = herbal supplement; Acid-Zap = propionic acid.

when lactic acid was fed alone, feed conversion was similar to the combination treatment above as well as several of the alternate treatments and, thus, produced intermediate response. Finally, several of the additives (Bio-Mos, Herb-Stat, FOS, and Acid-Zap) did not improve feed conversion when compared to the challenged controls, indicating they had little effect in reducing infections by *C. perfringens*.

At 28 d, total gross intestinal NE lesion scores were highest in the group given the fructose oligosaccharide with little practical difference occurring among any of the other treatments, including bacitracin (Figure 4). The lack of marked differences in the lesion scores among the treatments was most likely influenced by the high mortality observed among the NE challenged birds before the 28-d lesion scores were taken.

Given the pressure exerted by regulations of consumer and international export markets to eliminate use of antibiotics in food animal diets, it has become necessary for the poultry industry to find alternatives for preventing NE [13, 23]. The experience of the EU once the growth-promotant antibiotics were no longer available for use in poultry diets indicated that clinical and subclinical NE became an economically significant problem [8].

The results of this study indicate that use of a CE culture, such as All-Lac, either alone or in

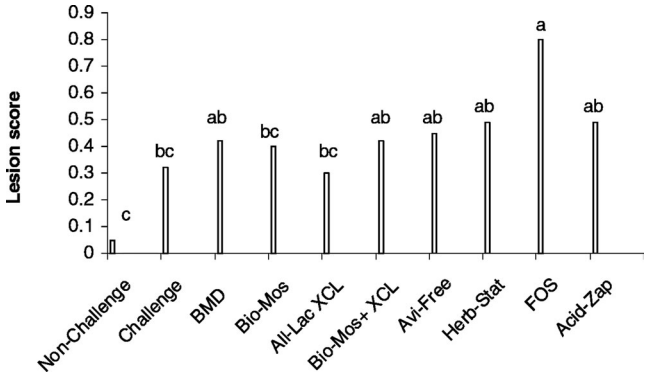

FIGURE 4. Average necrotic enteritis lesion score of 28-d-old broilers given an antibiotic, various feed additives, and competitive exclusion cultures. [a–c]Bars with different letters are significantly different ($P < 0.05$). BMD = bacitracin methylene disalicylate; Bio-Mos = mannan-oligosaccharide; All-Lac XCL = lactic acid bacterial preparation; Avi-Free = undefined competitive exclusion culture; FOS = fructose oligosaccharide; Herb-Stat = herbal supplement; Acid-Zap = propionic acid. Necrotic enteritis lesion scores were 0 = none, 1 = mild lesions, 2 = moderate lesions, 3 = marked to severe lesions.

combination with a mannan-oligosaccharide, such as Bio-Mos, can effectively reduce mortality as well as the subclinical effects of *C. perfringens* on feed efficiency. It is possible that feeding birds complex carbohydrates derived from the cell walls of yeasts may prevent colonization by the bacteria *C. perfringens*, as it has been demonstrated to prevent intestinal colonization by *Salmonella* and *Campylobacter* [24].

The results of our work with CE cultures are in agreement with the findings of other researchers [9, 11]. It appeared that the acidifier (Acid-Zap) did not produce any protective effects from the *C. perfringens* in the intestine, as mortality and feed conversion were similar to those for challenged controls. Normally, the crop is the only location that would be significantly affected by the pH of an intestinal acidifier. Because this challenge model is quite severe, it might have exceeded any benefit of lowering the upper intestinal pH. Future studies should investigate the use of these types of products alone and in combination to reduce or prevent NE in broilers raised under commercial conditions.

CONCLUSIONS AND APPLICATIONS

1. The necrotic enteritis challenge model used in this study was effective in inducing death, intestinal lesions, poor feed utilization, and reduced body weight gain in broiler chickens.
2. Under the conditions of this study, the lactic-acid-producing bacterial culture, alone or combined with the mannan-oligosaccharide, was effective in reducing *C. perfringens*-associated mortality and the subclinical effects on feed efficiency.
3. *C. perfringens* is an opportunistic bacterium that takes advantage of alterations in the normal bacterial flora of the intestine. Under the conditions of this study, the use of complex carbohydrates (Bio-Mos) in the feed combined with a CE culture (All-Lac XCL) may be an alternative to the use of growth-promotant antibiotics in prevention of NE.

REFERENCES AND NOTES

1. Van der Sluis, W. 2000. Clostridial enteritis is an often underestimated problem. World's Poult. Sci. J. 16:42–43.

2. Ficken, M. D., and D. P. Wages. 1997. Necrotic enteritis. Pages 261–264 in Diseases of Poultry. 10th ed. B. W. Calnek, ed. Iowa State University Press, Ames, IA.

3. Stutz, H. W., and G. C. Lawton. 1984. Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens*, and ideal weight of broiler chicks. Poult. Sci. 63:2036–2042.

4. George, B. A., C. L. Quarles, and D. J. Fagerberg. 1982. Virginiamycin effects on controlling necrotic enteritis infection in chickens. Poult. Sci. 61:447–450.

5. Hafshagen, M., and M. Kaldhusdal. 1992. Barley inclusion and avoparcin supplementation in broiler diets. 1. Effect on small intestinal bacteria flora and performance. Poult. Sci. 71:959–969.

6. Maxey, B. W., and R. K. Page. 1977. Efficacy of lincomycin feed medication for the control of necrotic enteritis in broiler-type chickens. Poult. Sci. 56:1909–1913.

7. Watkins, K. L., T. R. Shryrock, R. N. Dearth, and Y. M. Saif. 1997. *In vitro* antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. Vet. Microbiol. 54:195–200.

8. Kaldhusdal, M., and A. L. Lovland. 2000. The economical impact of *Clostridium perfringens* is greater than anticipated. Worlds Poult. 16:50–51.

9. Fukata, T., Y. Hadate, E. Baba, and A. Arakawa. 1991. Influence of bacteria on *Clostridium perfringens* infections in chickens. Avian Dis. 35:224–227.

10. Elwinger, K., C. Schneitz, F. Berndtson, O. Fossum, B. Teglof, and B. Bagstrom. 1992. Factors affecting the incidence of necrotic enteritis, cecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta Vet. Scand. 33:369–378.

11. Hofacre, C. L., R. Froyman, B. Gautrais, B. George, M. A. Goodwin, and J. Brown. 1998. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens*-associated necrotizing enteritis in broiler chickens. Avian Dis. 42:579–584.

12. Shane, S. M. 2001. Mannan-oligosaccharides in poultry nutrition: Mechanisms and benefits. Pages 65–77 in Proc. 17th Alltech Symp., Louisville, KY.

13. Kaldhusdal, M. I. 2000. Necrotic enteritis as effected by dietary ingredients. Worlds Poult. 16:42–43.

14. BMD-50, Alpharma, Fort Lee, NJ.

15. Bio-Mos, Alltech, Inc., Nicholasville, KY.

16. All-Lac XCL, Alltech, Nicholasville, KY.

17. Avi-Free, Alltech, Nicholasville, KY.

18. FOS, Alltech Nicholasville, KY.

19. Acid zap, Alltech, Nicholasville, KY.

20. Herb-Stat, Alltech, Inc., Nicholasville, KY.

21. Cobb, Siloam Springs, AR.

22. Thayer, S. 1996. Department of Avian Medicine, University of Georgia, Athens, GA. Personal communication.

23. Mlot, C. 2001. Antimicrobials in food production: Resistance and alternatives. ASM News 67:196–200.

24. Line, J. E. J. S. Bailey, N. A. Cox, N. J. Stern, and T. Tompkins. 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. Poult. Sci. 77:405–410.