Subclinical Necrotic Enteritis and Dysbacteriosis in Broilers Induced by Diet Manipulation in the Presence of Coccidia Cycling in the System

Marco Quiroz, Julia J. Dibner, Francis Yan and Chris D. Knight
Novus International, Inc., 20 Research Park Drive, Missouri Research Park,
St. Charles, Missouri 63304

ABSTRACT

The move toward drug-free poultry feeds has increased use of coccidial vaccines; however, the curtailing of feed and therapeutic antibiotic use worldwide has increased enteric diseases, including necrotic enteritis (NE). NE, caused by *Clostridium perfringens* (Cp) is one of the most economically important enteric diseases of broilers. Coccidial vaccination may predispose birds to NE. The purpose of this presentation is to place necrotic enteritis within the context of digestive health and microbial ecology by testing the hypothesis that coccidial vaccination alone does not lead to Cp overgrowth. The studies were designed to test whether diet and dietary additives also play a role in Cp overgrowth. To study the role of diet in the development of dysbacteriosis, a model has been developed that does not include a Cp challenge. Rather, the model uses a high viscosity diet that has been associated with Cp dysbacteriosis. Using this model, a series of experiments were conducted to study factors that can contribute to or mitigate the effects of subclinical enteritis. Results indicated that diet played a significant role in Cp growth while coccidiosis challenge had no significant effect. This model is being used to test novel feed additives. Determining the dietary and enteric conditions that precede clinical NE are essential in the development of dietary and feed solutions for sustainable drug free agriculture. The purpose of the research described here is to examine the relationship between coccidial cycling and dysbacteriosis involving *C. perfringens* in broiler chickens with the goal of identifying nutrition guidelines and feed additives that reduce the incidence of Cp overgrowth in the distal ileum of broiler chicks.

INTRODUCTION

Systematic study of poor gut health associated with dysbacteriosis is complicated by the fact that a variety of interacting factors contribute to its etiology. In many cases the initiating factors may be mild parasitic infection, exogenous pathogens or toxins. The host response to these insults contributes significantly to the course of the problem and the ultimate impact on flock performance; however, it is quite common that no clear clinical manifestations are associated with it. Therefore, the term that has been used to describe this form of poor gut health is subclinical enteritis (Hoerr, 1998) which, because it is not obvious, goes untreated and continues to reduce production efficiency. The purpose of the current report is to describe a reproducible experimental model that provides a means of creating subclinical enteritis such that the interacting factors that contribute to and mitigate this syndrome can be systematically studied.

MATERIALS AND METHODS

Trial 1. A 28-day broiler trial was conducted in which a 22% CP, 1.21%/1.07% total/digestible lysine mash diet was fed that contained 33% rye, 25% wheat, and 31% soybean meal. Treatments were in a 2X3 factorial arrangement that included two factors of cycling Eimeria as a 3X overdose of a 3-species live oocyst vaccine (ADVENT® coccidiosis control) or nothing, and 3 feed additive factors: a negative control, an antibiotic (bacitracin methylene disalicylate, 60 ppm:BMD) or an NSP enzyme mixture containing xylanase, glucanase and glycosidase (CIBENZA® CSM feed additive).

Trial 2. To examine the impact of dietary protein on ileal Cp and intestinal barrier function we used two diets, one with no animal protein and formulated to 22% CP and 1.38%/1.21% total/digestible lysine, the second diet was nearly identical to the first with the exception that it contained 14% poultry by-product meal (PBM) and was formulated to provide an excess of CP (30%) and total/digestible lysine (1.65%/1.38) Each diet was tested with or without 0.5% of a *B. licheniformis* derived protease (CIBENZA® DP100). All birds were subjected to an overdose of the same live oocyst coccidiosis vaccine with the exception that this occurred on day 7 of the study instead of day 1.

RESULTS

The overdose of coccidiosis vaccine in this high viscosity diet resulted in a 4-5% reduction ($P<.01$) in the efficiency of gain, however, there were no interactions with any of the feed additive effects (Trial 1). Therefore, results are presented as main effects averaged across coccidiosis challenge. Addition of the feed additives improved 28-day Performance Index [(period gain*period livability/period feed efficiency), PI] from 145 for control to 188 and 284 for antibiotic and NSP enzymes, respectively (Fig 1; $P<.01$). The PI improvement was more consistent for NSP enzymes than that of the antibiotic and was also associated with a significant reduction in digesta viscosity ($P<.01$) throughout the trial. NSP enzymes were also associated with a 1.5 to 2.5 log reduction in cultured Cp from the hindgut and lower ileum (Fig 2; $P<.01$), consistent with a previous report (Choct et al, 2006), however, the antibiotic did not have a significant effect on Cp number. Overall livability for the trial was in excess of 95%, there were no treatment related differences and no Clostridium-related deaths.

Figure 1.

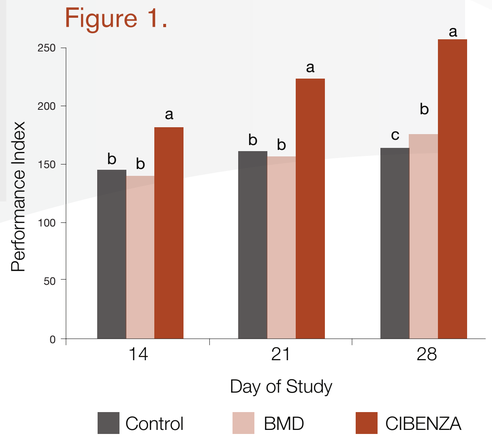


Figure 1: NSP Enzymes (CIBENZA® CSM) and to a lesser extent antibiotic (BMD) improved performance index in high NSP diets.

Figure 2.

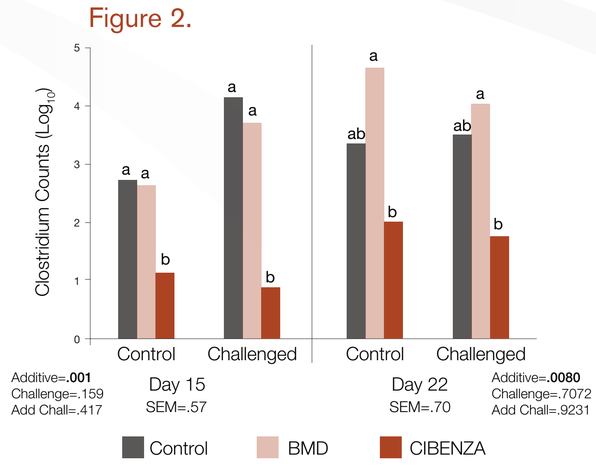
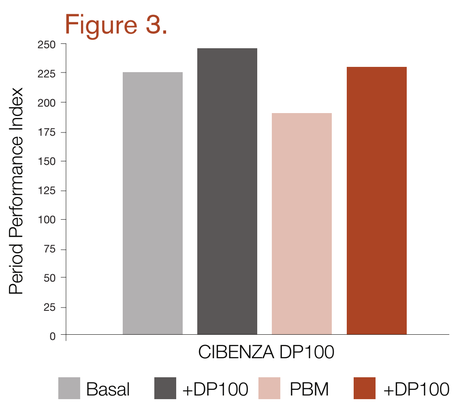


Figure 2: Addition of NSP enzymes reduced *C. perfringens* in lower intestinal tract (Ileum + Ceca).

Intestinal morphometry at day 15 and 22 of the study was used to assess intestinal health with respect to the various treatments employed in the trial (data not shown). Addition of the NSP enzymes improved intestinal morphometry (duodenum and mid-small intestine) as represented by reduced crypt/villus ratio, indicating that at least a portion of the improved performance was related to improved gut health and reduced demands on the crypt stem cell proliferation. These results indicate that subjecting broilers to this challenge of dietary NSP-containing ingredients created intestinal inflammation and stimulated Cp growth in the lower GIT. Furthermore, addition of NSP enzymes and to a lesser extent an antibiotic improved performance while the NSP enzymes alone mitigated intestinal.

The purpose of Trial 2 was to examine the impact of animal protein on performance and subclinical enteritis. In this case the protein from PBM was in excess of requirement. The 28-day results indicated a reduction in PI with the 30% protein PBM diet that was increased to that of the normal protein diets with the addition of protease (DP100; Fig 3). While these results were not significant, they are directionally consistent with the potential for excess protein in the hindgut to have a promoting influence on bacterial overgrowth and consequently a negative influence on performance. The ileal Cp levels for the 30% CP PBM diet were increased approximately 2 log units compared to the normal protein diet (Fig 4; $P<.01$). Addition of the protease to the normal protein diet had no impact on ileal Cp levels however, protease addition to the high protein diet resulted in a 2 log reduction in Cp. Addition of protease resulted in reductions of serum AGP levels (Fig 4; $P<.10$) regardless of dietary protein level. These results indicate that minimizing flow of digestible animal protein into the hindgut in the face of cycling *Eimeria* will reduce Cp levels whether this is done with lower dietary protein or the addition of a protease to increase digestibility in the upper GIT thereby minimizing protein flow to the hindgut. Furthermore, addition of the protease in this gut health challenge model improved intestinal barrier function as measured by systemic acute phase protein response, irrespective of dietary protein level.

Figure 3.



Analysis	
PBM	P=0.260
DP100	P=0.171
PBM*DP100	P=0.607
CV=27.3%	

Figure 3: The high protein diet (30% CP) containing 14% poultry by-product meal (PBM) tended to reduce performance index while addition of protease (DP100) tended to maintain it at levels comparable to normal protein (22% CP).

Figure 4.

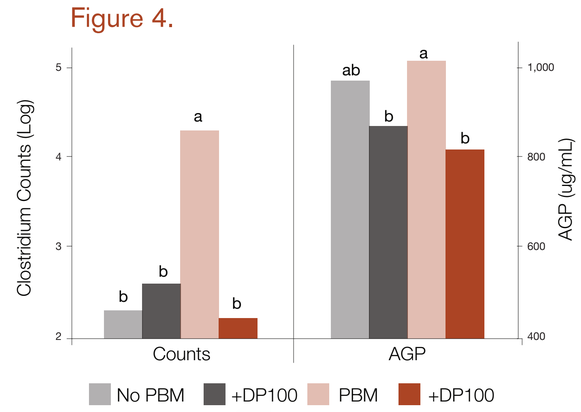


Figure 4: Addition of protease (DP100) reduced the elevated ileal Cp levels resulting from feeding the 30% CP diet (a,b; $P<.05$) and reduced systemic acute phase response (AGP) irrespective of dietary protein level (a,b; $P<.10$).
*AGP; Alpha-lacid glycoprotein

SUMMARY AND CONCLUSIONS

These studies focused primarily on the influence of increased nutrient flow to the hindgut on the stimulation of Cp growth and demonstrated the role that substrate specific digestive enzymes play in minimizing Cp growth and supporting maintenance of a healthy gut. We have also observed that while Cp is a reproducible marker of intestinal overgrowth of a potential pathogen, there is no need to challenge the birds with exogenous Cp in order to produce these effects. In fact, it's possible that this approach more closely mimics what occurs in the normal commercial environment. This model system will be used in the future to evaluate additional factors that can support the maintenance of intestinal barrier function, modulate the intestinal inflammatory response and resulting oxidative stress, and promote the maintenance of the appropriate microbiota in the gastrointestinal tract of the chicken.

References Available on Request