COMPETITIVE EXCLUSION: PROBIOTIC PREPARATIONS FOR POULTRY

J.M. COX and B.L. CHUNG

Summary

Competitive exclusion, involving the administration of single strains or mixtures of microorganisms to young poultry, is a strategy to minimise or prevent colonisation by enteric pathogens of veterinary and/or public health concern. The best competitive exclusion preparations are undefined microbial consortia, produced through fermentation of caecal or faecal material from adult chickens, or semi-defined microbial consortia prepared from faecal, caecal or mucosal cultures, and contain a diversity of microorganisms, including Gram-negative and Gram-positive, facultative and obligate anaerobes. Competitive exclusion preparations, alone or with amendments, can be applied to poultry in several ways, but early administration is paramount. While not a panacea, competitive exclusion is a useful part of an integrated pathogen control strategy in poultry production.

I. INTRODUCTION

Today, more than ever, food safety, and particularly the threat to public health posed by pathogenic microorganisms, is a major global issue. Worldwide, poultry is considered to be a significant vehicle of transmission of a range of enteric microbial pathogens, most notably *Salmonella* and *Campylobacter*; hence, management of enteropathogens during poultry production is crucial, and various approaches, singly or as integrated strategies, have been proposed. These include use of pathogen-free stock, feed and water, strict biosecurity, a comprehensive cleaning and sanitation program, and the topic of this paper, competitive exclusion (CE).

The concept of probiotics, the administration of microorganisms to animals (including humans) to enhance gastrointestinal balance and resistance to colonisation by enteric pathogens, is not new (Payne, 1999). Last century, Metschnikov promoted the consumption of yoghurt as a means to maintain gut health. More recently and specifically, the use of probiotics in poultry was first demonstrated in the early 1970s, and the term 'competitive exclusion' was coined (Nurmi and Rantala, 1973). The aim of this paper is to provide an overview of competitive exclusion for the control of enteropathogens in poultry.

II. DYNAMICS OF THE GASTROINTESTINAL MICROFLORA

To gain an insight into how probiotic strains or CE mixtures work, it is necessary to consider how enteric pathogens, the normal gut microflora and the host interact, and how the latter two minimise or prevent colonisation by the former. Pathogens have to contend with a hostile environment within the gastrointestinal tract of the healthy adult host, including low pH of the stomach, mucin trapping, peristaltic clearing, villus sweep, immune exclusion, toxic bile acids, secretory antibodies, cell mediated immunity, oxygen tension, and nutrient availability (Stern and Meinersmann, 1989). In addition, the normal gut microflora is believed to enhance exclusion through several mechanisms including, broadly, indirect and direct antagonistic effects (Rolfe, 1991). Direct effects include modification (deconjugation) of bile salts (Barrow, 1992), induction of immunological processes and stimulation of peristalsis. Direct antagonism involves depletion of or competition for essential nutrients.

Department of Food Science and Technology, University of New South Wales, Sydney NSW 2052.
(Hume et al., 1997), competition for bacterial receptor sites, creation of a restrictive physiological environment and elaboration of antibiotic-like substances.

Mucosal attachment is a prerequisite for colonisation of the intestinal tract by both the indigenous or beneficial microflora and pathogens (Fuller and Gibson, 1997). Colonisation involves interaction between cell wall polysaccharides of bacteria and heteropolysaccharides on the epithelium, and evidence suggests that the affinity of the former for the latter is generally greater among indigenous gut microorganisms than pathogens (Snoeyenbos et al., 1979; Pivnick and Nurmi, 1982). Physical hindrance through prior occupation of receptor sites is considered a major mechanism of competitive exclusion (Drasar and Barrow, 1985; Stavric et al., 1987).

Creation of a restrictive physiological environment is believed to be associated primarily with the production of weak organic acids, both non-volatile and volatile fatty acids (VFAs), and the consequent reduction in pH and prevalence of undissociated species of the VFAs (Barrow, 1992; Corrier et al., 1995). In this form, VFAs concentrate within the microbial cell until cellular energy is insufficient to drive their efflux, at which point cytoplasmic pH decreases dramatically and the cell dies. In addition, some microorganisms among the indigenous gut microflora and present in CE mixtures are able to produce other potentially inhibitory substances ranging from hydrogen sulphide to bacteriocins (Barrow, 1992). For example, strains of Enterobacteriaceae have been shown to produce substances specifically inhibitory toward Campylobacter jejuni (Schoeni and Doyle, 1992; Schoeni and Wong, 1994).

III. APPROACHES TO PREPARATION OF CE PRODUCTS

Different research groups have taken significantly different approaches in attempting to produce efficacious CE preparations. Broadly, these can be classified as use of:

- single microbial strains
- undefined preparations or mixtures
- semi-defined preparations or mixtures
- fully defined preparations or mixtures

Regardless of approach, the efficacy of CE preparations should be assessed using a standard method. To that end, Mead et al. (1989) developed a standard in vivo assay, involving challenge with an enteropathogen of two groups of day-old chicks, one of which serves as an untreated control, while the other receives the putative CE preparation one day prior to challenge. Five days post-challenge, following sacrifice, the mean average log population, or infection factor (IF) of the pathogen in each group, is determined through culture of caecal material. The ratio of IF values between the control group and the treated group yields the protection factor (PF); the higher the PF, the more protective the CE preparation. A PF of >4 is considered necessary for efficacious application under commercial rearing conditions.

Single or limited mixtures of strains from the bacterial genera Bacteroides (Barnes et al., 1979), Bifidobacterium (Barnes et al., 1979), Clostridium (Rigby and Pettit, 1980), Enterococcus (Hinton et al., 1991a,c), Lactobacillus (Barnes et al., 1980; Soerjadi et al., 1981b; Impey et al., 1982; Stavric et al., 1992; Jin et al., 1998), Streptococcus (Soerjadi et al., 1981b) and Veillonella (Hinton et al., 1991a,c), as well as the yeast, Saccharomyces boulardii (Line et al., 1997), have been evaluated as CE agents, with little, or at best temporary, exclusion effect.
Undefined CE preparations have been produced from whole caeca, caecal contents, mucosal scrapings from caeca, or faeces, used directly, in suspension or after a cultural process such as continuous-flow fermentation. Unlike single strains or limited mixtures, undefined preparations appear to offer the best protection against colonisation by enteropathogens. However, undefined CE preparations may inadvertently introduce other avian or even human pathogens into stock, unless carefully screened. (Corrier et al., 1993; Methner et al., 1997). From a commercial perspective, the composition often cannot be standardised, although strategies such as passage through gnotobiotic animals should facilitate consistency.

As a compromise between efficacy on the one hand and safety and consistency on the other, 'fully' and semi-defined CE mixtures have been developed, using as source material whole caeca, caecal contents, mucosal scrapings from caeca, or faeces. While some of these preparations are claimed to be fully defined, the lack of identification of some consortium members to species or even genus level suggests 'fully' is a misnomer. Fully defined preparations have also been prepared from known probiotic microorganisms, although the source of the strains is not necessarily poultry.

The microorganisms involved in CE preparations, other than those based on single strains, represent a diversity of genera and species, including a number of organisms that remain incompletely identified (Table 1). The organisms represent Gram-negative and Gram-positive bacteria, both facultative and obligate anaerobes, and most are typical of the types of microorganisms associated with the gut microflora of many animals. Efficacious CE mixtures, whether relatively simple or complex, contain significant numbers of lactic acid bacteria, reflecting the central role of this group of organisms in maintaining gastrointestinal stability.

IV. CURRENT PRODUCTS

In relation to commercial CE products, Nurmi (1983) considered they should have five vital characteristics. The product should: be safe, free from microbes constituting hazards to human or animal health; prevent infection and spread of salmonellae and other pathogens in growers; not impair growth, but rather promote growth and general health; be easy to transport, preserve and apply; and the price should be moderate and in proportion to the benefits conferred.

There are several commercial CE products currently on the market. Broilact® (Finland), Avigail (UK), CF3/Pre-Empt (USA; DeLoach 29 in Japan), AviFree (USA) and Lactobacillus reuteri (USA) are all derived from caecal material of poultry, while Protaxin (UK), is a mixture of fully defined organisms derived from and identified as probiotics in a range of non-avian animal hosts.

Apart from L. reuteri, which is essentially a probiotic, all products in the first group are undefined to some degree. Avigail and AviFree are non-selected, mixed cultures derived from the entire caecal contents of an adult chicken, whereas Broilact® undergoes a selection process to screen out any poultry or human pathogens.

Broilact®, based on a mixture of 32 pure cultures of partially to fully identified intestinal bacterial strains isolated from adult chickens (Nurmi, 1983), was the first commercial CE product, developed in Finland. It was launched in Finland and Sweden in 1987 as a liquid product, but since 1994 it has been sold as a lyophilised product. Schneitz et al. (1998) found that the bacterial population of Broilact® was able to attach to epithelial cells and protect chicks against Salmonella colonisation, improve the degradation of β-glucans and arabinoxylans by supplementing enzyme activity in the feed, resulting in lower
viscosity of ileal contents, improve nutrient digestibility, and increase the propionic acid concentration of caecal contents.

Table 1. Bacterial composition of 'defined' CE treatments, which provide protection against a single challenge of 10 cfu of *Salmonella* (after Stavric et al., 1991b).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Number of strains in CE treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 3 3 5 8 8 10 18 28 50</td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>3 1 1 7 8</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>2 3 6 11</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>2 3 2</td>
</tr>
<tr>
<td><em>Fusobacterium</em></td>
<td>8 3 18 4 10</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>3 1</td>
</tr>
<tr>
<td><em>Eubacterium</em></td>
<td>1 1</td>
</tr>
<tr>
<td><em>Propionibacterium</em></td>
<td>3 2</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>3 5 8 4 1</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>1 1</td>
</tr>
<tr>
<td>Gram-positive anaerobic rods</td>
<td>1 4 6</td>
</tr>
</tbody>
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Schneitz (1998) assessed the protective efficacy of three CE products, CF3, Aviguard and Broilact®, finding they yielded Infection Factor (IF) values of 3.0, 2.8 and 0.3, respectively. Although PF values are not mentioned, the last figure suggests Broilact® is efficacious. Palmu and Camelin (1997) found Broilact® treatment significantly reduced *Salmonella* contamination of birds both on-farm and at the processing plant. The administration of Broilact® in the hatchery via a modified vaccination cabinet was shown to be an effective and safe means of treating commercial broilers. In addition, birds pretreated with Broilact® were largely protected against strains of *S. Enteritidis* and *S. Typhimurium* at a challenge dose of 10⁴ cfu/bird (Methner et al., 1997).

PREEMPT (also known as CF3, Pre-Empt), is the latest commercial product, and has recently been approved for use in the poultry industry by the US FDA (Douglas, 1998). The product is a 'fully' defined culture of 29 facultative and obligate anaerobes that is sprayed on newly hatched chicks to establish an environment in the gut of the chicken to effectively prevent the establishment and growth of *Salmonella*. This product was developed by Dr. John DeLoach and his research team over a period of 10 years, initiated by the USDA/ARS Food Animal Protection Research Laboratory at Texas A & M University. Recent reports report the product to provide excellent protection against *Salmonella* colonisation in commercial layers, as demonstrated by the virtual elimination of *Salmonella* in layers in Japan (Glaser, 1998; Stephenson, 1998).

Avian Pac Plus, a commercial product containing *Lactobacillus acidophilus*, *Streptococcus faecium* and *S. Typhimurium*-specific antibodies, was found to significantly reduce colonisation by *S. Typhimurium* in market-aged broilers when administered at the hatchery and at the farm from day 1 to day 3 (Promsopone et al., 1998). However, more work is needed to determine if *S. Typhimurium*-specific antibodies alone have a beneficial effect to reduce *S. Typhimurium* colonisation.

Despite the reports of successful exclusion of enteropathogens, particularly *Salmonella*, by a range of CE products, Schneitz (1998) stated that it is unlikely that any efficacious CE product originating from caecal material could be wholly defined, and that only complex undefined treatment cultures are likely to provide adequate and consistent
protection to chicks against colonisation by salmonellae. The difficulty in producing an efficacious, defined preparation is made worse by the use of current, inadequate isolation techniques or insufficient knowledge of the normal intestinal microflora.

V. ADMINISTRATION

A number of methods have been assessed for their practicality and relationship to efficacy in administration of CE preparations to poultry. The most common method involves supply in drinking water, although chicks may refuse to drink for significant periods, or experience difficulty in locating drinkers in commercial broiler sheds (Schneitz, 1992; Schneitz et al., 1992). Overarching all methods is the desire to expose poultry to the beneficial microflora as early in life as possible. To this end, while delivery may be achieved through administration to freshly hatched chicks via water, spray or droplet application, in ovo inoculation has also been investigated (Schneitz, 1992), ensuring inoculation of the CE microflora, before chickens are exposed to the environment and consequently, potential exposure to enteropathogens. The efficacy of CE decreases significantly when a preparation is administered simultaneously with the challenge pathogen, and is ineffective if the challenge is administered first (Seuna, 1979; Soerjadi et al., 1981b; Hinton et al., 1990). In the commercial context, administration at the hatchery is advocated, but only if the challenge is likely to occur in the broiler shed. If contamination with pathogens occurs through vertical transmission, or at the hatchery, CE is likely to prove ineffective.

VI. AMENDMENTS

Several approaches to enhancing the efficacy of CE preparations have been described. The most common of these is inclusion of carbohydrates, most notably lactose, among others (Corrier et al., 1990, 1997; Bailey et al., 1991; Schoeni and Wong, 1994), in the diet. In the case of lactose, it is likely to be utilised readily by a range of gut microorganisms, most importantly lactic acid bacteria, thereby enhancing the population of these organisms, reducing gut pH, and increasing both production and the proportion of undissociated forms of volatile fatty acids. Other sugars such as mannose may interfere with adherence of pathogens to epithelial tissue (McSweegan et al., 1986). The immune system may also be stimulated, generally or specifically. In the former case, organisms such as Lactobacillus casei enhance IgA secretion (Perdigon et al., 1990), while in the latter, administration of attenuated vaccine strains (Methner et al., 1997), antigens derived from specific pathogens (Methner et al., 1997), or direct administration of anti-pathogen antibodies (Stern et al., 1990; Brandt et al., 1997) may enhance the effects of CE. Although these amendments offer promise in laboratory trials, use in the field is problematic, not only in the technical, but in a practical, sense in that any amendment adds to the cost of use, and may not offer a significant benefit.

VII. FACTORS AFFECTING EFFICACY

The source of cultures may have a profound effect on efficacy of CE. While it might seem obvious that the most protective cultures derive from poultry rather than non-poultry sources, the nature of the source poultry and the conditions under which they are reared profoundly influences their microflora. Several studies have shown that the microflora from SPF birds is far less protective than that derived from conventionally reared poultry (Stavric and D'Aoust, 1993). In addition, breed, diet, growth conditions and geographic location may influence the microflora (Salanitro et al., 1974).
A range of stressors, including extremes of temperature, starvation, thirst (Brownell et al., 1969), transport (Bailey, 1988), and other disease conditions such as coccidiosis (Arakawa et al., 1992), have been found to increase colonisation by and shedding of enteropathogens, particularly Salmonella. Presumably, the stressors impact on the indigenous gut microflora, and are thus likely to influence the efficacy of any CE treatment.

The therapeutic or prophylactic use of antimicrobial substances has been reported to have a positive, little (Smith and Tucker, 1978; Barrow, 1992), or negative (Impey et al., 1982; Bailey et al., 1988) impact on the efficacy of CE. The type of impact depends very much on the substance used, particularly its effect on any enteropathogen present compared to its effect on the indigenous microflora. Barrow (1992) suggested that the use of certain antimicrobials or growth promotants may lead to resistance among indigenous organisms, facilitating manipulation of the native gut microflora.

Lastly, the target pathogen can be considered to influence efficacy. Many studies (Nurmi and Rantala, 1973; Lloyd et al., 1977; Seuna, 1978; Rigby and Pettit, 1980; Soerjadi et al., 1981a; Sthersky et al., 1981; Impey et al., 1982; Stavric et al., 1985, 1987; Impey and Mead, 1988; Hinton et al., 1991b; Stavric et al., 1991a; Bailey, 1993; Blankenship et al., 1993; Nisbet et al., 1994; Corrier et al., 1995; Hume et al., 1996, 1998) have shown that Salmonella is excluded by undefined CE preparations, somewhat irrespective of the source material and its treatment. This is not the case for Campylobacter (James and Kaplan, 1998), which relates to fundamental differences in the ecology of the two pathogens in the avian gastrointestinal tract. While organisms such as Salmonella and pathotypes of E. coli colonise through attachment to the epithelium, Campylobacter tends to exist in a free-swimming state within mucin in the crypts of the lower gastrointestinal tract (Beery et al., 1988). For any given pathogen, including specific serovars of Salmonella, strain differences relating to expression of virulence or colonisation factors may influence the efficacy of CE.

VIII. CONCLUSIONS

Despite the expenditure of substantial time and effort, a highly efficacious CE mixture is yet to be developed, particularly one that proves highly effective in excluding enteropathogens from poultry under commercial production conditions. It appears that current products are best integrated into a holistic pathogen control strategy that also includes stringent biosecurity (exclusion of animate vehicles of pathogen transmission), selection of pathogen-free stock, feed and water, and maintenance of a clean production environment.

ACKNOWLEDGEMENTS

The authors thank the Chicken Meat and Egg Industry programs of RIRDC for support of BLC and research into competitive exclusion.

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