

# Control strategies for *Salmonella* colonisation of poultry: the probiotic perspective

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## Abstract

Zoonotic transmission of *Salmonella enterica* from poultry to man, particularly from chicken meat and egg production, is a major public health issue. *Salmonella* Enteritidis and *Salmonella* Typhimurium infections in poultry are often asymptomatic and therefore difficult to identify without rigorous screening. A number of control strategies are currently in place for the control of *Salmonella* in poultry including vaccination and biosecurity measures. However, additional and supplementary strategies are sought and the application of probiotics is promising. Probiotics have been shown to inhibit a range of *Salmonella enterica* isolates in poultry. These organisms may offer an additional tool in the arsenal of current control strategies to prevent zoonotic *Salmonella* transmission to humans. Currently, there are five key mechanisms by which the inhibition of pathogens is thought to occur, including immunomodulation. The use of probiotics in poultry to modulate the host immune system has been shown to aid the clearance of *Salmonella*. This article will review current understanding of probiotic inhibitory mechanisms, the interactions between the host and *Salmonella* and the practical use of probiotics *in vivo* to reduce/inhibit *Salmonella* in poultry.

**Keywords:** *Salmonella*, probiotics, prebiotics, zoonotic, competitive exclusion, poultry, chickens, immunomodulation

## 1. *Salmonella* and poultry farming: an economic and public health problem

As of 2005, the *Salmonella* genus consists of two species, *S. bongori* and *S. enterica*, of which the later is subdivided into six subspecies including *enterica* (which contain the pathogenic species of warm blooded animals), *salamae*, *arizonae*, *diarizona*, *houtenae* and *indica* (Tindall *et al.* 2005). Within these subspecies, more than 2500 serovars are known, of which fewer than 100 are of epidemiological significance. Colonisation of commercial poultry layer and meat flocks with *Salmonella* is considered endemic within many areas of the world, with *Salmonella* Enteritidis predominating as the most prevalent serovar. Recent studies indicate that between 23.7 and 37% of broilers raised within the European Union (EU) were positive for *Salmonella* (EFSA 2007a, 2007b).

*Salmonella*-infected poultry may present with clinical diarrhoea, general malaise, impotence and increased mortality. Unlike most other *Salmonella* serovars, *Salmonella* Enteritidis in poultry has the ability to disseminate from the gastrointestinal tract into other tissues such as the immune system tissue and reproductive organs (Cox 1995; Deng *et al.* 2008). Chicks infected within a couple of days post-hatch are highly susceptible to colonisation by *Salmonella* Enteritidis and are unable to provide an effective immune response, resulting in persistent infections (Gast and Holt 1998; Holt *et al.* 1999; Sadeyen *et al.* 2004; Beal *et al.* 2005). Chicks can become infected vertically (from adults via the egg to the chick) or horizontally (from the environment, pests or from feed; van de Giessen *et al.* 1994). Infection in the reproductive tissues leads to the incorporation of *Salmonella* Enteritidis into intact eggs due to shedding of the pathogen from the isthmus and magnum glands prior to egg shell formation. Particular virulence factors enable *Salmonella* Enteritidis to persist in poultry and provide a niche for this organism to persist as a potential source for human pathogens. For example, to aid persistence in eggs, *Salmonella* Enteritidis demonstrates

motility due to the presence of curli fimbriae expressed in the exponential phase of growth, which allows the organism to traverse the low-iron-containing egg albumin and access the iron- and nutrient-rich yolk (Cogan *et al.* 2004). The production of lipopolysaccharide (LPS) has also been closely linked to the organism's virulence, particularly its ability to infect a large number of tissue types, such as the spleen. Although *Salmonella* Enteritidis infection of poultry may lead to pathology, it is common for the disease to remain asymptomatic. Silent infection, coupled with the wide tissue distribution of *Salmonella* Enteritidis in the chick, presents particular problems in preventing zoonotic transmission to humans.

Colonisation with *Salmonella* Enteritidis often does not affect poultry weight gain or performance; asymptomatic infection thus can increase the likelihood of zoonotic transmission to humans through the food chain. In both developed and developing countries, *Salmonella* is a leading cause of bacterial food-borne disease (White *et al.* 1997; Cardinale *et al.* 2004). During 2006, there were 160 649 reported cases of human *Salmonella* food poisoning in the EU (EFSA 2007a). The young, old and immunocompromised are particularly vulnerable and infection may, on rare occasions, contribute to mortality. Symptoms of human *Salmonella* Enteritidis infection include diarrhoea, nausea and vomiting, stomach pains and cramps, fever, headache and general malaise. Although loss of poultry performance due to *Salmonella* Enteritidis occurs, the major concern is with public health and control of zoonosis. Thus, the reduction of poultry related infection has implications for both the economy and public health.

Several strategies are employed to ensure that commercial flocks are *Salmonella* free. The most important aspect of *Salmonella* control in commercial flocks is good animal husbandry and high standards of hygiene in bird houses, including vermin control and appropriate disinfection. The increasing problem of antibiotic resistance has led to the withdrawal of antibiotics in animal feed which were often used as growth promoters but which also reduced *Salmonella* colonisation (EU Commission 1998). Other strategies have been employed to control *Salmonella* in poultry including breeding of genetically resistant birds, the use of competitive exclusion (CE) organisms, and vaccination (Babu *et al.* 2003; Piao *et al.* 2007). Currently, two types of *Salmonella* vaccine exist, an attenuated live vaccine and an inactivated vaccine. These vaccines are often administered to both breeder and layer flocks but the effectiveness is dependent upon the targeted serovar, host species and also whether reduction rather than eradication is the objective (for a comprehensive review read Doyle and Erickson 2006). With the need to replace antibiotic supplements with effective alternatives, attention has turned to the development of probiotics which reduce the gastrointestinal carriage of *Salmonella*.

## 2. Subversion of the host by *Salmonella enterica*

Certain *Salmonella enterica* serotypes are host adapted pathogens, such as *Salmonella* Typhi in man, *Salmonella* Cholerae-suis in pigs, *Salmonella* Dublin in cattle and *Salmonella* Gallinarum in poultry, whilst others such as *Salmonella* Typhimurium and *Salmonella* Enteritidis are promiscuous with regard to host species. All, however, employ a variety of mechanisms for host function modulation, whereby cellular processes are hijacked by the pathogen to aid attachment, invasion and survival within the target organism. *Salmonella* species manipulate cell functions for colonisation and survival purposes with a variety of virulence factors and modulator effector proteins. *Salmonella* infection is characterised by the attachment of the bacteria to the intestinal epithelia, tissue invasion and, in the case of *Salmonella* Enteritidis in poultry, dissemination to peripheral tissues such as the spleen, liver and caecal tonsils.

Attachment of *Salmonella* to the epithelium and enhanced dissemination to peripheral tissues is mediated by the presence of fimbriae and flagellae located on the bacterial cell wall (Cox 1995; Baumler *et al.* 1996; Dobb-Fuller *et al.* 1999). *Salmonella* have been shown to preferentially attach to and invade M-cells, although entry through enterocytes also occurs (Jepson and Clark 2001; van Asten *et al.* 2005). M-cells perform the function of antigen sampling of the luminal contents by pinocytosis and are located primarily in Peyer's patches which are most abundant in the intestinal ileum. Once attached to target cells, invasion of the epithelium is aided by the complex process of host manipulation resulting in *Salmonella* uptake by endocytosis. *Salmonella* can subsequently translocate across the epithelium into the basolateral tissue and disseminate to peripheral tissues.

The major components of this molecular highjacking system are predominantly found in two pathogenicity islands: *Salmonella* pathogenicity island 1 (SPI1) and *Salmonella* pathogenicity island 2 (SPI2). SPI1 encodes for more than 20 proteins that construct a molecular injection tube called the 'needle complex'. This needle complex injects an assortment of effector proteins that manipulate host cell functions which are guided to host targets by chaperone proteins (for a comprehensive review read Kimbrough and Miller 2002). The effector proteins result in destabilisation of the cellular cytoskeleton forming classical membrane ruffles (Figure 1) and subsequent uptake by endocytosis.

Once *Salmonella* have been translocated across the epithelium into the basolateral tissue they can be recycled back into the lumen of the gut via epithelial cell replacement from the intestinal crypts. *Salmonella* may also infect CD18, expressing phagocytes by macropinocytosis directly at the epithelial surface or in the basolateral tissue and subsequently disseminate to deep tissue via the reticu-

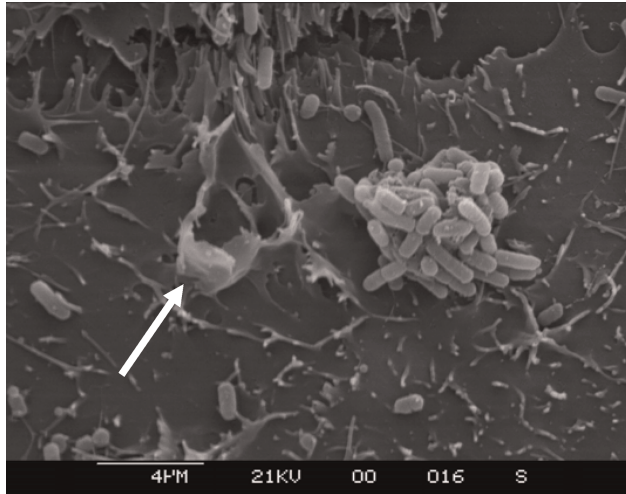


Figure 1. *Salmonella* Enteritidis association to HEp-2 cell line visualised using scanning electron microscopy results. Scanning electron microscopy of *Salmonella* Enteritidis association after 3 h incubation at 37°C to HEp-2 cells. Arrow indicates *Salmonella* Enteritidis-induced membrane ruffling. Figure taken with permission from Carter (2008).

loendothelial system (Vazquez-Torres *et al.* 1999; Worley *et al.* 2006; Figure 2). The pathology of the intestinal tract is thought to occur due to disruption of tight junctions and the recruitment of polymorphonuclear lymphocytes to the

site of infection and the subsequent release of cytotoxic substances such as oxygen free radicals and lysozyme.

### 3. Defining probiotics, prebiotics and synbiotics

In 1965, the term probiotic was first used by Lilly and Stillwell to describe an excreted product from one protozoan that resulted in the promotion of growth of another protozoan (Lilly and Stillwell 1965). Subsequently, the term probiotic was used to describe numerous beneficial biological interactions including the promotion of microbial growth by tissue extracts (Shortt 1999). In 1974, Parker was the first to describe probiotics as beneficial food supplements that promoted the production of a healthy gut flora. Parker's description of probiotics was too general due to the inclusion of antibiotics in his definition (Parker 1974). Fuller suggested the generally accepted definition of probiotics given in 1989 as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller 1989). As the science of probiotics matured, the concept of prebiotics was introduced in 1995 by Gibson and Roberfroid. Prebiotics are non-digestible food ingredients that promote the growth/activity of natural intestinal bacterial species within the gastrointestinal tract (Gibson and Roberfroid 1995). The concept of using prebiotics and probiotics in conjunction as a mixed preparation was termed synbiotics (Gibson and Roberfroid 1995).

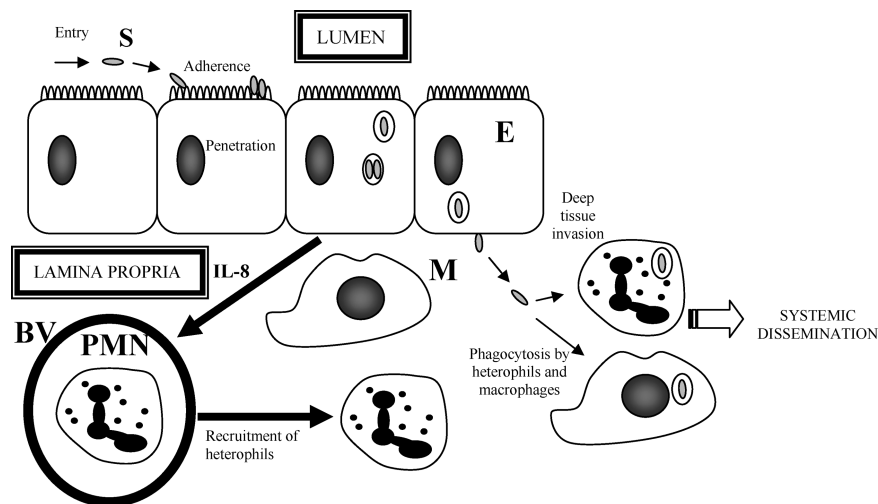


Figure 2. Diagrammatic representations of *Salmonella* invasion and the innate immune response. *Salmonella* (S) adhere to the epithelium (E) of the gastrointestinal tract via the action of adhesion factors including flagellae and fimbriae. Penetration into the epithelium is facilitated by the injection of virulence factors into the target cell mediated by a type three secretion system. Upon infection, the epithelium releases chemokines such as IL-8. Heterophils (PMN) are subsequently recruited from blood vessels (BV) into the lamina propria in response to chemokine signalling. *Salmonella* migrate into the lamina propria and are phagocytosed by heterophils and resident macrophages (M). Phagolysosome formation is inhibited and *Salmonella* are subsequently disseminated systemically by infected heterophils and macrophages. Figure adapted from Dibb-Fuller *et al.* 1999; Santos *et al.* 2003.

**Table 1. Summary of beneficial probiotic effects in poultry that have been experimentally demonstrated *in vivo***

Beneficial effects of probiotic bacteria in poultry	Probiotic strain/product	Reference
Enrichment of host microflora due to increased numbers of lactic acid bacteria (LAB) and decreased numbers of coliforms	<i>Enterococcus faecium</i> NCIMB 10415 LAB preparation <sup>a</sup>	(Samli <i>et al.</i> 2007) (Mountzouris <i>et al.</i> 2007)
Competitive exclusion of pathogens such as <i>Salmonella</i>	Aviguard <sup>®b</sup>	(Nakamura <i>et al.</i> 2002)
Improved poultry weight gain and feed conversion ratios	<i>Enterococcus faecium</i> NCIMB 10415	(Samli <i>et al.</i> 2007)
Increased production of mucin in the small intestine	PrimaLac <sup>®c</sup>	(Smirnov <i>et al.</i> 2005)
Improved total and protective antibody production	Interbac <sup>®d</sup> Aviguard <sup>®</sup>	(Haghighi <i>et al.</i> 2006) (Nakamura <i>et al.</i> 2002)
Reduction in meat cholesterol after culling	<i>Rhodobacter capsulatus</i>	(Salma <i>et al.</i> 2007)
Improve GI tract integrity and architecture, i.e. increased villus length	PrimaLac <sup>®</sup>	(Smirnov <i>et al.</i> 2005)
Improved quality and quantity of egg production	Dried <i>Bacillus subtilis</i>	(Li <i>et al.</i> 2006)

<sup>a</sup>Two *Lactobacillus* strains, one *Bifidobacterium* strain, one *Enterococcus* strain, and one *Pediococcus* strain

<sup>b</sup>Aviguard<sup>®</sup> is an undefined probiotic product

<sup>c</sup>PrimaLac<sup>®</sup> composed of 11 *Lactobacillus* spp.

<sup>d</sup>Interbac<sup>®</sup> consists of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus faecalis*.

#### 4. Design and selection of probiotics

There are over 20 criteria for the selection of a safe and functional probiotic product. These can be grouped into four categories: appropriateness; technological suitability; competitiveness; and performance and functionality (Klaenhammer and Kullen 1999). Performance and functionality is of particular interest due to the lack of understanding of the mechanisms involved. Some claimed beneficial probiotic effects include the interference and exclusion of pathogens, reduction of carcinogenic and mutagenic activity of gut metabolites, improvement of host blood pressure, reduction in incidence and duration of diarrhoea, prevention of vaginitis and maintenance of mucosal integrity (Cremonini *et al.* 2002; Marotta *et al.* 2003; Reid *et al.* 2003; Tanida *et al.* 2005; Falagas *et al.* 2008). Specific probiotic effects in poultry include improved feed conversion ratios and weight gain, increased performance of layer hens and quality of eggs, and enrichment of intestinal microflora (Table 1). Because of the lack of data describing probiotic mechanisms, some of the claims for positive benefits must be interpreted with caution although some beneficial effects have been described and are generally accepted.

Examples of proven benefits include a study involving 64 healthy women where, after oral administration of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 over 2 months, the vaginal flora was improved due to increased presence of lactobacilli and decreased numbers of vaginal coliforms and yeast. A possible mechanism of action is by ascending colonisation of the vagina with the probiotic, which has passed from the rectal area, with consequent reduction of localised pH, although immuno-

modulation could also be possible (Reid *et al.* 2003; Avonts *et al.* 2004). *Bifidobacterium lactis* LKM512 has also been shown to have positive affects when ingested by healthy adults by reducing gut mutagenicity. It appears that yoghurt containing LKM512 increases gut spermidine levels and that this increase results in desmutagenicity (Matsumoto and Benno 2004). Other scientifically validated probiotic effects include improvement of the mucosal barrier integrity by the administration of *Lactobacillus brevis*, reduction of rotavirus-induced infantile gastroenteritis by *Lactobacillus rhamnosus*, and the prevention of antibiotic associated diarrhoea through the use of *Saccharomyces cerevisiae* (boulardii; Surawicz *et al.* 1989; Shornikova *et al.* 1997; Garcia-Lafuente *et al.* 2001).

Another particular probiotic selection criterion of interest is appropriateness; this encompasses aspects of probiotic selection based on the safety of the product. Safety of probiotics is of particular concern as these agents may persist in the environment and may be introduced into the human food chain. The EU has recently introduced new directives in order to regulate the use of probiotics as animal feed additives in accordance with guidelines proposed by the Scientific Committee on Animal Nutrition (SCAN; Wright 2005). Regulation 1831/2003 EU regulates the use of animal feed additives, while Council Directive 87/153/EEC stipulates the assessment guidelines for feed additives. Council Directive 87/153 EEC requires that probiotic feed supplements must fulfil five important criteria:

- Safety has to be assessed in accordance with the test set out in the Directive guidelines
- Strains that produce toxins are not allowed



- Strains that have known virulence factors are not allowed
- Strains that produce antibiotic substances of clinical or veterinary significance are not allowed
- Strains that carry transmissible resistance determinants against antibiotics are not allowed.

## 5. Administration of probiotic products to poultry

For poultry, a major concern is the exclusion of pathogens and the early work of Nurmi and Rantala (1973) suggested that a mature flora out-competes pathogens. This is an accepted dogma and so probiotics are often used as CE agents. Commercial probiotic products for poultry that are available today can be separated into two categories, products that are defined and those that are undefined. In defined products, the microorganisms that compose the product have been identified. In contrast, undefined CE products, such as Aviguard and BROILACT<sup>®</sup>, are products where the bacterial cultures are either partially or completely undefined (Carita 1992; Nakamura *et al.* 2002). Particular problems arise when trying to evaluate the effectiveness of undefined products as the active organisms and often mechanisms of action are unknown.

The dose and administration of commercial probiotics is an important factor in their effective use (Votava *et al.* 1987; Carita 1992). The recommended dose of each microorganism or mixture of organisms varies between products due to the 'strength of probiotic action' and industrial production limitations. Recommended doses usually fall within the range of  $1 \times 10^8$  cfu of bacteria per kg of feed and  $1 \times 10^{10}$  cfu of bacteria per kg of feed. Examples include Toyocerin<sup>®</sup> which has approximately  $1 \times 10^8$  cfu of *Bacillus cereus* var. *toyoi* per kg of feed for use in poultry whereas AICare<sup>™</sup>, a probiotic product for swine, is administered at  $1 \times 10^9$  to  $1 \times 10^{10}$  cfu of *B. cereus* var. *toyoi* per kg of feed (Ricca *et al.* 2004). As discussed earlier, Nurmi and Rantala developed the first CE product to be administered to chickens (Nurmi and Rantala 1973). This was administered by oral gavage directly into the stomach of the chicks. This method was particularly crude and extremely impractical for broiler farmers who would have to administer the product to thousands of birds. Over the years, other methods have been developed to administer probiotic supplements into animal feed which include pellets, capsules, paste, powder or granules (Fuller 1989). The form in which the probiotic is administered depends on the use of the product (e.g. as a prophylactic) and also on the animals being dosed (Fuller 1989). The preferred method of dosing chickens with probiotic products has been via drinking water, although problems have arisen due to the refusal of chicks to drink the water containing unpalatable probiotic products (Carita 1992). More recently, the use of droplet spray application

systems have been developed that can improve the administration of probiotics to chicks. These systems range from the use of simple hand-held garden sprayers to modified bronchitis vaccination apparatus (Carita 1992).

## 6. Mechanisms of probiotic competitive exclusion

The CE of pathogenic bacteria by a probiotic product is thought to occur through the action of one or more of five key mechanisms: competition for nutrients, immunomodulation of the host, production of bacteriocins, production of inhibitory metabolites such as volatile fatty acids, and competition for binding receptors (Klaenhammer and Kullen 1999; Sanz *et al.* 2007). These inhibitory effects can be instigated via direct or indirect mechanisms. Direct inhibition occurs when the probiotic inhibits the pathogen by production of inhibitory substances and direct competition of receptor sites. Indirect exclusion can occur through improvement of host responses to the pathogen and subsequent host-instigated clearance or enrichment of intestinal bacteria that subsequently result in direct pathogen inhibition. Probably the most obvious mechanism for CE is competition for substrates and nutrients, although the fluidity and complexity of nutrient and substrate utilisation in the gastrointestinal tract makes defining the specifics of this mechanism difficult. The second mechanism is immunomodulation of the gut mucosa. Several organisms including *Bacillus* spp. and *Bifidobacterium* spp. have been shown to modulate the immune system, although the organisms that have been studied most extensively are members of the *Lactobacillus* genus (Medici *et al.* 2004; Gill and Prasad 2008; Zhang *et al.* 2008; Schierack *et al.* 2009). Another mechanism of action by probiotic microorganisms is the production of bacteriocins, of which several classes exist. Bacteriocins have been shown to be produced by several probiotic species, most notably *Lactobacillus* and *Enterococcus* species (Ouweland *et al.* 1999; Avonts *et al.* 2004; Franz *et al.* 2007). Lactic acid bacteria (LAB) have been shown to exclude Gram positive bacteria including *Listeria* species, possibly due to environmental pressures on these bacteria to out-compete closely related Gram positive organisms in the gut (Corr *et al.* 2007; Lemos Miguel *et al.* 2008). Inhibition of pathogenic bacteria by the production of volatile fatty acids or reduction of intestinal pH by the production of lactic acid has also been proposed as an exclusion mechanism (Klaenhammer and Kullen 1999). Several authors have demonstrated that reduced growth of *Escherichia coli* and *Salmonella* in cell cultures and in the chick gut is directly related to increased lactic acid production (Fuller 1977; Garriga *et al.* 1998; Makras *et al.* 2006). The final mechanism for CE is competition with both commensal and pathogenic bacteria for receptor sites. Adherence to the mucosal epithelium is considered to be an important characteristic

of a probiotic (Klaenhammer and Kullen 1999; Wagner *et al.* 2002). Probiotic LAB have been shown to antagonise *E. coli* and *Salmonella* binding to eukaryotic cell lines although the demonstration of this inhibitory mechanism is harder to elucidate unambiguously *in vivo* (Lee and Puong 2002; Mukai *et al.* 2002).

## 7. Other food supplements as intervention agents to control *Salmonella* in poultry

In recent years, the use of prebiotics in the prevention of poultry infection has become a popular area of research. Disaccharides, oligosaccharides and polysaccharides are thought to be good prebiotic candidates for use in poultry as many bind to host cell receptor sites, notably mannans (Patterson and Burkholder 2003; Chung and Day 2004; Donalson *et al.* 2008). Prebiotics are non-digestible (by the host) but are digested by a minority of the gastrointestinal microbial population with, for example, galactooligosaccharides being digested by bifidobacterial species. Prebiotics may bind to the host gut epithelium, blocking receptor sites or binding target pathogens directly, or more commonly may be utilised by the intestinal flora, resulting in the production of various metabolites, such as volatiles and bacteriocins. Importantly, the numbers of desired beneficial bacteria are increased. Mannose is a monosaccharide often used as a prebiotic due to Type 1 (F1) fimbriae of *Salmonella* binding to mannose residues on the epithelial glycoproteins. It should be noted that mannose is not considered a prebiotic as it can be metabolised by the host. However, free mannose and prebiotic preparations of yeast mannanoligosaccharide are thought to interfere with *Salmonella* binding to host cells (Allen *et al.* 1997; Fernandez *et al.* 2002). Fructooligosaccharides have been shown to promote the growth of *Enterococcus faecium*, *Lactobacillus lactis* and *Pediococcus* species *in vitro* (Oyarzabal and Conner 1995). Modification of metabolic activity of the intestinal flora has also been proposed due to the fermentation of indigestible saccharides into volatile fatty acids, lactate, carbon dioxide, methane and hydrogen (van Immerseel *et al.* 2002). Recent reports by Tzortzis *et al.* (2005) elegantly showed the activity of these two mechanisms by galactooligosaccharide mixtures *in vitro* and *in vivo*. The oligosaccharide mixture inhibited *Salmonella* binding to HT29 cells, presumably by the saturation of *Salmonella* cell binding receptors, and also promoted the growth of *Bifidobacterium* species in a continuous culture model and also *in vitro* (Tzortzis *et al.* 2005). Furthermore, recent reports have shown the use of isomaltooligosaccharides in poultry to promote *Bifidobacterium* growth *ex vivo* and have demonstrated the ability of this oligosaccharide to inhibit *Salmonella* growth *in vitro* (Chung and Day 2004). Recent research into the use of prebiotics to enhance clearance of *Salmonella* in poultry

opens new possibilities for the effective clearance of these zoonotic pathogens. The use of prebiotics and probiotics offers another tool for the control of *Salmonella* Enteritidis in poultry and may one day become an integrated part of pathogen control in commercial poultry production.

## 8. Competitive exclusion of *Salmonella* using probiotics in poultry

Although small number of antibiotics have been used to improve weight gain in poultry and act as bacterial prophylactics, this has led to rising antibiotic resistance of bacteria in poultry. With the withdrawal of antibiotics from animal feed in 2006, scientists are looking at probiotics as a serious alternative. Several pathogens including *Eimeria* spp. and *Campylobacter jejuni* have been inhibited by probiotic bacteria in poultry (Table 2). Particular success has been achieved with undefined avian caecal cultures in the CE of *Salmonella* species from poultry, which has resulted in the production of several commercial products (Table 3). Monocultures of probiotics have historically been thought to be less effective at excluding *Salmonella* Enteritidis from poultry but several studies in recent years have shown that these probiotic preparations show promise for use as effective CE products (Table 3).

The use of intestinal content preparations from adult chickens to prevent infection with *Salmonella* was first described by Nurmi and Rantala in 1973, who demonstrated a marked decrease in *Salmonella* infection of chicks. Caecal bacterial culture application to newly hatched chicks was subsequently shown to prevent infection by *Salmonella* Enteritidis in numerous chick models. This probiotic product could be administered in several ways such as in water, by direct spray or inclusion in feed slurry (Corrier *et al.* 1994). With the success of undefined CE preparations in the 1970s and 1980s, the first commercial avian caecal products were marketed in the 1990s. BROILACT<sup>®</sup>, a commercial undefined caecal CE preparation, has been shown to protect broiler chickens from oral challenge by *Salmonella* Enteritidis PT4 with significant reductions in *Salmonella* Enteritidis numbers in caecal contents (Nuotio *et al.* 1992; Schneitz 1992). Aviguard, another commercial undefined product, was designed to be used as a spray treatment or administered in drinking water. Aviguard, like BROILACT<sup>®</sup>, was designed to exclude *Salmonella* Enteritidis and *Salmonella* Typhimurium from chickens. Aviguard was also successful at reducing the persistence of *Salmonella* species with the effect of reducing tissue colonisation and death in the chicks (Nakamura *et al.* 2002). The treatment of chicks with enrofloxacin for *Salmonella* Enteritidis infection was also greatly improved when a competitive exclusion culture was administered after completion of a course of the antibiotic (Seo *et al.* 2000).

**Table 2. Pathogens inhibited by probiotic bacteria in poultry *in vivo***

Pathogen inhibited	Probiotic strain/product	Mechanism of action	Reference
<i>Salmonella</i> Enteritidis	<i>Lactobacillus reuteri</i> R-17485 and <i>L. johnsonii</i> R-17504	Suggested lactic acid production involved	(Van Coillie <i>et al.</i> 2007)
<i>Salmonella</i> Typhimurium	Milk product fermented with <i>L. helveticus</i> R389 Culture caecal contents	Improved inflammatory response Butyrate production	(Vinderolla <i>et al.</i> 2007a, 2007b) (Waters <i>et al.</i> 2005)
<i>Salmonella</i> Pullinorum/ <i>Gallinarum</i>	<i>E. faecium</i> J96 (protective effect only)	Suggested bacteriocin interference	(Audisio <i>et al.</i> 1999; Carina <i>et al.</i> 2000)
<i>Escherichia. coli</i>	<i>L. salivarius</i> 59	Reduction of crop pH	(Fuller 1977)
<i>Campylobacter jejuni</i>	PrimaLac <sup>®a</sup>	Not determined	(Willis <i>et al.</i> 2008)
<i>Clostridium perfringens</i>	Aviguard <sup>®b</sup> / <i>L. johnsonii</i> FI9785	Not determined	(Hofacre <i>et al.</i> 1998; La Ragione <i>et al.</i> 2003)
<i>Eimeria</i> spp.	MitoMax <sup>®c</sup> PrimaLac <sup>®</sup>	Improved humoral response Improved cell mediated immune response	(Lee <i>et al.</i> 2007) (Dalloul <i>et al.</i> 2003)
Newcastle disease virus	PrimaLac <sup>®</sup>	Improved humoral response to vaccine	(Talebi <i>et al.</i> 2008)

<sup>a</sup>PrimaLac<sup>®</sup> composed of 11 *Lactobacillus* spp.

<sup>b</sup>Aviguard<sup>®</sup> is an undefined probiotic product

<sup>c</sup>MitoMax<sup>®</sup> is composed of *Pediococcus acidilactici* and *Saccharomyces boulardii*.

Generally, the applications of multi-species probiotic cultures are significantly more effective at reducing *Salmonella* infection (Timmerman *et al.* 2004). However, several recent reports show potential to overturn this common theory. Carina Audisio *et al.* (2000) demonstrated that pre-treatment of broilers with *E. faecium* J96 reduced mortality caused by *Salmonella* Pullorum from 50 to 25% (Carina Audisio *et al.* 2000). A significant 1 log (10 fold) reduction in *Salmonella* Dusseldorf isolated from the caeca of Japanese quails was also observed 168 h post inoculation with *E. faecium* J96 (Laukova *et al.* 2003). Edens *et al.* (1997) demonstrated that pure cultures of

*Lactobacillus reuteri* decreased *Salmonella* and *E. coli* colonisation in chicks and turkey poults (Edens *et al.* 1997). Studies conducted by La Ragione and Woodward (2003) described the reduction of colonisation and persistence of *Salmonella* Enteritidis in a 1 day chick model after pre-dosing with *Bacillus subtilis* PY79. In the pre-dosed birds, 15% showed no shedding of *Salmonella* Enteritidis, with the remaining 85% shedding low numbers of *Salmonella*. Additionally, *B. subtilis* appeared to reduce infection by *Clostridium perfringens* (the aetiological agent of necrotic enteritis in poultry) over extended periods of time, suggesting immunomodulation or possibly

**Table 3. Commercial, multi- and single-component probiotic products that have proven efficacy for inhibition of *Salmonella* Enteritidis or *Salmonella* Typhimurium *in vivo***

Probiotic strain/product	Composition (Defined/undefined)	Reference
<i>Lactobacillus salivarius</i> CTC2197	Defined	(Pascula <i>et al.</i> 1999)
<i>Bacillus subtilis</i> PY79 <sup>a</sup>	Defined	(La Ragione <i>et al.</i> 2003)
Milk product fermented with <i>L. helveticus</i> R389	Defined	(Vinderola <i>et al.</i> 2007a, 2007b)
<i>L. reuteri</i> R-17485 and <i>L. johnsonii</i> R-17504	Defined	(Van Coillie <i>et al.</i> 2007)
<i>L. salivarius</i> 59 and <i>E. faecium</i> PXN-33	Defined	(Carter 2008)
<i>Enterococcus faecalis</i> and <i>Pediococcus pentosaceus</i>	Defined	(Waters <i>et al.</i> 2005)
FM-B11 <sup>b</sup>	Defined	(Higgins <i>et al.</i> 2007, 2008)
Aviguard <sup>®</sup>	Undefined	(Nakamura <i>et al.</i> 2002; Ferreira <i>et al.</i> 2003)
BROILACT <sup>®</sup>	Undefined	(Nuotio <i>et al.</i> 1992; Schneitz 1992)
Mucosal Starter Culture <sup>®</sup>	Undefined	(Ferreira <i>et al.</i> 2003)
Cultured caecal contents (Nurmi-type culture)	Undefined	(Nurmi <i>et al.</i> 1973; Waters <i>et al.</i> 2005)

<sup>a</sup>Study conducted in a 1 day chick model

<sup>b</sup>FM-B11 contains 11 different *Lactobacillus* spp.

spore germination and resulting in delayed exclusion effects (La Ragione and Woodward 2003). La Ragione *et al.* (2004) also demonstrated that, after a single oral dose of *Lactobacillus johnsonii* F19785, the colonisation and persistence of *Clostridium perfringens* in 1 day old chicks was suppressed.

Several recent reports have shown the use of lactobacilli to inhibit *Salmonella* Enteritidis in poultry. Vicente *et al.* (2008) and Higgins *et al.* (2008) both reported the use of commercial *Lactobacillus* species probiotic preparation FM-B11 to inhibit *Salmonella* Enteritidis *in vivo* (Higgins *et al.* 2007, 2008; Vicente *et al.* 2008). These studies used day-old commercial broilers infected with *Salmonella* on day 1 and treated with FM-B11 on day 2. Reduction in the recovery of *Salmonella* from the chicks was reduced but not eliminated. Van Coillie *et al.* (2007) also reported similar observations with *L. reuteri* R-17485 and R-17753 in a specific pathogen free (SPF) 6 day old chick model, although in this study the birds were treated with the probiotics prophylactically. Caution should be taken in the extrapolation of the results from these studies for use in a commercial environment. First, they were conducted in young birds over short periods of time. The developmental maturity in terms of immune competence of the birds plays a role in susceptibility, and the transmission of *Salmonella* among flocks is far from uniform. Additionally, the cyclic nature of infection from environmental sources may, and often does, ensure persistent colonisation of birds at various stages. Because of host development and environmental cycling, studies that simulate these conditions, at least in part, are required for the development of effective products for use in the commercial poultry industry.

Studies have been conducted over longer periods of time which have more closely modelled probiotic inhibition of *Salmonella* Enteritidis in poultry. A model used by Pascual *et al.* (1999) showed *L. salivarius* CTC2197 cleared the caeca of *Salmonella* Enteritidis C-114 by day 21 post-infection. It should be noted that Pascual *et al.* used a non-invasive strain of *Salmonella* Enteritidis but the experimental design included a period of chick development which reflected maturation of the host immune system. Administration of *B. cereus* and *Saccharomyces* species to commercial broilers during a 47 day period that were subsequently challenged at age 12 days with *Salmonella* Enteritidis showed improved weight and feed conversion as compared to the control group (Gil de los Santos *et al.* 2005). Although this model was specifically designed to evaluate weight gain rather than reduction in *Salmonella* carriage, it provides robust data of the efficacy of probiotic bacteria to improve host morbidity during *Salmonella* colonisation. It should be borne in mind that gastrointestinal clearance is likely to be mitigated by *Salmonella* Enteritidis, an invasive serotype, reseeding the gut from dee-

per tissues. Hence, the experiments described by Pascual *et al.* only reflect immediate effects at the gut level.

## 9. Probiotic immunomodulation

The majority of research into probiotic immunomodulation has focused on the anti-inflammatory effect of these organisms for attenuation of diseases such as irritable-bowl syndrome (Pathmakanthan *et al.* 2004; Rioux and Fedorak 2006). In contrast to these diseases, one possible target for probiotic immunomodulation for host clearance of *Salmonella* is improvement of the pro-inflammatory immune response. The immediate innate immune response, termed the acute phase response, and the cell mediated acquired response is comprised of cellular components which are controlled via cytokine and chemokine signals. It should also be noted that induction of Th-2 responses result in increased antibody dependent immunity and that the induction of this response may improve long-term protection against *Salmonella* colonisation (Haghighi *et al.* 2005).

During the early stage of *Salmonella* colonisation of poultry, the innate immune response mobilises in order to control infection. Several acute phase response cytokines have been implicated in *Salmonella* clearance including tumour necrosis factor (TNF)- $\alpha$ , IL-6 and IL-1 $\beta$ . Several reports have shown that these cytokines are expressed upon *Salmonella* contact. Withanage *et al.* (2005) has shown that *Salmonella* clearance in SPF Rhode Island red chicks is dependent upon the expression of inflammatory mediators IL-6 and MIP. Kogut *et al.* (2005) showed that the priming of heterophils by recombinant interferon (INF)- $\gamma$  resulted in increased expression of several pro-inflammatory cytokines including IL-1 $\beta$  and IL-6 in response to *Salmonella* challenge (Kogut *et al.* 2005). It has also been shown that depletion of TNF- $\alpha$  with antibodies reduces the effectiveness of vaccination in mice (Mastroeni *et al.* 1992). These studies indicate that the expression of inflammatory cytokines is required to eliminate *Salmonella* from the host.

The acquired immune response also plays an important role in *Salmonella* clearance. The carrier state of *Salmonella* Enteritidis in the caecal tonsils of young and mature 6 week old birds was dependent upon the ability to express INF- $\gamma$ , a potent Th-1 cytokine. The bird line 6<sub>1</sub> had a higher bacterial load of *Salmonella* Enteritidis in the caeca and also lower expression of INF- $\gamma$  in the same tissue as compared to bird line 15I (Sadeyen *et al.* 2004). Higher numbers of *Salmonella* Typhimurium in peripheral tissues have been attributed to age-related INF- $\gamma$  expression; pups showed significantly lower expression of INF- $\gamma$  and high *Salmonella* infection when compared with adult mice (Rhee *et al.* 2005). The importance of INF- $\gamma$  expression was also shown by Withanage *et al.* (2005), where clearance of *Salmonella* in Rhode Island chickens was dependent upon the expres-



sion of the cell mediated immune response cytokine INF- $\gamma$  and IgG, IgM and IgA (Withanage *et al.* 2005). From the reports earlier, it is clear that induction of the cell mediated response for *Salmonella* clearance is dependent upon INF- $\gamma$  expression. Thus, the induction of improved acute phase response and cell mediated immunity response by probiotic bacteria in chickens may offer a mechanism for the control of *Salmonella* Enteritidis in commercial poultry production.

Several species of LAB have been shown to induce both acute phase responses and cell mediated responses. The resultant immunomodulation mediated by these products may aid the clearance of intracellular pathogens such as *Salmonella* Enteritidis. Furthermore, previous studies showed the induction of IL-6 and TNF- $\alpha$  production in *in vitro* macrophage assays by the LAB *S. thermophilus* strain 133 (Marin *et al.* 1998). Previous reports by Maassen *et al.* (2000) have shown that administration of *L. reuteri* and *L. brevis* increased TNF- $\alpha$  producing cells to Chikungunya virus in mice (Maassen *et al.* 2000). It was suggested that this increase could lead to a Th-1 biased immune response resulting in preferential expression of IgG2a (Maassen *et al.*, 2000). Mohamadzadeh *et al.* (2005) also demonstrated the ability of *L. gasseri*, *L. johnsonii* and *L. reuteri* to induce the production of pro-inflammatory cytokines IL-12 and IL-18, moving macrophages responses towards a Th-1 response (Mohamadzadeh *et al.* 2005). They suggested that the production of pro-inflammatory cytokine could promote a 'robust' inflammatory response directed towards pathogens.

Recent studies have suggested that probiotic bacteria can stimulate cells of the immune system such as T-cells and macrophages and improve clearance of *Salmonella* in poultry (Noujaim *et al.* 2008). The induction of host acute pro-inflammatory and T-cell responses to *Salmonella* infection by probiotic bacteria has been shown to prevent *Salmonella* Typhimurium colonisation of mice. It has been shown that the administration of milk fermented with *L. helveticus* R389 to mice prevented colonisation by *Salmonella* Typhimurium (Vinderola *et al.* 2007a). Subsequent studies by the same group demonstrated that the administration of the milk fermentation product to mice increased IL-2 and TNF- $\alpha$  expression in the small intestine as observed by histological examination (Vinderola *et al.* 2007b). IL-2 causes the expansion of T-cell populations which initiates the development of the acquired immune responses. The studies earlier suggest that the induction of pro-inflammatory cytokines and cytokines important in T-cell population expansion are important in *Salmonella* Typhimurium clearance.

## 10. Conclusions

Prevention of zoonotic *Salmonella* infection poses significant problems for the poultry industry due to its preva-

lence in commercial flocks. *Salmonella* is well adapted for survival within the poultry gastrointestinal and reproductive tissues and has developed complex molecular systems to manipulate host cell functions to disseminate and persist in peripheral tissues. The need for alternative control strategies, due to asymptomatic infection and the ban on antibiotic growth supplements, has renewed interest in pre- and probiotic feed supplements as control strategies of *Salmonella* in birds.

Improvement of probiotic administration, dosing and a greater understanding of the mechanisms of probiotic CE are paramount for improvement of probiotic inhibition of *Salmonella* in poultry. The administration of probiotic bacteria in the commercial sector should be considered and thus studies designed to reflect the prophylactic use of organisms as CE products are required urgently. The practical application of commercially defined cultures should be a pragmatic approach regarding the ability of probiotics to inhibit the colonisation and persistence of *Salmonella* in poultry. Probiotic feed supplements are an integral part of a multi-factorial control strategy that includes stringent bio-security, regular *Salmonella* screening of layer and broiler flocks, good husbandry and vaccination programmes.

One area where research has been particularly fruitful for the understanding of probiotics mechanisms is the modulation of host immune responses to *Salmonella*. Targets for probiotic immunomodulation of poultry for the exclusion of *Salmonella* include the acute phase response and cell mediated immune response. Improved inflammatory responses aid clearance of *Salmonella* from infected poultry. Several studies have demonstrated that the induction of acute phase response and cell mediated response cytokines such as IL-1 $\beta$ , TNF $\alpha$ , IL-6, IFN $\gamma$  and IL-2 is required for *Salmonella* clearance. These cytokines drive the immune system to induce effector cell responses that target the pathogen, leading to subsequent clearance. The use of probiotics to manipulate these systems has been successful in the attenuation of *Salmonella* colonisation of poultry. It might be argued that some probiotics generate a general immunomodulatory effect that may mitigate against a broad range of pathogens. This is probably due to the indirect effects on host responses. However, for direct effects, such as production of inhibitory substances or blocking receptors, it is possible that there is a need to select specific probiotics for specific pathogens. It seems unlikely that direct effects are generic and effective against a wide range of pathogens. These two hypotheses deserve further consideration. As scientific understanding of probiotic mechanisms improve, particularly in areas such as immunomodulation of the host, the ability to select more effective prebiotic and probiotic supplements that prevent *Salmonella* colonisation of poultry will dramatically improve.

## 11. References

- Allen, V.M., Fernandez, F. and Hinton, M.H. 1997. Evaluation of the influence of supplementing the diet with mannose or palm kernel meal on *Salmonella* colonisation in poultry. *British Poultry Science* **38**(5): 485-488.
- Audisio, M.C., Oliver, G. and Apella, M.C. 1999. Antagonistic effect of *Enterococcus faecium* J96 against human and poultry pathogenic *Salmonella* spp. *Journal of Food Protection* **62**(7): 751-755.
- Avonts, L., Van Uytven, E. and De Vuyst, L. 2004. Cell growth and bacteriocin production of probiotic *Lactobacillus* strains in different media. *International Dairy Journal* **14**: 947-955.
- Babu, U., Scott, M., Myers, M.J. *et al.* 2003. Effects of live attenuated and killed *Salmonella* vaccine on T-lymphocyte mediated immunity in laying hens. *Veterinary Immunology and Immunopathology* **91**(1): 39-44.
- Baumler, A.J., Tsolis, R.M. and Heffron, F. 1996. The Ipf fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. *Proceedings of the National Academy of Science USA* **93**(1): 279-283.
- Beal, R.K., Powers, C., Wigley, P., Barrow, P.A., Kaiser, P. and Smith, A.L. 2005. A strong antigen-specific T-cell response is associated with age and genetically dependent resistance to avian enteric salmonellosis. *Infection and Immunity* **73**(11): 7509-7516.
- Cardinale, E., Tall, F., Gueye, E.F., Cisse, M. and Salvat, G. 2004. Risk factors for *Salmonella enterica* subsp. *enterica* infection in senegalese broiler-chicken flocks. *Preventive Veterinary Medicine* **63**: 151-161.
- Carina Audisio, M., Oliver, G. and Apella, M.C. 2000. Protective effect of *Enterococcus faecium* J96, a potential probiotic strain, on chicks infected with *Salmonella Pullorum*. *Journal of Food Protection* **63**(10): 1333-1337.
- Carita, S. 1992. Research Note: automated droplet application of competitive exclusion preparation. *Poultry Science* **71**: 2125-2128.
- Carter, A.J. 2008. *An evaluation of the efficacy and safety of probiotic lactic acid bacteria*. PhD thesis, University of Surrey, UK.
- Chung, C.H. and Day, D.F. 2004. Efficacy of *Leuconostoc mesenteroides* (ATCC 13146) isomaltooligosaccharides as a poultry prebiotic. *Poultry Science* **83**(8): 1302-1306.
- Cogan, T.A., Jorgensen, F., Lappin-Scott, H.M., Benson, C.E., Woodward, M.J. and Humphrey, T.J. 2004. Flagella and curli fimbriae are important for the growth of *Salmonella enterica* serovars in hen eggs. *Microbiology* **150**: 1063-1071.
- Corr, S.C., Li, Y., Riedel, C.U., O'Toole, P.W., Hill, C. and Gahan, C.G. 2007. Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proceedings of the National Academy of Science USA* **104**(18): 7617-7621.
- Corrier, D. E., Nisbet, D.J., Hollister, A.G. *et al.* 1994. Resistance against *Salmonella enteritidis* cecal colonisation in Leghorn chicks by vent lip application of cecal bacterial culture. *Poultry Science* **73**: 648-652.
- Cox, J.M. 1995. *Salmonella enteritidis*: virulence factors and invasive infection in poultry. *Trends in Food Science and Technology* **6**: 407-410.
- Cremonini, F., Di Caro, S., Santarelli, L. *et al.* 2002. Probiotics in antibiotic-associated diarrhoea. *Digestive and Liver Disease* **34**: (Suppl 2): S78-S80.
- Dalloul, R.A., Lillehoj, H.S., Shellem, T.A. and Doerr, J.A. 2003. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. *Poultry Science* **82**(1): 62-66.
- Deng, S.X., Cheng, A.C., Wang, M.S. *et al.* 2008. A study of the distribution patterns and levels of *Salmonella enteritidis* in the immune organs of ducklings after oral challenge by serovar-specific real-time PCR. *Avian Disease* **52**(3): 507-512.
- Dibb-Fuller, M.P., Allen-Vercoe, E., Thorns, C.J. and Woodward, M.J. 1999. Fimbriae- and flagella-mediated association with the invasion of cultured epithelial cells of *Salmonella enteritidis*. *Microbiology* **145**(5): 1023-31.
- Donalson, L.M., McReynolds, J.L., Kim, W.K. *et al.* 2008. The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract fermentation, *Salmonella enteritidis* infection, and intestinal shedding in laying hens. *Poultry Science* **87**(7): 1253-1262.
- Doyle, M.P. and Erickson, M.C. 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. *Poultry Science* **85**(6): 960-973.
- Edens, F.W., Parkhurst, C.R., Casas, I. and Dobrogosz, W.J. 1997. Principles of *ex ovo* competitive exclusion and *in ovo* administration of *Lactobacillus reuteri*. *Poultry Science* **76**: 179-196.
- EFSA. 2007a. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. *The EFSA Journal* **130**: 24-240.
- EFSA. 2007b. Report of the task force on zoonoses data collection on the analysis of the baseline survey on the prevalence of *Salmonella* in broiler flocks of *Gallus gallus*, Part A. *The EFSA Journal* **98**: 1-85.
- European Commission. 1998. Commission regulation of amending council directive 70/524/DEC concerning additives in feedingstuffs as regards withdrawal of authorization of certain antibiotics. No VI/7767/98. Brussels, Belgium.
- Falagas, M.E., Rafailidis, P.I. and Makris, G.C. 2008. Bacterial interference for the prevention and treatment of infections. *International Journal of Antimicrobial Agents* **31**(6): 518-522.
- Fernandez, F., Hinton, M. and Van Gils, B. 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella enteritidis* colonization. *Avian Pathology* **31**(1): 49-58.
- Ferreira, A.J., Ferreira, C.S., Knobl, T. *et al.* 2003. Comparison of three commercial competitive-exclusion products for controlling *Salmonella* colonization of broilers in Brazil. *Journal of Food Protection* **66**(3): 490-492.
- Franz, C.M., van Belkum, M.J., Holzapfel, W.H., Abriouel, H. and Galvez, A. 2007. Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. *FEMS Microbiology Reviews* **31**(3): 293-310.
- Fuller, R. 1977. The importance of *Lactobacilli* in maintaining normal microbial balance in the crop. *British Poultry Science* **18**: 85-94.
- Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology* **66**(5): 365-378.
- Garcia-Lafuente, A., Antolin, M., Guarner, F., Crespo, E. and Malagelada, J.-R. 2001. Modulation of colonic barrier function by the composition of the commensal flora in the rat. *Gut* **48**(4): 503-507.
- Garriga, M., Pascual, M., Monfort, J.M. and Hugas, M. 1998. Selection of lactobacilli for chicken probiotic adjuncts. *Journal of Applied Microbiology* **84**(1): 125-132.
- Gast, R.K. and Holt, P. 1998. Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. *Poultry Science* **77**: 1759-1762.
- Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* **125**(6): 1401-1412.

- Gil de los Santos, J.R., Storch, O.B. and Gil-Turnes, C. 2005. *Bacillus cereus* var. *toyoi* and *Saccharomyces boulardii* increased feed efficiency in broilers infected with *Salmonella* Enteritidis. *British Poultry Science* **46**(4): 494-497.
- Gill, H. and Prasad, J. 2008. Probiotics, immunomodulation, and health benefits. *Advances in Experimental Medicine and Biology* **606**: 423-454.
- Haghighi, H.R., Gong, J., Gyles, C.L. *et al.* 2005. Modulation of antibody-mediated immune response by probiotics in chickens. *Clinical and Diagnostic Laboratory Immunology* **12**(12): 1387-1392.
- Haghighi, H.R., Gong, J., Gyles, C.L. *et al.* 2006. Probiotics stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology* **13**(9): 975-980.
- Higgins, J.P., Higgins, S.E., Vicente, J.L., Wolfenden, A.D., Tellez, G. and Hargis, B.M. 2007. Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. *Poultry Science* **86**(8): 1662-1666.
- Higgins, S.E., Higgins, J.P., Wolfenden, A.D. *et al.* 2008. Evaluation of a *Lactobacillus*-Based Probiotic Culture for the Reduction of *Salmonella* Enteritidis in Neonatal Broiler Chicks. *Poultry Science* **87**(1): 27-31.
- Hofacre, C.L., Froyman, R., Gautrias, B., George, B., Goodwin, M.A. and Brown, J. 1998. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens*-associated necrotizing enteritis in broiler chickens. *Avian Disease* **42**(3): 579-584.
- Holt, P., Gast, R.K., Porter, J. and Stone, H.D. 1999. Hyporesponsiveness of the systemic and mucosal humoral immune system in chickens infected with *Salmonella enterica* serovar enteritidis at one day of age. *Poultry Science* **78**: 1510-1517.
- Jepson, M.A. and Clark, A. 2001. The role of M cells in *Salmonella* infection. *Microbes and Infection* **3**: 1183-1190.
- Kimbrough, T.G. and Miller, S.I. 2002. Assembly of the type III secretion needle complex of *Salmonella typhimurium*. *Microbes and Infection* **4**(1): 75-82.
- Klaenhammer, T. R. and Kullen, M.J. 1999. Selection and design of probiotics. *International Journal of Food Microbiology* **50**(1-2): 45-57.
- Kogut, M.H., Rothwell, L. and Kaiser, P. 2005. IFN- $\gamma$  priming of chicken heterophils upregulates the expression of proinflammatory and Th1 cytokine mRNA following receptor-mediated phagocytosis of *Salmonella enterica* serovar Enteritidis. *Journal of Interferon and Cytokine Research* **25**(2): 73-81.
- La Ragione, R.M., Narbad, A., Gasson, M.J. and Woodward, M.J. 2004. *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. *Letters in Applied Microbiology* **38**(3): 197-205.
- La Ragione, R.M. and Woodward, M.J. 2003. Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype enteritidis and *Clostridium perfringens* in young chickens. *Veterinary Microbiology* **94**(3): 245-56.
- Laukova, A., Guba, P., Nemcova, R. and Vasilkova, Z. 2003. Reduction of *Salmonella* in Gnotobiotic Japanese Quails Caused by the Enterocin A-producing EK13 Strain of *Enterococcus faecium*. *Veterinary Research Communications* **27**: 275-280.
- Lee, Y.K. and Puong, K.Y. 2002. Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate. *British Journal of Nutrition* **88**: (Suppl 1): S101-S108.
- Lemos Miguel, M.A., Dias de Castro, A.C. and Ferreira Gomes Leite, S. 2008. Inhibition of vancomycin and high-level aminoglycoside-resistant enterococci strains and *Listeria monocytogenes* by bacteriocin-like substance produced by *Enterococcus faecium* E86. *Current Microbiology* **57**(5): 429-436.
- Li, L., Xu, C.L., Ji, C. *et al.* 2006. Effects of a dried *Bacillus subtilis* culture on egg quality. *Poultry Science* **85**(2): 364-368.
- Lilly, D.M. and Stillwell, R.H. 1965. Probiotics: growth-promoting factors produced by microorganisms. *Science* **147**: 747-8.
- Maassen, C.B., van Holten-Neelen, C., Balk, F. *et al.* 2000. Strain-dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. *Vaccine* **18**(23): 2613-2623.
- Makras, L., Triantafyllou, V., Fayol-Messaoudi, D. *et al.* 2006. Kinetic analysis of the antimicrobial activity of probiotic lactobacilli towards *Salmonella enterica* serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds. *Research in Microbiology* **157**: 241-247.
- Marin, M.L., Tejada-Simon, M.V., Lee, J.H., Murtha, J., Ustunol, Z. and Pestka, J.J. 1998. Stimulation of cytokine production in clonal macrophage cell and T-cell models by *Streptococcus thermophilus*: comparison with *Bifidobacterium* sp. and *Lactobacillus bulgaricus*. *Journal of Food Protection* **61**: 859-864.
- Marotta, F., Naito, Y., Minelli, E. *et al.* 2003. Chemopreventive effect of a probiotic preparation on the development of preneoplastic and neoplastic colonic lesions: an experimental study. *Hepatology* **50**(54): 1914-1918.
- Mastroeni, P., Villarreal-Ramos, B. and Hormaeche, C.E. 1992. Role of T cells, TNF alpha and IFN gamma in recall of immunity to oral challenge with virulent salmonellae in mice vaccinated with live attenuated aro- *Salmonella* vaccines. *Microbiology and Pathogenesis* **13**(6): 477-491.
- Matsumoto, M. and Benno, Y. 2004. Consumption of *Bifidobacterium lactis* LKM512 yogurt reduces gut mutagenicity by increasing gut polyamine contents in healthy adult subjects. *Mutation Research* **568**(2): 147-53.
- Medici, M., Vinderola, C.G. and Perdigon, G. 2004. Gut mucosal immunomodulation by probiotic fresh cheese. *International Dairy Journal* **14**: 611-618.
- Mohamadzadeh, M., Olson, S., Kalina, W.V. *et al.* 2005. *Lactobacilli* activate human dendritic cells that skew T cells toward T helper 1 polarization. *Proceedings of the National Academy of Science USA* **102**(8): 2880-2885.
- Mountzouris, K.C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G. and Fegeros, K. 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science* **86**(2): 309-317.
- Mukai, T., Asasaka, T., Sato, E., Mori, K., Matsumoto, M. and Ohori, H. 2002. Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic *Lactobacillus reuteri*. *FEMS Immunology and Medical Microbiology* **32**(2): 105-110.
- Nakamura, A., Ota, Y., Mizukami, A., Ito, T., Ngwai, Y.B. and Adachi, Y. 2002. Evaluation of Aviguard, a commercial competitive exclusion product for efficacy and alter-effect on the antibody response of chicks to *Salmonella*. *Poultry Science* **81**(11): 1653-1660.
- Noujaim, J.C., Andreatti Filho, R.L., Lima, E.T., Okamoto, A.S., Amorim, R.L. and Neto, R.T. 2008. Detection of T lymphocytes in intestine of broiler chicks treated with *Lactobacillus* spp. and challenged with *Salmonella enterica* serovar Enteritidis. *Poultry Science* **87**(5): 927-933.
- Nuotio, L., Schneitz, C., Halonen, U. and Nurmi, E. 1992. Use of competitive exclusion to protect newly-hatched chicks against intestinal colonisation and invasion by *Salmonella enteritidis* PT4. *British Poultry Science* **33**: 775-779.



- Nurmi, E. and Rantala, M. 1973. New aspects of *Salmonella* infection in broiler production. *Nature* **241**: 210-211.
- Ouweland, A.P., Kirjavainen, P., Shortt, C. and Salminen, S. 1999. Probiotics: mechanisms and established effects. *International Dairy Journal* **9**: 43-52.
- Oyarzabal, O.A. and Conner, D.E. 1995. In vitro fructooligosaccharide utilization and inhibition of *Salmonella* spp. by selected bacteria. *Poultry Science* **74**(9): 1418-1425.
- Parker, R.B. 1974. Probiotics: the other half of the antibiotics story. *Animal Nutrition and Health* **29**: 4-8.
- Pascual, M., Hugas, M., Badiola, J.I., Monfort, J.M. and Garriga, M. 1999. *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonisation in chickens. *Applied and Environmental Microbiology* **65**(11): 4981-4986.
- Pathmakanthan, S., Li, C.K., Cowie, J. and Hawkey, C.J. 2004. *Lactobacillus plantarum* 299: beneficial *in vitro* immunomodulation in cells extracted from inflamed human colon. *Journal of Gastroenterology and Hepatology* **19**(2): 166-173.
- Patterson, J.A. and Burkholder, K.M. 2003. Application of prebiotics and probiotics in poultry production. *Poultry Science* **82**(4): 627-631.
- Piao, Z., Toyota-Hanatani, Y., Ohta, H., Sasai, K., Tani, H. and Baba, E. 2007. Effects of *Salmonella enterica* subsp. *enterica* serovar Enteritidis vaccination in layer hens subjected to *S. Enteritidis* challenge and various feed withdrawal regimens. *Veterinary Microbiology* **125**(1-2): 111-119.
- Reid, G., Charbonneau, D., Erb, J. *et al.* 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunology and Medical Microbiology* **35**(2): 131-134.
- Rhee, S.J., Walker, W.A. and Cherayil, B.J. 2005. Developmentally regulated intestinal expression of IFN- $\gamma$  and its target genes and the age-specific response to enteric *Salmonella* infection. *Journal of Immunology* **175**(2): 1127-1136.
- Ricca, E., Henriques, O.A. and Cutting, S.M. 2004. *Spore probiotics as animal feed supplements. Bacterial spore formers: probiotics and emerging applications*, Horizon Bioscience Norfolk.
- Rioux, K.P. and Fedorak, R.N. 2006. Probiotics in the treatment of inflammatory bowel disease. *Journal of Clinical Gastroenterology* **40**(3): 260-263.
- Sadeyen, J.R., Trotter, J., Velge, P. *et al.* 2004. *Salmonella* carrier state in chicken: comparison of expression of immune response genes between susceptible and resistant animals. *Microbes and Infection* **6**(14): 1278-1286.
- Salma, U., Miah, A.G., Maki, T., Nishimura, M. and Tsujii, H. 2007. Effect of dietary *Rhodobacter capsulatus* on cholesterol concentration and fatty acid composition in broiler meat. *Poultry Science* **86**(9): 1920-1926.
- Samli, H.E., Senkoylu, N., Koc, F., Kanter, M. and Agma, A. 2007. Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. *Archives of Animal Nutrition* **61**(1): 42-49.
- Santos, R.L., Tsois, R.M., Baumler, A.J. and Adams, L.G. 2003. Pathogenesis of *Salmonella* - induced enteritis. *Brazilian Journal of Medical Biology Research* **36**: 3-12.
- Sanz, Y., Nadal, I. and Sanchez, E. 2007. Probiotics as drugs against human gastrointestinal infections. *Recent Patents in Anti-Infective Drug Discovery* **2**(2): 148-156.
- Schierack, P., Filter, M., Scharek, L. *et al.* 2009. Effects of *Bacillus cereus* var. *toyoi* on immune parameters of pregnant sows. *Veterinary Immunology and Immunopathology* **127**(1-2): 26-37.
- Schneitz, C. 1992. Research note: Automated droplet application of a competitive exclusion preparation. *Poultry Science* **71**: 2125-2128.
- Seo, K.H., Holt, P., Gast, R.K. and Hofacret, C.L. 2000. Elimination of early *Salmonella enteritidis* infection after treatment with competitive-exclusion culture and enrofloxacin in experimentally infected birds. *Poultry Science* **79**: 1408-1413.
- Shornikova, A.V., Casas, A.I., Mykkanen, H., Salo, E. and Vesikari, T. 1997. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. *Pediatric Infectious Disease Journal* **16**(12): 1103-1107.
- Shortt, C. 1999. The probiotic century: historical and current perspectives. *Trends in Food Science and Technology* **10**: 411-417.
- Smirnov, A., Perez, R., Amit-Romach, E., Sklan, D. and Uni, Z. 2005. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. *Journal of Nutrition* **135**(2): 187-192.
- Surawicz, C.M., Elmer, G.W., Speelman, P., McFarland, L.V., Chinn, J. and van Belle, G. 1989. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*: a prospective study. *Gastroenterology* **96**(4): 981-988.
- Talebi, A., Amirzadeh, B., Mokhtari, B. and Gahri, H. 2008. Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathology* **37**(5): 509-512.
- Tanida, M., Yamano, T., Maeda, K., Okumura, N., Fukushima, Y. and Nagai, K. 2005. Effects of intraduodenal injection of *Lactobacillus johnsonii* La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. *Neuroscience Letters* **389**(2): 109-114.
- Timmerman, H.M., Koning, C.J., Mulder, L., Rombouts, F.M. and Beynen, A.C. 2004. Monostrain, multistain and multispecies probiotics—A comparison of functionality and efficacy. *International Journal of Food Microbiology* **96**(3): 219-33.
- Tindall, B.J., Grimont, P.A., Garrity, G.M. and Euzéby, J.P. 2005. Nomenclature and taxonomy of the genus *Salmonella*. *International Journal of Systematic and Evolutionary Microbiology* **55**(1): 521-524.
- Tzortzis, G., Goulas, A.K., Gee, J.M. and Gibson, G.R. 2005. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous *in vitro* fermentation system and in the proximal colonic contents of pigs *in vivo*. *Journal of Nutrition* **135**(7): 1726-1731.
- van Asten, A.J., Koninkx, J.F. and van Dijk, J.E. 2005. *Salmonella* entry: M cells versus absorptive enterocytes. *Veterinary Microbiology* **108**(1-2): 149-152.
- van de Giessen, A.W., Ament, A.J.H.A. and Notermans, S.H.W. 1994. Intervention strategies for *Salmonella enteritidis* in poultry flocks: a basic approach. *International Journal of Food Microbiology* **21**: 145-154.
- van Coillie, E., Goris, J., Cleenwerck, I. *et al.* 2007. Identification of lactobacilli isolated from the cloaca and vagina of laying hens and characterization for potential use as probiotics to control *Salmonella* Enteritidis. *Journal of Applied Microbiology* **102**(4): 1095-1106.
- van Immerseel, F., Cauwerts, K., Devriese, L.A., Haesebrouck, F. and Ducatelle, R. 2002. Feed additives to control *Salmonella* in poultry. *World's Poultry Science Journal* **58**: 501-511.
- Vazquez-Torres, A., Jones-Carson, J., Baumler, A.J. *et al.* 1999. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. *Nature* **401**(6755): 804-808.
- Vicente, J.L., Torres-Rodriguez, A., Higgins, S.E. *et al.* 2008. Effect of a selected *Lactobacillus* spp.-based probiotic on *Salmonella enterica* serovar enteritidis-infected broiler chicks. *Avian Disease* **52**(1): 143-146.



- Vinderola, G., Matar, C. and Perdigon, G. 2007a. Milk fermented by *Lactobacillus helveticus* R389 and its non-bacterial fraction confer enhanced protection against *Salmonella enteritidis* serovar Typhimurium infection in mice. *Immunobiology* **212**(2): 107-118.
- Vinderola, G., Matar, C. and Perdigon, G. 2007b. Milk fermentation products of *L. helveticus* R389 activate calcineurin as a signal to promote gut mucosal immunity. *BMC Immunology* **8**: 19.
- Votava, J., Kumprecht, I. and Borovan, L. 1987. Radioactive labelling of bacteria in probiotic preparations Lactiferm and Microferm and their single use in broilers. *Sbor. ved. prac. VUVZ Pohorelice* **20**: 257-276.
- Wagner, D.R., Holland, M. and Cerniglia, C.E. 2002. An *in vitro* assay to evaluate competitive exclusion products for poultry. *Journal of Food Protection* **65**(5): 746-751.
- Waters, S.M., Murphy, R.A. and Power, R.F. 2005. Assessment of the effects of Nurmi-type cultures and a defined probiotic preparation on a *Salmonella typhimurium* 29E challenge *in vivo*. *Journal of Food Protection* **68**(6): 1222-1227.
- White, P.L., Baker, A.R. and James, W.O. 1997. Strategies to control *Salmonella* and *Campylobacter* in raw poultry products. *Rev. Sci. Tech. Off. Int. Epiz.* **16**(2): 525-541.
- Willis, W.L. and Reid, L. 2008. Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and *Campylobacter jejuni* presence. *Poultry Science* **87**(4): 606-611.
- Withanage, G.S., Wigley, P., Kaiser, P. *et al.* 2005. Cytokine and chemokine responses associated with clearance of a primary *Salmonella enterica* serovar Typhimurium infection in the chicken and in protective immunity to rechallenge. *Infection and Immunity* **73**(8): 5173-5182.
- Worley, M.J., Nieman, S.N., Geddes, K. and Heffron, F. 2006. *Salmonella typhimurium* disseminates within its host by manipulating the motility of infected cells. *Proceedings of the National Academy of Science USA* **103**(47): 17915-17920.
- Wright, A. 2005. Regulating the safety of probiotics-the European approach. *Current Pharmaceutical Design* **11**: 17-23.
- Zhang, L., Su, P., Henriksson, A., O'Rourke, J. and Mitchell, H. 2008. Investigation of the immunomodulatory effects of *Lactobacillus casei* and *Bifidobacterium lactis* on *Helicobacter pylori* infection. *Helicobacter* **13**(3): 183-190.

### About the corresponding author

Alun Carter was based at the VLA and affiliated with the University of Surrey conducting a PhD into the efficacy and safety of probiotics for use in poultry. The PhD investigated the use of *L. salivarius* and *E. faecium* to prevent *Salmonella* Enteritidis infection of poultry. Of particular interest was the interaction of the probiotic strains with the host and subsequent reduction of *Salmonella* colonisation. Specific areas of interest include probiotic immunomodulation, antibiotic and toxicological safety and mechanisms of *Salmonella* host interactions. Alun Carter is currently based at the Defence Science and Technology Laboratory (Dstl) in the UK as a post-doctoral researcher in applied trauma immunology.