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Metabolic and Physiological Impact of Probiotics or Direct-Fed-Microbials on Poultry: A Brief Review of Current Knowledge[1]

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Abstract: The poultry industry is facing a ban on the use of antibiotic feed additives in many parts of the world. Consequently, there is a growing interest in finding viable alternatives for disease prevention and growth enhancing supplements. The effects of probiotics or direct fed microbials (DFM) on gut health and performance in poultry as well as other species are presented. The interactions between intestinal microbiota, the gut epithelium and the immune system are important in the competitive exclusion process. The mechanisms by which probiotics operate include spatial exclusion, micro-environmental alterations, production of antimicrobial substances and epithelial barrier integrity. The preponderance of research data in this field suggests the likelihood of a small but additive series of beneficial changes from an animal's exposure to probiotics. Further investigations will be needed to fully characterize the effects and sustained outcomes of probiotic and DFM treatments in poultry.

Key words: Direct fed microbial, probiotic, review, poultry, competitive exclusion

Introduction

The metabolic activity and energy requirements of the intestinal microbiota is comparable to that which takes place in the liver, the most metabolically active organ (Isolauri *et al*., 2004). In vertebrates, there are more microbial cells within the gastrointestinal (GI) tract than total cells within the body-proper (Hove *et al*., 1999; Mai, 2004). The microorganisms most commonly observed are bacteria and yeast.

There are two populations of microorganisms that are found within the GI tract of poultry. The first, the *autochthonous* bacteria, colonize the gut by inoculation resulting from environmental exposure and normal feeding activities of the bird (Gusils *et al*., 1999). The second, *allocthonous* bacteria, are exogenous in nature and are introduced as a dietary supplement into the GI tract through the feed or drinking water as direct fed microbials (DFM) or probiotics (Patterson and Burkholder, 2003). Modern nutritionists use the terms probiotics and DFM interchangeably, however, currently the term probiotic is most often used. For the purposes of this review, the term, probiotic, will be used to denote the use of either probiotics or direct fed microbials.

Some data in the literature indicate that allocthonous bacteria introduced via probiotics may prevent infection and colonization of the GI tract by opportunistic pathogens (Fuller, 1989). Introduction of such probiotics is believed to prevent or attenuate the growth of clinical enteric pathogens in poultry, resulting in enhanced growth and performance of the host bird. This phenomenon has prompted a widespread interest in the poultry industry of probiotic usage as an alternative to the prophylactic use of antibiotics for the prevention of disease within poultry flocks (Salminen *et al*., 1998). This interest has arisen as the result of growing concerns about prophylactic usage of antibiotics in poultry and other animal production systems in Europe and the US (Davis and Anderson, 2002; Mai, 2004).

Unfortunately, failure to consistently reproduce various mechanistic changes in animal physiology and beneficial alterations in production parameters with the usage of probiotics has failed to result in a universal acceptance of the efficacy of these supplements amongst the poultry science community (Bird *et al*., 2002). There are several reasons for this failure. First, the mode of action of probiotics is poorly understood. The majority of papers published on their biological activity employ a wide variety of probiotic organisms without a confirmed genotype. Additionally, the species of poultry as well as the age, physiological state and diets vary. This has resulted in a body of literature that is hard to integrate and hinders the formulation of a specific hypothesis regarding the modes of action that result in beneficial effects often associated with the usage of probiotics. Once these mechanisms are elucidated, it may be possible to use modern molecular biology techniques to develop more efficacious probiotic organisms and to predict under which production conditions the use of a particular probiotic consortium may be of value.

This review will briefly describe traditional definitions of probiotics and their reported beneficial effects on poultry production systems and summarize what is currently understood about their colonization in the GI tract, their metabolism and their mechanisms of action in altering host animal health and performance. From this body of information, we will propose a paradigm that might be of use in future poultry probiotic studies that will facilitate integration of the findings of one study with another.

Probiotics defined: Probiotics are "live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance" (Isolauri *et al.*, 2004) or "a live microbial feed that is beneficial to health" (Fooks and Gibson, 2002; Netherwood *et al.*, 1999; Patterson and Burkholder, 2003). They may contain only one, or several (a consortium) different bacterial species. The mechanisms of action of different bacterial strains in a probiotic consortium may differ (Bomba *et al.*, 2002). Additionally, different genotypes (subtypes) within the same species may have different biological effects that can be either synergestic or antagonistic. For instance, isolates within the same species can be unique and may have differing areas of adherence, specific immunological effects and other biological actions (Isolauri *et al.*, 2004). Hence, probiotics containing similar species of bacteria may, in fact, differ in their efficacy.

Although many articles and reviews in the current scientific and popular literature refer to the "beneficial effects" of probiotics, these articles are often vague as to exactly what benefits are conferred upon the animal by the probiotic (Bouzaine *et al.*, 2005). In poultry production systems, benefits and efficacy can be specifically defined. Any feed supplement or therapy that enhances poultry health and performance as measured by enhanced animal health, growth and/or feed efficiency. These can be defined as beneficial or efficacious. In the case of probiotics, the preponderance of literature suggests that these production endpoints such as growth and feed efficiency are beneficially impacted by probiotics.

Probiotic colonization and attachment within the gastrointestinal tract: Successful probiotic colonization depends on the survival and stability of the probiotic strain, specificity of the strain relative to host, dose and frequency of administration, health and nutritional status of the host, effect of age, stress and genetics of the host. (Mason *et al.*, 2005). In general, probiotic bacteria are anaerobes or facultative anaerobes (Salanitro *et al.*, 1978). In poultry, probiotic bacterial colonization, as measured by colony forming units (CFU), increase in number beginning at the beak, progressing distally to the colon (Simon *et al.*, 2004).

The crop, proventriculus and gizzard have very low anaerobic bacteria numbers due to the presence of the oxygen consumed with the feed as well as the low luminal pH, primarily associated with the hydrochloric acid within the proventriculus (Rastall, 2004). The small intestine has large bacterial numbers consisting of facultative anaerobes such as *Lactobacilli*, *Streptococci* and *Enterobacteria* as well as anaerobes such as *Bifidobacterium* spp., *Bacteroides* spp. and *Clostridia* spp. at levels ranging from 10^4 to 10^8 CFU/ml (Gaskins, 2003). The most heavily colonized regions of the GI tract are the colon and cecum with colonization of 10^{10} to 10^{13} CFU/ml (Heczko *et al.*, 2000; Klaasen *et al.*, 1992). Autocthonous and allocthonous (probiotic) bacteria colonize three different areas within the GI intestinal tract, the enterocyte surface, the cecal epithelia surface and the colonic epithelia surfaces (Yamauchi and Snel, 2000). Each of these areas generally includes three microenvironment components. The digesta, the surfaces of the enterocytes and the cecum and colon and the mucous blanket covering the epithelial surface as well as the epithelial cells of the cecum and colon.

The digesta, which is created by the consumption of a rich milieu of feed nutrients and water, is an ideal environmental niche within which many bacterial species flourish. Probiotic bacteria can be found attached to individual feed particles such as starch granules (Fig. 1A and 1B). Other bacteria are not associated with the feed particles, but simply exist within the aqueous matrix of the digesta. The second microenvironment of the GI tract where microbes are found is within the mucous blanket that covers the epithelial lining of the GI tract including the intestinal villi (Fig. 1C and 1D) and cecal and colonic surfaces. The mucous not only serves as an environment within which these microbes exist, but also serves as a source of nutrients for bacteria (Jacobsen *et al.*, 1999). Finally, bacteria can also exist associated with or attached to the surface of apical plasmalemma of the epithelial cells lining these areas (Fig. 2A and 2B). Fig. 2B depicts a rod-shaped organism associated with a goblet cell on the ileal villus of a chick at d 21. Additionally, Fig. 1C depicts a cluster of segmented filamentous bacteria (SFB) or segmented fusiform bacteria attached and penetrating into the cytoplasm of the enterocytes of the ileum (Marteau *et al.*, 2004).

The functional relationship of bacteria associated with the three GI micro environments described above and its biological significance has not been established (Kankaanpaa *et al.*, 2004). The ability of many strains of probiotic bacteria to physically adhere to portions of the GI micro environments may speak to their ability to effect changes in enteric health (Sarem-Damerdji *et al.*, 1995). Attachment to the enterocyte's plasmalemma is considered a very first step in the colonization of the host enterocyte surfaces. This permits probiotic organisms to resist both peristalsis and mixing with the digesta and mucus layer and subsequent removal from the gut. However, adherent probiotic bacteria usually do not colonize the intestinal epithelium permanently and are normally eliminated from the GI tract a few days after cessation of supplementation (Gusils *et al.*, 1999).

The ability of a probiotic strain to adhere to mucus and epithelial cell surfaces is one of the main selection criteria for a candidate probiotic (Rojas and Conway, 1996). Very few studies have investigated adhesion and colonization of probiotic bacteria because of the complexity of the intestinal enterocytes and the extensive interaction amongst intestinal cell types within the intestinal tract (Henriksson *et al.*, 1991). *Lactobacilli*,

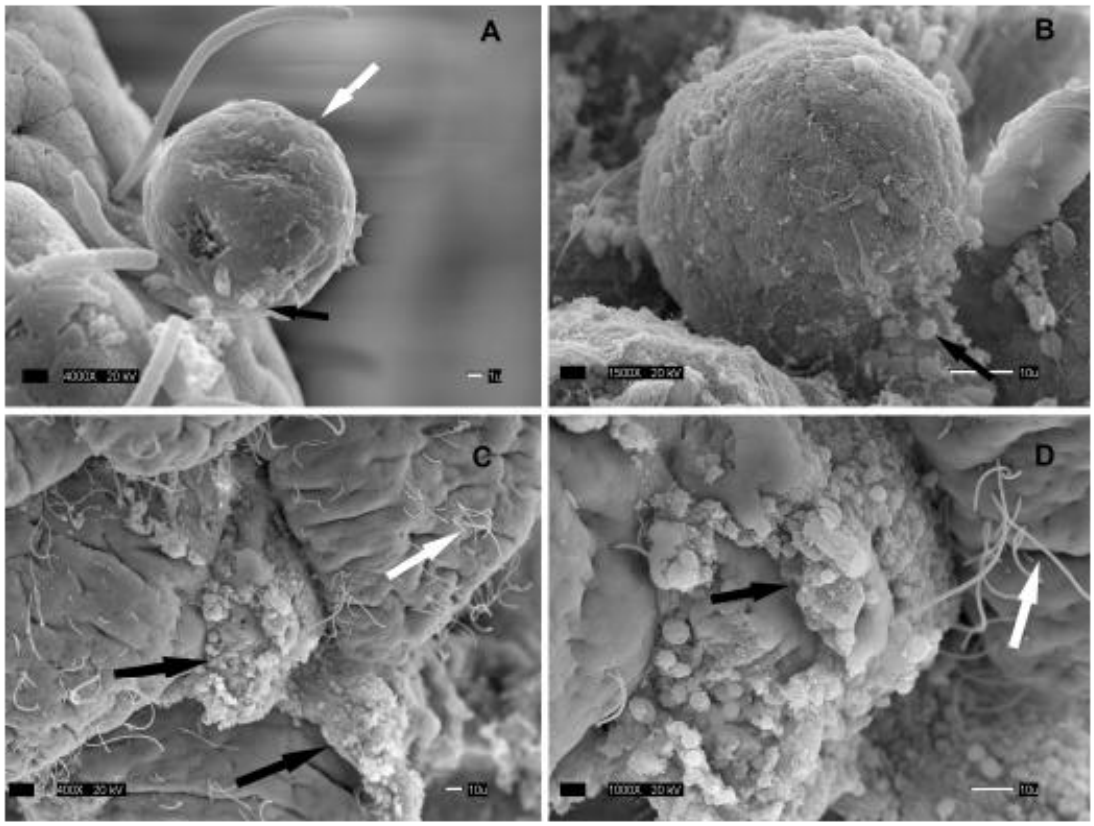

Fig. 1: SEM micrograph of chicken ileal enterocytes at d 21 after hatch. Tissues were fixed in 1%OsO_4 and examined using JEOL 5900LV microscope at 20kV. A, B: several microorganisms (black arrows) are visible attached to starch granules (white arrow) in the ileal lumen; C, D: mucous blanket seen between ileal villi (black arrows)

whether shed from epithelial surfaces or multiplying in ingested food, permeate all regions of the digestive tract in poultry (Servin and Coconnier, 2003). It has been reported that *Lactobacilli* can colonize non-secretory, gastric epithelium, in non-avian species, by attaching to epithelial cells from which they can inoculate gastric contents and the lower regions of the intestinal tract (Servin and Coconnier, 2003). Henriksson *et al.* (1991) propose that *Lactobacilli* adhere to the stomach of porcine epithelial cells through proteinaceous components located on the bacterial surface. It has been postulated that lactic acid bacteria display various surface determinants and that these are involved in their interaction with enterocytes and other epithelial cells. Those determinants include passive forces, electrostatic interactions, hydrophobic forces, steric forces, lipoteichoic acids and specific structures such as external appendages covered by lectins (Gusils *et al.*, 1999). Not all strains of *Lactobacillus* adhere to enterocytes, indicating that this property is strain specific (Kankaanpaa *et al.*, 2004). Additionally, *Lactobacilli* have also been demonstrated to prevent the adherence of pathogenic bacteria. A dose-dependent inhibition of adherence of enterotoxigenic *E. coli*, enteropathogenic *E. coli* (EPEC) and *S. typhimurium* to Caco-2 cells, a line of immortalized intestinal enterocytes, by strains of *Bifidobacteria* and *Lactobacillus* has been reported (Briandet *et al.*, 1999). *Lactobacillus animalis* has been demonstrated to inhibit growth, *in vitro* adhesion of various *Salmonella* strains as well as the production of antimicrobial substances (Kankaanpaa *et al.*, 2004).

Since it is very difficult to study bacterial adhesion *in vivo*, most experiments use *in vitro* models. Microbial Adhesion to Solvents (MATS) is one technique that has been used to investigate bacterial cell affinities for polar and non-polar solvents (Wadstrom *et al.*, 1987). Non-polar solvents have been used to estimate their hydrophobic properties, while polar solvents have been used to help estimate Lewis acid/base properties (Gusils *et al.*, 1999). The low affinities of *Lactobacilli* for non-polar solvents suggest that these bacteria possess a hydrophilic rather than a hydrophobic cellular surface (Huang and Adams, 2003). It has also been demonstrated that a higher percentage of hydrophobic bacteria adhere to intestinal epithelial cells than do hydrophilic strains (Bomba *et al.*, 2002). The highest

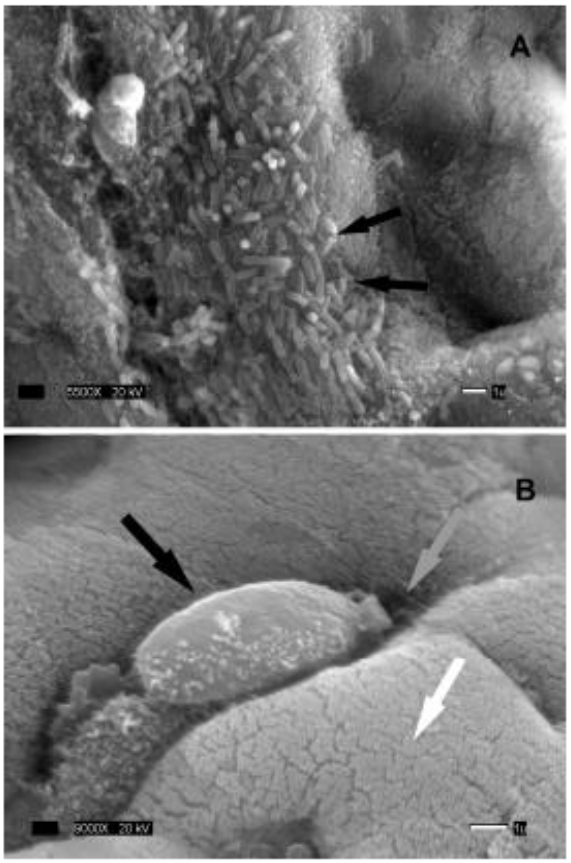

Fig. 2: SEM micrograph of chicken ileal enterocytes at d 21 after hatch. Tissues were fixed in 1%OsO_4 and examined using JEOL 5900LV microscope at 20kV. A: SEM micrograph of cecal epithelium, multiple microbes are visible and are attached to the surface of the enterocyte microvilli (black arrows); B: Microorganism (black arrow) seen near a goblet cell (gray arrow) in the chicken ileum; white arrow indicates epithelial brush border

adhesion values were obtained at pH 7 (Bomba *et al.*, 2002). When those microorganisms were cultured with free polyunsaturated fatty acids (PUFA), hydrophobicity was diminished.

Other *in vitro* studies have used various cell lines to study probiotic bacterial adhesion. Huang and Adams (2003) used a human intestinal epithelial cell line to study probiotic bacterial adhesion. This cell line spontaneously differentiates under standard culture conditions and the differentiated cells then express characteristics of mature enterocytes. This study utilized *Lactobacillus acidophilus* (which readily adheres to enterocytes) and *Bifidobacterium lactis* (does not adhere to enterocytes), as positive and negative controls, respectively, while testing the adhesion properties of various strains of propionibacteria. Huang and Adams (2003) observed large numbers of *L. acidophilus* adhering to the surface of the human enterocyte cell line by scanning electron microscopy (SEM); while very few *B. lactis* were observed on cell surfaces. Polyunsaturated fatty acids (defined above) were reported to alter bacterial adhesion sites on Caco-2 cells by (Fooks and Gibson, 2002). This suggests that dietary PUFA affects the attachment sites for the GI microbiota, possibly by modifying the composition of fatty acids in the intestinal wall, thus affecting its hydrophobicity. The stimulatory effect of PUFA upon adhesion of *Lactobacilli* may be useful for enhancing the effectiveness of probiotics in inhibiting digestive tract pathogen colonization (Wagner and Cerniglia, 2005).

Nutrient metabolism of common probiotic bacteria: As mentioned above, probiotic organisms can be divided into two general groups based on their tolerance to oxygen, anaerobes and facultative anaerobes. The facultative anaerobe genera, *Bifidobacterium* and *Lactobacillus* are frequently included in probiotic bacterial consortia (Fooks and Gibson, 2002; Jozefiak *et al.*, 2004). They reduce the redox potential in the gut and render the environment suitable for obligate anaerobes (Cummings and Macfarlane, 1997).

Obligate anaerobic bacteria are those species only capable of anaerobic fermentation (Cummings and Macfarlane, 1997). The anaerobic fermentation of carbohydrates and proteins yield metabolic intermediates that act as electron acceptors via substrate level phosphorylation (Macfarlane and Cummings, 1999). Anaerobic fermentation results in the production of the metabolic end-products such as lactate, succinate and the short chain volatile fatty acids (VFA), acetate, propionate and butyrate, H_2,CO_2 and CH_4 as well as bacterial biomass (de Vries and Stouthammer, 1968). Most of the VFA formed by intestinal bacteria are absorbed and metabolized by the bird thus contributing to host energy requirements (Fooks and Gibson, 2002). Some bacterial metabolites, such as ammonia, phenols and amines, resulting from the breakdown and fermentation of proteins, are toxic (Dommett *et al.*, 2005).

Bifidobacteria make a significant contribution to fermentation in the colon. *Bifidobacteria* ferment carbohydrates to fructose-6-phosphate, via fructose-6-phosphate phosphoketolase (Marteau, 2000). The principal end products of fermentation by *Bifidobacteria* are acetate and lactate which are produced in a 3:2 molar ratio (Gill *et al.*, 2001). Additionally, *Bifidobacteria* produce a wide-range of antimicrobial agents that are effective against both gram-positive and gram-negative organisms. These agents might include antimicrobial peptides, such as defensins, cathelicidins and lyzozyme (Fooks and Gibson, 2002). *Bifidobacteria* have been

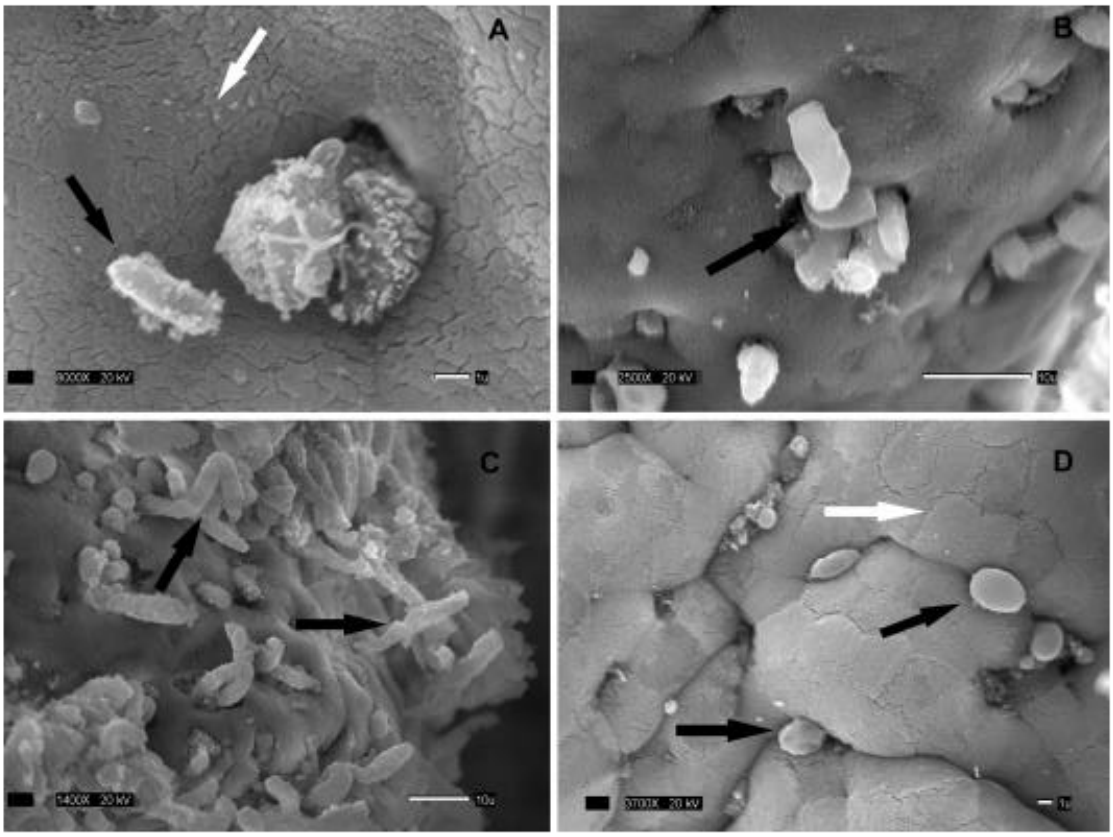

Fig. 3: SEM micrograph of chicken ileal enterocytes at d 21 after hatch. Tissues were fixed in 1%OsO_4 and examined using JEOL 5900LV microscope at 20kV. A, B and C: various probiotic organisms (black arrows) attached to epithelial tissue in the chicken cecum; white arrows indicates epithelial brush border; D: ileal microvilli surface with several organisms (black arrows, attached in the transversal furrows of the villus, white arrow shows enterocyte border in the mid villus

reported to alter fecal bacterial enzyme activities, reduce antibiotic induced side-effects, inhibit mammary and liver tumors and, in conjunction with oligofructose, reduce 1,2-dimethylhydrazine induced colonic carcinogenesis in mice (Marteau *et al.*, 2004). Oral supplementation of *Bifidobacterium lactis* to elderly subjects increased the production of total, helper $CD4^+$ and activated $CD25^+$ T lymphocytes and natural killer cells (NK); and increase the phagocytic activity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of NK (Bauer *et al.*, 2006; Chichlowski *et al.*, 2007a; Chichlowski *et al.*, 2007b; Hugo *et al.*, 2006).

Lactobacilli are involved in both homolactic and heterolactic fermentation. Homolactic fermentation involves splitting of hexoses into C_3 moieties using fructose-1,6-bisphosphate via the glycolytic pathway. This process yields two pyruvate molecules which are then converted into lactate. Two moles of ATP are generated per mole of glucose with this type of fermentation. Heterolactic fermentation proceeds via the pentose phosphate pathway, to produce lactate, CO_2 and ethanol, generating one mole of ATP per mole of glucose. Phosphoketolase is the key enzyme involved in this process (Chichlowski *et al.*, 2007a; Cummings and Macfarlane, 1997; Galdeano and Perdigon, 2006; Ichikawa *et al.*, 1999; Mead, 1989). *Lactobacilli* have also been reported to produce antibacterial proteins and bacteriocins (Bird *et al.*, 2002; Fooks and Gibson, 2002). Some *Lactobacillus* bacteriocins display a wide antibacterial spectrum against gram-positive bacteria. Others speculate that beside competitive exclusion, there exist a number of mechanisms the increase both enteric and whole-bird health (Edens *et al.*, 1997). Virtually all actions of probiotics, both physical and chemotaxic, attenuate or eliminate the ability of pathogens to foster diseased states in the intestinal tract and other body tissues. Actions of probiotics include alterations in the microbial micro environments, alteration of the host animal's metabolism,

modifications of the host's immune system, improvement in feed digestion and absorption and the production of antimicrobial compounds (Klose *et al.*, 2006).

Postulated mechanisms of probiotic enhancement of poultry health and productivity

Prevention of pathogen colonization: Enhanced health, as well as both enteric and systemic disease reduction, has been ascribed to probiotics when used in poultry. The mechanisms of action associated with the beneficial effects of probiotics are still unclear (Chichlowski *et al.*, 2007ab). The single most frequently described mechanism is "competitive exclusion" (Mack *et al.*, 1999). Competitive exclusion refers to the physical blocking of opportunistic pathogen colonization by probiotic bacteria via their ability to physically colonize environmental niches within the intestinal tract such as intestinal villus and colonic crypts which are favorite colonization sites of enteric pathogens such as *Salmonella* (Duggan *et al.*, 2002); (Fig. 3A-3D). Additionally, work in our laboratory suggests that probiotics may also selectively colonize areas around the opening to villus goblet cells (Chichlowski *et al.*, 2007b; Forestier *et al.*, 2001; Pochapin, 2000).

Probiotics also exclude the colonization of pathogens by preventing their adhesion to gastrointestinal epithelium. The exact mechanism by which probiotic bacteria prevent the attachment and colonization of pathogens can vary from organism to organism. *Lactobacillus plantarum* inhibits pathogen adhesion without competing for binding sites. This probiotic bacterium induces the transcription and excretion of the mucins MUC2 and MUC3 from goblet cells and thereby inhibits the adherence of enteropathogenic *E. coli* to the intestinal surface (Fooks and Gibson, 2002). Another example is a *Lactobacillus* species which directly inhibit the attachment of *Salmonella*, *E. coli* and other food borne pathogens (Mead, 1989). *Lactobacilli* have also been reported to suppress the growth of *Shigella flexneri*, *Salmonella typhimurium*, *Clostridium difficile* and other pathogens (Isolauri *et al.*, 2004). The exact mechanisms of this inhibition are unknown.

Probiotics can alter the physical microenvironment of the intestinal tract in such a manner that opportunistic pathogens cannot survive. Changes in the physical microenvironment inhibit pathogen growth in two ways. First, probiotic organisms compete with pathogens for nutrients thus preventing them from acquiring energy to grow and function in the gut environment (Cummings and Macfarlane, 1997). Second, probiotics produce a variety of organic acid end products, such as VFA and lactic acid as a part of their metabolism of nutrients in the gut digesta (Gibson, 1999). These weak organic acids lower the pH of the gut environment below that essential for the survival of such pathogenic bacteria as *E. coli* and *Salmonella* (Marteau *et al.*, 2004).

These changes in the physical environment can affect intestinal epithelial function and whole-body metabolism as well. Volatile fatty acids can affect colonic epithelial cell transport, colonocyte metabolism and growth and differentiation. Volatile fatty acids are rapidly absorbed from the small intestinal tract and colon, stimulate electrolyte and water absorption within the intestinal tract and have a major effect on the growth of epithelial cells. The colonic epithelium derives 60-70% of its energy from bacterial fermentation products (Marteau *et al.*, 2004). Volatile fatty acids also serve as energy yielding substrates to the host bird. Volatile fatty acids are involved in the hepatic regulation of lipid and carbohydrates and act as substrates to the muscle, kidney, heart and brain (Meghrous *et al.*, 1990).

Probiotic bacteria are known to produce a class of small, antimicrobial molecules that are collectively known as *bacteriocins* (Kohler *et al.*, 2002). These bacteriocins can kill pathogenic bacteria or impede their colonization (Bar-Shira and Friedman, 2006). They are proteins, or protein complexes which can have antagonistic actions against species related to the producer bacterium. Additionally, other non-bacteriocin compounds are produced by probiotics which inhibit the growth of pathogens (Madsen *et al.*, 2001). The polyamine derivate piperidine, which is produced by intestinal microflora as the result of amino acid degradation, has been shown to inhibit the binding and internalization of *Salmonella* and *Shigella* to intestinal epithelial cells *in vitro* (Gusils *et al.*, 2003).

Maintenance of epithelial barrier integrity: Another of the major functions of enterocytes is to act as a protective barrier shielding the body from organisms and substances that do not serve as nutrients (Metcalfe *et al.*, 1991). Probiotics have been reported to enhance the maintenance and function of the epithelial barrier. For example, Madsen *et al.* (2001) have shown that a commercial mixture of various *Bifidobacterium* and *Lactobacilli* strains can enhance the epithelial barrier in IL-10 knock-out mice. There are two major mechanisms by which the epithelial barrier maintains functional integrity. The first is the enterocyte "mucous blanket" (Fig. 1D), a relatively thick layer of mucus that is secreted by the goblet cells dispersed throughout the luminal epithelium in the small intestine. This mucus consists of mucin, many small associated proteins, glycoproteins, lipids and glycolipids (Caballero-Franco *et al.*, 2007). It also contains soluble receptors that recognize specific adhesion proteins that facilitate bacterial attachment (Chichlowski *et al.*, 2007b). Intestinal bacteria can trigger enterocyte inflammation. It has been shown, however, that administration of probiotics can alter these effects (Mack *et al.*, 2003). Caballero-Franco *et al.* (2007) reported a 60% increase in basal luminal mucin content after treatment with the

probiotic mixture VSL#3. These authors suggest that non-pathogenic bacteria up-regulated the mucin, *MUC2*, gene expression. They indicate, however, that the presence of multiple species of probiotic bacteria tested did not result in an additive increase in mucin secretion. In addition, they speculate an increased number of goblet cells as an effect of probiotic treatment. Chichlowski *et al.* (2007b) observed a greater number of goblet cells on chicken intestinal villi after probiotic treatment. It is possible that metabolites produced during bacterial fermentation may play a role in the growth and maturation of goblet cells. Additionally, *in vitro* studies have shown increased production of mucin, especially MUC3, after treatment with several strains of *Lactobacillus* (Montalto *et al.*, 2004).

The second mechanism that ensures epithelial barrier integrity is associated with tight junctions (*zonula occludens*) via which the intestinal epithelial cells attach to one another to form an unbroken, contiguous biological barrier which prevents the entrance of bacteria and large molecules from the digesta mileux. The tight junction and *zonula adherens* are collectively referred to as the *apical junction complex* (Vogelmann and Nelson, 2005). Tight junctions permeability can be modulated by zonulin, a molecule which is involved in the movement of fluid, macromolecules and leukocytes from the bloodstream to and from the intestinal lumen (Shen *et al.*, 2006). There is a very little data regarding effects of probiotics on tight junction structure. Since these dynamic structures are involved in developmental, physiological and pathological processes (Marteau *et al.*, 2004), it is possible that they are affected by action of probiotic organisms. A protective action of *Lactobacillus* on zonulin was reported after treatment with non-steroidal inflammatory drugs *in vitro* (Buts *et al.*, 2002). Also, Shen *et al.* (2006) using electron microscopy demonstrated more intact epithelial cell tight junctions after probiotic treatment but stated the mechanisms responsible for this observation were not clear.

Intestinal nutrient transport: The trophic effects of probiotics include increases in the specific and total activities of the brush-border membrane enzymes in the jejunal enterocytes of growing rats. After oral treatment of rats with *S. boulardii*, there was a marked stimulation of sodium dependent D-glucose uptake into brush border membrane vesicles with a corresponding increase of the sodium D-glucose cotransporter-1, SGLT-1 (Eberl, 2005). It has also been reported that the oral administration of *L. casei* increased the crypt cell production rates of the jejunum, ileum, cecum and distal colon in rats (Kohler *et al.*, 2003). Additionally, Chichlowski *et al.* (unpublished observations) have reported that a probiotic consortium increases passive absorption of glucose in the chicken ileum.

Enhancement of intestinal immune function: Although it is well known that probiotic organisms may stimulate or interact with the host immune system, a general understanding of this relationship is complicated by our limited knowledge of how the avian immune system is regulated in the gut and how the host differentiates between "good" and "bad" bacteria. The intestinal enterocytes presents a unique challenge to the immune system. It needs to allow for digestion and nutrient uptake without mounting specific responses to food or the commensal organisms living in the gut, which help with these processes. At the same time, intestinal enterocytes are monitoring this expansive surface area of the epithelial cells for the presence of potential pathogens. As a result, there is a constant interaction amongst different cell types, including members of the innate and the adaptive immune systems and bacteria in the gut lumen, epithelium or lamina propria (He *et al.*, 2002; Kohler *et al.*, 2003; Madsen *et al.*, 2001; Perdigon *et al.*, 2002; Vidal *et al.*, 2002). The balance between a hyper-response and no response is primarily achieved through specific organization of the intestine and the inter-digitation of immune cells throughout the epithelial tissue. The gut is often referred to as the largest immune organ of the body as more lymphocytes reside in the gut than in any other tissue. This is a reflection of the size of the gut and the amount of surface area in contact with the external environment. The enterocytes of the intestinal epithelium provide a barrier both to prevent the passive loss of nutrients and to prevent the access of pathogens to the body proper. The same barrier limits, however, the immune system's ability to detect potential pathogens in the lumen. To circumvent this, pathogenic bacteria expressing appropriate genes penetrate the gut through M cells. M cells, are scattered throughout the intestinal tract and comprise approximately 1% of total intestinal epithelia. The M cells are above regions of the lamina propria enriched for lymphocytes, macrophages, heterophils and dendritic cells. M cells have phagocytic properties and sample antigens originating from the gut lumen and transport them to nearby immune cells beneath.

The question is how do probiotics fit into this paradigm? Several studies have described the use of probiotics to enhance specific aspects of intestinal and/or systemic immunity. Numerous studies targeted at the specific effects of various probiotic organisms on the immune system have been conducted in mammals These studies have suggested many different potential mechanisms for interactions and outcomes. Probiotics may have the ability to directly influence the inflammatory response elicited by pathogens by down regulating specific signaling pathways (Yurong *et al.*, 2005). There are several pathways proposed for activation of immune response by gut microflora or when cells are infected by a variety of pathogens including those utilizing MAP

kinase and NF-*kappa* beta pathways (Neish *et al.*, 2000). Probiotics modulate the expression of various pro- and anti-inflammatory cytokines. The results of these various studies are similar to those investigating the physiologic effects of probiotics in that biologic outcomes can vary greatly between strains of bacteria and even between species or genotype of the host.

While the reports of probiotic effects on the immune response in chickens are more limited compared with mammals, similar results have been described. It has to be mentioned that the majority of these studies have examined effects on systemic immunity. Yurong *et al.* (2005) have described that the use of probiotics increased the amount of IgA found in the lumen, the numbers of IgA, IgM and IgG producing cells as well as the numbers of T cells in the cecal tonsils. These increases in immune parameters were accompanied by increased density of the microvilli and length of the cecal tonsils. Haghighi *et al.* (2005) reported that oral administration of probiotic organisms increases natural antibodies (Abs) against several different antigens (Ag) in both the gut and the serum. Similarly, Zulkifli *et al.* (2000) described an increased Ag-specific Ab response following probiotic treatment and Newcastle disease vaccination. The potential affects probiotics have on systemic immunity is not limited to just Ab responses. Farnell *et al.* (2006) noted that the treatment of chickens with probiotics lead to a significant increase in the oxidative burst and degranulation of heterophils as compared to controls.

In spite of these reports there is still much we do not understand about how these organisms interact with the host or the factors which dictate their efficacy. Studies by Balvei *et al.* (2001) found no significant difference in any immune parameter measured. Additionally studies by Haghighi *et al.* (2006) indicate that differences in the Ag used to test the immune stimulatory responses of probiotics can affect the significance of the results. Likewise, studies by Koenen *et al.* (2004) described the same strain of bacteria had different effects on the GI tract and immune system of birds depending on their genetics and age, suggesting that the different types of birds (layer versus broiler) may require different doses of probiotics at different intervals.

Summary

It is likely that the beneficial effects of probiotics are the result of the summation of a complex, multi-variate series of alterations in gut microbial and whole body metabolism. Those alterations might include whole body and immune function, feed consumption, absorption of nutrients and beneficial changes in intestinal architecture. The data described demonstrate also that some probiotic species can communicate with the epithelial cells and/or the immune system, modulating tissue physiology and response to host's infection. Probiotic products may be alternatives to growth promoting antibiotics. More research, however, is needed to fully define the mechanisms of probiotic effects on the body before they can be employed by the industry in a consistently efficacious manner.

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Abbreviation Key: DFM-direct-fed microbial, GI-gastrointestinal, IL-interleukin, PUFA-polyunsaturated fatty acid, SEM-scanning electron microscopy, SAL-salinomycin, SFB-segmented filamentous bacteria, VFA-short chain volatile fatty acids