

The role of the commensal gut microbial community in broiler chickens

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To understand the relationship between the gastrointestinal inhabiting microbial community and broiler health, a literature review is presented. The available information on the development of gut microbial community, the relationship between commensal microflora and digestive function, the role of gut microorganisms on competitive exclusion of chickens against pathogens, and modulation of the gut microbial community by addition of prebiotics to the diet is summarized. Gut dominant microbial communities become more complex as broilers grow older. The establishment of the dominant bacterial community is affected by dietary and host-related factors. Dietary prebiotics can modulate bacterial community shift towards non-harmful bacteria, which is beneficial for the health of broiler chickens. Gut commensal microorganisms play an important role in the prevention of colonization by pathogens in the gastrointestinal tract of chickens, a process known as competitive exclusion. In conclusion, the dilemma caused by the forthcoming ban of antibiotics feed additives and need to maintain the intestinal health of broiler chickens, has produced an enormous interest in finding alternatives. Modulating the intestinal microbial community in a healthy direction, by dietary ingredients such as prebiotics, could be a good solution.

Keywords: gut microbial community; competitive exclusion; gut mucosal cell proliferation; prebiotic; broiler chickens

Introduction

The selection of broilers for increased growth rate may have inadvertently resulted in changes in gastro-intestinal development during growth of the animal. Some other negative effects can also occur at times, such as metabolic disorders, poor immunocompetence and increased susceptibility to pathogens (Tottori *et al.*, 1997). For these fast growing animals, it is generally accepted that the use of a sub-therapeutic dose of antibiotics added to the diet led to improvements both in health and productivity. However, such additives have also resulted in some other unexpected consequences.

Gut microbial community and broiler chickens: Y. Lan et al.

There have been reports about increases in the incidence of antibiotic-resistant microbial pathogens (van der Waaij and Nord, 2000). Potentially, such findings could have severe negative effects on public health (Shea, 2003).

The gut lumen and mucosal surface of the intestines and caecae of broiler chickens are major sites for colonization by bacteria and other microbes. The presence of antibiotics may alter the gastrointestinal tract (GIT) ecosystem. The use of sub-therapeutic doses of antibiotics in a broiler diet may possibly affect the stability of intestinal microflora (Ohya and Sato, 1983), and can reduce the population of *Lactobacillus* in the intestines (Engberg *et al.*, 2000; Ane Knarreborg *et al.*, 2002).

In poultry production, the ban on the use of antibacterial feed additives is increasing interest in realizing the role of commensal microbial community on health. Understanding the role of gut microorganisms in nutrition and health is vital for achieving future sustainability and for improving the efficiency and environmental acceptability of poultry production. Replacements for unacceptable chemical (*e.g.* antibiotic) based approaches are particularly needed. Research results of development of gut microbial community, the relationship between commensal microflora and digestive function, the role of gut microorganisms on competitive exclusion of chickens against pathogens, and modulation of the gut microbial community by addition of prebiotics to the diet are summarized in this review.

Gut microorganisms development

The intestinal microflora comprises a diverse collection of microbial species in broiler chickens. For many years, the assessment and identification of commensal bacteria has been based on traditional culturing techniques. Culturing techniques used to profile a bacterial community inadvertently select for some organisms while excluding others. To overcome the difficulties in culturing of individual microbes, modern approaches analyze the structure of bacterial communities by determining the characteristic features of the microbial DNA extracted from the community samples (Apajalahti *et al.*, 2001; Tannock, 2001; Knarreborg *et al.*, 2002; Gong *et al.*, 2002; Lan *et al.*, 2002; van der Wielen *et al.*, 2002; Zhu *et al.*, 2002; Hume *et al.*, 2003; Zhu and Joerger, 2003; Amit-Romach *et al.*, 2004). Using such techniques, it has been found that 90% of the bacteria in the chicken gastrointestinal tract represent previously unknown species (Lan *et al.*, 2002).

Earlier culture-based studies found that host factors such as age influence the composition of the caecal microbiota (Mead and Adams, 1975). Knowledge based on these methods indicates that the normal intestinal microflora of the small intestine, caecum and large intestine in chickens develop after hatching. Bacteria have not been detected in any of GI sites at hatching (Day 1), but by Day 3, significant numbers of *faecal streptococci* and *coliforms* have been isolated from all sites of the GIT (Barnes *et al.*, 1980; Coloe *et al.*, 1984). The microbial community is established in the small intestine within approximately two weeks. The community of the small intestine is limited to *faecal streptococci* and *coliforms* for the first 40 days, and then *lactobacilli* become established and dominant (Smith, 1965; Coloe *et al.*, 1984). In contrast, the caecal microbial community is established at a later age than that in the small intestine. Microbial communities establishment in the caecum needs 6-7 weeks (Coloe *et al.*, 1984). A large variety of both facultative and strictly anaerobic organisms colonize the caecum. *Faecal streptococci*, *clostridia*, *enterobacteria*, *pediococci*, and occasionally *Pseudomonas aeruginosa* have all been isolated. After the chick has been fed for one day, the numbers of lactobacilli in the caeca are quite variable; by the third day, however, large numbers of bacteria are present throughout the alimentary tract. Many of these species are only

Gut microbial community and broiler chickens: Y. Lan et al.

transient; after 40 days the flora becomes stable and consists predominantly of *faecal streptococci*, *Escherichia coli*, *Bacteroides spp.* and *Lactobacillus spp.* (Barnes *et al.*, 1980; Coloe *et al.*, 1984).

Molecular assessment results are in agreement with the observations obtained from culture techniques, with regard to population increased and became more complex when broiler chickens grew older (van der Wielen *et al.*, 2002). Analysis of caecal contents based on molecular techniques showed that the microbial community is somewhat different to the contents based on culture analyses. As for adult broilers, *Clostridiaceae*-related sequences (65%) were the most abundant group detected in the caecum, with the other most abundant sequences being related to *Fusobacterium* (14%), *Lactobacillus* (8%), and *Bacteroides* (5%). Sequences of members of the *Bacteroides* group, the *Bifidobacterium infantis* subgroup, and of *Pseudomonas spp.* each accounted for less than 2 % of the total. Microflora are also harboured in the ileum. Nearly 70% of sequences from the ileum were related to those of *Lactobacillus*, with the majority of the rest being related to *Clostridiaceae* (11%), *Streptococcus* (6.5%), and *Enterococcus* (6.5%) (Xiang *et al.*, 2002; Jiangrang *et al.*, 2003). As for young chicks (under 14 d) the major species present in the small intestines and caeca was *Lactobacilli* (Amit-Romach *et al.*, 2004). This is quite different from the results of traditional culturing techniques (Smith, 1965; Coloe *et al.*, 1984). As for *Lactobacillus*, the sequence analysis results of different study had some diversity (Lan *et al.*, 2002; Zhu and Joerger, 2003; Amit-Romach *et al.*, 2004). This could be caused by host, environment and diet factors (Apajalahti *et al.*, 2001; van der Wielen *et al.*, 2002).

In summary, the early stage of post-hatch is a critical period for the establishment of the gut microbial community in broiler chickens. This process starts from a nearly sterile gastrointestinal environment at the moment of hatching. The advances in ribosomal DNA-based molecular techniques make it possible to identify different bacterial populations in intestinal contents and mucosal samples without cultivation, and helpful on monitoring the effect of dietary ingredients on the changes of digestive bacterial communities in broiler chickens.

Relation of commensal gut microorganisms with the nutrient digestion, digestive enzymes and gut mucosal proliferation

The microbial community is distributed along the entire gastrointestinal tract (GIT) of broiler chickens, and the caecum is the major region for anaerobic bacteria (Barnes *et al.*, 1980). The fermentative part of the microbial activity yields mainly lactic acid and short-chain fatty acids (SCFA). The microbial populations which are in the caeca stimulate reabsorption of water (Mead, 1989). Here also, non-protein nitrogen may be present in the caeca by means of retrograde peristalsis. Such compounds are degraded by and incorporated in caecal microorganisms (Braun and Campbell, 1989; Mead, 1989), and urinary nitrogen is likely to be recovered (Mattocks, 1971).

The bacterial activities of the GIT can affect the nutrient utilization of birds. The large numbers of GIT microbes need and consume energy. This normally comes from feed and they may therefore use some of the energy which would otherwise be available for the chicken. The microflora can also benefit the host bird by producing energy in the form of SCFA. This release of SCFA from fermentation of non-hydrolysable oligo- and polysaccharides, may provide extra energy to the bird, which was otherwise unavailable, and this can mean a better feed conversion ratio. This would be particularly so when the birds are fed a high-fibre diet which may otherwise be limiting in energy (Hegde *et al.*, 1982; Muramatsu *et al.*, 1991). However, when dietary energy is supplied in the form of

Gut microbial community and broiler chickens: Y. Lan et al.

substrates which are easily digestible by the animal itself, the efficiency of energy utilization may be reduced due to the presence of the GIT microflora. This competes with the host enzymes for substrates (Muramatsu *et al.*, 1994). Therefore, the presence of microorganisms can either improve or limit metabolizable energy (ME) values of the diets. Not all energy digested in conventional chicks is necessarily utilized for growth, because the microflora will use a part of it for its own metabolism (Furuse and Yokota, 1984b). This suggests that although the gut microflora may have beneficial effects on the digestion of certain dietary components, it may also have a negative effect on utilization of ME by the host. Thus, it can be expected, chicks that harbour an active GIT microflora apparently have an increased energy requirement for maintenance. Meanwhile, the intestinal microbial community also affects dietary amino acid digestion. There was no difference in the residual methionine in the distal ileum between germ-free broilers and conventional birds. But intestinal bacteria significantly reduce the apparent absorption of L-2-hydroxy-4-methylthiobutoanic acid from the intestinal tract of broiler chickens (Drew *et al.*, 2003). Conventional birds have been shown to excrete higher amounts of endogenous amino acids than germ-free birds when fed high-fibre diet. This indicates that there is a substantial microbial synthesis of amino acids in the gut. (Parsons *et al.*, 1983). Faecal digestibilities of organic matter, crude fat, starch and amino acids, nitrogen retention and metabolizable energy were reduced when conventional chicks were fed on the highly methylated citrus pectin diet. Feeding the highly methylated citrus pectin diet to germ-free birds hardly affected faecal digestibility of nutrients and nitrogen retention (Langhout *et al.*, 2000).

The presence of the GIT bacteria can affect the activity of digestive enzymes. This influence relates to the bacterial metabolic end-products, and their effect on gut mucosal morphology and on intestinal cell proliferation in chicken (Palmer and Rolls, 1983). For example, the activity of alkaline phosphatase is greater in the mucosal homogenates of conventional chicks compared with the GF birds. The concentrations of acid phosphatase and *iso*-citric dehydrogenase are however similar in the two groups (Palmer and Rolls, 1983). There is a tendency that proteolytic activity is increased and the amylase activity is decreased in conventional chicks compared with GF animals (Philips and Fuller, 1983). On the other hand dietary prebiotic may improve the activities of amylase and total protease via affecting gut microbial communities (Xu *et al.*, 2003). This might be because the *Bifidobacterium* and *Lactobacillus* which were stimulated by dietary prebiotic delivered enzymes, such as increasing the intestine digestive enzyme activity (Sissons, 1989).

The dietary composition, and microflora, as well as the interaction between the diet and microflora can affect the intestinal development, mucosal architecture and the mucus composition of the GIT. Therefore, it appears that the functional characteristics of the intestinal tract are altered by the microflora. The absolute and relative weight (weight/kg body-weight) of all intestinal sections are greater in conventional birds compared with their GF counterparts, when chickens receive average and higher concentrations of protein in the diet (Furuse and Yokota, 1984a; Muramatsu *et al.*, 1988; Muramatsu *et al.*, 1993). Protein synthesis in the intestines is enhanced by the presence of the gut microflora in terms of both fractional (%/d) and absolute rates [mg/ (100g BW.d)] (Muramatsu *et al.*, 1988). Fermentable carbohydrates can increase the weight of the gut; this is associated with their stimulating role on a normal microflora (Komai and Kimura, 1980; Goodlad *et al.*, 1989; Muramatsu *et al.*, 1993). Considerable amounts of SCFA are produced by microbial fermentation in the hindgut. Most of the anions in the caecal contents are SCFA, mainly acetate, propionate and butyrate. It has been suggested that SCFA can accelerate gut epithelial cell proliferation, thereby increasing intestinal tissue weight, which will result in changes of mucosal morphology (Le Blay *et al.*, 2000; Fukunaga *et al.*, 2003). Several mechanisms are involved in the growth-stimulating role of SCFA on animal

Gut microbial community and broiler chickens: Y. Lan et al.

intestines. Luminal and systemic SCFA stimulate mucosal proliferation by increasing plasma glucagon-like peptide 2 (GLP-2) and ileal pro-glucagon mRNA, glucose transporter (GLUT2) expression and protein expression, which are all signals which can potentially mediate SCFA-induced mucosal proliferation (Tappenden and McBurney, 1998). The trophic effects of SCFA infused in the colon on the jejunum are mediated in part by gastrin (Reilly *et al.*, 1995). SCFA infused into the colon act systemically to improve jejunal structure and increase jejunal gastrin concentrations in rats. Gastrin receptor blockade abolished the effects of SCFA on jejunal DNA, protein, crypt cell proliferation, and gastrin. Gastrin blockade does not reduce SCFA-induced augmentation of villous height or crypt depth. Le Blay *et al.* (2000) reported that at a low concentrations (0.05 and 0.1 mM) butyrate mildly stimulated cell proliferation. At 0.5 mM no modulation was observed, and ≥ 1 mM-butyrates dose-dependently inhibited cell proliferation. In an established cell line ≥ 0.05 mM-butyrates inhibited cell proliferation. Collagenous and non-collagenous protein synthesis in mucosa are both stimulated by butyrate. Thus, butyrate, which is produced by dietary fibre fermentation, may affect intestinal muscles by directly acting at the molecular level on myocytes. Luminal pH has a significant inverse correlation with the percentage of cells in the S phase (a cell activity phase in which DNA is actively synthesized) in the caecum, proximal colon, and distal colon (Lupton *et al.*, 1985). Transepithelial gradients of SCFA generate pH gradients across the colonic epithelium. Extracellular pH regulation occurs in two separate microdomain surroundings: (a) colonic crypts: the crypt lumen and (b) the sub epithelial tissue adjacent to crypt colonocytes. Physiological SCFA gradients cause polarized extracellular pH regulation because epithelial architecture and vectorial transport work together to establish properly regulated microenvironments (Chu and Montrose, 1995).

As living beings, the GIT bacteria need nutrients for maintenance and development, and therefore compete with the host animal for some available nutrients. However, their unique role of metabolizing non-digestible carbohydrates cannot be taken over by the animals' own intestinal enzymes. So this can be of help for the animals energy supply because fermentation end-products, such as some SCFA, can be of benefit for the host animal.

Modulation of the intestinal microbial community by dietary prebiotics

The intestinal ecosystem contains a large diversity of bacterial cells that perform different functions which influence the host. Some specific components of the intestinal microflora, especially *lactobacilli* and *bifidobacteria*, have been associated with beneficial effects for the host, such as: (1) promotion of gut maturation, (2) gut integrity, (3) antagonisms against pathogens, and (4) immune modulation (Carter and Pollard, 1971; Berg and Savage, 1975; Tlaskalova-Hogenova *et al.*, 1980; Tlaskalova-Hogenova *et al.*, 1983; Tlaskalova-Hogenova *et al.*, 1994). In addition, the microflora seems to play a significant role in the maintenance of intestinal immune homeostasis and prevention of inflammation (Powrie, 1995). Young birds obtain microorganisms from the outside environment via feed and water, and this microflora is constantly, though indirectly, exposed to the external environment. Diet turned out to be the strongest determinant of the gut bacterial community structure. Both the source of feed and local feed amendment changed the bacteriological profile significantly, whereas profiles of individual farms with identical feed regimens hardly differed from each other (Apajalahti *et al.*, 2001; Hume *et al.*, 2003). Therefore it should be possible to shift the microbial community from harmful to non-harmful bacteria by dietary ingredient (Gibson and Roberfroid, 1995; Gibson, 1998; Collins and Gibson, 1999; McBain and MacFarlane, 2001; Glunder, 2002; Schiffrin

Gut microbial community and broiler chickens: Y. Lan et al.

and Blum, 2002). “Prebiotics” are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and /or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health (Gibson and Roberfroid, 1995). Any food ingredient that enters the large intestine is therefore a potential prebiotic. However, to be effective, selectivity of fermentation is essential. Most current attention and successes have been derived using non-digestible oligosaccharides, especially those that contain fructose, xylose, galactose, glucose and mannose (Gibson and Roberfroid, 1995; Gibson, 1998). It has been reported that oligosaccharides and polysaccharides are specifically utilizable by bifidobacteria (Yazawa *et al.*, 1978). And the prebiotic characteristics of fructooligosaccharide (FOS) in this regard for humans, have been well demonstrated (Gibson, 1998). In broiler chickens, the numbers of gut *Bifidobacterium* and *Lactobacillus* are increased by the administration of prebiotics in the diet (Xu *et al.*, 2003). Dietary soy soluble carbohydrates can increase the survival population of lactic acid bacteria in caecal contents after the birds were infected with *E. tenella* (Lan *et al.*, 2004).

The competitive exclusion role of GIT commensal microorganisms against invading pathogens

In this review, “competitive exclusion” is defined as any action by the GIT commensal microorganisms which resist colonization by invading pathogens, such as occupation of attachment sites on the intestine mucosal surface, competition for nutrients, or the release of bacteriocins.

Newly hatched chicks are particularly vulnerable to invasion by pathogens before their intestinal microbial communities become established. Therefore, administration of intestinal microflora of adult chicken to chicks during the early period after hatching is applied worldwide as a prophylactic method to control some pathogens infection in poultry. This practice was introduced some decades ago (Weinack *et al.*, 1979). The microbiological strategies using adult intestinal microflora are such that they may increase resistance to pathogens in young chicks, but may be ineffective in older hens (Corrier *et al.*, 1993). In young chicks, administration of gut microflora has been shown to be effective against several pathogen species, such as *Yersinia enterocolitica* (Soerjadi-Liem *et al.*, 1984), *Campylobacter fetus subsp. jejuni* (Stern, 1994), *Salmonella typhimurium* (Mead, 2000), *Salmonella kedougou* (Ferreira *et al.*, 2003), *Salmonella infantis* (Goren *et al.*, 1984), *Escherichia coli* (Snoeyenbos *et al.*, 1982) and *Listeria monocytogenes* (Hume *et al.*, 1998). The safety and efficacy of competitive exclusion products have been proven in large-scale trials (Salvat *et al.*, 1992). However, this practice is not effective for the practical control of fowl typhoid (Silva *et al.*, 1981). It is effective to use competitive exclusion products which either derived from a suspension or anaerobic cultures of gut contents of healthy adult birds as a prophylactic treatment rather than as therapeutic agents (Watkins and Miller, 1983).

Dietary ingredients, such as some fermentable carbohydrates, can stimulate the growth of the intestinal microflora and can promote the competitive exclusion abilities of chicks (Schoeni and Wong, 1994; Kubena *et al.*, 2001; Lan *et al.*, 2004).

Prevention of colonization by pathogens in the GIT by a commensal or administrated microflora can be achieved through several different mechanisms. Firstly, pathogens that invade the gastrointestinal tract of chicks must penetrate the epithelial layer before infecting the birds. Administration of bacterial communities to newly hatched chicks can lead to early colonization of adherent bacteria on the gut mucosal surface, forming a mat of microflora and occupying the adhering niches (Soerjadi *et al.*, 1982; Stavric *et al.*,

Gut microbial community and broiler chickens: Y. Lan et al.

1991). Furthermore, undefined microbial populations and strains freshly isolated from washed caeca, show hydrophobic properties, which improves the efficacy of protective mixtures (Stavric *et al.*, 1991). Secondly, competition for nutrients between native bacteria and invading pathogens is a limiting factor for the colonization of the invading pathogens. Thirdly, some commensal bacteria produce bactericidal or bacteriostatic products. Finally, the endogenous microflora seems to have a capacity to modulate cellular mechanisms related to host defences and homeostasis. Of these possible mechanisms, only the first one is tested and proven in chicks, while the others need further research.

Furthermore, the gastro-intestinal tract is an area by which the host animal is in very close contact with its environment. Therefore, the gut-associated lymphoid tissue (GALT) is the first line of defence by which animals resist invasion of pathogens from that environment. GALT is distributed along the entire intestine of broilers. It plays an important role in making specific antibodies for broilers (Befus *et al.*, 1980). Microbial communities, which inhabit the intestinal tract, can stimulate these immune responses and therefore act to strengthen the host's defence mechanisms. Also gut lactic acid bacteria have been shown to have immunostimulatory properties by cell wall components such as peptidoglycans, polysaccharide and teichoic acid (Takahashi *et al.*, 1993; Haller *et al.*, 1999). Therefore, an increased lactic acid bacterial population by means of using prebiotics in the diet of broiler chickens would enhance the natural immune competence ability of the animals by increasing the natural antibody levels, and further to promote the immune defence responsibility. The possible effect of prebiotics on the natural immune competence ability and the possibility of alternating antibiotics with prebiotics in diet of broiler chickens need further study.

For a long time, broiler chickens have been selected by growth rate and feed intake, but they have a weak immune system. The dilemma caused by the forthcoming ban of antibiotics feed additives and the need to maintain the intestinal health of broiler chickens, has sparked off an enormous interest in finding alternatives. Modulating the intestinal microbial community in a healthy direction by dietary ingredients, such as prebiotics, could be a good solution.

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Gut microbial community and broiler chickens: Y. Lan et al.

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Gut microbial community and broiler chickens: Y. Lan et al.

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Gut microbial community and broiler chickens: Y. Lan et al.

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