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Impact and Manipulation of Gut Microflora in Poultry: A Review

S. Adil and S.N. Magray
Division of Livestock Production and Management,
Faculty of Veterinary Science and Animal Husbandry,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shuhama, Alusteng, 190006 Srinagar, India

Abstract: Gut microbial population of chicken mainly comprises of gram positive bacteria, most of which are facultative anaerobes from crop to terminal ileum while caeca additionally contain strict anaerobes. In chickens, the main site of bacterial activity are the crop and the caeca and to a lesser extent, the small intestines. Bacterial fermentation of most non-digestible carbohydrates occurs in the crop and caeca resulting in the production of Short-Chain Fatty Acids (SCFAs) which may provide extra energy to the bird. SCFAs can accelerate gut epithelial cell proliferation thereby increasing intestinal tissue weight resulting in changes of mucosal morphology. Certain beneficial bacteria create a microenvironment hostile to other bacterial species by producing antimicrobial metabolites, a process known as competitive exclusion. The digestive tract of birds also contains pathogenic such as Salmonella, *Escherichia coli*, *Clostridium perfringens*, etc. which produce harmful substances like endotoxins. These endotoxins cause fever and the release of endogenous pyrogens which act on thermoregulation centres in the hypothalamus. The development of gut and the competitiveness of beneficial and harmful bacteria can be altered by dietary manipulation (enzymes, prebiotics, probiotics, mannan oligosaccharides and symbiotics) which can alter not only gut dynamics but also many physiologic processes due to the end products metabilosed by symbiotic gut flora.

Key words: Chicken, gut microflora, dietary manipulation, physiologic process, bacterial, India

INTRODUCTION

The gut flora are the microorganisms that normally live in the digestive tract and can perform a number of useful functions for their host. The unicellular micro organisms viz. bacteria (which are paramount), fungi and protozoans comprise the digestive flora of poultry. From the microbiologist's standpoint, the intestine can be divided into three sections: the duodenum and small intestine where the numbers of bacteria are relatively low, generally $<10^8$ g^{-1}; the caeca where a considerable microbial fermentation occurs and the number of bacteria present is approximately 10^{11} g^{-1} (wet weight) and the large intestine which in most birds is relatively short and is a posterior extension of the small intestine beginning at the level of the caeca and opening into the cloaca. The organisms present include those from both the small intestine and the caeca. Large populations of chicken gut bacteria are gram positive and mainly include facultative anaerobes from the crop to the terminal ileum while caeca additionally contain strict anaerobes which are dominant (Fuller, 1984; Gong *et al*., 2002). In chickens, the main site of bacterial activity are the crop and the caeca and to a lesser extent, the small intestines. The pH and transit time of different sections of chicken selectively allow establishment of a specific microbial population in chicken. The microorganisms of the digestive flora may be located in the gut lumen, buried in the mucus layer or adhering to the digestive mucosa where they can form very important cell layers (Fuller, 1984). Bacteria have not been detected in any of gastro-intestinal sites at hatching (day 1) but by day 3, significant numbers of faecal streptococci and coliforms have been isolated from all sites of the gastro intestinal tract (Barnes *et al*., 1980; Coloe *et al*., 1984). The microbial community is established in the small intestine within approximately 2 weeks. The caecal as well as intestinal flora undergoes changes and diversifies with age (Knarreborg *et al*., 2002; Lu *et al*., 2003). The microbial community of the small intestine is limited to faecal streptococci and coliforms for

Corresponding Author: S. Adil, Division of Livestock Production and Management,
Faculty of Veterinary Science and Animal Husbandry,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng,
190006 Srinagar, India

the 1st 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 stage than that in the small intestine and needs 6-7 weeks (Coloe *et al*., 1984).

IMPACT OF GUT FLORA COMMUNITIES

Digestive effects: The conventional birds have been shown to excrete higher amounts of endogenous amino acids than germ-free chicken when fed high fibre diet, indicating substantial microbial synthesis of amino acids in the gut (Parsons *et al*., 1983). Most non-digestible carbohydrates are fermented by the microflora in the crop but mainly in the caeca (Mead, 1989) resulting in the production of Short-Chain Fatty Acids (SCFAs) which may provide extra energy to the bird which was otherwise unavailable, this can mean a better feed conversion ratio (Gabriel *et al*., 2003). The microorganisms of the digestive tract have a positive effect by releasing nutrients that the host can absorb in the intestine and the caeca, the latter also being able to transport carbohydrates and amino acids. Gut microbes cause deconjugation of bile salts which results in reduced digestibility of saturated fatty acids such as palmitic and stearic fatty acids but have no effect on unsaturated fatty acids such as oleic and linoleic acids (Boyd and Edwards, 1976). The conventional chicken usually show a reduced growth compared to germ-free animals (Kussaibati *et al*., 1982; Furuse and Okumura, 1994) because gut microorganisms are in competition with the host for the use of available nutrients in the digestive tract particularly the feedstuffs that are poorly digested by the host. Because of the production of SCFAs, microflora contributes to the absorption of minerals, like sodium in the caeca and colon (Braun, 2003) and also synthesizes vitamin B, K and E but it is thought that only folic acid is available for the host (Coates, 1980).

Trophic effects: SCFAs can accelerate gut epithelial cell proliferation thereby increasing intestinal tissue weight resulting in changes of mucosal morphology. In the caeca, the presence of microorganisms induces a higher relative weight and a thicker wall (Furuse and Yokota, 1984). In conventional birds, intestinal villi are higher in the jejunum and ileum compared to germ free birds (Gabriel *et al*., 2003). 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 which are all signals which can potentially mediate SCFA-induced mucosal proliferation.

Suppression of pathogenic bacteria: Certain beneficial bacteria create a microenvironment hostile to other bacterial species by producing antimicrobial metabolites (competitive exclusion). The term Competitive Exclusion (CE) is used to describe the inability of one population of microorganisms to colonize the gut because of the presence of another population of microorganisms and was first described as a method of preventing pathogen colonization of the avian gastrointestinal tract in 1973 (Nurmi and Rantala, 1973). In the crop, lactobacilli produce a large amount of lactic acid beneficial to them but deleterious to coliforms and most other bacteria (Fuller, 1984). Reuterin, secreted by *Lactobacillus reuteri* is effective against salmonellae, coliforms and campylobacters (Mulder *et al*., 1997). Beneficial bacteria also have an effect by modifying the receptors used by adverse bacteria or their toxins thereby hampering their development in the digestive tract (Rolfe, 1991).

Effect on immune system: The beneficial flora intervenes through the competitive use of essential nutrients (Rolfe, 1991) and participates in the development and maintenance of an effective intestinal immune system (Salminen *et al*., 1998). The chicken microbiota contributes to this because of their ability to activate both innate defense mechanisms and adaptive immune responses (Brisbin *et al*., 2008).

Harmful effects: The digestive tract of birds can house pathogenic such as Salmonella, some *Escherichia coli*, *Clostridium perfringens*, etc. Gram-negative bacteria produce endotoxins that are released during the lysis of the lipopolysaccharides that are part of their cell walls. These endotoxins cause fever and the release of endogenous pyrogens which act on thermoregulation centres in the hypothalamus. Other toxins may affect intestinal motility thereby causing diarrhoea. Fermentations, particularly of the amino acids by the digestive flora lead to the production of irritating components, like ammonia which cause conjunctivitis and results in respiratory problems in chicken (Thomke and Elwinger, 1998). Ammonia is considered as the most harmful gas in a poultry house. Broiler feed consumption and feed efficiency has been shown to decrease during exposure to levels of NH_3, ranging from 25-125 ppm. Both pathogenic and supposedly commensal organisms can cause disease through initiation of an inflammatory response (Kelly *et al*., 2004). The host surveillance system is activated and inflammation occurs when certain organisms or their metabolites (enterotoxins) attach to or transgress the flora/host barrier. The extent of the disease process that follows an initial inflammatory response is influenced by several determinants. While any antigen

can cause gastrointestinal disease by initiating an inflammatory response, pathogenic organisms have evolved three specific disease-causing mechanisms:

- Enterotoxigenic organisms produce toxins that kill cells (e.g., α-toxin from *Clostridium perfringens*) or upset cell function (e.g., shiga toxins from *Escherichia coli*)
- Enteroinvasive organisms like *E. coli*, some *Salmonella* species and Listeria monocytogenes are able to transgress the flora/host barrier by stimulating endocytosis by epithelial cells, initiation of inflammatory cytokine release and causing cell death
- Enteropathogenic *E. coli* causes typical Attaching and Effacing (A/E) lesions characterized by adhesion, microvillus destruction and gross cytoskeleton reorganization (Frankel *et al*., 1998; Donnenberg *et al*., 1997; Donnenberg, 2000)

MANIPULATION OF GUT FLORA

By dietary means it is possible to affect the development of the gut and the competitiveness of beneficial and harmful which can alter not only gut dynamics but also many physiologic processes due to the end products metabilosed by symbiotic gut flora. Additives such as organic acids, enzymes, prebiotics, probiotics, mannan oligosaccharides and symbiotics are now extensively used throughout the world. These dietary additives prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance of the bird are improved (Ravindran, 2006).

Ricke (2003) reported that organic acids affect the integrity of microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism causing bactericidal effect. Addition of supplemental organic acids to the diets of broiler chicken cause a significant reduction in caecal viable and coliform counts (Adil *et al*., 2011).

The effects of enzymes on gut microflora were classified by Bedford (2000) into two phases: an ileal phase and a caecal phase. In the ileum, enzymes simply reduce the number of bacteria by increasing the rate of digestion and limiting the amounts of substrates available to the microflora. In the caecal phase, enzymes produce soluble, poorly absorbed sugars which feed beneficial bacteria. The Volatile Fatty Acids (VFAs) produced by such bacteria may be of benefit not only in controlling populations of *Salmonella* species and perhaps, *Campylobacter* species but also in providing an energy source for the bird (Snel *et al*., 2002).

Two basic mechanisms by which probiotics act to maintain a beneficial microbial population include competitive exclusion and immune modulation. Competitive exclusion involves competition for substrates, production of antimicrobial metabolites that inhibit pathogens and competition for attachment sites. By directly interacting with gut mucosal immune system, probiotics can modulate either innate or acquired immunity or both (Dugas *et al*., 1999). Further, specific immune modulatory effects of probiotics are dependent on the strain or species of bacteria included in the probiotics (Endens, 2003; Huang *et al*., 2004).

Gibson and Roberfroid (1995) defined a prebiotic as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract thus, improving the host's microbial balance. The growth of endogenous microbial population groups such as bifidobacteria and lactobacilli is specifically stimulated and these bacteria species are perceived as beneficial to animal health. Prebiotics have the advantage, compared with probiotics that bacteria are stimulated which are normally present in the GIT of that individual animal and therefore already adapted to that environment (Snel *et al*., 2002).

Mannanoligosaccharides, derived from yeast cell wall are components of the outer layer of yeast cell walls and their components include proteins, glucans and phosphate radicals as well as mannose (Klis *et al*., 2002). Three major modes of action by which broiler performance is improved by MOS are proposed: control of pathogenic or potential pathogenic bacteria which possess type-1 fimbriae (mannosesensitive lectin), immune modulation and modulation of intestinal morphology and expression of mucin and brush border enzymes (Ferket, 2004).

A symbiotic is in its simplest definition, a combination of probiotics and prebiotics (Collins and Gibson, 1999; Schrezenmeir and de Vrese, 2001). Bengmark (2001) regards symbiotics as products of fermentation. This combination could improve the survival of the probiotic organism because its specific substrate is available for fermentation. This could result in advantages to the host through the availability of the live micro-organism and the prebiotic.

CONCLUSION

In this study, the intestinal flora undergoes changes and diversifies with age. The gastrointestinal tract contains within it a microenvironment of bacteria that influences the host animal in many ways. The microflora can metabolize several nutrients that the host cannot

digest and converts these to end products (such as short-chain fatty acids), a process that has a direct impact on digestive physiology. Useful microbes in gut play a positive role in controlling the gut flora and stimulate the development of the gut wall. The microbiota helps educate the immune system, affects the integrity of the intestinal mucosal barrier, modulates proliferation and differentiation of its epithelial lineages and plays a key role in extracting and processing nutrients consumed in the diet. By dietary additives like enzymes, prebiotics, probiotics, mannan oligosaccharides and symbiotics, it is possible to affect the development of the gut and the competitiveness of beneficial and harmful bacteria which can alter not only gut dynamics but also many physiologic processes due to the end products metabilosed by symbiotic gut flora.

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