ROLE OF GUT MICROBIOME IN IMPROVING ANIMAL HEALTH AND PRODUCTIVITY

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Abstract

The gut microbiome comprises of a complex combination of microbes which includes archaea, bacteria, fungi, protozoa, helminths and viruses. The mammalian gastro-intestinal tract harbors this complex ecosystem in equilibrium with the host immune system. This organized and long-lasting process of colonization by microorganisms has developed into flourishing relationships creating diverse environment within the host gut. The most magnificent feature of this commensal relationship is that the host requires commensal microorganisms to get colonized in gastrointestinal tract (GIT) for its own development and health. The present review article will focus on various aspects of gut microbial colonization and their influence on the host organ systems in relation to its role in various morphological changes occurring within the host leading to improvement in health and productivity of animals.

Key words: Gut microbiome, Gut-organ axis, Morphological changes, Productivity

Introduction

A diverse microbial population colonizes the in-utero sterile mammalian gastrointestinal tract during and after the birth. This organized and long-lasting process of colonization by microorganisms has developed into flourishing relationships creating diverse environment within the host gut (Mazmanian *et al.*, 2005). There are various factors that affect the optimum colonization of gut microbiome and their normal functioning on various organ systems of the host body directly or indirectly. The gut microbiome comprises of a complex combination of microbes (Mezouar *et al.*, 2018). The mammalian gastro-intestinal tract harbors this complex ecosystem in equilibrium with the host immune system. The most magnificent feature of this commensal

relationship is that the host requires the commensal microorganism to get colonized in their gastrointestinal tract for their own development and health. The process of colonization is a two-way interaction between the host and the microorganisms.

Colonization of gut microbiota in the host gastrointestinal tract

During intra-uterine life, the gastrointestinal tract (GIT) of the developing fetus is almost sterile that gets colonized by microorganisms gradually during and after birth. This colonization is a two-way interaction between the host and the microbiome. The host factors such as intestinal pH, retention of food particles in the gut and host immune system and the

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microbial factors like adhesion with the gut wall, survivality in the diverse environment within the gut and nutrient dependence as well as external factors such as diet and antibiotic treatment during early life, all combine to affect the gut colonization. Gut microbes of ruminants, specially the rumen microbiome, provide 70% of their daily energy requirement from the fermentation of indigestible dietary substrates. Therefore, studies in ruminant gut microbiota have focused mainly on the rumen to understand how this microbiome affects meat and milk production (Malmuthuge *et al.*, 2015).

Rumen microbiota is involved in the fermentation of complex carbohydrates, and their composition is influenced by a number of factors. Rumen microbial composition varies significantly depending on the species, diet, host age, season, and geographic region. Bacteria dominate the ruminal microbial population and responsible for the production of volatile fatty acids (VFAs) and microbial proteins. Anaerobic bacteria dominate the rumen of neonatal ruminants by the second day of life (10^9 CFU/mL of rumen fluid) and the density of cellulolytic bacteria is stabilized (10^7 CFU/mL of rumen fluid) within the first week of life. The establishment of other microbial groups is delayed until after bacterial establishment (Fonty *et al.*, 1987).

Anaerobic fungi and methanogens appear in the neonatal rumen between 8 and 10 days postpartum (Fonty *et al.*, 1987), while protozoa appear only after 15 days postpartum (Fonty *et al.*, 1988). The early rumen microbiote consists of bacterial species from *Propionibacterium*, *Clostridium*, *Peptostreptococcus* and *Bifidobacterium*, while *Ruminococcus* species dominates the cellulolytic bacterial population (Fonty *et al.*, 1987). When bacterial population throughout the gut was analyzed, the rumen and large intestinal regions consisted primarily of *Bacteroidetes* and *Firmicutes*, while >95%

of the bacteria in the small intestine contents were composed of *Firmicutes*. In contrast, the mucosa-associated bacterial community in the small intestine is composed of primarily *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, including 17 genera that are unique to this region of the intestine (Malmuthuge *et al.*, 2014).

Keynote factors affecting gut microbiome colonization

In animals, the host and the microbiome can have a symbiotic as well as pathogenic association. Symbiotic association is beneficial to both the host and the microbes. The symbionts need not to completely depend on the host and on the other hand, the host can have different symbionts at different times. This host-microbe interaction is affected by a number of factors that involves the immune system of the host, nutrition, reproduction and many others (Bahrndorff *et al.*, 2016). The climate and seasonal changes also influence the gut microbiome. A strong variation in the gut microbiota of chicken has been observed depending upon the season where the microbial population in summer was double than that of in winter (Carrasco *et al.*, 2019). On the other hand, many studies reported differences in the gut microbiota profiles of poultry according to geographic location (Pandit *et al.*, 2018). The farm managemental factors also play a key role in influencing the gut microbiome population.

Gut microbiome and host digestive system

The main function of the gastrointestinal tract microbes is digestion of ingested food substrates. The symbiotic relationship between the microbes and their host primarily involves acquisition of nutrients. However, beyond digestion, GIT microbes perform other functions that potentially contribute to the overall health status of the host (Hanning and Diaz-Sanchez, 2015). Hooper *et al.* (2001) reported that *Bacteroides thetaiotaomicron*

modulates 71 intestinal genes involved in various processes including intestinal maturation and nutrient absorption. Experiments have suggested the role of gut microbiome in digestion and metabolism of nutrients and thus helps in weight management in human and animals. *Lactobacillus* spp. are found to be involved in the digestion of complex carbohydrates not digested by the host and also participate in the degradation of lipids and simple sugars. Moreover, *Lactobacillus* sp. are able to survive in the gut at low pH and bile and further produce certain antimicrobial agents with the help of which they reduce the number of bacteria in the gut, thus significantly affect the gut microbiome and consequently on weight change (Drissi *et al.*, 2017).

Drastic differences are observed in germfree animals. Rekecki *et al.* (2009) reported reduction of mucosal linings due to absence of microbial stimulation of mucosa production in germfree sea bass (*Centropomus striata*). The intestinal villi of the germfree dog have been reported to be of same length as the normal dog but with thinner and pointer tips (Heneghan *et al.*, 1979). The lamina propria of germfree mice had a sparse stroma, with few lymphocytes and macrophages, and the Peyer's patches were smaller. In all germfree animals studied as reported by authors, the turnover rate of intestinal epithelium was decreased. These changes in the histology of intestine are the results of a reduced interaction with the bacteria that stimulate the immune system and histological development (Fasano and Sheadonoghue, 2005). Apart from histological changes in the intestine, changes in other organs have also been reported. The germfree rat had a smaller heart while the cecum was four to six times larger than rats with normal microbiomes (Gordon *et al.*, 1963). This change has been explained by an accumulation of substances normally degraded by the microbiome (Ishikawa *et al.*, 1989). In germfree chicks, a

change in the normal body temperature was noted which was slightly higher than that of chicks with normal microflora (Harrison and Hewitt, 1978).

These experiments suggest that the microbiome play a complex role in the host development. Age of the host also play a critical role in changing the microbial population. For example, the chicken gastrointestinal tract is sterile at hatch but gets quickly inhabited by aerobic *Proteobacteria* and after 12 days it gets dominated by anaerobic *Firmicutes*. Initially, the *Proteobacteria* stimulate the histological maturation of the gastrointestinal tract and thus provide an ideal environment for the *Firmicutes* to establish within the gut. The *Proteobacteria* grow well in the immature gastrointestinal tract but are poor competitors and are replaced after a mature and anaerobic gut environment is established. The successive colonization of bacteria also depends on nutrition and gut bacteria population selection. This establishment can also be selected by feeding specific foods. This was demonstrated in rabbits fed only milk for the first 42 days of life. These animals lacked the cellulolytic bacteria in the cecum and could not digest plant feeds (Padilha *et al.*, 1999).

Modification of intestinal microbiome can promote growth in many agricultural animals. Delivery of probiotic cultures to animals can enhance the development of the intestinal tract (Callaway *et al.*, 2008). The mature gastrointestinal tract improves nutrient uptake and increases the growth rate of the animal. Careful selection of probiotic bacteria is crucial as it can have a lifelong impact on intestinal health. Shirkey *et al.* (2006) reported differences in the gut histology and immune marker responses dependent on which bacteria initially colonized the gut of the pigs. The authors reported that the microbial diversity is important for healthy maturation of the entire intestinal tract.

Gut-microbiome-immunity axis in health

The mammalian immune system is a dynamic and remarkable organ which recognizes, responds and adapts countless foreign and self molecules thus acts as a primary force to the process of health and disease (Janeway *et al.*, 2001). Commensal bacteria have positive impact on development of gut-associated lymphoid tissues (GALT), intraepithelial lymphocytes and production of mucosal IgA (Hooper, 2004). Dendritic cells loaded with commensal bacteria that induce responses to commensals do not penetrate further than the draining mesenteric lymph nodes, immune induction to commensals is confined to mucosa, and thus the protective mucosal immunity to commensals is separated from systemic immunity (Macpherson *et al.* 2005). In this way the systemic immune system is kept completely ignorant of commensals.

The gastrointestinal tract represents a major gateway for infection by potential pathogens. Accordingly, the gut mucosa has evolved multiple layers of protection against invading pathogens. Epithelial cells have tight junctions and are covered with layers of mucus produced by goblet cells. The intestinal epithelium contains a large population of intra-epithelial lymphocytes (IELs), which recognize and eliminate infected or damaged epithelial cells. Paneth cells recognize microbial products via pattern recognition receptors, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors and contribute to innate immunity by secreting a diverse repertoire of antimicrobial proteins. In the lamina propria, dendritic cells (DCs) extend processes between the epithelial cells to continuously monitor the content of the gut lumen and activate lymphocytes. The lamina propria contains large numbers of effector lymphocytes even in the absence of disease. These include immunoglobulin A (IgA)-

producing plasma cells and $\gamma\delta$ T cells. In addition, recent studies have revealed that CD4⁺ T cells in the intestinal mucosa comprise significant numbers of Interleukin (IL)-17-expressing (Th17) cells. Furthermore, the mucosal immune system contains other unusual lymphocytes, such as IL-22-producing natural killer-like cells, which are positive for NK cell markers including Nkp46 but do not have the killing activity of MHC class I negative cells and CD4⁺CD3⁻ lymphoid tissue inducer cells (LTi cells), which are known to orchestrate the development of programmed and induced intestinal lymphoid tissues (Tanoue *et al.*, 2010).

All these cells remain in sufficient amount in the intestinal mucosa even under normal conditions as there is always a chance of deleterious responses to commensal microbiota. Shortly after birth, the bacterial microflora forms an ecosystem in dynamic equilibrium. This beneficial relationship can be maintained only when the host immune system discriminates the commensal organism from the pathogenic one, and thereby exerts immune regulation by inducing inhibitory molecules for innate immune signaling, as well as inducing the accumulation of regulatory T (Treg) cells.

Microbiota-gut-brain axis

It has been reported by Li *et al.* (2018) that the microorganisms and circadian clock genes can interact with each other and can regulate insomnia, circadian rhythm disorders and depression, host sleep and mental states through the microbiome-gut-brain axis. Emotion and physiological stress can also affect the composition of the gut microorganisms. The gut microbiome and inflammation may be linked to sleep loss, circadian clock misalignment, affective disorders, and metabolic disease. The microbiome-gut-brain (MGB) axis (Bercik, 2011) affects brain function through three pathways that produce a bidirectional flow of

information. The first of these is the immunoregulatory pathway, in which the microbiota interacts with immune cells in such a way as to affect the levels of cytokines, cytokinetic reaction factor, and prostaglandin E2 (Feng *et al.*, 2018). As a result, brain function is affected. The second is the neuroendocrine pathway. Raybould (2010) reported that there are more than 20 types of enteroendocrine cells in the intestine, which constitutes one of the largest endocrine organs in the body. Powley *et al.* (2008) observed that the gut microbiota regulates the secretion of neurotransmitters such as cortisol, tryptophan, and serotonin (5-HT) and thereby affect the hypothalamic-pituitary-adrenal (HPA) axis and the central nervous system (CNS). The third is the vagus nerve pathway, in which the enteric nervous system plays an important role. The sensory neurons of the intestinal myenteric plexus are exposed to the gut microbiota; these neurons form synaptic contacts with motor neurons in the intestine and thus regulate the intestinal motility and gut hormone secretion. The intestinal nervous system also forms synapses with the vagus nerve, which connects the intestine to the brain (Powley *et al.*, 2008) and constitutes an information transmission pathway that could be described as the gut microbiota-enteric nervous system (ENS)-vagus-brain pathway. Wang and kasper (2014) reported that the gut microbiome produces neurotoxic metabolites such as D-lactic acid and ammonia that may pass through the vagus nerve into the CNS and may effect the brain function, stress responses, and sleep structure. Similarly, the CNS can also regulate the intestinal microbiota composition through these three pathways.

Microbial interaction with the gut-lung Axis

Trompette *et al.* (2014) reported that dysbiosis in gut microbiota is associated with lung disorders and respiratory infections. Asthma in early life has been correlated with reduction in

genus *Bifido* bacteria and increase in Clostridia in the intestine (Kalliomaki *et al.*, 2001). Further studies have shown that the antibiotic intake depletes the population of certain species within the gut microbiota and thus influences lung diseases and allergic inflammation (Metsälä *et al.*, 2015). Looft and Allen (2012) further reported that removal of sensitive gut bacteria after administration of neomycin in mouse leads to an increase in the susceptibility to influenza virus infection in lungs.

Vice-versa phenomenon has also been reported where changes in lung microbiota may also influence the composition of gut microbiota. Looft and Allen (2012) had reported that influenza virus infection in the respiratory tract in mice increases *Enterobacteriaceae* and reduces *Lactobacilli* and *Lactococci* in the intestinal microbiota. Similarly, Sze *et al.* (2014) had reported that upon administration of lipopolysaccharide (LPS) in mice, the lung microbiota changes that further affects the gut microbiota due to movement of bacteria from lung into the bloodstream. Harris *et al.* (2006) stated that IgA positive plasma cells are in lesser numbers in neonates and germ-free animals. The immunity and IgA production enhances after colonization by gut bacteria. The T and B cells, induced in Peyer's patches, can move into the circulation and migrate to intestinal epithelium, bronchial epithelium and lymphoid tissues (Matsuno *et al.*, 2010). Such B-cells produce IgA which is transported to the mucosal surface and passes immunological information between different organs (Bingula *et al.*, 2017). Thus, the lymph and/or bloodstream acts as a link between the gut (where primary sensitization occurs) and the affected site on the lung (He *et al.*, 2017)

The role of gut microbiome as a virtual endocrine organ

The gut microbiome affects the host physiology. Manipulation of its composition influences the

host metabolism and body composition. The virtual endocrine organs also play a role in the regulation of the plasma concentrations of tryptophan, a key neurotransmitter and precursor to serotonin, within both the enteric and central nervous systems. The gut microbiota also controls the hypothalamic-pituitary-adrenal axis. This is evident from studies in microbiota-deficient germ-free animals which showed exaggerated responses to psychological stress that was further normalized by monocolonization with certain bacterial species including *Bifidobacterium infantis*. Therapeutic targeting of the gut microbiota may thus be useful in treating or preventing stress-related microbiome-gut-brain axis disorders and metabolic diseases (O'Collaghan *et al.*, 2016).

It has been reported that the gut microbiota plays a critical role in the production of norepinephrine and dopamine in the gut (Asano *et al.*, 2012). This production is due to the expression of β -glucuronidases by commensal gut bacteria, generating dopamine and norepinephrine through the cleavage of their inactive conjugated forms. Gut microbes can also produce non-noradrenergic, non-cholinergic transmitters such as nitric oxide, which plays a pivotal role in the regulation of gastric emptying (Orihata and Sarna, 1994), through the anaerobic reduction of nitrate to nitrogen (Cutruzzola, 2012). The inhibitory transmitter gamma-aminobutyric acid can be generated by *Lactobacillus brevis* and *Bifidobacterium dentium* (Barrett *et al.*, 2012); both of these organisms were isolated from humans (Ventura *et al.*, 2009). Researchers have shown that the gut microbiota plays a role in the development of diabetes (Wen *et al.*, 2008). The gut microbiota, through LPS, has been shown to exert a pro-inflammatory effect and when combined with a high-fat diet it leads to the onset of insulin resistance and, therefore, the development of type 2 diabetes had been observed in a mouse model (Cani *et al.*, 2008).

Studies have suggested the role of gut microorganism in modulating the levels of the serotonin precursor tryptophan and hence the microbiota has a control over serotonin levels in the host. Studies have shown that germ-free rats have depleted levels of tryptophan, but upon administration of certain bacteria, such as *Bifidobacterium* species, tryptophan levels are increased (Desbonnet *et al.*, 2008), thus suggests that bacteria can alter the available serotonin pool and ultimately elicit communication between the gut and the brain. Some microbes, such as *Candida* spp., *Streptococcus* spp., *Escherichia* spp. and *Enterococcus* spp., have been shown to directly produce serotonin (Cryan and Dinan, 2012).

Role of gut microbiome in animal productivity

The gut microbial colony contains cellulolytic, hemicellulolytic, amylolytic, proteolytic, and lipolytic species, exhibiting a high level of functional redundancy, and is capable of effectively degrading host-indigestible plant fiber. Volatile fatty acids (VFAs), principally acetate, propionate, and butyrate, are the major products of rumen microbial fermentation and are absorbed and used as energy sources by the host. Ruminally derived VFAs can meet up to 70% of the host's energy needs, and thus their production is essential to animal performance. Metabolism of nitrogen-containing compounds (including peptides, ammonia and urea) by the rumen microbiota is also vital in the provision of microbial proteins to the host for muscle and milk synthesis. Ingested fiber, carbohydrates and protein are first hydrolyzed to shorter chains (oligomers) and monomers (glucose, amino acids) by primary members of the microbiota and subsequently used as substrates by various members of the microbial community (Hara *et al.*, 2020).

Livestock industries are a significant source of environmentally harmful green house gases

(GHG), with CO₂, CH₄, and N₂O being the major green-house gases emitted from food and agricultural production chains. The potent global warming potential of CH₄ is the most extensively studied GHG in terms of ruminant emissions, and reducing rates of enteric methanogenesis is desirable in terms of both improved animal productivity and environmental stability. The CH₄ is produced in the rumen by methanogenic archaea, which are estimated to account for 0.3–3.3% of the rumen microbial population, based on 16S ribosomal RNA (rRNA) gene analysis (Janssen and Kirs, 2008). Three major pathways of ruminal methanogenesis have been reported (Hungate, 1967; Leahy *et al.*, 2013) out of which, hydrogenotrophic methanogenesis is the predominant pathway in the rumen and is carried out mainly by *Methanobrevibacter* species. In relation to this, the most effective mitigation strategy demonstrated to date is basal dietary supplementation with 3-nitroproxy-propanol (3-NOP). Developed in 2012, 3-NOP acts by inhibiting the methyl coenzyme-M reductase (MCR) enzyme in the terminal step of methanogenesis (Duval and Kindermann, 2012). Supplementation of 3-NOP has been shown to dramatically reduce ruminal CH₄ production in lactating dairy cows and crucially does not have any adverse effect on milk yield (Hristov *et al.*, 2015), though an increase in milk fat has been reported (Lopes *et al.*, 2016). It has also been proven to be an effective CH₄ inhibitor in sheep (Martínez-Fernández *et al.*, 2014) and beef cattle (Romero-Perez *et al.*, 2015).

Conclusion

Extensive studies have been reported on the

association between the host and gut in humans but the information regarding host-microbe relationship in animals is still in infant stage. However, the studies reviewed till date is generating fruitful results. The gut microbial composition and its influence on animal health and productivity needs a vast indepth identification of this complex ecosystem which will provide an insight into the diagnosis and treatment of autoimmune and auto inflammatory diseases in animals. The study of this complex interaction between the host and gut microbiota will further help to design new therapies for diseases arising due to physical and psychological stress. A substantial step forward will be helpful to explain the role of the gut microbiome in host physiology and to understand the metabolic capacity of the early microbiome. Isolation of metabolically active rumen microbiota may be important to enhance our understanding of their roles in monocultures and mixed populations. This information will provide the basis for future strategies to manipulate the microbiome and improve both production and health. The study of this interaction can further help the researchers to develop animal models that can provide information regarding microbial changes within specific regions of gastrointestinal tract and analyze local effects on mucosal immune functions. It can also be helpful to establish the information of effect of dysbiosis of microbiome during early colonization in newborn gut both locally and systemically as well as whether the restoration of gut flora during later stage can reverse the effect of early microbial dysbiosis that impacts the animal health and production.

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