



Dr. Sailendra Kumar Das
Retd. Professor and Head,
Department of Veterinary Microbiology,
Assam Agricultural University, Khanapara
Guwahati -781022, Assam, India

Overview

Gut microbiome: The paramount research programmes concerning animal productivity and health

In recent times, research on gut microbiome and gut health has started to draw attention of the scientists working in the field of animal and fishery sciences globally. The scientists of these two sectors are entrusted with the target of increasing food of animal and fish origin. It is an established fact that only healthy animals and fishes are more productive. In the present scenario the livestock or fish are prone to virulent pathogens, sometime of unknown origin. Till few years back, the gastrointestinal tract, popularly known as “gut” was considered as organ of food digestion and absorption only. It was also known that the “gut” is the organ where large numbers of residential micro flora, most of whom are beneficial inhabit and indiscriminate use of antimicrobial agents results in imbalance of the population of beneficial gut micro flora. To balance these imbalances 'Probiotics', 'Prebiotics' and 'Synbiotics' are used by the human and animal health practitioners. In earlier days our knowledge on gut microbiome was limited to only few cultivable pathogenic or harmless residential micro floras. It is now established that traditional culture methods can recover only 10% to 30% of gut microbes. The advances in genomic technologies have provided new opportunities for large scale in depth analysis of entire microbial communities. The technologies like 16S sequencing (phylogenetic composition), metagenomics (functional capability), metatranscriptomics (functional intent) and metabolomics (metabolic impact) are providing in depth information of the intestinal microbiota including their composition and their metabolic activities. Although we are now aware of existence of large number of microbiome in the gut of an animal, we are quite ignorant of the role played by them individually or collectively. Our knowledge on gut microbiome in animals and fish is still limited when compared with human counterpart. Since gut microbiome and gut health are said to be interlinked and gut health in turn plays an important role in overall health of an individual, the time has come to diverse our research target to gut microbiome and gut health. In fact the gastrointestinal tract itself is a wonderful organ. Although in true sense it is not a vital organ from physiological and anatomical point of view, but it is playing a vital role from immunological point of view. The attention of immunologists are diverted to gut immunity because of its role in maintaining sound health of any individual, be it human or animal or fish. The gastro intestinal tract is considered to be the largest organ of the immune system as more than 70% of the cells of the immune system are present in the tract. The world of gut microbiome still remains an unexplored area in the fishery and animal sciences. The microbes present in the gut may possess some genes which may play a direct or indirect role in increasing production or food quality improvement. In spite of promising roles of gut microbiome in protecting animal health and food production, the number of studies on this aspect in our country is very limited. In case of poultry production, some works on therapeutic uses of selected

gut microbes as an alternative to antibiotic are practiced. Similarly, limited works on rumen microbes are conducted to study the negative environmental impact due to emission of green house gases such as methane. The rumen microbiologists should also do experimental study to minimise the production of major harmful green house gases from food animals. Indian Journal of Animal Health has taken an appreciable step to publish a special issue on “Gut microbiome and health for improving productivity in animal and fish” for encouraging the young scientists to take up research project on different aspects of gut microbiome, gut health in order to increase the animal and fish production.

There are many challenges ahead for the scientists working in this field. But challenges open windows of opportunities also. Data generation, manipulation and engineering of the gut microbes for improving health and production of animals are the main challenges. To a scientist, each and every microbe present in the gut of any animals, birds or fish is an asset and it is a challenge to exploit them for beneficial purpose. Since there is diverse population of gut microbiome, the million dollar question is “What is the ideal gut microbiome?” Can it even be determined if one exists? And if so, can a gut microbial community be modulated effectively enough to ensure the desired community modulated effectively enough to ensure the desired community becomes established?” A group of expert in this field rightly opined that whole gut microbiome should be the paramount research programmes concerning animal productivity and health, which has huge potential to make valuable contributions to efficient and regenerative livestock production globally.

Dr. Sailendra Kumar Das



Dr. N. P. Sahu
Principal Scientist & HOD
Fish Nutrition, Biochemistry and Physiology Division
&
Dean (Academic)
ICAR-Central Institute of Fisheries Education,
Mumbai- 400061, India

Overview

Role of gut microbes on growth and health of fish

N. P. Sahu and Manish Jayant

Fish Nutrition, Biochemistry and Physiology Division,
ICAR-Central Institute of Fisheries Education, Versova, Mumbai-400 061, India

Study on gut microbiota in fish is in rudimentary stage as it has not been studied in detail. Though extensive studies have been done for the ruminant microbes where microbial digestion plays a major role, microbial digestion in non-ruminants especially in fish is very much negligible. During 1950 to 1970 researchers initiated the work on effect of dietary composition on the gut microbes of fish, because gut is the major port of entry of pathogens into fish body. Hence, rather than digestion, the gut microbes in fish contribute more to health and immunity of fish.

The structure of the gut varies with the species and hence their feeding habits. Accordingly, the microbes present in the different segment of the gut also equally vary. Beside, many fluids secret into the lumen of the gut which is also greatly influence the microbial composition of the gut. The pH of the stomach is acidic which turns gradually into alkaline when the gut contents moves towards the distal end of the intestine. This also influences the gut microbial composition in comparison to the stomach less fishes.

The microbial composition is of paramount importance when animal protein sources are replaced with the plant protein sources in aqua feed. Inconsistent supply of fish meal and fish oil and the use of plant-protein based ingredients to feed the fish have attenuated the fish immune system. Plant-protein based ingredients are nurtured with good nutritional profile but also comprised of inherent anti-nutritional factors (ANFs) such as tannin, phytates, trypsin inhibitors, lectin, saponin, alkaloid and glucosinolates etc. (Francis *et al.*, 2001; Mohanta, 2012; Ghosh *et al.*, 2019). These ANFs negatively affect the fish growth and health by interfering with the digestive enzyme activities, nutrients bioavailability and the interference in gut microbiome (Ringø *et al.*, 2016). In freshwater fishes, the major genera of gut microbiota are *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Lactococcus*, *Acinetobacter* and *Clostridium* while, *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Vibrio* and *Micrococcus* are the dominating species in the gut of marine fishes (Gomez and Balcazar, 2008). These microorganisms have a symbiotic relationship by receiving nutrients from the host and providing aid in digestion, metabolism, growth and immunity to the host. Generally, nutrient rich gut-environment is considered as most favourable sites for microbes (108 microorganism per g tissue) to attach and flourish (Ray *et al.*, 2012; Wong *et al.*, 2013; Merrifield and Rodiles, 2015). These gastro-intestinal (GI) tracts associated microbes secrete the digestive enzymes and aid in digestion (Ringø *et al.*, 2012; Hoseinifar *et al.*, 2015; Banerjee *et al.*, 2019).

The gut microbiota regulates the appetite, feed intake, digestion, absorption and finally growth as stated

by Fetissov (2017) and Read and Holmes (2017). Duca *et al.* (2012) reported that mice having gut microbiome attained better growth than mice without gut microbiome. The author also revealed that mice without intestinal microbiota had lower ghrelin and leptin, suggesting the role in regulation of appetite and feed intake. No changes were noticed in feed intake of Zebrafish when fed with *Lactobacillus rhamnosus*. While in rohu, *Labeo rohita* experienced the change in gut microbiota when fed with fructo-oligosaccharide (Falcinelli *et al.*, 2018; Kim and de La Serre, 2018). Gut microbiota influence the metabolism in grass carp, zebrafish and Japanese flounder. These microorganisms enhance the lipid (fatty acid) absorption, transportation and metabolism (Semova *et al.*, 2012; Sheng *et al.*, 2018). Fish gut microbiome is dependent on their developmental stages. For instances, Proteobacteria and Bacteroidetes are the most dominating colonizing groups in eggs and larval stages of grass carp, respectively but the diversity increases as fish grows from larvae to adult in different fishes and dominated by Fusobacteria, Proteobacteria, Bacteroidetes and Firmicutes (Wang *et al.*, 2015; Yan *et al.*, 2016).

Immune responses

Non-pathogenic, pathogenic and commensal bacteria invade the fish intestine and their equilibrium influences the fish immune system. The gut microbiota regulates the energetic-homeostasis and defense against pathogens therefore, known as an extra organ for the host (O'Hara and Shanahan, 2006). Mucus secreted by the gastro-intestinal tract invades the microbiome at mucosal layer and act as first line of defense and aid to build host-immune system. Banerjee and Ray (2017) stated that gut microbiota produces different types of enzymes, compete for attachment sites with pathogens and secrete different bacteriocins. Microorganisms of exogenous origin and antigens interact with host at mucosal layer containing inherent microbiome. In response, this mucosal layer along with epithelium secretes antimicrobial substances such as complement proteins, enzymes like mucins, and antimicrobial peptides such as piscidins and defensins etc. (Silphaduang *et al.*, 2006; Zou *et al.*, 2007). Pérez *et al.* (2010) reported that adhesion of *Lactobacillus* in GI tract lowered the furunculosis in rainbow trout. Similarly, an establishment of beneficial bacteria in fish intestine such as *Paenibacillus*, *Bacillus* and *Staphylococcus* reduced the pathogenic bacterial community such as *Aeromonas hydrophilla*, *A. salmonicida* and *Vibrio alginolyticus* improved nutrition and physiology (Yee *et al.*, 2013).

Gut-immunity is regulated by gut-associated lymphoid tissues (GALT). Rekecki *et al.* (2009) revealed that synthesis of glycoprotein in GI tract of European sea bass (*Dicentrarchus labrax*) is regulated by autochthonous microbial community. According to Rawls *et al.* (2004), gut microbial community regulate the expression of different genes responsible for innate immunity (for e.g. genes related to complement system, C-reactive proteins, myeloperoxidase and glutathione peroxidase etc.). The group of Actinobacteriaregulates, the secretion of interleukin-10, is responsible for inflammatory responses (Lee and Mazmanian, 2010). Ibrahim (2015) stated that feeding probiotics improve the fish gut microbiota community and positively influence the fish growth and health. Possible suggested probiotics for fish diets are *B. subtilis*, *B. cereus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *B. Megaterium*, and *Arthrobacter* sp. etc. (Dahiya *et al.*, 2012; Bhatnagar and Lamba 2014; Efendi and Yusra, 2014; Vand *et al.* 2014).

Shifting of plant based feed from fish meal based feed has shown alterations in their intestinal morphology including disruption to the lamina propria and mucosal folds, which may modify attachment sites for commensal bacteria, and can therefore impact microbial composition. Understanding microbial-host-environmental interactions are crucial for aquaculture. As has already been discussed, *Bacillus* sp. and *Lactobacillus* sp. have beneficial effect on immunity and are suggested to provide an alternative approach for controlling disease in aquaculture. Therefore, identifying imbalances in the gut microbiome or dysbiosis, could be a useful predictor of stress-related syndromes, which could ultimately lead to mortality. The teleost gut microbiome has a clear role in the future of aquaculture. Although research has come a long way in recent decades; there are still many areas of gut microbiome research that require further attention.

Conclusion

As we foresee plant based feed for aquaculture, microbial biomass will definitely play a greater role for complete adoption to new feeding habit. Therefore, in-depth study pertaining to gut microbiome of fish is required for a sustainable aquaculture. Both biotic and abiotic factors are the predisposing factors which significantly affect the gut microbiota. Hence, a compatible microbiota suitable for a species and its environment is a researchable issue for the future aquaculture. Some researchers have already initiated the study on gut microbiota when trouts are fed with plant based diet. On the other hand, a desired group of microbiota can be developed by feeding a specific diet, which may be beneficial for enhancing growth and immunity in fish.

References

- Banerjee G and Ray AK, 2017. The advancement of probiotics research and its application in fish farming industries. *Res Vet Sci*, 115: 66-77, doi: 10.1016/j.rvsc.2017.01.016
- Banerjee S, Mukherjee A, Dutta D and Ghosh K, 2019. Evaluation of chitinolytic gutmicrobiota in some carps and optimization of culture conditions for chitinase production by the selected bacteria. *J Microbiol Biotechnol Food Sci*, 5(1): 12-19, doi: 10.15414/jmbfs .2015.5.1.12-19
- Bhatnagar A and Lamba R, 2015. Antimicrobial ability and growth promoting effects of feed supplemented with probiotic bacterium isolated from gut microflora of *Cirrhinus mrigala*. *J Integr Agric*, 14(3): 583-592, doi: 10.1016/S2095-3119(14) 60836-4
- Dahiya T, Gahlawat SK and Sihag RC, 2012. Elimination of pathogenic bacterium (*Micrococcus* sp.) by the use of probiotics. *Turkish J Fish Aquat Sci*, 12(1): 185-187, doi: 10.4194/1303-2712-v12_1_21
- Duca FA, Swartz TD, Sakar Y and Covasa M, 2012. Increased oral detection, but decreased intestinal signaling for fats in mice lacking gut microbiota. *PLoS One*, 7(6): e39748, doi: 10.1371/journal.pone.0039748
- Efendi Y and Yusra Y, 2014. *Bacillus subtilis* strain VITNJ1 potential probiotic bacteria in the gut of tilapia (*Oreochromis niloticus*) are cultured in floating net, Maninjaulake, West Sumatra Pak J Nutr, 13(12): 710-715, doi: 10.3923/ pjn.2014.710.715
- Falcinelli S, Rodiles A, Hatef A, Picchietti S, Cossignani L *et al.*, 2018. Influence of probiotics administration on gut microbiota core: a review on the effects on appetite control, glucose, and lipid metabolism. *J Clin Gastroenterol*, 52 (Suppl1): S50-S56, doi: 10.1097/MCG.0000000000001064
- Fetissov SO, 2017. Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour. *Nat Rev Endocrinol*, 13:1125, doi: 10.1038/nrendo.2016.150
- Francis G, Makkar HPS and Becker K, 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquac*, 199(3-4): 197-227, doi: 10.1016/S0044-8486(01)00526-9
- Ghosh K, Ray AK and Ringø E, 2019. Applications of plant ingredients for tropical and subtropical freshwater finfish: possibilities and challenges. *Rev Aquac*, 11(3): 793-815, doi: 10.1111/raq.12258
- Gomez GD and Balcazar JL, 2008. A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunol Med Microbiol*, 52:145154, doi: 10.1111/j.1574-695X.2007.00343.x
- Hoseinifar SH, Esteban MÁ, Cuesta A and Sun YZ, 2015. Prebiotics and fish immune response: A review of current knowledge and future perspectives. *Rev Fish Sci Aquac*, 23(4): 315-328, doi: 10.1080/23308249.2015.1052365
- Ibrahim MD, 2015. Evolution of probiotics in aquatic world: potential effects, the current status in Egypt and recent prospectives. *J adv Res*, 6(6): 765-791, doi: 10.1016/j.jare.2013.12.004
- Kim JS and de La Serre CB, 2018. Diet, gut microbiota composition and feeding behavior. *Physiol Behav*, 192: 177-181, doi: 10.1016/j. physbeh.2018.03.026
- Lee YK and Mazmanian SK, 2010. Has the microbiota

- played a critical role in the evolution of the adaptive immune system? *Science*, 330(6012): 1768-1773, doi: 10.1126/science.1195568
- Merrifield DL and Rodiles A, 2015. The fish microbiome and its interactions with mucosal tissues. In: Beck BH, Peatman E (eds) *Mucosal Health Aquac*, pp. 273-295. Elsevier, Amsterdam, The Netherlands, doi: 10.1016/B978-0-12-417186-2.00010-8
- Mohanta KN, 2012. Plant feed resources of India. In: Sogbesan OA, Mohanta KN, Sahoo PK, Mitra G, Jayasankar P (eds) *Invited Papers on Application of Solid State Fermentation Technology in Aquaculture*, Central Institute of Freshwater Aquaculture, Bhubaneswar, India, pp. 100-113
- O'Hara AM and Shanahan F, 2006. The gut flora as a forgotten organ. *EMBO Rep*, 7(7): 688-693, doi: 10.1038/sj.embor.7400731
- Pérez T, Balcázar JL, Ruiz-Zarzuela I, Halaihel N, Vendrell D *et al.*, 2010. Hostmicrobiota interactions within the fish intestinal ecosystem. *Mucosal Immunol*, 3(4): 355-360, doi: 10.1038/mi.2010.12
- Rawls JF, Samuel BS and Gordon JI, 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proceedings of the National Academy of Sciences*, 101(13): 4596-4601, doi: 10.1073/pnas.0400706101
- Ray AK, Ghosh K and Ringø E, 2012. Enzyme-producing bacteria isolated from fish gut: A review. *Aquac Nutr*, 18(5): 465-492, doi: 10.1111/j.1365-2095.2012.00943.x
- Read MN and Holmes AJ, 2017. Towards an integrative understanding of diethostgut microbiome interactions. *Front Immunol*, 8: 538, doi: 10.3389/fimmu.2017.00538
- Rekecki A, Dierckens K, Laureau S, Boon N, Bossier P *et al.*, 2009. Effect of germ-free rearing environment on gut development of larval sea bass (*Dicentrarchus labrax* L.). *Aquaculture*, 293 (1-2): 8-15, doi: 10.1016/j.aquaculture. 2009.04.001
- Ringø E, Zhou Z, Olsen RE and Song SK, 2012. Use of chitin and krill in aquaculture: the effect on gut microbiota and the immune system: A review. *Aquac Nutr*, 18(2): 117-131, doi: 10.1111/j.1365-2095.2011.00919.x
- Ringø E, Zhou Z, Vecino JLG, Wadsworth S, Romero J *et al.*, 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquac Nutr*, 22(2): 219-282, doi: 10.1111/anu.12346
- Semova I, Carten JD, Stombaugh J, Mackey LC, Knight R *et al.*, 2012. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host Microbe*, 12(3): 277-288, doi: 10.1016/j.chom.2012.08.003
- Sheng Y, Ren H, Limbu SM, Sun Y, Qiao F *et al.*, 2018. The presence or absence of intestinal microbiota affects lipid deposition and related genes expression in zebrafish (*Danio rerio*). *Front Microbiol*, 9: 1124, doi: 10.3389/fmicb.2018.01124
- Silphaduang U, Colorni A and Noga EJ, 2006. Evidence for widespread distribution of piscidin antimicrobial peptides in teleost fish. *Dis Aquat Organ*, 72(3): 241-252, doi: 10.3354/dao072241
- Vand ZDA, Alishahi M and Tabande MR, 2014. Effects of different levels of *Lactobacillus casei* as probiotic on growth performance and digestive enzymes activity of *Barbus grypus*. *Int J Biosci*, 4 (7): 106-116, doi: 10.12692/ijb/4.7.106-116
- Wang W, Wu S, Zheng Y, Cheng Y, Li W *et al.*, 2015. Characterization of the bacterial community associated with early developmental stages of grass carp (*Ctenopharyngodon idella*). *Aquac Res*, 46(11): 2728-2735, doi:10.1111/are. 12428
- Wong S, Waldrop T, Summerfelt S, Davidson J, Barrows F *et al.*, 2013. Aquacultured rainbow trout (*Oncorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. *Appl Environ Microbiol*, 79(16): 4974-4984, doi: 10.1128/AEM.00924-13
- Yan Q, Li J, Yu Y, Wang J, He Z *et al.*, 2016. Environmental filtering decreases with fish development for the assembly of gut microbiota. *Environ Microbiol*, 18(12): 4739-4754, doi: 10.1111/1462-2920.13365
- Yee LC, Ina-Salwany MY and Abdelhadi YM, 2013. Antibacterial ability and molecular characterization of probionts isolated from gut microflora of cultured red tilapia. *Asian J Anim Vet Adv*, 8(1): 116-123, doi: 10.3923/ajava. 2013.116.123



Dr. Arup Kumar Samanta
Professor and Head
Department of Animal Nutrition
CVSc & AH, CAU, Selesih, Aizawl, Mizoram, India

Overview

Gut microbiome of ruminant and non-ruminants: crucial consideration for animal production

The integrity of gastrointestinal tract (GIT) and gut microbes play very important role in the nutrient absorption, development of innate immunity and resistance to various ailments. Alterations in the type and species of the gut microbes pose serious adverse effects on production and productivity of livestock, poultry and fish. In earlier days, it was very difficult to identify and characterise the GIT microbes by conventional selective culture based techniques. At present, with the advent of molecular techniques, our knowledge and understanding of microbial diversity of GIT microbes have been improved dramatically. The GIT harbours a diverse microbial community. An imbalanced gut microbiota i.e. dysbiosis may lead to reduced intestinal barrier function, poor nutrient digestibility and poor performance of livestock, poultry and fish. Rumen microbial ecosystem consists of bacteria, ciliate protozoa, fungi, bacteriophage and virus. The host-microbes symbiosis that exists in the rumen is crucial to meet the nutrient requirement of the ruminants fed high fibrous diets. Understanding rumen microbes is important to increase the profitability, reduce methane production and produce quality products (meat and milk). GIT microbiota are also of critical importance in non ruminants like pigs, horse and poultry when it comes to gut health, immunological development, performance and overall health status of animal. Many challenges faced by swine producers like post weaning diarrhoea, poor growth performance and poor product quality may be addressed by understanding GIT microbiota and their management. Beneficial gut microbiota of poultry plays a key role in maintenance of intestinal health through competitive exclusion of detrimental microorganisms and thereby improves the avian productivity. The gut microbiota of fish is responsible for key physiological functions which help maintain health status of its host. Knowledge of microbial composition in health and disease is vital for the growth of aquaculture industry.

This special issue of Indian Journal of Animal Health explores the importance of GI microbiota of livestock, poultry and fish with application to host gut health, performance, immunity and overall health status. I wish every success of this special issue and its wide circulation.

Prof. Arup Kumar Samanta



Dr. Mahmoud Alagawany
Assistant Professor
Poultry Department, Agriculture Faculty
Zagazig University
Zagazig, Egypt

Overview

It is well known that healthy gut microbiota is essential to promote host health and well-being. The intestinal microbiota of endothermic animals as well as fish are classified as autochthonous or indigenous, when they are able to colonize the host's epithelial surface or are associated with the microvilli, or as allochthonous or transient. Furthermore, the gut microbiota of aquatic animals is more fluidic than that of terrestrial vertebrates and is highly sensitive to dietary changes. Furthermore, some information is available on bacterial colonization of the gut enterocyte surface as a result of dietary manipulation which indicates that changes in indigenous microbial populations may have repercussion on secondary host-microbe interactions. The impact of dietary components on the gut microbiota is important to investigate, as the gastrointestinal tract has been suggested as one of the major routes of infection in animal and fish. Possible interactions between dietary components and the protective microbiota colonizing the digestive tract will be discussed in this special issue. Since, supplementation of some feed additives in the diet have the ability to increase animal health and performance; through contributions to gut health and nutrient use. For instance, supplementation of feed additives has been demonstrated to benefit farm animals in immune modulation, structural modulation and increased cytokine production, which positively affect the intestinal mucosal lining against pathogens.

This special issue is interested in both reviews and research papers on all aspects of gut microbiome and health for improving productivity in animal, poultry and fish. It is also interested in all feed additives and their effects on gut health, performance and public health. Also, the objective of this special issue is intended to describe unique and distinct advances in nutritional strategies in animal and poultry industry. Also, this issue highlights various natural feed supplements (probiotics, prebiotics, enzymes, organic acids, etc.) that are used in livestock and their possible beneficial effects on the consumer's health. The present special issue will be very much useful for nutritionists, veterinarians, pharmacists, researchers, animal and poultry farmers, public health experts, and pharmaceutical industry. This special issue will accept submissions of review papers on above mentioned topics / theme as well as comprehensive review articles.

Dr. Mahmoud Alagawany