MANAGING THE MICROBIAL ECOLOGY OF THE GASTROINTESTINAL TRACT OF FISH WITH THE HELP OF PROBIOTICS AND PREBIOTICS IN AQUACULTURE

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Abstract

Aquaculture Industry now becomes much intensified so higher stocking rate and high quality feed has been given to the cultured organisms for meeting the high demand of aquaculture products. Due to high intensity culture there are chances of disease outbreak which can be overcome by using antibiotics and vaccination. But the therapeutic agents have limitations like antibiotic residual effects, development of antibiotic resistant and destroying of beneficial bacteria. Due to these reasons many countries ban on these therapeutic agents so alternate strategy has been applied to overcome these problems by using Probiotics and Prebiotics. The results of probiotics and prebiotics have encouraging results as in terrestrial animals and to some extent in fishes and crustaceans too. The both prebiotics and probiotics has beneficial effect on GI tract of fishes like altering the micro flora with beneficial bacteria ,alter the pH , increase the incidence of adhesion of beneficial bacteria, absorption of trace elements and also some time provides extra energy and last but least increase the immunity of the host. The micro flora of culture aquaculture organism has not been fully understood especially anaerobic bacteria. In this context more comprehensive study of microbial strata of GI tract of aquatic organism is to be undertaken so that more effective use of prebiotics and probiotics supplementation. It is an attempt to summarize the knowledge of intestinal microbial flora of aquatic organism and potential use of prebiotics and probiotics in aquaculture system.

Introduction

There is steady demand in aquaculture products due continuous increase in population throughout the world and there are limitations on harvesting the fishes from the capture fisheries resources. The capture fisheries are not manageable as aquaculture facilities and so there are limitations on production of fisheries product from capture fisheries. In aquaculture system there is high intensity of stocking of desired aquaculture animal and these are fed with prepared diet to provide all required nutrients for their overall development. Due to this there is increase in chances of disease outbreak due to poor water quality, left over feed, high intensity stress, decrease of food quality, increase in bacterial, viral and parasitic infection etc. Traditionally dealing with bacterial infection in aquaculture is done by administration of antibiotics. But excessive use of antibiotics in aquaculture system leads to antibiotic residue in the aquaculture animals, development of antibiotic resistant bacteria, destruction of environmental beneficial microbial flora etc. Use of antibiotics or vaccination for controlling the disease in farming system is expensive and also unavailable at that time of disease out breaks. To overcome these problem considerable attention is been paid on use of probiotics and prebiotics for control of diseases which are environmentally safe as compare to antibiotics are concerned.

There are several definitions to explain the term Prebiotics and Prebiotics by different workers

As per Gismondo etal.,1999 the term Probiotics means “for life”, originating from Greek words ” pro” and ” bios” .

As per Fuller 1989 Probiotics as a live microbial feed supplement which beneficially affects host animal by improving its intestinal balance.

Gatesoupe, 1999 probiotics plays many beneficial roles like competition with pathogenic bacteria for nutrients, for adhesion site in Gastrointestinal tract and stimulate immune system.

As per Manning and Gibson, 2004 Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating growth and activate limited number of beneficial bacteria in gastro intestinal tract (GI) such as *Lactobacillus* and *Bifidobacter* species while limiting the potentially pathogenic bacteria such as *Salmonella, Listeria* and *Escherichia coli* Some common prebiotics are fructooligosaccharide (FOS), transgalactooligosaccharide **(TOS),** and inulin (Vulevic et al. 2004). Prebiotics have been used in humans (reviewed by

Gibson and Roberfroid 1995; Manning and Gibson 2004; Rastall2004), poultry (Patterson and Burkholder 2003), and pigs (Smiricky- Tjardes et al. 2003; Konstantinov et al. 2004).

The prebiotics have several advantages, but the main advantage of prebiotics over probiotics is that they are natural feed ingredients. Their incorporation in the diet does not require particular precautions and their authorization as feed additives may be more easily obtained, in spite of some concerns about their safety and efficacy. Originally, prebiotics were chosen to stimulate bifidobacteria and lactobacilli in human microbiota (Gatesoupe,2005).

Inclusion of prebiotics in the diets leads to increase in uptake of glucose (Breves etal.,2001), bioavailability of trace elements (Bongers and van den Heuvels,2003),increase in absorption of minerals such as calcium, magnesium, and iron as these are not absorbed in the small intestine .

Prebiotics are selectively fermented by probiotic bacteria e.g. *Bifidobacteria*, *Lactobacillus* and *Bacteroides* to produce short chain fatty acids (acetate, butyrate, propionate) and lactate. It has been demonstrated that short chain fatty acids are absorbed through the intestinal epithelium, thus becoming an energy source for the host, whereas lactate enters the liver and is used as precursor for gluconeogenesis (Smiricky-Tjardes et al., 2003; Gibson et al., 1995; Burr et al., 2005). Reducing the pH of the colon resulting from the production of Short chain Fatty Acids (SCFA) is another prebiotic properties. Lower pH values inhibit the growth of certain pathogenic bacterial species while stimulating the growth of the bifidobacteria and other lactic acid species (Mussatto and Mancilha, 2007).

Manipulation of Fish GI microbiota with Probiotics

Recently it has been established that feeding potentially beneficial bacteria to terrestrial animals as probiotics alter the intestinal environment of GI tract and favour the growth of beneficial microorganism .Use of probiotics are extensively studied in pigs (Sakata et al. 2003; Gardiner et al. 2004), chickens (Nisbet 2002; Patterson and Burkholder 2003), and humans (Fioramonti et al. 2003), but to a more limited extent in fishes (reviewed by Gatesoupe 1999; Verschuere et al. 2000; Irianto and Austin 2002a). The GI tract microbial community of the host organism fed a probiotic becomes readily dominated by the probiont; however, the probiont typically disappears within days after withdraw1 of the probiotic as demonstrated in chickens (Netherwood et al. 1999). Probiotics have been shown to have numerous favorable effects on the host including increased nutrient digestion. For example, probiotics have been used to aid in the digestion of lactose by people without lactase (Jiang and Savaiano 1997). In juvenile turbot *Scophthalmus maximus*, growth was significantly increased with the addition of *Lactobacillus* spp. to the diet (Gatesoupe 1991). Nitrogen retention of turbot also was reported to increase when the diet was supplemented with *Vibrio proteolyticus* (De Schrijver and Ollevier 2000). Probiotics also have been reported to inhibit diseases of the GI tract (Ma0 et al. 1996; Ichikawa et al. 1999) and aid in the development of the GI tract immune system (Fukushima et al. 1999; Rodrigues et al. 2000). Probiotics also may provide benefits for the GI tract itself by impeding degradation of the intestinal mucus (Rojas and Conway 1996; Zhou et al. 2001). In livestock production, probiotics mainly have been used to enhance the disease resistance of the host to bacterial pathogens by modifying the microbial community of the GI tract (Patterson and Burkholder 2003). Pathogenic microorganisms infect terrestrial animals through the GI tract, and competitive exclusion cultures have been reported to inhibit diseases in both swine and poultry (Nisbet 2002), including inhibition of *Campylobacter jejuni* colonization in chicks (Schoeni and Wong 1994). Lactic acid bacteria have been the most commonly used probiont in humans (reviewed in Fioramonti et al. 2003), poultry (reviewed in Patterson and Burkholder 2003), and swine (Ohashi et al. 2004). Lactic acid bacteria also have received considerable attention as probiotics in fishes (Ring0 and Gatesoupe 1998; Gildberg and Mikkelsen 1998; Hagi et al. 2004). For example, lactic acid bacteria included in the diet of Atlantic cod *Gadus morhua* was found to increase the survival of the host when challenged with the bacterial pathogen *Vibrio angullarum* (Gildberg and Mikkelsen 1998). Production of acetate and lactate by lactic acid bacteria has been shown to inhibit the growth of several species of *Vibrio* (Vazquez et al. 2005). Enhanced survival and increased specific and non-specific immune responses have been demonstrated in rainbow trout (Nikoskelainen et al. 2003; Panigrahi et al. 2005) and gilthead seabream (Salinas et al. 2005) fed lactic acid bacteria. Although lactic acid bacteria have been most widely studied probiotic, *Aeromonas media* has been reported to decrease saprolegniosis in challenged eels *Anguilla australis* (Lategen et al. 2004). While probiotics have been shown to successfully decrease mortality in larval and pathogen challenged fishes, as well as provide additional enzymes to potentially aid the host in digestion, the use of probiotics is potentially limited for several reasons. In particular, the viability of these probiotic microbes may be affected by the harsh conditions of extrusion or pellet manufacturing. There also may be possible regulatory issues to limit microbial supplements in the diet. Thus, prebiotic supplements have received heightened attention as potentially offering the same benefits of probiotics without the addition of live bacteria to the diet.

Manipulation of Fish GI microbiota with prebiotics

In the gastrointestinal track, the bacterial community is affected by the substances and vice verse. On the other hand, there are positive and/or interaction between the bacterial and substance in gastrointestinal track. Flickinger et al. (2003), explained these phenomenon such a way that, the GI tract of invertebrates and vertebrates provide habitat for a diverse ecosystem of microorganisms. The colonic microflora is of crucial importance to any consideration of the role of feed ingredients in health and disease because many physiological effects of such compounds influence their activities. Prebiotic oligosaccharides such as inulin and oligofructose are fermented in the colon where they promote the growth of bacterial populations associated with a healthy, well-functioning colon. This selective stimulation occurs because oligosaccharides are readily fermented by beneficial types of colonic bacteria and are not used effectively by potentially pathogenic bacterial species. In general, we may divide the bacteria in two groups. Some bacteria are hazardous and the others are beneficial for fish. Due to activity of the first group, the hazard effect or toxin material may be produced. As Flickinger etal. (2003) explained, a number of these bacteria are pathogenic whereas health-promoting, or pathogen suppressing, properties have been attributed to particular bacteria (e.g., *Bifidobacterium, Lactobacillus*). A number of adverse consequences result from toxic metabolites formed during fermentation of food/feed in the large bowel. Toxic compounds formed at that site include ammonia (a liver toxin), amines (liver toxins), nitrosoamines (carcinogens), phenols and cresols (cancer promoters), indole and skatole (carcinogens), estrogens (suspected carcinogens/ breast cancer promoters), secondary bile acids (carcinogens /active colon cancer promoters) and a glycones (mutagenic substances) (Flickinger et al., 2003). In case of beneficial bacteria, Merri¢eld et al. (2009)_ by study of a couple of articles, suggested that the beneficial bacteria plays a role as a defensive barrier against pathogenic species in addition to contributing towards digestive function via the production of a range of vitamins and enzymes (Rimmer and Wiebe, 1987; Moriarty, 1990; Sugita et al., 1997; Sugita et al., 1998; Ramirez and Dixon, 2003). Gastric bacterial populations may also play an important role with regard to immunostimulation and development of gut-associated lymphoid tissues (Picchietti et al., 2007). Furthermore, several researches have demonstrated the influence of mucosal bacterial populations on the integrity of the epithelial surface (Ringø et al., 2003; Ringø et al. 2007). It is demonstrated that the lactic acid bacteria (e.g., *Bifidobacterium, Lactobacillus*) have the ability to tolerate the acidic and bile environment of the intestinal tract.

Lactic acid bacteria (LAB) also functions to convert lactose into lactic acid, thereby reducing the pH in the GIT and naturally preventing the colonization by many bacteria (Mombelli and Gismondo, 2000; Klewicki and Klewicka, 2004). In aquaculture, few reports are available on the influence of prebiotics on growth and intestinal microflora in fish. In the earliest of studies with fish, certain nutrients such as linoleic acid, linolenic acid and soluble carbohydrate were investigated mainly by Ringo and his colleagues their effects on the aerobic/facultative anaerobic intestinal microbiota of Arctic char *Salvelinus afpinus*. When linoleic acid was supplemented to the diet of Artic char, the total viable counts increased by an order of magnitude (10 fold) as compared with fish fed a diet without linoleic acid (Ringø, 1993; Ringø et al., 1998; Ringø and Olsen, 1999). Adding linoleic acid to the diet altered the intestinal microbial community by inhibiting the growth of Lactobacillus sp. and enhancing the growth of *Aeromonas sp., Pseudomonas sp. and Vibrio* sp. Polyunsaturated fatty acids of the n-3 and n-6 series also were shown to alter the microbial population of Arctic char, with the lactic acid bacteria *Carnobacterium* spp. being the dominant facultative anaerobe cultivated (Ringø et al., 1998). Lactosucrose has been shown to increase the thickness of intestinal tunica muscularis of red sea bream, while this dietary supplement was used as substrate by the intestinal microflora (Kihara et al.,1995). However, lactosucrose was poorly used by trout (Kihara and Sakata, 2001a) and carp microbiota (Kiharaand Sakata, 2001b). Olsen et al. (2001), have observed a damaging effect of inulin on enterocytes of Arctic charr, when the amount of the prebiotic in the diet was 15% ofthe diet. In another investigation using dextrin instead, researchers reported that substituting dextrin with 15% inulin reduced the bacterial population from 4.8×10^5 to 3.56×10^4 level in the hindgut of Arctic charr, however the composition of bacteria colonizing the hindgut of Arctic charr fed inulin were dominated by Gram-positive bacteria of the genera Staphylococcus, Streptococcus, *Carnobacterium and Bacillus* (Ringø et al., 2006). Supplementation of Beluga's (*Huso huso*) diet with 1, 2 and 3% inulin showed that all bacteria levels increased during the first 4 weeks and started to decrease in inulin fed fish during the next 4 weeks and there were no significant differences between all treatments, but the intestinal lactic acid bacteria (LAB) increased in the 1% inulin group. Olsen et al. (2001) observed that a diet supplemented with 15% inulin caused harmful effects on enterocytes to Arctic charr, *Salvelinus alpinus*. Dietary supplementation of 2% inulin significantly changed GI microflora in turbot *Psseta maxima* larvae by increasing *Bacillus* species to 14% and decreasing *Vibrio* species (Mahious et al.,2006). In summary, prebiotics have been reported to have numerous beneficial effects in fish such as increased disease resistance and improved nutrient availability. The reasons for the different results are not clear yet. It may be due to the different basal diet, inclusion level, type of monosaccharide, adaptation period, chemical structure (degree of polymerization, linear or branched, type of linkages between monometric sugars), origin of prebiotic, animal characteristics (species, age, and stage of production), duration of use and hygienic conditions of the experiment. If beneficial effects of prebiotics are manifested in fishes, then prebiotics have much potential to increase the efficiency and sustainability of aquacultural production. Therefore, comprehensive research to more fully characterize the intestinal microbiota of prominent fish species and their responses to prebiotics is warranted.

Conclusion

Prebiotics and probiotics has innumerable benefits as in terrestrial animal is concerned but it is not yet cleared the role in fishes. There is an limited knowledge about the microbial community in the GI tract of various species. There unanswered questions about lactic acid bacteria are beneficial to fishes and is Biofidobacterium present in fishes? So comprehensive research has to be undertaken to understand the microbial flora of fishes GI tract and also the benefits of using prebiotics and probiotics. Prebiotics have been reported to have numerous beneficial effects in fish such as increased disease resistance and improved nutrient availability. The reasons for the different results are not clear yet. It may be due to the different basal diet, inclusion level, type of monosaccharide, adaptation period, chemical structure (degree of polymerization, linear or branched, type of linkages between monometric sugars), origin of prebiotic, animal characteristics (species, age, and stage of production), duration of use and hygienic conditions of the experiment. If beneficial effects of prebiotics are manifested in fishes, then prebiotics have much potential to increase the efficiency and sustainability of aqua cultural production. Therefore, comprehensive research to more fully characterize the intestinal micro biota of prominent fish species and their responses to prebiotics is warranted. There are several questions that must be answered by more comprehensively evaluating In summary, prebiotics have been reported to have numerous beneficial effects in terrestrial animals such as increased disease resistance and improved nutrient availability. If these types of responses are manifested in fishes, then prebiotics have much potential to increase the efficiency and sustainability of aquacultural production. Therefore, comprehensive research is to be undertaken to more fully characterize the intestinal microbiota of prominent fish species and their responses to prebiotics is warranted.

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