Review Article

Fermentation of feed ingredients as potential strategy to improve health and reduce opportunistic infection in fish farming

> Comment [D1]: The title should be better off this way: Fermentation of feed ingredients as potential strategy to improve health status and reduce opportunistic pathogens in fish farming.

ABSTRACT

The rapid increase in fish farming has been affected by outbreak of diseases and erratic feed costs. These challenges have stimulated increase in the use of antibiotics to rear fish. Unfortunately, excessive use of antibiotics inhibits or kills beneficial gut microbiota and makes antibiotic residues to accumulate in fish products, which are harmful for human consumption. The use of biological strategies has therefore, been adopted to improve health status, growth performance and reduce predisposition of fish to diseases. This has become necessary in view of the EU ban on most antibiotics used as growth promoters in animal husbandry due to their roles in the production of antibiotic resistant bacteria. Moreover, use of the natural fermentation process, which utilizes functional and safe microbes to transform large and potentially harmful chemical constituents in fish feed to less harmful or safe states have been contemplated in aquaculture. In the present review, lactic acid bacteria (LAB) activity during feed fermentation to mediate positive effects in farmed fish is highlighted, including; modulation of gastrointestinal pH, production of bacteriocins, competitive inhibition and translocation of pathogenic bacteria in the GIT. Other potentials of fermentation to promote feed efficiency and growth performance in fish are also discussed.

Keywords: Aquaculture, Antibiotics, Fermentation, Lactic acid bacteria, Porbiotics, Resistance bacteria.

> Comment [D2]: Fish farming is better to be used as keyword here since aquaculture involves general term of culturing different aquatic organisms.

> Comment [D3]: Probiotics not porbiotics. Please correct it.

1. INTRODUCTION

Worldwide, aquaculture has grown tremendously during the last 30 years to become the fastest growing food-production sector, with the greatest potential to meet the growing demand for aquatic food [1, 2]. However, the rapid global growth of fish aquaculture is threatened by several factors, including the outbreak of numerous fish diseases, high cost of feed, species nutrition and relatively slow flesh growth. Inadequate nutrition of farm animals and poor hygiene could have significant implications that may likely translate to slow growth, diseases outbreak, thus leading to high stock mortalities [3].

> Comment [D4]: Globally is better phrase to be used here than worldwide.

Prevention and control of diseases in fish farming has led to significant increase in the use of antibiotics in recent years, which have resulted in the selective survival of resistant species or strains of bacteria [4, 5, 6]. Resistance to infection could be transferred to previously susceptible bacteria and constitute serious hazards to both animal and human health [5]. Furthermore, antibiotics also inhibit or kill beneficial microbiota in the gut microflora, leading to the accumulation of antibiotic residues in fish products that are harmful for human consumption [7]. In recognition of these dangers, the use of sub-therapeutic doses of antibiotics as growth-promoting agents in rearing animals was banned by the European Union since 2006 [8] and the evaluation for alternative strategies are mandatory.

Consequently, new strategies for feeding and health management during fish farming continue to receive attention [9]. The global demand for safe food has prompted the search for natural alternatives to Antibiotic growth promoters (AGPs) for feeding farmed animals. The alternatives contemplated and being tested includes the use of probiotics, organic acids, prebiotics, minerals, enzymes, herbs, phenolic aromatic components and fermented foods (FF) [10, 11, 12, 13, 14, 15]. Although the consumption of FF is popular among different cultures around the world and has been adopted in different animal husbandry practices, it has unfortunately, not been fully adopted on feeds for rearing fish.

The present review highlights the benefits of fermentation of feed ingredients as alternative strategy to improve fish health through improvement in feed quality, digestibility, promotion of increased nutrients absorption and enhancing the activities of antioxidant enzymes. The improvement of fish immune system following the consumption of fermented feeds are also highlighted and discussed.

2. Purpose and Benefits of Feed Fermentation

The primary purpose and benefit of fermentation is the conversion of sugars and other carbohydrates to usable end products [16]. Naturally fermented foods and beverages contain both functional and non-functional microorganisms [17]. Functional microorganisms transform the chemical constituents of raw materials from plant and animal sources during fermentation, thereby enhancing the bio-availability of constituent nutrients, enriching sensory quality of the feed, imparting bio-preservative potentials and improving feed safety. Toxic components and anti-nutritive factors are also degraded, antioxidant and antimicrobial compounds are produced, probiotic functions are stimulated and the feed is also fortified with health-promoting bioactive compounds [18, 19, 20, 21, 17].

Among bacteria associated with fermented feeds and alcoholic beverages, are mostly species of *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Weissella*, etc. These are reported to be present in sufficient quantities in many fermented feeds and beverages [22, 23]. Furthermore, Lv et al. [24] reported that the genera and species of yeasts isolated from fermented foods, alcoholic beverages and non-food mixed amylolytic starters mostly include *Candida, Debaiyomyces, Geotrichum, Hansenula, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, Saccharomycopsis, Schizosaccharomyces, Torulopsis, Wickerhamomyces*, and *Zygosaccharomyces*. These microorganisms exhibit diverse functional properties that may form important criteria for their selection in the starter cultures to be used in the manufacture of functional feeds via fermentation [25]. Some of these genera and species of microorganisms are used as commercial starters in food fermentation, where some of the products have been commercialized and marketed globally as functional, health promoting, therapeutic and nutraceuticals foods [26, 20, 21].

2.1 Advantages of Food Fermentation

Fermentation makes foods more palatable by enhancing their organoleptic properties [27]. Higher organoleptic properties make fermented foods more popular than their unfermented counterparts in terms of consumer acceptance [28]. A number of foods especially cereals, which constitute the main staple diet of low income populations, have poor nutritional value [27]. Consequently, LAB fermentation has been shown to improve the nutritional value and digestibility of these foods [29]. The enzymes which the fermenting microorganisms produce, including amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively [30]. The quantity and quality of the proteins in food and often, the content of water soluble vitamins are generally increased. On the other hand, the constituent anti-nutrient factors (ANFs) such as phytic acid and tannins in food decline during fermentation, leading to increased bioavailability of minerals such as calcium, phosphorus, zinc, iron, amino acids and simple sugars [31, 32, 33].

The preservative activity of local fermentation such as lowering of the pH to below 4 through acid production inhibits the growth of pathogenic organisms which cause food spoilage, food poisoning and diseases and by doing this, the shelf life of fermented food is prolonged [34, 35]. It makes food safe for consumers in terms of stability, transportation and storage [27].

Food and feeds are often contaminated with a number of toxins like fumonisins, ocratoxin A, zearalenone and aflatoxins (mycotoxins) either naturally or through infestation by microorganisms such as moulds, yeast, bacteria and viruses [36]. Using LAB in fermentation detoxifies toxins and is more advantageous, because it is a milder method which preserves the nutritive value and flavor of foods [27]. In addition to this, fermentation irreversibly degrades mycotoxins without adversely affecting the nutritional value of the food [36] and without leaving any toxic residues [37].

Lactic Acid Bacteria are applied as barrier against non-acid tolerant bacteria, which are ecologically eliminated from the medium due to their sensitivity to acidic environment [38]. Fermentation has also been demonstrated to be more effective in the removal of Gram negative than the Gram-positive bacteria, which are more resistant to fermentation processes. As such, fermented foods can control diarrhoeal diseases in children [37]. Furthermore, Lactic Acid Bacteria are also known to produce antimicrobial agents such as bacteriocins, peptides, etc, that elicit antimicrobial activity against food spoilage organisms and food borne pathogens, but do not affect the producing organisms [37].

2.2 Health Benefits of Fermented Foods

Many of the fermented products consumed by different ethnic groups have therapeutic values. Some of the most widely known are fermented milks (i.e., yoghurt, curds and *Nono)* which contain high concentrations of probiotic bacteria that can lower the cholesterol level [39], improve nutrients absorption and digestion, restores the balance of bacteria in the gut to hinder constipation, abdominal cramps, asthma, allergies, lactose and gluten intolerance [34]. The slurries of carbohydrate based fermented Nigerian foods such as *ogi*, *fufu* and *wara* have been known to exhibit health promoting properties such as control of gastroenteritis in animals and human [40, 35]. Raw fermented foods are rich in enzymes. Age decreases the production of enzymes, therefore, animals and humans need enzymes to properly digest, absorb and make full use of food [41].

2.3 Microorganisms Involved in Fermented Food Production

The commonest organisms responsible for fermentation of foods are acid-forming bacteria such as *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Aerococcus* and *Pediococcus* [27, 38] known as obligate fermenters, flavorful organisms (aromatic compound microorganisms) and *Propionibacterium* species [42]. The yeasts are mainly of the species *Saccharomyces, Candida, Kluyeromyces* and *Debaryomyces* [43, 27]. Moulds have been used mainly in milk and cheese fermentation [44] and these include *Penicillium, Mucor, Geotrichium,* and *Rhizopus* species [27]. Microorganisms of higher economic importance are the LAB.

LAB are a group of Gram positive bacteria, non-respiring, non-spore forming, cocci or rods, the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main species that play a key role in safety and acceptability of the products of carbohydrates in tropical climate [45]. Most pathogenic microorganisms found in-food cannot survive the low pH, hence, Lactic acid fermentation of food has been used to reduce the risk of pathogenic microorganisms growth in the food [34]. Alkaline fermentation causes the hydrolysis of protein to amino acids and peptides and releases ammonia, which increases the alkalinity by the *Bacillus* species such as *Bacillus subtilis* (dominant species), *B. licheniformis* and *B. pumilius* [46, 27].

Indigenous natural fermentation takes place in a mixed colony of microorganisms such as moulds, bacteria and yeasts [44]. These bacteria are not harmful to the consumers and have enzymes such as proteases, amylases and lipases that hydrolyze food complexes into simple nontoxic products with desirable textures, aroma that makes them palatable for consumption [45]. Thus, fermentation products in food substrates are based on the microorganisms involved in the fermentation. Some of the compounds formed during fermentation include organic acids (palmitic, pyruvic, lactic, acetic, propionic, malic, succinic, formic and butyric acids), alcohols (mainly ethanol) aldehydes and Ketones (acetaldehyde, acetoin, 2-methyl butanol) [36].

2.4 Nigeria Fermented Foods

The deliberate fermentation of foods by man through the use of microbes is possibly the oldest method of preserving perishable foods [16]. Traditional fermentation of foods serves several functions, which include the following;

- enhancement of diet through development of flavour, aroma, and texture in food substrates
- preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation
- enhancement of food quality with protein, essential amino acids, essential fatty acids and vitamins
- improving digestibility and nutrient availability
- detoxification of anti-nutrient through food fermentation processes, and
- decrease in cooking time and fuel requirement [47, 16].

In Nigeria, the popular fermented foods include the following:

Ugba, is an indigenous fermented food and a popular staple in the Eastern part of Nigeria. It is rich in protein (44%) and other minerals [16]. *Bacillus* spp. and *Lactobacillus* spp. were found to be responsible for the fermentation of African oil bean seeds to *ugba* [48]. In some West African

Comment [D5]: You don’t have to italized those word as they are not scientific words countries, especially Nigeria, the production of *gari* and *fufu* (fermented cassava product), *ogi* (fermented maize, sorghum, or millet gruel), *fura da nono* (fresh cow's milk with fermented millet gruel), and *pito, kunun-zaki* and *Burukutu* (cereal-based alcoholic beverages) are largely brought about by lactic acid bacteria and yeast, with *L. plantarum* predominating [49, 16]. In another study, *L. plantarum* and *Lactobacillus brevis* were the dominant lactic acid bacteria isolated in different batches of *pito* and *burukutu* collected from local producers in Nigeria [50]. Some *Bacillus* and *Enterococcus* strains, isolated from traditional *okpehe* fermentations, have been studied for their suitability as starter cultures in laboratory-scale fermentations of *Prosopis Africana* seeds for the production of *okpehe,* a traditional fermented vegetable product of Nigeria. The bacteriocin produced by *B. subtilis* from *okpehe* was identified as subtilisin [51].

Dadawa/Iru is one of the most important food condiments in Nigeria and many countries of West and Central Africa. It is used in much the same way as bouillon cubes are used in the Western world as nutritious flavouring additives along with cereal grains sauce and may serve as meat substitute. *dadawa (iru)* is prepared from the seeds of African locust beans *(Parkia biglobosa)* thus are rich in fat (39 to 40%) and protein (31 to 40%) [52] and contributes significantly to the energy intake, protein and vitamins, especially riboflavin [16]. The major fermenting organisms are the *Bacillus* and *Staphylococcus* [16]. *Dadawa* fermentation is very similar to that of *okpehe* prepared from the seeds of *Prosopis africana*, *ogiri* prepared from melon seeds (*Citrullus vulgaria*) and castor oil bean *(Ricinus cummunis*) [16]. Although, the organisms involved in the fermentation of these foods condiments varies. Other biochemical changes that occur during *dadawa* fermentation include the hydrolysis of indigestible oligosaccharide present in African locust beans notably stachyose and raffinose, to simple sugars

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by alpha and beta galactosidase, the synthesis of B-vitamins (thiamin and riboflavin),vitamin C and the reduction of anti-nutritional factors(oxalates and phytates) [16].

3. Probiotics in Aquaculture

In recent years, there has been an upsurge in research into probiotics, as well as growing commercial interest in the probiotic concept [8]. This increased research has resulted in significant advances in our understanding and ability to characterize specific probiotic organisms, as well as attempts to verify their attributed health benefits [8]. The use of probiotics and prebiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming an integral part of the aquaculture practices for improving growth and disease resistance [53]. This strategy offers innumerable advantages to overcome the limitations and side effects of antibiotics and other drugs and also lead to high production [54, 55].

The term "probiotic" (or beneficial bacteria) comes from the Greek words "pro" and "bios" meaning *"for life"*. It is opposed to the term "antibiotic" meaning "*against life*" [56]. Probiotics are often defined as applications of entire or component(s) of a micro-organism which are beneficial to the health of the host [57]. Other probiotic definitions are more encompassing, for example, Verschuere et al. [58] suggested the definition "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by increasing the host response towards disease, or by improving the quality of its environment". Although there is some dispute about what an aquatic probiotic actually is, all definitions differ to that of Fuller [59] in that there is no longer the requisite for the probiotic to be acting in the gastrointestinal

Comment [D7]: et al should be italicized please tract [60]. Therefore, modes of action such as competition for nutrients and production of inhibitory substances could occur in the culture water. Additional effects of probiotic action should also be considered, given the modified definition, including change of the water quality and interaction with phytoplankton [58].

Probiotics that are currently used in aquaculture industry include a wide range of taxa- from *Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus and Carnobacterium spp. to Bacillus, Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Aeromonas, Enterococcus, Nitrosomonas, Nitrobacter and Vibrio spp., yeast (Saccharomyces, Debaryomyces)* etc. [57, 61, 55].

3.1 Mechanisms or Modes of Action of Probiotics

Recently, there has been a growing interest in understanding the mechanisms of action of probiotics, especially in humans and other mammals [8]. Probiotics activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration, and route and frequency of delivery [8]. The mechanisms of actions of probiotics, as reported in the literature are as summarized in Table 1.0

Table 1. Mechanisms of Action of Probiotics and likely Benefits to Host [62]

Antimicrobial Activity
Decrease luminal pH
Secrets antimicrobial peptides
Inhibit bacterial invasion
Block bacterial adhesion to epithelial cells
Enhancement of Barrier
Increase mucus production
Enhance barrier integrity
Immunomodulation
Effects on epithelial cells
Effects on dendritic cells
Effects on monocytes/macrophage
Effects on lymphocytes
-B lymphocytes
-NK cells
-T cells
-T cells redistribution

As shown in Table 1, some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses, actively participating in the fight against infections. Others, on the other hand, inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules or modulating the inflammatory/immune response. Several studies have demonstrated that pattern recognition receptors [PRRs, such as toll-like receptors (TLRs)], signaling pathways, immune responses and the secretion of antimicrobial peptides such as defensins and chemokines by the epithelium play important roles in these mechanisms [63, 64].

These alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immune responses. However, other effects not directly immune related have been observed, such as improved growth performance, feed utilization, digestive enzyme activity, antioxidant enzyme activity, gene expression, disease resistance, larval survival, gut morphology, alteration of the gut microbiota, mediation of stress response, improvement in nutrition, reduced risk of certain cancers (colon, bladder), production of lactase, alleviation of symptoms of lactose intolerance and malabsorption [65, 53, 66, 67, 68, 69].

3.2 Gastrointestinal Tract Microbiota of Fish

Gastrointestinal (GI) microbiota of fish, like that of mammals, can be classified as either autochthonous or allochthonous populations [70]. The autochthonous bacteria are those able to colonize the host's epithelial surface or are associated with the microvilli, which can be considered as potentially resident populations, while allochthonous populations are transient visitors present in the lumen [70]. There are differences in micro-organism found in the gut microflora with respect to fish from both sea water and fresh water. Thus salinity and differences in species may play a role in the GI microbiota [71].

Numerous surveys of the bacterial flora in the GI tract of fish are made during the last twenty years. Many reports demonstrated that Gram-negative, facultative anaerobic bacteria such as *Acinetobacter*, *Alteromonas*, *Aerotnonas*, *Bacteroides*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Pseudornonas*, *Proteobacterium* and *Vibrio* spp. constitute the predominant endogenous microbiota of a variety of species of marine fish [72, 73, 74]. In contrast to saltwater fish, the endogenous microbiota of freshwater fish species tends to be dominated by members of the genera *Aeromonas*, *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Pseudornonas* representatives of the family *Enterobacteriaceae*, and obligate anaerobic bacteria of the genera *Bacteroides*, *Clostridium* and *Fusobacterium* [75, 76, 77, 78]. Various species of LAB *(Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, and *Carnobacterium* spp.) have also demonstrated to comprise part of this microbiota [79, 77, 80, 81]. They are not dominant in the normal intestinal microbiota of fish, but some strains can colonize the gut [82, 83] or inhibit adhesion of several fish pathogens [81].

3.3 Probiotics as Immunomodulatory Agents

Probiotic bacteria have multiple and diverse influences on the host (Table 1.0) [62]. Different organisms can influence the intestinal luminal environment, epithelial and mucosal barrier function, and the mucosal immune system [62]. They exert their effects on numerous cell types involved in the innate and adaptive immune responses, such as epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, including T cells with regulatory properties, and NK cells [62]. Figure 1.0 provides a simplified illustration of the main mechanisms of action of probiotics and likely benefits to host [84, 85].

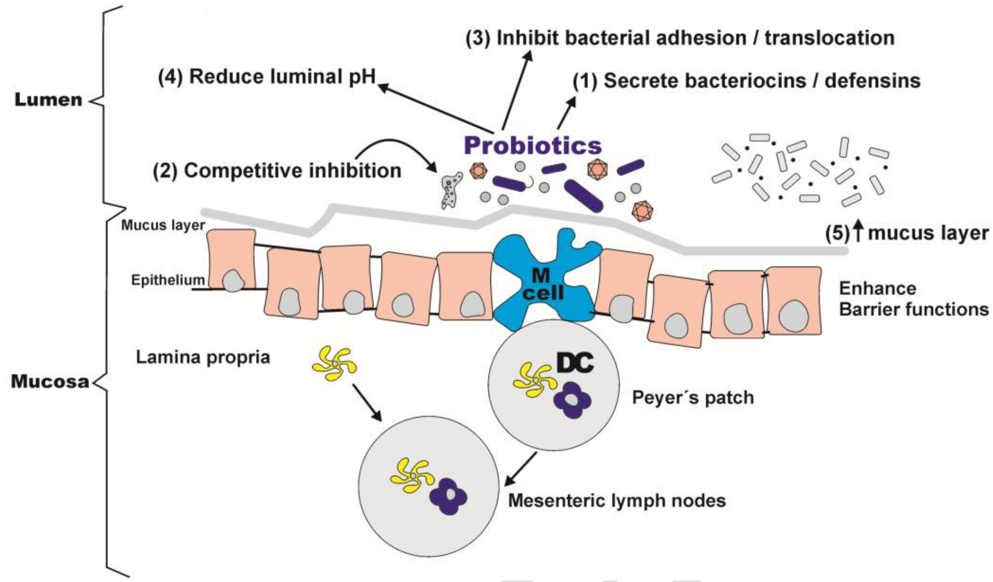

Figure 1. lnhibition of enteric bacteria and enhancement of barrier function by probiotic bacteria. Schematic representation of the crosstalk between probiotic bacteria and the intestinal mucosa. Antimicrobial activities of probiotics include the (1) production of bacteriocins/defensins, (2) competitive inhibition with pathogenic bacteria, (3) inhibition of bacterial adherence or translocation, and (4) reduction of luminal pH. Probiotic bacteria can also enhance intestinal barrier function by (5) increasing mucus production. [Color figure can be viewed in the online issue, which is available at www.interscience.wilev.com. [62].

The normal microbiota in the GI ecosystem influences the innate immune system, which is of vital importance for the disease resistance of fish and is divided into physical barriers, humoral and cellular components [8]. Several studies have shown that probiotics improves the growth rate of fish by improving their immune status. The use of Probiotics to displace pathogenic bacteria by competitive process is a better remedy than administering AGPs [8].

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Probiotics can interact with the host's immune cells such as mononuclear phagocytic cells (monocytes, macrophages), poly-morphonuclear leucocytes (neutrophils) and natural killer cells to enhance innate immune responses. Experiments report influences in the organism phagocytic activity, respiratory burst activity, lysozyme levels, peroxidases activity and complement system activity [86]. More detailed approaches mention cytokines modulation [66]. Within probiotic bacteria, *Lactobacillus* and *Enterococcus* genera appear to be the most influent in the immune system modulations [8]. It's most common action appears to be the improvement of complement system activity [87], peroxidase [88] and cytokine expression [89].

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The first line of defense within the GIT is the mucosa that separates the gut microbiota from direct contact with the epithelial cells of the GIT [90]. It is because of this direct contact with the mucus that the immune system of the GIT, often referred to as gut-associated lymphoid tissue or GALT, has developed mechanisms to distinguish between potentially pathogenic bacteria and the normal, commensal autochthonous bacteria [90]. Consequently, the GALT can determine whether to mount an attack or tolerate a specific bacteria's presence [90]. If potentially pathogenic bacteria are detected, the cellular and humoral mechanisms of the GALT activate the innate immune system and, subsequently, the adaptive immune system (via antibodies) to prevent bacteria from causing and/or spreading infection [91]. However, Simon [92] argued that bacterial probiotics do not have a mode of action but act on species specific or even strain-specific and immune responses of the animal, and their interaction with intestinal bacterial communities plays a key role. Probiotics produce inhibitory substances that could be antagonistic to the growth of pathogens in the intestine. The ability of some probiotics to adhere to the intestinal mucus may block the intestinal infection route common to many pathogens [93, 67].

Components of the innate or non-specific immune response include such factors as blood neutrophil oxidative radical production, serum lysozyme, and superoxide anion production in activated macrophages [90]. Other Innate humoral parameters include antimicrobial peptides, lysozyme, complement components, transferrin, pentraxins, lectins, antiproteases and natural antibodies, whereas nonspecific cytotoxic cells and phagocytes (monocytes/macrophages and neutrophils) constitute innate cellular immune effectors [8]. Cytokines comprise an integral component of the adaptive and innate immune response, particularly IL-1β, interferon, tumor necrosis factor-α, transforming growth factor-β and several chemokines regulate innate immunity [91]. These various responses are intended to kill a wide variety of foreign or invading microorganisms, and enhancing them could significantly reduce the mortality of the aquatic organism when exposed to various pathogens [90].

Previous studies have demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout (*Oncorhynchus mykiss)* enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes [94]. Rengpipat et al. [95] reported that the use of *Bacillus* spp. (S11) has provided disease protection by activating both cellular and humoral immune defenses in fish. Nikoskelainen et al. [96] showed that administration of *Lactobacillus rhamnosus* (ATCC 53103) at a level of 105 cfu/g feed stimulated the respiratory burst in rainbow trout. Mona et al. [97] indicated that dietary administration of garlic and C. *dactylon* (as immunostimulants) enhanced all the growth performance and survival rates of *P. clarkii* after 6 weeks. Dietary administration of Biogen improved immune response of *P. clarkii* juveniles due to an increase in phagocytic activity of granulocytes under the effect of Bacillus [98]. A higher immune response was reported to be induced when *lactobacillus* was used as a probiotic. This observation is also supported by Salinas et al. [99] and Picchietti et al. [100], who claimed that

Comment [D10]: Italicized the et al please phagocytosis and cytotoxic activity were increased in seabream when *L. delbrueckii* and *Bacillus subtilis* were used as probiotic agents. Al-Douhail et al. [101] concluded that fish immunoglobulin concentration increases with probiotic Lactobacillus in the diet, irrespective of the species and the study situation. Increased total immunoglobulin concentration could be due to an increased immune response in the probiotic group, induced by the presence of *L. acidophilus*, as suggested by Panigrahia et al. [102]. The authors reported higher immunoglobulin levels in the blood plasma of rainbow trout when lactic acid bacteria *L. rhamnosus* JCM 1136 were supplemented in the diet of the fish. This also supports the fact that fish fed the probiotic diet were healthier, as also reported by Gabriel et al. [103].

3.4 Effects of Probiotics on Antioxidant Parameters

Probiotic supplementation has been correlated with antioxidant parameters modulation. Although not completely understood, possibilities encompass two major theories: improved diet utilization, hence increasing the assimilation of dietary antioxidants from feed, and also, an active role in antioxidants activity or availability. Antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase are considered the first line of antioxidant defense and served as sensitive biomarkers of oxidative stress [104]. Superoxide dismutase is considered the first enzyme responsible for scavenging reactive oxygen species (ROS) and protecting cells from damage by free radicals process [105].

3.5 Effects of Probiotics on Fish Growth Performance and Feed Utilization

Previous studies with fish showed an improvement in growth performance, survival and feed efficiency when a probiotic (either commercial or isolated from fish gut) was used, could be due to better nutrient digestibility, high-quality absorption and increased enzyme activities caused by a proper balance of the intestinal microbial flora [59] or exoenzyme secretion as suggested by Moriarty [106]. The author reported that bacteria of the, genus Lactobacillus secrete a wide range of exoenzymes that aid in nutrient digestibility. Similarly, Tovar et al. [107], Wang and Zirong [108], and Suzer et al. [109] all reported that digestive enzyme activities were increased when fish was fed with a probiotic-supplemented diet. The exoenzymes can also stimulate the appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and breakdown of indigestible components [34]. Additionally, better growth performance and nutrient efficiency could possibly be related to lower stressor levels in fish fed the probiotic diet. Decreased Cortisol levels have been reported by Carnevali et al. [110] when fish was fed a diet supplemented with *L. delbrueckii*. The authors claimed that the decreased cortisol levels affected the transcription of two genes, insulin-like growth factor (IGF-1) and myostatin (MSTN), both of which regulate growth performance. IGF-1 transcription increased and MSTN transcription was inhibited in the groups treated with probiotic, leading to a drastic increase in body weight of the fish compared with the control.

Mona et al. [97] reported that feeding *Procambarus clarkii* juveniles with diet containing, Biogen® (as probiotics), showed a significant increase in specific growth rate (SGR) after 6 weeks. Incorporation of *L. acidophilus* as probiotic in diet of African catfish resulted in higher growth rate and better nutrient utilization [101]. Enhanced growth has been observed in channel catfish subjected to *B. subtilis* probiotics feed [111]. Dennis and Uchenna [112] indicated significant growth of larval African catfish by the use of *L. acidophilus, L. bulgaricus, S. thermophilic* and *S. cereviciae* compared to artemia.

Fish feeds supplemented with probiotics such as *Bacillus* spp., *Bacillus subtilis* (ATCC 6633), *Lactobacillus acidophilus, Enteroccus faecium* ZJ4, *Lactobacillus delbrueckii* subsp. *Delbrueckii* (AS13B), *Micrococcus luteus, Pseudomonas* spp., *Streptococcus faecium,* Live yeasts, when fed to common carp, rainbow trout, Nile tilapia and European sea bass yield better digestive enzyme activities, better growth performance and feed efficiency, and body-weight gain [113, 110, 114, 115, 88, 116].

The incorporation of sesame seed meal fermented with *L. acidophilus* into diets of *Labeo rohita* improved their growth and nutritional performances [117]. An improved growth rate was observed in *O. mossambicus* when fed with diets like *Lactobacillus, Vibrio* sp, *Aeromonas* and *E. coli* [118]. The addition of probiotics to larval starter diets enhances soybean meal utilization in rainbow trout [119]. The incorporation of yeast *S. cerevisiae* in the diets of Nile tilapia produced better growth [120]. Similarly, improved growth performances were noted when *S. cerevisiae* was used in diets of sea bass [121], hybrid striped bass [122] and Japanese flounder [123]. The beneficial effects of yeast could be associated with its beneficial compounds like nucleic acid, β-glucans, mannan oligosaccharides and proteins [124]. Yeast naturally occurs in the gastrointestinal tract of healthy fish and constitutes an important part of the gut microbiota [125]. Yeast is able to stand pelletizing and retains its quality after pelleting [112]. Harikrishnan et al. [126] reported that yeast supplemented diets have effects of stimulating growth, feed efficiency, blood biochemistry, survival rate, and non- specific immune responses in olive flounder (*Paralichthys olivaceus)* challenged with *Uronema marinum* infection. Mixing of probiotic can be beneficial than using single probiotic strain. In the diets of rainbow trout juveniles challenged with *Yersinia ruckeri* administration of *S. cerevisiae* treated with beta- mercaptoethanol was better than whole cell yeast and n-3 highly unsaturated fatty acids (HUFA)-enriched yeast, in enhancing immune system and growth stimulation [127].

Within the tested probiotic blend, *Bacillus* and *Lactobacillus* genera seem to be the most correlated with growth improvement, either by influencing appetite, conversion ratio or reducing myostatin transcription [82, 110, 128] a protein responsible for mitigating muscle growth and development [86].

3.6 Probiotics for Nutritional Improvement and Pathogen Prevention

The intestinal microbiota has important and specific metabolic, trophic, and protective functions [129, 130]. The normal (resident) microbiota of the gut confers many benefits to the intestinal physiology of the host. Some of these benefits include the metabolism of nutrients, contribution of the colonization resistance, antagonistic activity against pathogens, immunomodulation etc. [129]. The intestinal microbiota has a profound impact on the anatomical, physiological and immunological development of the host [131]. Thus, establishing a healthy microbiota plays an important role in the generation of immuno-physiologic regulation by providing crucial signals for the development and maintenance of the immune system [132]. Understanding how the fish immune system generally responds to gut microbiota may be an important basis for targeting manipulation of the microbial composition. This might be of special interest to design adequate strategies for fish disease prevention and treatment [91]. The intestinal microbiota possesses antagonistic activity against many fish pathogens and participates in infection-protective reactions [133, 134, 135, 136]. Yoshimizu and Ezura [137] reported that fish intestinal bacteria such as *Aeromonas* and *Vibrio* spp. produced antiviral substances.

The bacterial flora of the GI tract of fishes in general, represents a very important and diversified enzymatic potential. It is capable of producing proteolytic, amylolytic, cellulolytic, lipolytic, and chitinolytic enzymes, which is important for digestion of proteins, carbohydrates, cellulose, lipids and chitin respectively [138, 133]. The enzyme producing microbiota can be beneficially used as probiotic supplements while formulating the fish diet, especially in the larval stages. It presents a scope for fish nutritionists to use the enzyme producing isolates as a probiotic in formulating cost-effective fish diets.

The useful microbiota sometimes serves as a supplementary source of food and microbial activity in the digestive tract and also is a source of vitamins or essential amino acids [139]. It has been seen that Bacteroides and Clostridium species contribute to the host's nutrition, especially by supplying fatty acids and vitamins [140].

The enzymes liberated by probionts helps in increasing the digestive utilization of feed or detoxifying injurious metabolites liberated by the harmful micro-flora. The alteration of microbial metabolism is however affected either by increased or decreased enzymatic activity. Amylase and lipase are the major enzymes related to carbohydrate and fat digestion, respectively. Tovar et al. [141] reported an increase in amylase and trypsin secretion in sea bass *(Dicentrarchus labrax*) larvae after being fed with live yeast *Debaryomyces hansenii.* Moreover, Mohapatra et al. [139] noted elevated level of digestive enzyme (protease, amylase and lipase) activities in *Labeo rohita* when fed with a mixture of *Bacillus subtilis*, *Lactococcus lactis* and *Saccharomyces cerevisiae.* Bacteria also secrete proteases to digest the peptide bonds in proteins and therefore break down the proteins into their constituent monomers and free amino acids, which can benefit the nutritional status of the animal. Higher alkaline phosphatase activity was observed in probiotic fed Nile tilapia *(Oreochromis niloticus)*, thereby reflecting a possible development of brush border membrane of enterocytes, and hence, indicating that the carbohydrate and lipid absorption has been enhanced due to probiotic supplementation [142]. Bacillus sp. Isolated from *Cyprinus carpio* demonstrated considerable extracellular amylolytic, cellulolytic, proteolytic and lipolytic activities [138]. Probiotics also play a very positive effect on the digestive processes as well as the assimilation of food components [57]. This increase in the nutrient digestibility maybe because of better availability of exoenzymes produced by probiotics [143] or better health condition [139].

4. CONCLUSION

Fermentation process transforms many harmful substances in feeds to non-harmful states. This improves bioavailability of nutrients, imparts biopreservative qualities and improves feed safety. Fermentation also leads to the production of antioxidant and antimicrobial substances, which impart health benefits to fish.

Comment [D11]: Impact instead of impart is better use in the context

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