

Fermentation of feed ingredients as potential strategy to improve health and reduce opportunistic infection 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, Probiotics, Resistance bacteria.

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].

31 Prevention and control of diseases in fish farming has led to significant increase in the use of
32 antibiotics in recent years, which have resulted in the selective survival of resistant species or
33 strains of bacteria [4, 5, 6]. Resistance to infection could be transferred to previously
34 susceptible bacteria and constitute serious hazards to both animal and human health [5].
35 Furthermore, antibiotics also inhibit or kill beneficial microbiota in the gut microflora, leading
36 to the accumulation of antibiotic residues in fish products that are harmful for human
37 consumption [7]. In recognition of these dangers, the use of sub-therapeutic doses of antibiotics
38 as growth-promoting agents in rearing animals was banned by the European Union since 2006
39 [8] and the evaluation for alternative strategies are mandatory.

40 Consequently, new strategies for feeding and health management during fish farming continue
41 to receive attention [9]. The global demand for safe food has prompted the search for natural
42 alternatives to Antibiotic growth promoters (AGPs) for feeding farmed animals. The
43 alternatives contemplated and being tested includes the use of probiotics, organic acids,
44 prebiotics, minerals, enzymes, herbs, phenolic aromatic components and fermented foods (FF)
45 [10, 11, 12, 13, 14, 15]. Although the consumption of FF is popular among different cultures
46 around the world and has been adopted in different animal husbandry practices, it has
47 unfortunately, not been fully adopted on feeds for rearing fish.

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

53 2. Purpose and Benefits of Feed Fermentation

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

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

76 2.1 Advantages of Food Fermentation

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

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

93 Food and feeds are often contaminated with a number of toxins like fumonisins, ocratoxin A,
94 zearalenone and aflatoxins (mycotoxins) either naturally or through infestation by
95 microorganisms such as moulds, yeast, bacteria and viruses [36]. Using LAB in fermentation
96 detoxifies toxins and is more advantageous, because it is a milder method which preserves the
97 nutritive value and flavor of foods [27]. In addition to this, fermentation irreversibly degrades

98 mycotoxins without adversely affecting the nutritional value of the food [36] and without
99 leaving any toxic residues [37].

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

108 **2.2 Health Benefits of Fermented Foods**

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

119 **2.3 Microorganisms Involved in Fermented Food Production**

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

128 LAB are a group of Gram positive bacteria, non-respiring, non-spore forming, cocci or rods,
129 the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are the main species
130 that play a key role in safety and acceptability of the products of carbohydrates in tropical
131 climate [45]. Most pathogenic microorganisms found in-food cannot survive the low pH,
132 hence, Lactic acid fermentation of food has been used to reduce the risk of pathogenic
133 microorganisms growth in the food [34]. Alkaline fermentation causes the hydrolysis of protein
134 to amino acids and peptides and releases ammonia, which increases the alkalinity by the
135 *Bacillus* species such as *Bacillus subtilis* (dominant species), *B. licheniformis* and *B. pumilius*
136 [46, 27].

137 Indigenous natural fermentation takes place in a mixed colony of microorganisms such as
138 moulds, bacteria and yeasts [44]. These bacteria are not harmful to the consumers and have
139 enzymes such as proteases, amylases and lipases that hydrolyze food complexes into simple
140 nontoxic products with desirable textures, aroma that makes them palatable for consumption
141 [45]. Thus, fermentation products in food substrates are based on the microorganisms involved
142 in the fermentation. Some of the compounds formed during fermentation include organic acids

143 (palmitic, pyruvic, lactic, acetic, propionic, malic, succinic, formic and butyric acids), alcohols
144 (mainly ethanol) aldehydes and Ketones (acetaldehyde, acetoin, 2-methyl butanol) [36].

145 **2.4 Nigeria Fermented Foods**

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

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

158 *In Nigeria, the popular fermented foods include the following:*

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

162 countries, especially Nigeria, the production of *gari* and *fufu* (fermented cassava product), *ogi*
163 (fermented maize, sorghum, or millet gruel), *fura da nono* (fresh cow's milk with fermented
164 millet gruel), and *pito*, *kunun-zaki* and *Burukutu* (cereal-based alcoholic beverages) are largely
165 brought about by lactic acid bacteria and yeast, with *L. plantarum* predominating [49, 16]. In
166 another study, *L. plantarum* and *Lactobacillus brevis* were the dominant lactic acid bacteria
167 isolated in different batches of *pito* and *burukutu* collected from local producers in Nigeria [50].
168 Some *Bacillus* and *Enterococcus* strains, isolated from traditional *okpehe* fermentations, have
169 been studied for their suitability as starter cultures in laboratory-scale fermentations of *Prosopis*
170 *Africana* seeds for the production of *okpehe*, a traditional fermented vegetable product of
171 Nigeria. The bacteriocin produced by *B. subtilis* from *okpehe* was identified as subtilisin [51].

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

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

186 3. Probiotics in Aquaculture

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

196 The term "probiotic" (or beneficial bacteria) comes from the Greek words "pro" and "bios"
197 meaning "*for life*". It is opposed to the term "antibiotic" meaning "*against life*" [56]. Probiotics
198 are often defined as applications of entire or component(s) of a micro-organism which are
199 beneficial to the health of the host [57]. Other probiotic definitions are more encompassing, for
200 example, Verschuere et al. [58] suggested the definition "a live microbial adjunct which has a
201 beneficial effect on the host by modifying the host-associated or ambient microbial community,
202 by ensuring improved use of the feed or enhancing its nutritional value, by increasing the host
203 response towards disease, or by improving the quality of its environment". Although there is
204 some dispute about what an aquatic probiotic actually is, all definitions differ to that of Fuller
205 [59] in that there is no longer the requisite for the probiotic to be acting in the gastrointestinal

206 tract [60]. Therefore, modes of action such as competition for nutrients and production of
207 inhibitory substances could occur in the culture water. Additional effects of probiotic action
208 should also be considered, given the modified definition, including change of the water quality
209 and interaction with phytoplankton [58].

210 Probiotics that are currently used in aquaculture industry include a wide range of taxa- from
211 *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Streptococcus* and *Carnobacterium* spp. to
212 *Bacillus*, *Flavobacterium*, *Cytophaga*, *Pseudomonas*, *Alteromonas*, *Aeromonas*, *Enterococcus*,
213 *Nitrosomonas*, *Nitrobacter* and *Vibrio* spp., yeast (*Saccharomyces*, *Debaryomyces*) etc. [57, 61,
214 55].

215 **3.1 Mechanisms or Modes of Action of Probiotics**

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

221

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

223 **Antimicrobial Activity**

224 Decrease luminal pH

225 Secretes antimicrobial peptides

226 Inhibit bacterial invasion

227 Block bacterial adhesion to epithelial cells

228 **Enhancement of Barrier**

229 Increase mucus production

230 Enhance barrier integrity

231 **Immunomodulation**

232 Effects on epithelial cells

233 Effects on dendritic cells

234 Effects on monocytes/macrophage

235 Effects on lymphocytes

236 -B lymphocytes

237 -NK cells

238 -T cells

239 -T cells redistribution

240

241 As shown in Table 1, some probiotics exert their beneficial effects by elaborating antibacterial

242 molecules such as bacteriocins that directly inhibit other bacteria or viruses, actively

243 participating in the fight against infections. Others, on the other hand, inhibit bacterial

244 movement across the gut wall (translocation), enhance the mucosal barrier function by

245 increasing the production of innate immune molecules or modulating the inflammatory/immune
246 response. Several studies have demonstrated that pattern recognition receptors [PRRs, such as
247 toll-like receptors (TLRs)], signaling pathways, immune responses and the secretion of
248 antimicrobial peptides such as defensins and chemokines by the epithelium play important roles
249 in these mechanisms [63, 64].

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

258 **3.2 Gastrointestinal Tract Microbiota of Fish**

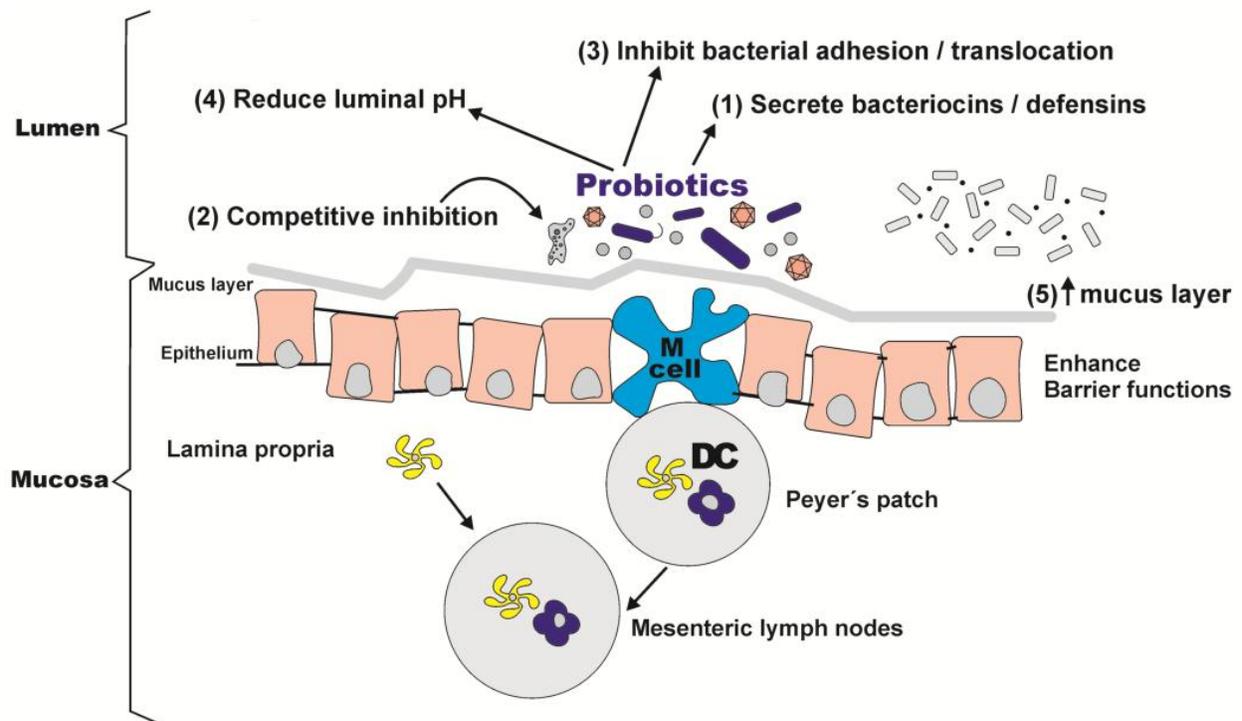
259 Gastrointestinal (GI) microbiota of fish, like that of mammals, can be classified as either
260 autochthonous or allochthonous populations [70]. The autochthonous bacteria are those able to
261 colonize the host's epithelial surface or are associated with the microvilli, which can be
262 considered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

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

279 3.3 Probiotics as Immunomodulatory Agents

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

287



288

289

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

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

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

310 The first line of defense within the GIT is the mucosa that separates the gut microbiota from
311 direct contact with the epithelial cells of the GIT [90]. It is because of this direct contact with the
312 mucus that the immune system of the GIT, often referred to as gut-associated lymphoid tissue or
313 GALT, has developed mechanisms to distinguish between potentially pathogenic bacteria and
314 the normal, commensal autochthonous bacteria [90]. Consequently, the GALT can determine
315 whether to mount an attack or tolerate a specific bacteria's presence [90]. If potentially
316 pathogenic bacteria are detected, the cellular and humoral mechanisms of the GALT activate the
317 innate immune system and, subsequently, the adaptive immune system (via antibodies) to
318 prevent bacteria from causing and/or spreading infection [91]. However, Simon [92] argued that
319 bacterial probiotics do not have a mode of action but act on species specific or even strain-
320 specific and immune responses of the animal, and their interaction with intestinal bacterial
321 communities plays a key role. Probiotics produce inhibitory substances that could be
322 antagonistic to the growth of pathogens in the intestine. The ability of some probiotics to adhere
323 to the intestinal mucus may block the intestinal infection route common to many pathogens [93,
324 67].

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

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

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

357 **3.4 Effects of Probiotics on Antioxidant Parameters**

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

366 **3.5 Effects of Probiotics on Fish Growth Performance and Feed Utilization**

367 Previous studies with fish showed an improvement in growth performance, survival and feed
368 efficiency when a probiotic (either commercial or isolated from fish gut) was used, could be
369 due to better nutrient digestibility, high-quality absorption and increased enzyme activities

370 caused by a proper balance of the intestinal microbial flora [59] or exoenzyme secretion as
371 suggested by Moriarty [106]. The author reported that bacteria of the, genus *Lactobacillus*
372 secrete a wide range of exoenzymes that aid in nutrient digestibility. Similarly, Tovar et al.
373 [107], Wang and Zirong [108], and Suzer et al. [109] all reported that digestive enzyme
374 activities were increased when fish was fed with a probiotic-supplemented diet. The
375 exoenzymes can also stimulate the appetite and improve nutrition by the production of
376 vitamins, detoxification of compounds in the diet and breakdown of indigestible components
377 [34]. Additionally, better growth performance and nutrient efficiency could possibly be related
378 to lower stressor levels in fish fed the probiotic diet. Decreased Cortisol levels have been
379 reported by Carnevali et al. [110] when fish was fed a diet supplemented with *L. delbrueckii*.
380 The authors claimed that the decreased cortisol levels affected the transcription of two genes,
381 insulin-like growth factor (IGF-1) and myostatin (MSTN), both of which regulate growth
382 performance. IGF-1 transcription increased and MSTN transcription was inhibited in the groups
383 treated with probiotic, leading to a drastic increase in body weight of the fish compared with the
384 control.

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

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

398 The incorporation of sesame seed meal fermented with *L. acidophilus* into diets of *Labeo*
399 *rohita* improved their growth and nutritional performances [117]. An improved growth rate
400 was observed in *O. mossambicus* when fed with diets like *Lactobacillus*, *Vibrio* sp, *Aeromonas*
401 and *E. coli* [118]. The addition of probiotics to larval starter diets enhances soybean meal
402 utilization in rainbow trout [119]. The incorporation of yeast *S. cerevisiae* in the diets of Nile
403 tilapia produced better growth [120]. Similarly, improved growth performances were noted
404 when *S. cerevisiae* was used in diets of sea bass [121], hybrid striped bass [122] and Japanese
405 flounder [123]. The beneficial effects of yeast could be associated with its beneficial
406 compounds like nucleic acid, β -glucans, mannan oligosaccharides and proteins [124]. Yeast
407 naturally occurs in the gastrointestinal tract of healthy fish and constitutes an important part of
408 the gut microbiota [125]. Yeast is able to stand pelletizing and retains its quality after pelleting
409 [112]. Harikrishnan et al. [126] reported that yeast supplemented diets have effects of
410 stimulating growth, feed efficiency, blood biochemistry, survival rate, and non- specific
411 immune responses in olive flounder (*Paralichthys olivaceus*) challenged with *Uronema*
412 *marinum* infection. Mixing of probiotic can be beneficial than using single probiotic strain. In
413 the diets of rainbow trout juveniles challenged with *Yersinia ruckeri* administration of *S.*
414 *cerevisiae* treated with beta- mercaptoethanol was better than whole cell yeast and n-3 highly

415 unsaturated fatty acids (HUFA)-enriched yeast, in enhancing immune system and growth
416 stimulation [127].

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

421 **3.6 Probiotics for Nutritional Improvement and Pathogen Prevention**

422 The intestinal microbiota has important and specific metabolic, trophic, and protective functions
423 [129, 130]. The normal (resident) microbiota of the gut confers many benefits to the intestinal
424 physiology of the host. Some of these benefits include the metabolism of nutrients, contribution
425 of the colonization resistance, antagonistic activity against pathogens, immunomodulation etc.
426 [129]. The intestinal microbiota has a profound impact on the anatomical, physiological and
427 immunological development of the host [131]. Thus, establishing a healthy microbiota plays an
428 important role in the generation of immuno-physiologic regulation by providing crucial signals
429 for the development and maintenance of the immune system [132]. Understanding how the fish
430 immune system generally responds to gut microbiota may be an important basis for targeting
431 manipulation of the microbial composition. This might be of special interest to design adequate
432 strategies for fish disease prevention and treatment [91]. The intestinal microbiota possesses
433 antagonistic activity against many fish pathogens and participates in infection-protective
434 reactions [133, 134, 135, 136]. Yoshimizu and Ezura [137] reported that fish intestinal bacteria
435 such as *Aeromonas* and *Vibrio* spp. produced antiviral substances.

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

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

447 The enzymes liberated by probionts helps in increasing the digestive utilization of feed or
448 detoxifying injurious metabolites liberated by the harmful micro-flora. The alteration of
449 microbial metabolism is however affected either by increased or decreased enzymatic activity.
450 Amylase and lipase are the major enzymes related to carbohydrate and fat digestion,
451 respectively. Tovar et al. [141] reported an increase in amylase and trypsin secretion in sea bass
452 (*Dicentrarchus labrax*) larvae after being fed with live yeast *Debaryomyces hansenii*. Moreover,
453 Mohapatra et al. [139] noted elevated level of digestive enzyme (protease, amylase and lipase)
454 activities in *Labeo rohita* when fed with a mixture of *Bacillus subtilis*, *Lactococcus lactis* and
455 *Saccharomyces cerevisiae*. Bacteria also secrete proteases to digest the peptide bonds in proteins
456 and therefore break down the proteins into their constituent monomers and free amino acids,
457 which can benefit the nutritional status of the animal. Higher alkaline phosphatase activity was
458 observed in probiotic fed Nile tilapia (*Oreochromis niloticus*), thereby reflecting a possible

459 development of brush border membrane of enterocytes, and hence, indicating that the
460 carbohydrate and lipid absorption has been enhanced due to probiotic supplementation [142].
461 *Bacillus* sp. Isolated from *Cyprinus carpio* demonstrated considerable extracellular amylolytic,
462 cellulolytic, proteolytic and lipolytic activities [138]. Probiotics also play a very positive effect
463 on the digestive processes as well as the assimilation of food components [57]. This increase in
464 the nutrient digestibility maybe because of better availability of exoenzymes produced by
465 probiotics [143] or better health condition [139].

466 4. CONCLUSION

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

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