1 **Research progress on gut health of farmed teleost fish: a viewpoint from the**

2 **damage of intestinal mucosal barrier**

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20 **Abstract**

21 Maintaining intestinal homeostasis and health is important to enhance the

22 performance of farmed teleost fish. However, the intestinal mucosa barrier is

23 challenged by a number of factors, which lead to damage of the mucosal barrier. The

24 occurrences of cell death, including apoptosis, pyroptosis, necroptosis as well as

25 secondary injury by cell lysis, are closely related to gut homeostasis and pathogenesis

26 of intestinal mucosa barrier damage in most farmed teleost fish. However, the

27 mechanisms behind intestinal mucosal barrier damage are not well studied. Here we

28 summarized the factors causing the damage of gut mucosal barrier. Further we

29 discussed the intracellular and interstitial signaling pathways potentially regulating

30 intestinal homeostasis and cell death, including the immunologically-silent apoptotic

31 forms and non-apoptotic inflammatory cell death, which may improve our

32 understanding of the damage progression and facilitate the development of

33 intervention strategies.

34 **Keywords**: teleost fish, gut health, intestinal mucosal barrier, damage, gut microbiota,

35 cell death

36   **Abbreviation:**

37   GI, gastrointestinal; IECs, intestinal epithelial cells; PCD, programmed cell death;

38   PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated

39   molecular patterns; PRRs, pattern recognition receptors.

40

41   **Introduction**

42   The global fishery and aquaculture data derived from the OECD-FAO

43   Agricultural Outlook 2017-2026 show that global fish production is projected to reach

44   194 million tonnes in 2026, and aquaculture will continue to be one of the fastest

45   growing food sectors (OECD/FAO, 2017). The sustainable increases in aquaculture

46   production require improved fish breeding and genetics, disease detection and control,

47   sustainable nutrition and feeds, as well as enhanced production systems (Finegold

48   2009; Pelletier et al. 2018).

49   Maintaining intestinal homeostasis is important to enhance the performance of

50   farmed teleost fish. The basic features of the gastrointestinal (GI) tract and its function

51   in teleost fish are similar as that of other vertebrates although teleost fish have many

52   different feeding habits, diet types, nutrient requirements and physiological conditions

53   (Day et al. 2014; Løkka and Koppang 2016; McCue et al. 2017; Zhao and Pack 2017).

54   In vertebrates, the GI tract is the largest surface of the body which is exposed to the

55   intestinal contents and consequently faces many challenges including bacteria,

56   parasites, viruses, antigens and toxins from the luminal contents (Gomez et al. 2013;

57   Løkka and Koppang 2016; Rombout et al. 2011). As the key determinant of gut health,

58   the intestinal mucosal barrier in teleost fish is made up of mucous layer, epithelium

59   and lamina propria, which separates the luminal contents from the underlying tissues.

60   It is the most important and selective barrier that functions in maintaining cellular and

61   tissue homeostasis (Chen et al. 2015; Gomez et al. 2013; Marjoram et al. 2015).

62   Like other animals, teleost fish are sensitive to exposure to the xenobiotics which

63   can induce cellular stress responses and mucosal barrier damage in the GI tract

64   (Hamilton et al. 2017; Løkka and Koppang 2016; Ringø et al. 2010). Moreover, the

65   time in which teleost fish must adjust to the internal and external environment is short

66   because the intestinal environment is always dynamic and complex in fish, and its

67   homeostasis is fragile (Benjamin and Eric 2015; Pérez et al. 2010). Particularly,

68   seawater fish have regular challenges in that they are constantly dehydrated and need

69   to drink seawater that can be contaminated by numerous pathogens and damaging

70   agents (Dehler et al. 2017). Disruptions of the fine-tuned intestinal mucosal barrier

71   resulted in permeability defects through intracellular, transcellular and paracellular

72   routes. However, the cause of formation as well as the cellular and molecular

73   mechanisms involved in the regulation of intestinal tissue damage in farmed teleost

74   fish (zebrafish regarded as representative of economically farmed teleost fish if

75   mentioned) are poorly understood, and here we review current knowledge about the

76   factors and the associated mechanisms.

77

78   **Part 1. Factors causing the damage of intestinal mucosal barrier in farmed**

79   **teleost fish**

80   The gut health of farmed teleost fish is challenged by a number of factors

81   including host, microbial and other environmental factors, which can disturb intestinal

82   homeostasis (Table 1). Once the host fails to resist or neutralize the negative effects of

83   external stimuli, the intestinal mucosa barrier loses its structural integrity

84   and dysfunction occurs (Marjoram et al. 2015; Ringø et al. 2007a, b; Xia et al. 2013).

85   The progress of intestinal mucosa barrier damage in teleost fish appears to be a

86   progressive convergence of diverse signaling, which derived from the regulatory

87   components underlying different overlapping cell layers (Fig. 1).

88

89   **Imbalance of host-microbiota symbiosis**

90   Host-microbiota interactions within the intestinal ecosystem are essential for

91   gastrointestinal homeostasis and pathogen defense (Bledsoe et al. 2018; Pérez et al.

92   2010; Ringø et al. 2016). The microbial communities, their metabolites and

93   components are necessary for immune responses and can regulate the susceptibility of

94   the host to gastrointestinal disorders (Butt and Volkoff 2019; Rooks and Garrett 2016).

95   In response to microbiota (*Pseudomonas aeruginosa* PA01) colonization in zebrafish

96   gut, a systemic signal intestinal Serum amyloid A (*Saa*) can be induced to decrease

97 inflammatory tone and bactericidal activity, and enhance damage repair by restricting

98 of the aberrant activation of neutrophils (Murdoch et al. 2019). Disturbance of the

99 balance of gut-microbiota might lead to dysbiosis, allowing translocation and invasion

100 of enteric bacteria, including *Aeromonas hydrophila* (*A. hydrophila*) *and Vibrio*

101 *anguillarum* (*V. anguillarum*) due to increased pathogen susceptibility of host (Gomez

102 et al. 2013; Liu et al. 2016; Yang et al. 2017). Tran et al. (2018) pointed out gut

103 microbiota alternations are associated with intestinal disease (enteritis) in grass

104 carp (*Ctenopharyngodon idellus*), due to the change of specific metabolic pathways

105 related to xenobiotics biodegradation and metabolism in diseased fish. Studies in

106 zebrafish model with human intestine inflammatory bowel disease

107 (IBD)-like enterocolitis have indicated that gut microbiota regulates intestinal

108 epithelial gene expression by suppressing a nuclear receptor transcription factor

109 Hepatocyte nuclear factor 4 alpha (*Hnf4a*) (Davison et al. 2017). Similar regulatory

110 mechanisms have been found in human and mice, suggesting that microbial

111 suppression of *Hnf4a* may be a conserved feature of transcriptional programs. It is

112 involved in the intestinal inflammatory networks and the progression of intestinal

113 mucosal barrier damage.

114

115 **Nutritional imbalance and environmental stimulus**

116 The gut health of teleost fish is affected by a variety of nutritional and

117 environmental factors, which regulate the composition of gut microbiota, the immune,

118   endocrine and nervous system (Piazzon et al. 2017). If the presence of influence

119   factors exceeds the tolerance limits, they may be harmful to intestinal homeostasis of

120   fish trough direct and/or indirect influences (Arias-jayo et al. 2018; Wang et al. 2019b;

121   Xia et al. 2018).

122   Nutritional imbalance with excessive or deficient dietary supplementation in fat,

123   energy, and sugar may cause local and systemic inflammation, resulting in disorders

124   of gut and metabolic health. Previous studies suggest that high supplementation of

125   plant-based alternative protein in fish feeds, such as dietary gossypol, soybean meal

126   (SBM) and broad bean, containing antinutritional factors (ANFs), can cause intestinal

127   barrier damage that is accompanied with apoptosis and necrosis in the intestinal

128   epithelial cells (IECs), and lead to intestinal oxidative stress, inflammation (enteritis)

129   in some farmed teleost fish species, such as Atlantic salmon (*Salmo salar*), juvenile

130   turbot (*Scophthalmus maximus*), rainbow trout (*Oncorhynchus mykiss*), yellow

131   catfish (*Pelteobagrus filvidraco*) and grass carp (*Ctenopharyngodon idella*) (Gajardo

132   et al. 2017; Green et al. 2013; Gu et al. 2018; Jiang et al. 2018; Li et al. 2018; Miao et

133   al. 2018; Mosberian-Tanha et al. 2016; Wang et al. 2019b). Similar consequences

134   were found in fish fed low or high-fat diets, which caused the damage of

135   intestinal physical structure and immune barrier function, and led to gut

136   microbiota dysbiosis and intestinal inflammation in fish (Arias-jayo et al. 2018; Feng

137   et al. 2017). Moreover, dietary deficiency of certain microelements, such as

138   phosphorus, magnesium and pyridoxine, which impair the intestinal integrity and

139   immune function in grass carp (*Ctenopharyngodon idella*) (Chen et al. 2018; Wei et al.

140   2018; Wu et al. 2018b).

141   Ecotoxicological studies have revealed that widespread pollutants of the aquatic

142   ecosystems adversely impact the intestinal homeostasis of freshwater and marine fish

143   through immunotoxicologic effects on enterocyte and macrophages. Chronic exposure

144   to chemical contaminants, such as the dissolved metal, organophosphorous pesticides

145   and microplastic particles, which can be ingested and accumulated in aquatic

146   organisms, cause gut dysbiosis, enterocyte damages, intestinal barrier dysfunction and

147   inflammation (Barišić et al. 2018; Lei et al. 2018; Mijošek et al. 2019). Begam and

148   Sengupta (2015) found that mercury at a sub-lethal concentration induced intestinal

149   inflammatory damage in the fresh water fish *Channa punctatus* Bloch. The intestinal

150   histopathological features were characterized by villi cracking, mucosal folding

151   lesions, epithelium fragmentation, decline in the number of goblet cells, and damage

152   of enterocyte and macrophage (Begam and Sengupta 2015).

153

Immune dysfunction

154   **Immune dysfunction**

155   Dysregulation of inflammatory cytokines, chemokines, and immune cell

156   recruitment and activation in the lamina propria may trigger intestinal barrier defects,

157   and additional exposure to diverse stimuli, such as pathogens and DAMPs released

158   from died cells, may result in a amplification loop of damage signals (Maloy and

159 Powrie 2011; Nunes et al. 2014). Overexpression of proinflammatory cytokines has

160 been detected in various intestinal cell types including immune cells and IECs in

161 human and mice, which is a key element in the development of intestinal

162 inflammatory diseases (Haines et al. 2016). Chronic proinflammatory milieu

163 accumulates high concentration of ROS as well as matrix metalloproteinases

164 (MMPs), that in turn exacerbate intestinal mucosal wounds by disruption of the

165 extracellular matrix and epithelial junction, and result in further invasion of

166 pathogens (Leoni et al. 2015). When fresh water teleost *Channa punctatus* were

167 exposed to mercuric chloride, the immunomodulation of intestinal macrophages is

168 compromised, leading to overexpression of pro-inflammatory cytokines

169 (e.g., TNF-α and IL-6), which may be involved in the inflammatory damage in the

170 intestinal epithelium (Begam and Sengupta 2015).

171

172 **Host-specific genetic/epigenetic/phenotypic factors**

173 The genetic background of teleost fish determines the susceptibility of the GI

174 tract, and influences the gut microbial composition, especially under the challenge of

175 various danger factors (Brown et al. 2019; Marancik et al. 2015). Deficiency in the

176 intestinal barrier-related genes, such as the class III PI3-kinase (phosphoinositide

177 3-kinase, PIK3C3), macrophage-stimulating protein (MSP) and its receptor RON

178 (Recepteur d'Origine Nantais), causes disorder of IECs polarity, results in

179 spontaneous intestinal inflammation with IBD-like features, and increases

180 susceptibility toward epithelial damage in zebrafish (Torraca and Mostowy 2018;

181 Witte et al. 2014; Zhao et al. 2018; Zhao and Pack 2017). Moreover, the evolution and

182 modification of epigenomic factors allow host cells to regulate gene expression

183 without altering the genetic code, and this leads to develop potent mechanisms by

184 which vertebrate cells can transcriptionally respond, quickly or stably, to

185 environmental signals (Marjoram et al. 2015; Stilling et al. 2014). Marjoram et al.

186 (2015) revealed that loss of function of epigenetic regulator ubiquitin-like protein

187 containing PHD and RING finger domains 1 (*uhrf1*) induced reduction of DNA

188 methylation and epigenetic repression at the *tnfa* promoter, resulting in intestinal

189 barrier loss, IECs shedding and apoptosis, chronic inflammation, and IBD-like

190 intestinal disease.

191

192 **Enteric nervous system dysfunction**

193 The enteric nervous system (ENS) is the largest and most complex part of the

194 peripheral nervous system, which modulates essential intestinal functions including its

195 motility, secretion and blood flow (Alonso et al. 2014; Kulkarni et al. 2018; Yoo and

196 Mazmanian 2017). It plays an important role in maintaining intestinal health by

197 sensing the dynamic ecosystem of the GI tract, and sustaining the balance of gut

198 microbiota composition (Ganz 2018; Rolig et al. 2017; Taylo et al. 2016). Zebrafish

199 has emerged as a powerful model to study gastrointestinal diseases that

200 associated with ENS disorders. The role of the ENS have been analyzed using

201   zebrafish mutant larvae, which are especially established by genetic mutation of

202   certain conserved regulatory factors, such as the glial cell line-derived neurotrophic

203   factor (GDNF)/RET proto-oncogene, the transcription factors sex-determining-region

204   Y-box 10 (SOX10) and paired-like homeobox 2b (PHOX2B) (Roy-Carson et al. 2017;

205   Taylor et al. 2016). In zebrafish model of humans hirschsprung disease (HSCR) with

206   ENS dysfunction, lack of the normal development of zebrafish ENS, that resulted

207   from a mutation in gene *sox10*, altered gut motility parameters and impaired

208   pathogen clearance, which led to bacterial overgrowth and dysbiosis, and then

209   intestinal inflammation (Rolig et al. 2017).

210

211   **Part 2. Cell death modes determining gut mucosal homeostasis in farmed teleost**

212   **fish**

213   Intestinal epithelial cells have evolved a series of well-regulated programs to

214   prevent penetration, pathogen translocation and tissue damage (Enyedi and

215   Niethammer 2015; Ramanan and Cadwell 2016). During the normal physiological

216   differentiation and maturation of IECs, high proliferative rates coexist with cellular

217   demise under tight regulation contributing to maintenance of epithelial barrier

218   functions (Delgado et al. 2016; Gudipaty and Rosenblatt 2017).

219   Under multiple abnormal physiological and pathological conditions, induction of

220   programmed cell death (PCD) in IECs is a defense mechanism for immune reactions

221 or local homeostasis (Fig. 2). According to the distinct morphological features,

222 signaling pathways and immunological effects, PCD includes different forms of cell

223 death, such as apoptosis, necroptosis and pyroptosis, which are associated with the

224 intestinal mucosal barrier as reported in mammals (Flieger et al. 2018; Nunes et al.

225 2014; Wen et al. 2017). Disturbance of these genetically regulated processes triggers

226 excessive cell death in the intestinal epithelial layer, and these signals in turn interact

227 with a range of cell-intrinsic and cell-extrinsic regulatory modules. Defects in these

228 homeostatic modules may impair the vital functions of intestinal mucosal barrier, and

229 induce the pathophysiological damage of the intestinal epithelium (Frank and Vince

230 2019; Gudipaty and Rosenblatt 2017; Nunes et al. 2014; Sharma and Kanneganti

231 2017).

232

233 **Apoptosis**

234 Apoptosis is a highly regulated programmed cell death under the regulation of

235 proteolytic caspases cascade, characterized by special morphological features that

236 differ from other cell death forms, but seems to be similar in higher eukaryotes

237 (Alberts et al. 2002; Fuchs and Steller 2011; Kale et al. 2012; Márquez-Jurado et al.

238 2018). Defects in the signaling pathways of apoptosis directly or indirectly trigger

239 pathological death signals, known as secondary necrosis (Günther and Seyfert 2018;

240 Oropesa-Ávila et al. 2015).

241   Most of the core regulatory molecules that are necessary for apoptosis are

242   evolutionarily conserved between teleost fish and mammals (AnvariFar et al. 2017;

243   dos Santos et al. 2008; Giri et al. 2018; Li et al. 2011; Sakamaki et al. 2007). The

244   signaling pathway of apoptotic IECs in fish can be categorized into

245   caspase-8-mediated death receptor pathway, caspase-9-mediated mitochondria

246   pathway, and ER stress-related pathway. The regulation of apoptosis in fish intestine

247   is related to gene expression of pro-apoptotic proteins (Apaf-1, Bax and FasL) and

248   anti-apoptotic proteins (Bcl-2, IAP and Mcl-1b), and the activation of caspases (-2, -3,

249   -7, -8 and -9), which may be linked with the c-terminal Jun Kinase (JNK) signaling

250   pathway (Ronza et al. 2011; Wei et al. 2018).

251

Necroptosis

253   In case of the failure of the apoptotic caspase activation, there is an alternative

254   programmed cell death called necroptosis (Galluzzi et al. 2018; Negroni et al. 2017).

255   Necroptosis plays an important role in control of tissue damage, inflammation and

256   epithelial homeostasis in response to a variety of physiological and pathological

257   conditions (Dannappel et al. 2014; Moerke et al. 2019; Negroni et al. 2017; Pasparakis

258   and Vandenabeele 2015).

259   Necroptosis is linked to the pathogenesis of fish diseases due to the release of

260   residual bacteria into permissive extracellular milieu. In zebrafish tuberculosis models,

261 high tumour-necrosis factor (TNF) promoted early macrophage resistance to

262 mycobacteria infection through mitochondrial ROS, and subsequently

263 infected-macrophage undergone necroptosis and lysis that contributed to extracellular

264 bacterial proliferation (Roca et al. 2013). Insight into the regulatory mechanisms of

265 necroptosis is helpful to develop some potential therapeutic interventions against

266 inflammatory diseases. Roca et al. (2013) reported that preventing necroptosis using

267 drug inhibition or combined genetic blockade of the key regulators conferred

268 resistance to pathogen infection. The necroptosis in fish cells is essentially dependent

269 on the activation of kinase domain of receptor interacting protein (RIP), and the

270 RIP1-RIP3-mediated pathway is highly conserved from human to teleost fish. One of

271 putative teleost RIP3 homologues has been cloned from liver of the half-smooth

272 tongue sole *Cynoglossus semilaevis*, designated CsRIP3. Ectopic CsRIP3

273 overexpression enhances the sensitivity of human HeLa cells to TNFα-induced

274 necroptosis through intracellular CsRIP3-MLKL interaction, suggesting that the

275 function of RIP3 is conserved in human and fish. In response to the pathogen *Vibrio*

276 and viral mimic poly (i:c) challenges, the expression of CsRIP3 is upregulated in

277 various tissues of *Cynoglossus semilaevis*, including liver, heart, head kidney, spleen

278 and gill, but it is unclear in intestine (Ge et al. 2018).

279

Pyroptosis

281 Pyroptosis is a necrotic form of regulated cell death and differs from necroptosis

282 because of the requirement of inflammatory caspases (Amarante-Mendes et al. 2018;

283 Frank and Vince 2019; Weinlich et al. 2017). During the pyroptosis process, both

284 canonical and noncanonical inflammasome activation promote the maturation of

285 proinflammatory cytokines IL-1β and IL-18, which in turn shows an inhibitory effect

286 on autophagy, and triggers the lytic form of inflammatory cell death, including

287 pyroptosis and necroptosis (Rathinam et al. 2016; Gutierrez et al. 2017). Pyroptosis

288 exerts pleiotropic effects in intestinal homeostasis or damage control (Blazejewski et

289 al. 2017; Bortolotti et al. 2018), playing important roles in defense against

290 intracellular pathogens, removal of compromised IECs, and regulation of mucosal

291 immune responses (Lei-Leston et al. 2017; Pellegrini et al. 2017; Sellin et al. 2015;

292 Strowig et al. 2012; Zmora et al. 2017). Aberrance of pyroptosis *in vivo* may

293 contribute to the pathogenesis of autoinflammatory diseases (Seveau et al. 2018;

294 Strowig et al. 2012).

295 Activation of inflammasome in zebrafish IECs has been involved in the

296 pathologies of intestinal inflammation and functional dysregulation induced by

297 high-cholesterol (HCDs) diets, which leads to Caspase-1 (*Dr*Caspase-A) activition

298 (Progatzky et al. 2014). Differently, activation of the caspy2-noncanonical

299 inflammasome in zebrafish intestine has been indicated to strengthen the defense

300 against enteric pathogens infection. Knockdown of caspy2 in zebrafish larvae results

301 in prominent pathological signs of gut inflammation, due to the loss of immune

302 defenses against bacterial infection (Yang et al. 2018). Certain aspects of the caspy2

303   non-canonical inflammasome pathway in zebrafish are complementary to the model

304   of mammalian enteritis.

305   The progression to pyroptotic or secondary necrotic cell death in mammal cells

306   can be mediated by an deafness autosomal dominant nonsyndromic sensorineural 5

307   (*DFNA5*) gene in a caspase-3-dependent manner, that resembles GSMD-mediated

308   pyroptotic pathways (Rogers et al. 2017). *DFNA5* is one of the most ancient

309   gasdermin members that has been found in diverse species from teleost fish to

310   humans (de Beeck et al. 2012; Tamura et al. 2007). In zebrafish, DFNA5 is also

311   defined as GSDME. Sequence alignment has shown that the gasdermin-N domain of

312   zebrafish GSDME is similar with human and mice. There are two forms of zebrafish

313   GSDME, named GSDME1 and GSDME2 (referred to here as DFNA5a and DFNA5b),

314   with each of them sharing about 50% sequence similarity with human GSDME.

315   Zebrafish GSDME1 can be specifically cleaved and activated by caspase-3, which

316   switches chemotherapy drugs- or TNF-induced apoptosis to pyroptosis. However, no

317   caspase-3 cleavage motif has been observed in zebrafish GSDME2 or lancelet

318   GSDME (Wang et al. 2017).

319

Secondary injury by cell lysis

320

321   Endogenous stress signals, known as damage-associated molecular patterns

322   (DAMPs) and bioactive inflammatory mediators that released from necrotic cells,

323   alone or in combination with PAMPs, may initiate a series of inflammatory responses

324   in intestine (Nanini et al. 2017; Sharma and Kanneganti 2017). The effects of

325   endogenous signal molecules are diverse in gut pathology. Certain inflammatory

326   mediators play an important role in reparative process of intestinal mucosal barrier by

327   promoting pathogen expulsion and maintain cellular homeostasis. However, extensive

328   inflammatory responses may aggravate the damage of intestinal mucosal barrier

329   through this feedback loop (Huang et al. 2018).

330   Progression to secondary necrosis is considered as a consequence of improper

331   clearance of apoptotic cells, resulting from either genetic anomalies and/or a

332   persistent disease state (Sachet et al. 2017; Szondy et al. 2014). In experimental and

333   natural pasteurellosis induced by the Gram negative *Photobacterium*

334   *damselae ssp. piscicida* (*Phdp*) infection in sea bass, fish enterocytes undergone

335   caspase 3-mediated apoptosis (anoikis) and detached from intestine mucosa, which

336   were terminated by secondary necrosis and lysis due to lack of elimination by

337   phagocytosis (do Vale et al. 2007a). This is because macrophages and neutrophils in

338   the spleen, head kidney and gut lamina propria were impaired by the

339   extensive secondary necrosis, especially as it was accompanied by the release of a

340   highly tissue-damaging enzyme neutrophil elastase (do Vale et al. 2007b; Silva et al.

341   2013). The released cytotoxic molecules by secondary necrosis have been implicated

342   in the pathogenesis of the necrotic tissue lesions observed in the diseased fish (do Vale

343   A et al. 2016).

344

Part 3. Assessment of intestinal mucosal damages in teleost fish models

346   The histopathology and signaling molecules of intestinal inflammation and tissue

347 injury in teleost fish have been studied in experimental enteritis models established by

348 chemical or biological means. The structural and functional damage of the intestinal

349 mucosa barrier is usually assessed from histopathological, hematological, biochemical

350 and bacteriological perspectives. Previous studies have established a series of

351 assessment indexes for description of the pathology of intestinal injury.

352   The morphological changes of the GI tract and its cells can be analyzed by

353 means of special staining techniques combined with light microscopy or electron

354 microscopy. Previous studies have summarized the histopathological features of fish

355 intestine, mainly including loss of epithelial integrity, edema, inflammatory cell

356 infiltration, disintegration of tight junctions, presence of cell debris in the lumen,

357 basal hydropic degeneration of enterocytes, disorganization of microvilli, extrusion of

358 epithelial cells, hydropic mitochondrial damage, and/or presence of bacteria-like

359 particles (Del-Pozo et al. 2010; Kong et al. 2017; Ringø et al. 2010). The

360 histopathological features of the GI tract of fish may vary depending on

361 different causative agents, fish species, intestinal segments and cell types (Salinas et

362 al. 2008; Ringø et al. 2007b).

363   Usually, the intestinal structural integrity of farmed teleost fish is evaluated by

364   tight junctional complexes and intestinal mucosal barrier permeability, which can be

365   assessed by serum bacterial endotoxin or DNA, d-Lactate and cytotoxins (Grant 2015;

366   Gu et al. 2018; Huang et al. 2015; Kong et al. 2017; Mosberian-Tanha et al. 2016).

367   Other serum parameters, such as malondialdehyde (MDA), superoxide dismutase

368   (SOD), alanine aminotransferase (ALT), diamine oxidase (DAO),

369   glutathione-peroxidase (GSH-Px), catalase (CAT) and antioxidant capacity (TAC)

370   activities, which have been speculated to be a secondary reaction to the intestinal

371   damage, indirectly indicate the pathological state of oxidative damage as well as

372   the severity of local tissue damage (Jiang et al. 2018).

373   Intestinal epithelial cells undergoing different forms of cell death show distinct

374   morphological features, which facilitate specific methods for identifying cell

375   characteristics. Annexin V-FITC and PI staining is an assessment method for cell

376   membrane integrity, and has been widely used to distinguish apoptosis from necrosis

377   (Goldsmith et al. 2013, 2016; Klöditz and Fadeel 2019). Apoptotic cells in the latest

378   stages are characterized by condensation of the cytoplasm and nucleus, and

379   internucleosomal cleavage of DNA, which can be tested by TUNEL assay and DNA

380   fragmented analysis. The positive cells or tissues with fluorescent labeling are

381   analyzed by fluorescence microscopy or flow cytometry (Goldsmith et al. 2016; Gu

382   et al. 2018). The ultra microstructure of damaged cells can be observed through

383 electron microscope imaging, showing different morphological features of the cells

384 (Del-Pozo et al. 2015; Huang et al. 2015).

385 In the past decades, zebrafish has been widely used as model organism

386 for studying intestinal damage and inflammation similar to gastrointestinal diseases in

387 human and farmed teleost fish (Butt and Volkoff 2019; Lickwar et al. 2017; Oehlers et

388 al. 2013; Torraca and Mostowy 2018). The zebrafish model allows functional

389 genomic analysis based on targeted genome-editing tools, such as mutagenesis with

390 zinc-finger nuclease (ZFNs), transcription activator-like effector nucleases (TALENs),

391 CRISPR/Cas9, siRNA and morpholino oligonucleotide-based knockdown assays

392 (Kawahara et al. 2016; Murdoch et al. 2019; Varshney et al. 2016). Furthermore, the

393 fish intestinal cell lines, such as fathead minnow (FHM, minnow epithelial cells)

394 and channel catfish enteric epithelial cells (Skirpstunas and Baldwin 2002) and

395 the rainbow trout (*Oncorhynchus mykiss*) cell line (RTgutGC) (Langan et al. 2017;

396 Minghetti et al. 2017; Pumputis et al. 2018; Wang et al. 2019a), possess the

397 functional features of intestinal epithelial cells, can be used as an efficient *in*

398 *vitro* model to reveal the molecular mechanisms of intestinal mucosal barrier

399 damage.

400

401 **Part 4. Conclusions and future perspectives**

402 To maintain the gut health in farmed teleost fish, the potential risks of

403 exposure to exogenous stimuli must be properly assessed. Furthermore, appropriate

404 preventive and protective practices are required to maintain the intestinal homeostasis

405 of farmed fish that are often confronted with adverse stimuli in the intensive

406 aquaculture.

407 Previous studies have suggested that dietary supplementation with probiotics

408 (Gisbert et al. 2013; Hao et al. 2017; Standen et al. 2015), prebiotics (Carbone and

409 Faggio 2016; Ringø et al. 2010) or postbiotics (Abid et al. 2013; Ringø et al. 2016)

410 are favorable to the intestinal health and homeostasis in various fish species

411 (Hoseinifar et al. 2018; Kuebutornye et al. 2019; Standen et al. 2016). Dietary

412 probiotics administration regulates intestinal homeostasis by reducing the

413 morphological and functional damage of the host's intestinal mucosal barrier and

414 inflammation (Kong et al. 2017). Optimal dietary supplementation with postbiotic

415 feed additives, such as sodium butyrate, improves the growth performance, disease

416 resistance, intestinal immune and physical barrier function in fish by inhibiting

417 oxidative damage and apoptosis of intestinal cells (Tian et al. 2017; Wu et al. 2018a).

418 These studies indicate that supplementation of selected probiotics, prebiotics, and/or

419 postbiotics can improve intestinal health of aquatic animals.

420 Further, integrative analysis of multiple complex regulatory networks associated

421 with intestinal mucosal damage, including protein-protein interaction networks,

422 transcriptional regulation networks, signal transduction networks, biochemical or

423 metabolic networks, will promote the illumination of the signaling pathways. Deeper

424 understanding of these signaling pathways may allow the design of effective

425 strategies in favor of early diagnosis and optimized therapeutic intervention against

426 structural and functional disorders of the intestinal mucosa barrier in farmed teleost

427 fish.

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434

435 **Conflict of interest**

436 The authors have declared no conflict of interest.

437

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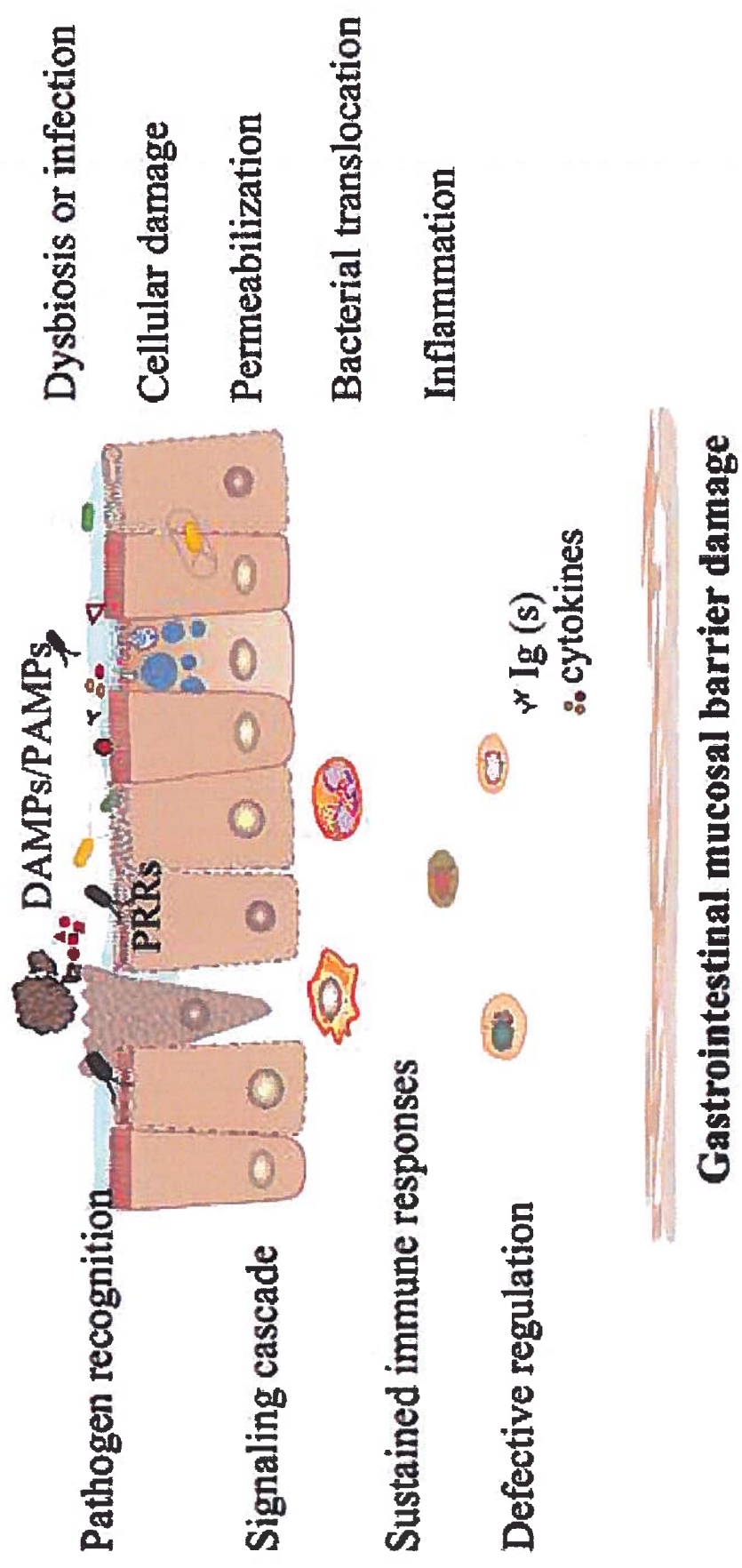896 inflammation. Mucosal Immunol 10:865–883

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898

899 **Figure Legends**

900 **Figure 1. Diagram of the pathogenesis of intestinal mucosal barrier damage**
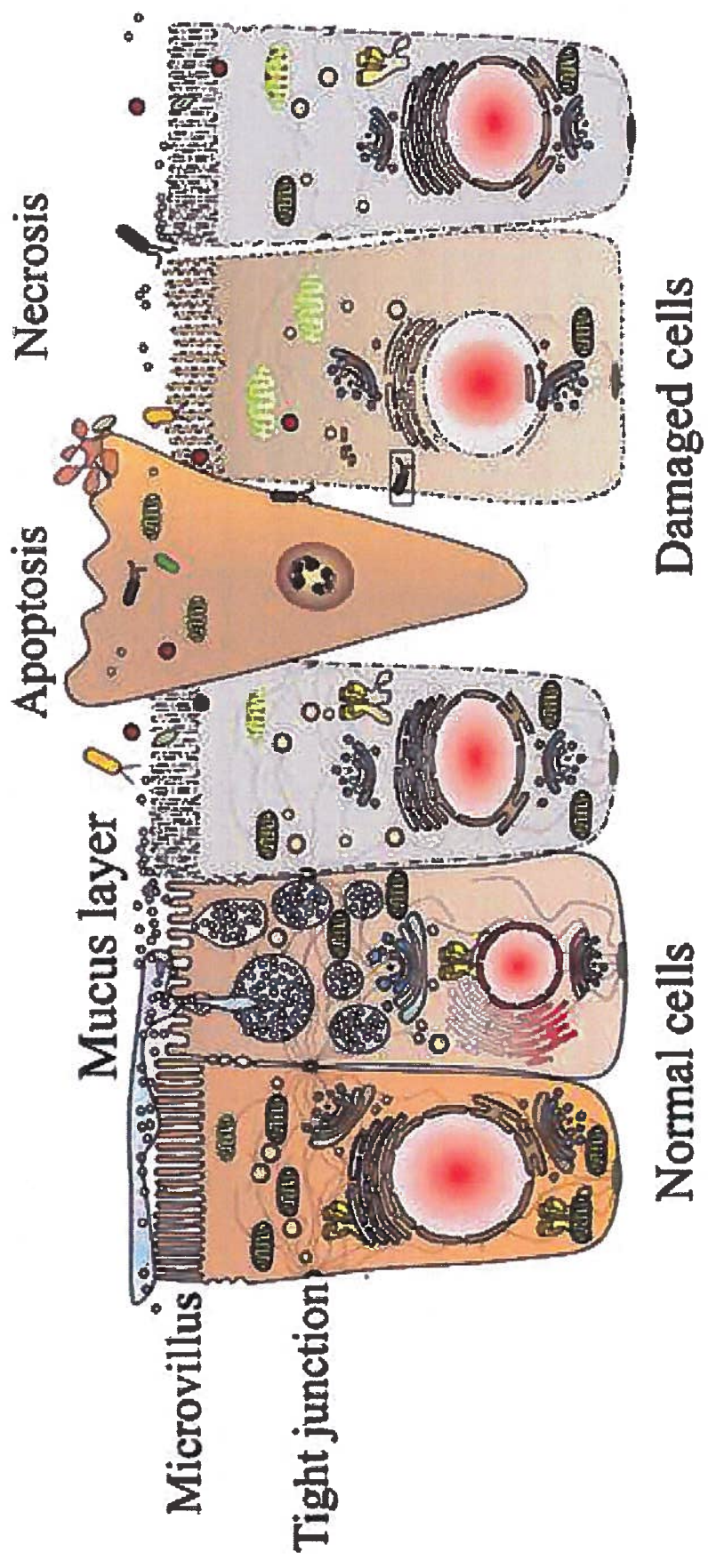
901   The development of intestinal mucosa barrier damage is associated with disorder

902 of diverse signals. Intestinal epithelial cells recognize potential risk factors, known as

903 extrinsic PAMPs or endogenous DAMPs, through specific pattern recognition

904 receptors (PRRs), that induce programmed cell death (PCD) in IECs. Disorder of the

905 cell death progress leads to excessive cell death which disrupts the intestinal integrity.

906 Defects of intestinal permeability potentially allow bacterial translocation through

907 intracellular, transcellular or paracellular routes, and dysbiosis or infection of gut

908 microbiota which may trigger sustained immune responses and complex cytokines

909 release. The progressive convergence of diverse signal modules with defective

910 regulation promotes the course of intestinal mucosal barrier damage. PAMPs,

911 pathogen-associated molecular patterns; DAMP, damage-associated molecular

912 patterns.

913

914 **Figure 2. Model of cellular damage in intestinal epithelium**

915   Cell death is closely related to the pathogenesis of intestinal mucosa barrier

916 damage in teleost fish. Disturbance of the genetically regulated processes triggers

917 excessive cell death in the intestinal epithelium. Signals from apoptosis, pyroptosis,

918 necroptosis, as well as secondary injury by cell lysis in turn interact with a range of

919 cell-intrinsic and cell-extrinsic regulatory modules.

Figure.tif

Dysbiosis or infection

Cellular damage

Permeabilization

Bacterial translocation

Inflammation

DAMPs/PAMPs

PRRs

Pathogen recognition

Signaling cascade

Sustained immune responses

Defective regulation

Ig (s)

cytokines

Gastrointestinal mucosal barrier damage

Figure

Table 1. Factors causing the damage of intestinal mucosal barrier in farmed teleost fish

Factors	Fish species	Histopathological alterations	Cellular responses	References
Pathogen infection (e.g., Aeromonas hydrophila, Vibrio anguillarum)	Grass carp (Ctenopharyngodon idella), Atlantic salmon (Salmo salar L.), turbot (Scophthalmus maximus), orange-spotted grouper (Epinephelus coioides), and ayu (Plecoglossus altivelis)	Intestinal mucosa barrier structural damage, functional disorder and inflammation, tight junction ultrastructure changes in IECs	Up-regulation of pro-inflammatory cytokines, ATPase activity	Gao et al. (2016), Gomez et al. (2013), Hao et al. (2017), Kong et al. (2017), Liu et al. (2016), Ringø et al. (2010), Yang et al. (2017)
Microbial components (e.g., LPS, flagellin and biotoxin)	Jian carp, Zebrafish (Danio rerio), silver pomfret (Pampus argenteus), and Juvenile grass carp (Ctenopharyngodon idella)	Disruption of intestinal physical barrier and oxidative damage, mucosal barrier dysfunction and inflammation	ROS-induced oxidative damage, autophagy inhibition, apoptosis and necrosis in IECs	Gao et al. (2018), Huang et al. (2018), Jiang et al. (2017), Li et al. (2017)
High plant-based alternative protein	Atlantic salmon (Salmo salar), juvenile Turbot (Scophthalmus maximus), rainbow trout	Increased intestinal barrier permeability, mucosal barrier dysfunction and inflammation or enteritis	ROS-induced oxidative damage, autophagy inhibition, apoptosis and necrosis in IECs	Gajardo et al. (2017), Green et al. (2013), Gu et al. (2018), Jiang et al. (2018), Li et al. (2018), Miao

Trigger	Species	Effects		References
	(Oncorhynchus mykiss), yellow Catfish (Pelteobagrus filvidraco) and grass carp (Ctenopharyngodon idella)			et al. (2018), Mosberian-Tanha et al. (2016), Ringø et al. (2016), Wang et al. (2019b)
Low or high-fat diets	Grass carp (Ctenopharyngodon idella), Zebrafish (Danio rerio)	Damage of intestinal physical structure and immune barrier function		Arias-jayo et al. (2018), Feng et al. (2017), Huang et al. (2015)
Dietary deficiency of microelements	Grass carp (Ctenopharyngodon idella)	Intestinal oxidative damage, disturbance of intestinal integrity and immune barrier function	Apoptosis in intestinal cells	Chen et al. (2018), Jiang et al. (2019), Wei et al. (2018), Wu et al. (2018b)
Chemical (e.g., DSS, TNBS, oxazolone and Glafenine)	Zebrafish (Danio rerio)	The intestinal architecture disruption, gut microbiota dysbiosis, increased recruitment of neutrophils with IBD-like colitis	Up-regulation of pro-inflammatory cytokines, organelle stress in IECs, apoptosis and cell shedding	Oehlers et al. (2013), He et al. (2014), Marjoram and Bagnat (2015)
Aquatic environmental contaminants	Brown trout (Salmo trutta Linnaeus); Channa punctatus Bloch, Zebrafish (Danio rerio)	Intestinal oxidative stress, mucosal lesions and inflammation, dysbiosis, damage of IECs and macrophages	Up-regulation of pro-inflammatory cytokines, enterocytes disruption	Barišić et al. (2018), Begam and Sengupta (2015), Lei et al. (2018), Mijošek et al. (2019)

Loss of epigenetic regulator	Zebrafish (*Danio rerio*)	Intestinal barrier loss, inflammation		Marjoram et al. (2015)
Immune dysfunction	*Channa punctatus*	Inflammatory damage in the intestinal epithelium and macrophages	Overexpression of proinflammatory cytokines	Begam and Sengupta (2015)
Enteric nervous system dysfunction	Zebrafish (*Danio rerio*)	Impairment of gut motility, dysbiosis, and intestinal inflammation	IECs shedding and apoptosis	Rolig et al. (2017)