Physiological roles of tryptophan in teleosts: current knowledge and perspectives for future studies

Hoseini, Seyyed Morteza; Pérez-Jiménez, Amelia; Costas, Benjamin; Azeredo, Rita; Gesto, Manuel

Published in:
Reviews in Aquaculture (Print)

Link to article, DOI:
[10.1111/raq.12223](https://doi.org/10.1111/raq.12223)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Hoseini, S. M., Pérez-Jiménez, A., Costas, B., Azeredo, R., & Gesto, M. (2019). Physiological roles of tryptophan in teleosts: current knowledge and perspectives for future studies. *Reviews in Aquaculture (Print)*, 11(1), 3-24. <https://doi.org/10.1111/raq.12223>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Physiological roles of tryptophan in teleosts: Current knowledge and perspectives for future studies

Short title: Roles of tryptophan in teleosts

Seyyed Morteza Hoseini^{a*}, Amalia Pérez-Jiménez^b, Benjamin Costas^{c,d}, Rita Azeredo^{d,e}, Manuel Gesto^f

^a Inland Waters Aquatic Stocks Research Center, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Gorgan, Iran

^b Departamento de Zoología, Universidad de Granada, Campus de Fuentenueva, 18071 Granada, Spain

^c Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS), Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.

^d Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4450-208 Matosinhos, Portugal.

^e Departamento de Biologia, Faculdade de Ciências da Universidade do Porto (FCUP), 4169-007 Porto, Portugal

^f Section for Aquaculture, The North Sea Research Centre, DTU Aqua, Technical University of Denmark, Hirtshals, Denmark

*Corresponding author: Tel.: +989112750713, POB: 139; Fax: +981732240290. E-mail address: seyyedmorteza.hoseini@gmail.com

Abstract

Tryptophan is an essential amino acid with a huge functional versatility, in addition to its participation in protein synthesis. Because of the complexity of its metabolism, and the functional relevance of several of its metabolites, it directly or indirectly participates in a wide array of physiological pathways. This amino acid is a precursor for the synthesis of the neurotransmitter/neuromodulator serotonin (5HT), the hormone melatonin and kynurenine and related compounds such as kynurenic acid, quinolinic acid or niacin. Because of this, it has a key role in the regulation of processes ranging from the neuroendocrine to the immune system in vertebrates. In aquaculture, extensive research has been performed to optimize the levels of tryptophan in the commercial diets for many fish species. Providing adequate levels of this amino acid is critically important for fish growth but also for fish welfare, since tryptophan has been shown to modulate fish behaviour, stress responses, and antioxidant and immune systems. Currently available data suggest a wide variation in tryptophan requirements of different species ranging 0.3-1.2 % of dietary protein level, but recent evidence also show that fish tryptophan requirements can greatly vary depending on the rearing conditions of the fish. We also review here the participation of tryptophan and related metabolites in different physiological functions that are crucial for fish welfare. The review covers the involvement of tryptophan in 5HT- and melatonin-mediated functions, along with its participation in the regulation of the immune system and its role as an antioxidant and antitoxic agent in fish.

Keywords: tryptophan metabolism, serotonin, melatonin, antioxidant response, immune mechanism

Introduction

Tryptophan is a neutral, aromatic amino acid that is essential for animals. As an essential amino acid, tryptophan should be supplied via diet (Wilson, 2002). The teleost requirements for the different amino acids is generally determined by means of growth dose-response curves. By using this method, the dietary tryptophan requirement for several teleost has been determined to range from 0.3 to -1.2 % of dietary protein

(Table 1). In the literature, however, there are some contradictory data regarding tryptophan requirement of a number of teleost fish species (Kim *et al.*, 1987; Walton *et al.*, 1986; Walton *et al.*, 1984; Johnston *et al.*, 1990; Dabrowski, 1981; Tang *et al.*, 2013; Fatma Abidi & Khan, 2010; Murthy & Varghese, 1997; Ahmed & Khan, 2005; Benakappa & Varghese, 2003), indicating that dietary tryptophan requirement may depend on some unknown factors. On the other hand, tryptophan is the least abundant amino acid in teleosts, indicating that the requirements of tryptophan for protein synthesis are low. This suggests that growth dose-response curve might not be a suitable method for the determination of tryptophan requirements in teleosts. Different observations in previous studies are in support of this (see below).

In vertebrates, following dietary uptake, tryptophan may be used for protein synthesis and/or may enter one of the three following pathways (Fig. 1): catabolism through the kynurenine-niacin pathway, serotonin (5HT) synthesis and melatonin synthesis (Le Floch *et al.*, 2011). The balance among these pathways depends on physiological conditions and/or external factors. While the routes from L-tryptophan to serotonin or melatonin are well established in fish and known to be well conserved among vertebrates, much less information is available regarding the fish kynurenine-niacin pathways. Some of the components of the machinery of the mammalian kynurenine pathway are known to be present in fish (see further sections of this review) and the general catabolic routes through kynurenine are assumed to be similar to those in mammals. However, unlike mammals, fish seem to be incapable of producing relevant amounts of niacin from L-tryptophan and thus kynurenine-niacin pathway is mainly directed to remove excess tryptophan, regulate the immune response and produce sexual pheromones (Cortés *et al.*, 2016; Ng *et al.*, 1997; Yambe *et al.*, 2006).

Being the precursor of 5HT and melatonin, tryptophan modulates behaviour, mood and stress responses in fish (Lepage *et al.*, 2005a; Lepage *et al.*, 2002; Winberg & Lepage, 1998; Winberg *et al.*, 2001; Azpeleta *et al.*, 2010; Herrero *et al.*, 2007; Lepage *et al.*, 2005b). Overall, the different tryptophan metabolites have a wide range of physiological roles including modulation of stress response, antioxidant system, immune response and behavioural responses (Cortés *et al.*, 2016; Höglund *et al.*, 2005; Hoseini & Hosseini,

2010; Hoseini *et al.*, 2016; Akhtar *et al.*, 2013a; Akhtar *et al.*, 2013b; Ciji *et al.*, 2013b; Ciji *et al.*, 2012; Ciji *et al.*, 2015; Tejpal *et al.*, 2009; Wolkers *et al.*, 2012; Hoseini *et al.*, 2012; Hosseini & Hoseini, 2013; Azeredo *et al.*, 2016). The knowledge regarding tryptophan effects on teleosts is currently scarce compared to that from mammals. Many studies in teleost fish focused in the modulation of the serotonergic system and the stress response following short-term tryptophan administration. Moreover, available data show several inconsistencies in results from different studies suggesting that there is a need for more in -depth studies on tryptophan effects in teleosts.

In this review, the current knowledge on the effects of tryptophan on teleost's serotonergic, melatonergic, kynurenine, antioxidant and immune systems was summarized in order to provide a general insight on the topic for future reference.

Tryptophan requirement, growth response and deficiency signs

As an essential amino acid, dietary uptake is the main source of tryptophan for teleosts. Feedstuffs have a wide range of tryptophan content. Wheat flour (2.13% of dietary protein), soybean oilcake (1.48% of dietary protein), barley meal (1.47% of dietary protein), wheat gluten (1.3% of dietary protein), cotton seed meal (1.27% of dietary protein), blood meal (1.14% of dietary protein) and fishmeal (0.93-1.16% of dietary protein) are feedstuffs with high tryptophan content; whereas, corn (0.68-0.82% of dietary protein), hydrolysed feather meal (0.66% of dietary protein), meat and/or bone meal (0.51-0.63% of dietary protein) and corn gluten (0.47% of dietary protein) have lower tryptophan content (NRC, 2011). Using standard dose-growth response plot method [purified or semi-purified diets with a range of tryptophan content; Wilson (2002)], tryptophan requirement has been determined in a variety of teleost species, ranging 0.30-1.30% of dietary protein (Table 1). Besides fish growth retardation, dietary tryptophan deficiency leads to scoliosis, lordosis, cataract, opercular shortage, caudal fin root and interference in mineral metabolism (Fagbenro & Nwanna, 1999; Murthy & Varghese, 1997; Poston & Rumsey, 1983; Walton *et al.*, 1984; Shanks *et al.*, 1962).

Whole body amino acid profile of teleosts is suggestive of their dietary requirement (Kaushik, 1998). It is strongly constant among fish with different size, and even among

different species (Kaushik, 1998; Mambrini & Kaushik, 1995; Wilson & Cowey, 1985). However, despite this relatively high similarity in the whole-body tryptophan content tryptophan among different fish studies (in certain species or among different species), reported dietary tryptophan requirements among different species show a wide variation (0.3-1.3% of dietary protein; Table 1). Moreover, there are some contradictions in the data on dietary tryptophan requirements for certain species. For instance, in *Cyprinus carpio* dietary tryptophan requirement has been determined to be 0.3 and 1.1% of dietary protein, which stands for 4-fold variation (Dabrowski, 1981; Tang *et al.*, 2013). Similarly in *Oncorhynchus mykiss*, dietary tryptophan requirement has been reported to be 0.40-0.71 % of dietary protein (Kim *et al.*, 1987; Walton *et al.*, 1986; Walton *et al.*, 1984; Johnston *et al.*, 1990). Dietary tryptophan levels of 0.9 and 1.13% of dietary protein (Fatma Abidi & Khan, 2010; Murthy & Varghese, 1997) have been recommended as required tryptophan levels for optimum growth performance in *Labeo rohita*. However, Akhtar *et al.* (2013a) and Ciji *et al.* (2015) showed that *L. rohita* fed on diets containing tryptophan levels of 3.05-5.17% of dietary protein have significantly improved weight gain compared to control fish (fed with diet containing 0.99% tryptophan). In *Cirrhinus mrigala*, dietary tryptophan levels of 0.95 and 1.2% of dietary protein were reported as optimum dietary tryptophan levels for optimum growth of the fish. Nevertheless, Tejpal *et al.* (2009) reported that this species showed elevated growth performance when fed diets supplemented with exogenous dietary tryptophan levels of 1.94 and 3.88% of dietary protein, regardless of basal dietary tryptophan content (estimated to be 1% of dietary protein). Also, when these fish are reared under stressful conditions (e.g. increased stocking density) higher exogenous tryptophan (7.75% of dietary protein) is required to assure maximum growth performance. Such inconsistencies among dietary tryptophan requirement levels may be due to the fact that tryptophan is a functional amino acid; i.e. tryptophan is required for some other vital processes beyond protein synthesis in fish (Wu, 2009). Tryptophan is necessary for the synthesis of 5HT and melatonin; it has antioxidant properties; it is necessary to counteract toxic substances; and participates in immune function (see the following sections). A study on *C. mrigala* (Tejpal *et al.*, 2009) suggests that stress may increase fish demand for dietary tryptophan. It could be partly due to increased serotonergic activity in teleosts under

stressful conditions as well as an increased requirement for antioxidant capacity (discussed below). Akhtar *et al.* (2013a) and Ciji *et al.* (2015) reported that growth performance of *L. rohita* exposed to stressful conditions and water pollution improves when fed diets containing 3.05-5.17% tryptophan (% of dietary protein) compared to that containing 0.99% tryptophan (discussed below).

Practically, tryptophan deficiency signs (scoliosis, lordosis, cataract, opercular shortage, caudal fin root and interference in mineral metabolism) are not reported in farmed fish; because these signs are not disease-specific. However, it is necessary to monitor the effects of dietary tryptophan supplementation on fish growth performance in farm, where they face a variety of stressors (crowding, environmental and pollutant) that may increase the need for dietary tryptophan (Fig. 2).

Effects of tryptophan on 5HT synthesis, behaviour and stress response

5HT is a monoamine which is simultaneously a neurotransmitter in the central nervous system and a paracrine or endocrine signal in the gut and blood (Fernstrom, 2016). 5HT pathway is relatively less significant in terms of tryptophan consumption, but 5HT synthesis is essential regarding particular organs' well-functioning. Tryptophan uptake to fish brain is somehow similar to mammals, which takes place through a stereospecific and saturable carrier, but this carrier is used for other amino acids uptake, too (Aldegunde *et al.*, 1998). Increased tryptophan intake results in blood tryptophan elevation which in turn increases tryptophan availability for uptake into the brain (Johnston *et al.*, 1990). Tryptophan is converted to 5HT by activity of tryptophan hydroxylase and aromatic L-amino acid decarboxylase in the presence of vitamin B₆ (Johnston *et al.*, 1990). Tryptophan hydroxylase is not saturated by its substrate, tryptophan; therefore, elevated tryptophan intake causes constant increase in the brain 5HT and also 5-hydroxyindole acetic acid (5HIAA), the major serotonin metabolite (Johnston *et al.*, 1990).

About 95 % of total body 5HT content is located in the gut (Le Floc'h *et al.*, 2011), particularly in the wall where the myenteric plexus is a major monoamine producer (Caamano-Tubio *et al.*, 2007). Moreover, some, but not all teleost species (Anderson &

Campbell, 1988; Yui *et al.*, 1988), possess enterochromaffin cells in the gastrointestinal mucosa which also account for this production thereby modulating surrounding cells functions (Muñoz-Pérez *et al.*, 2016). Peripheral 5HT synthesis is also complemented by gills epithelium and both the head- and trunk- portions of the kidney (Caamano-Tubio *et al.*, 2007) as well as liver and stomach (Nagai *et al.*, 1997). In mammals, platelets can internalize and store 5HT in granules by expressing the 5HT transporter (*Sert*). Upon activation, these cells release the indoleamine which then mediates several actions from vasodilation or vasoconstriction (Berger *et al.*, 2009) to neutrophil recruitment (Duerschmied *et al.*, 2013). In contrast to mammals, fish blood-brain barrier allows the passage of 5HT to the peripheral blood vessels but, to best of our knowledge, there is no report on the presence of 5HT in fish thrombocytes (Fritsche *et al.*, 1993). However, because 5HT concentration in *O. mykiss* plasma was equal to that measured in whole blood, it is assumed in fish that 5HT coming from peripheral tissues is stored in the extracellular plasma pool (Caamano-Tubio *et al.*, 2007). Fish intestinal contraction is mediated by 5HT which, similar to mammals (Fernstrom, 2016), is mainly produced in the myenteric plexus (Velarde *et al.*, 2010). Since the presence of enterochromaffin cells in teleosts is not transversal to all species, there is a serious lack of studies on the function of enterochromaffin cells-produced 5HT. Still, given the homology between the serotonergic systems of fish and mammals, it is likely that 5HT released by these cells might display the same pro-inflammatory and motility roles it does in higher vertebrates (Bertrand & Bertrand, 2010; Gershon, 2013).

In the brain, serotonergic activity plays a major role in the hypothalamus-pituitary-interrenal (HPI) axis. Serotonergic activity is usually measured by the rate of production of 5HIAA, the main 5HT metabolite, mediated by the monoamine oxidase (Caamano-Tubio *et al.*, 2007). Tryptophan administration stimulates brain 5HT synthesis; the synthesized 5HT can both stimulate and inhibit the HPI axis as previously observed in several studies with *O. mykiss* (Lepage *et al.*, 2002; Winberg & Lepage, 1998; Winberg *et al.*, 2001), modulating aspects related to appetite and aggression (Höglund *et al.*, 2005; Höglund *et al.*, 2007). In these studies, tryptophan supplementation indirectly [through adrenocorticotrophic hormone (ACTH) action] stimulated or inhibited cortisol production in unstressed or stressed fish, respectively, as observed by enhanced

serotonergic activity. Though this indirect effect induces the strongest responses, 5HT is also able to directly stimulate cortisol production in interrenal cells (Lim *et al.*, 2013). Interestingly, levels of 5HIAA increased quite simultaneously or even previously to the typical rise in plasma catecholamine concentration in stressed *O. mykiss*, suggesting that 5HT may trigger not only the HPI axis but also the response of the brain-sympathetic-chromaffin axis in fish (Gesto *et al.*, 2013). This effect is similar to that observed in turkeys (Denbow *et al.*, 1993) but not human (Rasmussen *et al.*, 1983; Benedict *et al.*, 1983).

Several studies in fish have shown that tryptophan administration increases serotonergic activity characterized by increased 5HT and/or 5HIAA (Basic *et al.*, 2013b; Herrero *et al.*, 2007; Höglund *et al.*, 2007; Hseu *et al.*, 2003; Lepage *et al.*, 2002; Lepage *et al.*, 2003), however, all these studies focused on the short-term tryptophan administration. 5HT alters voluntary feed intake, macronutrient selection, and stress response, and also suppresses aggression (Rubio *et al.*, 2006; Winberg & Nilsson, 1993; Winberg *et al.*, 1997; Øverli *et al.*, 1998). Several studies demonstrated that tryptophan administration increases 5HT and serotonergic activity and generates the effects similar to those caused by direct 5HT administration. *Gadus morhua* showed increased brain 5HIAA and 5HIAA/5HT ratio and decreased aggressive behaviour when treated with tryptophan for 7 days (Höglund *et al.*, 2005). Such an aggression-suppressive effect was also observed in *Brycon cephalus* after 7 days tryptophan administration, although serotonergic activity was not reported (Wolkers *et al.*, 2012). Similarly, *Epinephelus coioides* treated with tryptophan for 10 days showed suppressed aggressive behaviour and cannibalism along with brain 5HT elevation (Hseu *et al.*, 2003). Also, *Dicentrarchus labrax* showed increase in 5HT synthesis and altered voluntary locomotion after 7 days tryptophan administration (Herrero *et al.*, 2007). These studies suggest that tryptophan affects teleost behaviour via the serotonergic system; however, it is necessary to monitor long-term tryptophan administration on the fish serotonergic response and behaviours.

Tryptophan requirement increases under stressful condition as a decrease in plasma concentrations of tryptophan was observed in fish under stressful husbandry conditions when compared to non-stressed fish (Aragao *et al.*, 2008; Costas *et al.*, 2008), thus

231 dietary tryptophan supplementation may be a promising nutritional strategy for welfare
232 management in aquaculture (e.g. high rearing densities, transport and handling). Most of
233 the studies on tryptophan effects on the fish stress response have focused on short-term
234 tryptophan administration (7-15 days), but both acute and chronic stressors. In *O. mykiss*,
235 7 days tryptophan administration had no significant effects on blood cortisol, but
236 suppressed post-acute-stress cortisol elevation, and this change in stress response seems to
237 be modulated via serotonergic activity and ACTH release (Lepage *et al.*, 2002; Lepage *et*
238 *al.*, 2003). Such an effect was observed in *D. labrax* fed a tryptophan-supplemented diet
239 for 7 days (Herrero *et al.*, 2007). Also, in *Salmo trutta*, 7 days tryptophan administration
240 counteracted stress-induced anorexia (Höglund *et al.*, 2007). But there are some studies
241 showing different results. In *Oreochromis niloticus*, 7 days feeding with a tryptophan-
242 supplemented diet increased serotonergic activity, decreased basal cortisol, but had no
243 significant effects on post stress cortisol (Martins *et al.*, 2013). In *Salmo salar*, 7 days
244 tryptophan administration had no significant effects on basal and post stress (acute) brain
245 serotonin, 5HIAA and 5HIAA/5HT ratio, while it either stimulated or inhibited basal and
246 post stress plasma cortisol, depending on tryptophan level and time (Basic *et al.*, 2013a).
247 Using the same tryptophan levels in *G. morhua*, Basic *et al.* (2013b) found elevated
248 5HIAA/5HT ratio depending on tryptophan concentration. Tryptophan had no significant
249 effects on basal cortisol, but mitigated post stress cortisol response 6 days, but not 1 and
250 2 days, after tryptophan administration termination; suggesting long lasting effects
251 tryptophan on stress response. Similarly in *S. salar*, post stress cortisol levels decreased
252 8 and 21 days after a 7-day period tryptophan administration (Höglund *et al.*, 2017). The
253 importance of tryptophan administration time on serotonergic activity and cortisol
254 response has been also reported earlier in *O. mykiss* (Lepage *et al.*, 2003). Interestingly,
255 Hoseini *et al.* (2016) reported increase in basal and post stress cortisol levels in
256 *Acipenser persicus* after 15 days feeding with tryptophan-supplemented diet (0.5% of
257 diet). The same authors Hoseini and Hosseini (2010), however, reported a mitigation of
258 the stress response (serum cortisol and glucose) to a 7-days osmotic challenge in *C.*
259 *carpio* fed with similar tryptophan supplementation (0.5% of diet). On the other hand,
260 only a few studies have evaluated the effects of long-term tryptophan administration on
261 the fish stress response. Morandini *et al.* (2015) found suppressed basal cortisol and

increased brain serotonergic activity after 4 weeks tryptophan administration in *Cichlasoma dimerus*, with no gender-specific trend. Tejpal *et al.* (2009), Akhtar *et al.* (2013a) and Kumar *et al.* (2014) showed that 45-90 days tryptophan administration under constant stressful conditions significantly suppressed cortisol and glucose responses and improved growth performance in *C. mrigala* and *L. rohita*, suggesting the need for higher tryptophan intake under stressful conditions to support maximum growth (Fig. 2).

Altogether, these studies show that there is a need for further research to find important factors affecting tryptophan effects on the fish stress response, from brain serotonergic activation to the release of cortisol. As mentioned above, timing appears to be a critical factor; and due to time-dependent nature of serotonergic and cortisol responses to stress, administration and sampling time should be taken into account when tryptophan effects on acute stresses are investigated. Also, it seems that tryptophan administration level needs to be considered as the above mentioned studies used a wide range (0.3 % - 4.45 %) of tryptophan supplementation. The matter is even more complicated when species differences emerge, as two similar studies with similar tryptophan levels but different species and exposure times showed different outputs (Basic *et al.*, 2013a; Basic *et al.*, 2013b).

Tryptophan and melatonin synthesis

Melatonin is produced from 5HT and is a rhythmic hormonal signal involved in the synchronization of daily processes in which the pineal organ and retina play a central role. Therefore, the amino acid tryptophan is also an indirect precursor for the biosynthesis of the hormone melatonin (N-acetyl-5-methoxytryptamine), which could be partly responsible for the effects observed after tryptophan administration in vertebrates. In melatonin-producing cells, tryptophan is first transformed to 5HT (Fig. 1), which is subsequently transformed into melatonin through two enzymatic steps: the transformation of serotonin into N-acetylserotonin, catalyzed by aryl-alkylamine N-acetyl transferase (AANAT; EC 2.3.1.87), and the subsequent conversion of N-acetylserotonin into melatonin, catalyzed by the hydroxyindole-*O* -methyltransferase (HIOMT; EC 2.1.1.4) (Falcón *et al.*, 2010).

291 Pineal melatonin content elevates at night but decreases with light. Plus, because
292 melatonin is highly lipophilic and can therefore easily cross cell membranes, its plasma
293 levels oscillates parallel to pineal production (Falcón *et al.*, 2011). On the other hand,
294 melatonin produced in the gut and retina is considered to have paracrine actions in those
295 tissues. However, gut melatonin seems somehow to have contribution in circulating
296 melatonin level in fish. The gastrointestinal tract is a major melatonin production site.
297 Gastrointestinal cells are probably responsible for low, daytime levels of melatonin, while
298 pinealocytes light-sensitive melatonin production accounts for most of the night-time
299 hormone levels.

300 Other external and internal factors may account for melatonin activity modulation, though
301 instead of dictating rhythms, their action is limited to modifying rhythms amplitude
302 (Falcón *et al.*, 2011). These can be temperature, hormones (melatonin itself) and
303 catecholamines (Falcón *et al.*, 1991).

304 In teleosts, melatonin is engaged in several physiological processes other than those ruled
305 by circadian rhythms, from gut motility (Muñoz-Pérez *et al.*, 2016) to osmoregulation
306 (Kulczykowska *et al.*, 2006), immunity (Cuesta *et al.*, 2008; Cuesta *et al.*, 2007), stress
307 response (López-Patiño *et al.*, 2013) and antioxidant capacity (Maitra & Hasan, 2016),
308 feed intake (Pinillos *et al.*, 2001; Velarde *et al.*, 2010) and voluntary activity (Herrero *et al.*, 2007). Still, a large variability among responses, regulatory mechanisms and enzyme
309 distribution is observed in different teleost species.
310

311 Tryptophan administration has been shown to increase melatonin concentration in blood
312 and in other tissues such as the pineal gland or the gastrointestinal tract. For example,
313 oral administration of tryptophan has been shown to increase blood and tissue levels of
314 melatonin in birds and mammals (Paredes *et al.*, 2007a; Sanchez *et al.*, 2008; Huether *et al.*, 1992; Hajak *et al.*, 1991). However, the effect of tryptophan administration on tissue
315 melatonin content could vary with the moment of the day or the reproductive stage
316 (Reiter *et al.*, 1990; Guchhait & Haldar, 2001). In fish, few existing studies resulted in
317 ambiguous conclusions. As in mammals, and similarly to what happens with 5HT,
318 tryptophan administration leads to increased melatonin production in the *O. mykiss*
319 enterochromaffin cells (Lepage *et al.*, 2005b; Acuna-Castroviejo *et al.*, 2014). Lepage *et al.*
320 (2005b) observed that melatonin levels were not different at night in *O. mykiss* fed
321

322 tryptophan supplemented diets, in contrast to what happened during daytime. The authors
323 suggested two hypothesis; either i) intestinal melatonin production was decreased during
324 the night, or ii) melatonin itself acted in a negative feedback mechanism on pineal
325 hormone synthesis, the second being supported by observations in both birds (Wright &
326 Preslock, 1975) and fish (Yanez & Meissl, 1995). In contrast to *O. mykiss*, tryptophan
327 did not alter the hormone synthesis in the gut of the *D. labrax* (Herrero *et al.*, 2007).
328 The authors suggested that the lack of increase in melatonin levels could be related to
329 the low level of tryptophan supplementation utilized in this study (tryptophan content in
330 the experimental diet was increased 2-fold with respect to the control diet), compared to
331 the previous study in fish [Lepage *et al.* (2005b); a 8-fold increase in the tryptophan-
332 enriched diet compared to the control diet].

333 Melatonin administration in fish and other vertebrates has been shown to induce some
334 stress-mitigating effects that resemble some of the effects observed after tryptophan
335 treatment. In birds and mammals, melatonin has been demonstrated to act as a central
336 and peripheral inhibitor of the hypothalamus-pituitary-adrenal (HPA) axis. Animals treated
337 with melatonin usually display an attenuated glucocorticoid response to stress
338 (Konakchieva *et al.*, 1997; Torres-Farfan *et al.*, 2003; Saito *et al.*, 2005), in a similar
339 fashion to what is observed after tryptophan treatment [Dantzer and Moermede (1979);
340 Markus *et al.* (2002); also refer to previous section of this review]. In teleost fish, there
341 is also a body of evidence suggesting an anti-stress effect for melatonin at the level of
342 the HPI axis. In this regard, melatonin has been shown to inhibit and/or delay the
343 glucocorticoid response to stress in fish (Azpeleta *et al.*, 2010; López-Patiño *et al.*, 2013;
344 Conde-Sieira *et al.*, 2014; Gesto *et al.*, 2016). Again, this effect is similar to what is
345 observed after tryptophan treatment in fish (Herrero *et al.*, 2007; Lepage *et al.*, 2002;
346 Lepage *et al.*, 2003). The mechanisms behind the observed melatonin effects on the
347 HPA/HPI axis are not well known. Some evidences in mammals point to a direct effect
348 of melatonin on the adrenal gland (Appa Rao *et al.*, 2001; Torres-Farfan *et al.*, 2003),
349 but there is also evidence suggesting that melatonin could have a direct action at the
350 brain, modulating the central pathways controlling the HPA/HPI axis (Xu *et al.*, 1996;
351 Conde-Sieira *et al.*, 2014; Gesto *et al.*, 2016).

Based on the similitudes between the stress response-mitigating effects of tryptophan and melatonin treatment, and on the fact that tryptophan treatment increases melatonin levels in vertebrates, it seems logical to think that melatonin could be at least partly responsible for the stress-related effects of tryptophan, even when those effects are generally attributed to the modulation of the serotonergic system (see previous section of this review). However, the extent of melatonin participation in the effects induced by tryptophan administration is not well known. In an elegant series of studies, Lepage *et al.* (2005b) first suggested the possibility that melatonin could be mediating the stress- and aggression- related effects of tryptophan in *O. mykiss* but, in a subsequent study, they showed that most of the effect was probably being mediated by 5HT (Lepage *et al.*, 2005a). However, even when it seems clear that the involvement of melatonin is not required for tryptophan to exert its stress-mitigating effects, a complementary action of both serotonergic system and melatonin mediating the anti-stress action of tryptophan administration should not be ruled out. Since the increase of melatonin levels after tryptophan treatment seems to be dose-dependent (Herrero *et al.*, 2007; Lepage *et al.*, 2005a) a diet supplementation of tryptophan high enough to increase circulation/tissue melatonin levels could be acting on the stress axis by both 5HT- and melatonin-mediated mechanisms.

Melatonin could be also involved in other effects observed after tryptophan administration in fish. For example, it could be mediating part of the effects of tryptophan regarding its antioxidant properties and its effects on the immune system (see the following sections in this review).

Furthermore, the potential effects of a sustained elevation of melatonin levels after long-term tryptophan supplementation on fish growth and other physiological aspects are not known. Besides its known involvement in the circadian and seasonal regulation of different physiological processes (Falcón *et al.*, 2007), melatonin has been also shown to affect feeding and aggressive behaviour in fish (Munro, 1986; López-Olmeda *et al.*, 2006; Pinillos *et al.*, 2001). Further research would be needed to unveil whether any of those aspects could be affected by a long-term increase in circulating melatonin levels in fish.

Antioxidant capability of tryptophan

In aquaculture, fish are exposed to multiple stressors derived from farming management which can lead to reactive oxygen and nitrogen species (ROS and RNS) generation and then to an oxidative stress situation (Andrade *et al.*, 2015; Castro *et al.*, 2012; Morales *et al.*, 2004; Pérez-Jiménez *et al.*, 2012; Wu *et al.*, 2015). To counteract the possible negative effect of ROS overproduction, fish have evolved effective defence systems, consisting of both enzymatic defences such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), glutathione peroxidase (GPx; EC 1.11.1.9), glutathione reductase (GR; EC 1.8.1.7) and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), and non-enzymatic antioxidant defences including vitamins, carotenoids, glutathione, thioredoxins, among others (Halliwell & Gutteridge, 2015; Martínez-Álvarez *et al.*, 2005). When oxidant agents exceed antioxidant defences, oxidative stress takes place and macromolecules in cells are damaged (Halliwell & Gutteridge, 2015) and this fact might reduce growth performance and feed intake of animals, affect the quality and palatability of the final product, increase susceptibility to diseases or even lead to death, with the considerable economic loss for aquaculture producers (Andrade *et al.*, 2015; Castro *et al.*, 2012; Suárez *et al.*, 2009). Although different strategies to mitigate oxidative stress effects can be adopted, nutrition approach plays a central role in this struggle (Oliva-Teles, 2012).

In fish, dietary supplementation with amino acids have been shown to improve antioxidant capacity enhancing the activity of main antioxidant enzymes and glutathione and decreasing oxidative damage in lipids and proteins, in different fish species (Coutinho *et al.*, 2016; Giri *et al.*, 2015; Pérez Jiménez *et al.*, 2012). In the case of tryptophan, although there are many studies in different fish species focused on its nutritional requirements, role in behaviour, immunological properties or primary stress modulation (see the other sections of this review), very few reports are available about its effect on oxidative stress mitigation in fish (Akhtar *et al.*, 2013a; Ciji *et al.*, 2015; Jiang *et al.*, 2016a; Jiang *et al.*, 2015; Kumar *et al.*, 2014; Wen *et al.*, 2014).

It has been proposed that tryptophan exhibits antioxidant activity *per se*, since it reacts with highly reactive free radicals such as hydroxyl radicals and also modulates

antioxidant enzyme activities (Del Angel-Meza *et al.*, 2011; Peyrot & Ducrocq, 2008; Reyes-Gonzales *et al.*, 2009). This fact has been shown both *in vitro* and *in vivo* studies with terrestrial animals including mammals and birds. For example in rabbits, tryptophan was effective in protecting the animals from the generation of free radicals and lipid oxidative damage produced by a hypoxic myocardial injury (Narin *et al.*, 2010). In weaned piglets, increasing levels of dietary tryptophan ameliorated the hepatic oxidative stress situations induced by injection of diquat (Mao *et al.*, 2014). Liu *et al.* (2015) observed that dietary tryptophan supplementation improved antioxidant status of ducks. However, the mechanisms by which tryptophan exerts its antioxidant capacity are not clear and need to be elucidated.

It is known that many of the potential antioxidant properties of tryptophan have been attributed to its metabolites (Pérez-González *et al.*, 2014). In this sense, 5HT and melatonin, which are the main molecules synthesized from tryptophan, have been shown as important antioxidants, although compared to melatonin, 5HT antioxidant capacity has been less explored. Several studies, both *in vitro* and *in vivo*, have shown that 5HT can act as a direct free radical scavenger, mainly of superoxide anion, hydrogen peroxide and hydroxyl radicals and decrease lipids oxidative damage in several tissues (Daniels *et al.*, 1996; Gülçin, 2008; Park *et al.*, 2002). Moreover, 5HT has been shown to exert its antioxidant action through chelating activities of ferrous ions (Sarıkaya & Gulcin, 2013). Additionally, its direct metabolite, N-acetyl-serotonin, has been also indicated as antioxidant molecule by different authors (Lezoualc'h *et al.*, 1998; Moosmann *et al.*, 1997).

Melatonin has different physiological roles in fish (see previous section in this review) and it is the most important antioxidant molecule of those derived from tryptophan metabolism. Unlike other antioxidants, it can scavenge up to ten free radicals, including ROS and RNS, throughout a cascade of reactions that converts this indole in other secondary and tertiary metabolites which are also able to neutralize free radicals (Tan *et al.*, 2015). Moreover, melatonin is not only an effective oxidant agent scavenger, but also has the ability of activating main antioxidant enzymes such as SOD, CAT and GPx, fact that has been demonstrated in humans and different terrestrial animals including

mammals and birds (Barlow-Walden *et al.*, 1995; Cabeza *et al.*, 2001; Fjaerli *et al.*, 1999; Paredes *et al.*, 2007b).

In the same way, other studies about tryptophan metabolites, such as 5-hydroxytryptophan, indole-3-acetic acid, 3-hydroxyanthranilic acid, 3-hydroxykynurenine or xanthurenic acid have demonstrated their antioxidant capacities both as ROS scavengers and modulation of antioxidant enzymes (Christen *et al.*, 1990; Ferrari *et al.*, 2014; Matuszak *et al.*, 1997; Pérez-González *et al.*, 2015; Peyrot & Ducrocq, 2008). Similarly, some tryptophan-metabolizing enzymes, such as tryptophan 2,3-dioxygenase (TDO; EC 1.13.11.11) and indoleamine 2,3-dioxygenase (IDO; EC 1.13.11.52) has been considered as antioxidant since in their reactions to catalyse the oxidative degradation of tryptophan, they use the superoxide anion and hydrogen peroxide as cofactors (Batabyal & Yeh, 2007; Britan *et al.*, 2006; Hayaishi, 1996).

The general effects of dietary tryptophan supplementation (beyond to normal requirements) in oxidative status of cultured fish have been scarcely studied (to our best knowledge only in two fish species, *L. rohita* and *Ctenopharyngodon idella*). Akhtar *et al.* (2013a) studied the effect of different levels of dietary tryptophan supplementation (0, 0.7 and 1.4%) in *L. rohita* under stressful conditions of temperature and salinity, observing that SOD and CAT activities in liver and gills were reduced in tryptophan supplemented groups, concluding that this amino acid had a role in the oxidative stress-mitigation function. A later study in the same fish species under thermal stress and fed on dietary tryptophan supplemented levels of 0, 0.36, 0.72 and 1.42 %, confirmed the previous observed results, observing SOD and CAT activities in liver and gills significantly higher in the control groups that decreased with the increase of dietary tryptophan inclusion (Kumar *et al.*, 2014). In the same way, the activities observed for SOD and CAT in muscle, liver and brain of *L. rohita* kept under nitrite exposure-induced stress, increased in fish fed on 0% dietary tryptophan supplementation group, whereas the activity of these antioxidant enzymes was decreased when fish were fed on 0.75 and 1.5% of dietary tryptophan supplemented levels (Ciji *et al.*, 2015). These studies have used tryptophan levels higher than the species requirement suggesting that

tryptophan requirement increases under oxidative conditions caused by environmental stress and pollutants (Fig. 2).

In *C. idella*, Wen *et al.* (2014) evaluated the effects of dietary tryptophan (0.07, 0.17, 0.31, 0.4, 0.52 and 0.61% inclusion levels) in the intestinal oxidative status after stress induced by environmental copper exposure. The fish fed on dietary tryptophan supplementation increased CuZn-SOD and GPx activities and GSH contents and also decreased the oxidative damage biomarkers for protein and lipids determined as protein carbonyl and malondialdehyde (MDA) levels. Moreover, the relative gene expression levels of CuZn-SOD and GPx were up-regulated with increasing of dietary tryptophan up to a certain level, concluding that appropriate dietary levels of this amino acid improves antioxidant status in fish. Additionally, in this study, the authors determined that up-regulation of intestine antioxidant enzyme gene expression might be related to increase of mRNA levels of Nrf2 observed in fish fed on dietary tryptophan levels up to 0.44%. Nrf2 is a signal molecule which has been related to expression of genes that encode antioxidant enzymes (Lushchak, 2011). In this sense, a positive correlation between CuZn-SOD and GPx and gene expression level of Nrf2 was observed (Wen *et al.*, 2014).

In later studies, Jiang *et al.* (2015) determined the effect of dietary tryptophan supplementation on antioxidant defence mechanisms, oxidative damage biomarkers and related signalling molecules, Nrf2 of gill in *C. idella*. The results showed that adequate tryptophan levels improved gill oxidative status avoiding oxidative damage, since ROS level decreased in fish fed on this amino acid compared to un-supplemented group. These authors related the obtained results with the scavenger capacity of tryptophan *per se*, neutralizing superoxide and hydroxyl radicals. Additionally, oxidative status improvement was also justified by the results observed in GSH content, CuZn-SOD, CAT, GPx, GST and GR activities, which were increased with dietary tryptophan supplementation compared to the control group, and that were positively correlated to the mRNA levels for these enzymes. As for the case of *L. rohita*, in *C. idella*, Nrf2 expression was also up-regulated by dietary tryptophan, which could partially justify the increase in enzymatic antioxidant defenses. On the other hand, whereas in muscle of *C.*

idella, responses for MDA levels, protein carbonyl content, GPx activity and expression and GSH levels were similar to those previously observed in gills, no changes in either SOD activity or gene expression were observed (Jiang *et al.*, 2016b). Even more, CAT activity and gene expression decreased at optimal dietary tryptophan levels, fact that was justified by the complementary action of GPx.

Altogether, these results might suggest that amino acid supplementation in general, and tryptophan in particular, is a very positive practice to improve animal welfare throughout the optimization of the oxidative status. However, literature also includes studies with contradictory results that point out the necessity of researching the conditions in which the amino acid supplementation is performed (Deng *et al.*, 2014; Jiang *et al.*, 2016a; Jiang *et al.*, 2015; Jiang *et al.*, 2016b; Peyrot & Ducrocq, 2008). In fish species, Jiang *et al.* (2015) and Jiang *et al.* (2016b) observed that excessive tryptophan inclusion in diets leads to oxidative stress in gills and muscle of *C. idella*. So, when compared to the optimum levels of tryptophan (0.31-0.4%), excessive supplementation of this amino acid (0.61%) generate increased ROS content, MDA and protein carbonyl levels and low GSH content and SOD, CAT, GPx, GST and GR activities and gene expression levels. In these cases, the authors indicated that a possible motive for the generation of this oxidative stress situation might be related to the production of 2,3-pyridine dicarboxylic acid, a metabolite of tryptophan that has been demonstrated as stimulator of ROS production (Sadeghnia *et al.*, 2013; Santamaría *et al.*, 2001).

Tryptophan antitoxic effects

A few studies have been conducted to find if dietary tryptophan administration counteracts adverse effects of toxic substances in teleosts. Hoseini *et al.* (2012) showed that dietary tryptophan administration significantly improved *C. carpio* survival upon copper exposure. Also, tryptophan suppressed some adverse effects of copper toxicity on serum cortisol, glucose and alanine transaminase (ALT). Fatahi and Hoseini (2013) found that tryptophan supplementation significantly improves *Rutilus caspicus* survival following acute copper exposure. Ciji *et al.* (2013b) demonstrated that dietary tryptophan supplementation prevents nitrite-induced haematocrit, total protein, albumin and globulin demotion in *L. rohita*. Ciji *et al.* (2013a) observed that dietary tryptophan

supplementation in *L. rohita* mitigates growth deterioration, cortisol elevation, T₄, testosterone and estradiol demotion under nitrite exposure. Ciji *et al.* (2015) observed that dietary tryptophan mitigates nitrite-induced growth and feed efficiency deterioration in *L. rohita*. Also the added tryptophan significantly mitigated adverse effects of nitrite exposure on blood white blood cell count; serum total protein, albumin, globulin, glucose and lysozyme; liver CAT, SOD, alanine transaminase (ALT) and aspartate transaminase (AST); and muscle lactate dehydrogenase, ALT and AST.

Although the aforementioned studies suggest that tryptophan supplementation is beneficial for fish when exposed to toxicants, the underlying mechanisms have not been studied. It is known that some of those toxicants may interfere with brain serotonergic activity and suppress 5HT synthesis as reported in *C. carpio* exposed to ambient copper (De Boeck *et al.*, 1995), *Channa punctatus* exposed to carbofuran (Gopal & Ram, 1995) and *Oreochromis mossambicus* exposed to mercury (Tsai *et al.*, 1995). Therefore, the serotonergic activity-promoting ability of tryptophan administration could help to counteract the adverse effects of those toxic substances on the fish serotonergic activity. This hypothesis is yet to be explored in fish.

Another potential explanation for the positive effects of tryptophan in fish exposed to toxic substances might be mitigation of oxidative stress through its antioxidant property, as described above. This is because the toxic agents used in the commented studies are all known to cause oxidative damage in fish (Jia *et al.*, 2015; Ciji *et al.*, 2012; Vutukuru *et al.*, 2006; Wei & Yang, 2016; Roméo *et al.*, 2000; Sanchez *et al.*, 2005; Ciji *et al.*, 2015).

Tryptophan and immune response

There has been growing interest in recent years in the role of tryptophan in both stress and immune functions. The neuroendocrine-immune network is essential for homeostasis during stress and infection and thus sustains a balanced immune response to effectively clear the pathogen, while minimizing the damage to the host (Verburg-Van Kemenade *et al.*, 2009). A number of different hormones and cytokines, as well as their interactions, are involved (Dhabhar, 2009). The energetic costs of the stress condition will activate

other hormones involved in metabolic support, which may also have an influence on immune function. Therefore, leucocytes express glucocorticoid, adrenergic, cholinergic and opioid receptors, which make them sensitive to a wide repertoire of neuroendocrine responses (Verburg-Van Kemenade *et al.*, 2009). For instance, glucocorticoids are known to have anti-inflammatory and immunosuppression actions in vertebrates. In this regard, cortisol alone can influence multiple aspects of immune defence mechanisms in fish. Cortisol inhibits lipopolysaccharides (LPS)-induced expression of pro-inflammatory cytokines and inducible nitric oxide (NO) synthase (Verburg-van Kemenade *et al.*, 2011; Stolte *et al.*, 2008). Cortisol also induces apoptosis and inhibits proliferation of immune cells (Verburg-Van Kemenade *et al.*, 2009).

Metabolism and immunity are thus affected when fish are under stress and this together with sub-optimal dietary nitrogen formulations may affect fish welfare. A suitable balanced diet should provide adequate health and immune conditions to fish. However, little research has been done in optimizing the nitrogenous fraction of the diet in order to minimize welfare problems. Recent evidence shows that stressful husbandry conditions affect amino acid metabolism in fish, and under some stress situations an increase in the requirement of certain indispensable amino acid may occur (Conceição *et al.*, 2012). Protein or amino acid requirements may also increase as a direct consequence of metabolic changes associated with inflammation and infection (Melchior *et al.*, 2004; Sakkas *et al.*, 2013). In fact, the dependence of the immune system upon the availability of amino acid is related to their role as signalling molecules essential for cellular function (Li *et al.*, 2009). For instance, amino acids present important features in higher vertebrates as they regulate i) activation of T- and B-lymphocytes, natural-killer cells and macrophages; ii) cellular redox state, gene expression and lymphocyte proliferation; and iii) production of antibodies, cytokines and cytokines substances as NO and superoxide (Wu, 2013). Moreover, both innate and adaptive immune responses are dependent upon adequate provision of amino acids for the synthesis of antigen-presenting molecules, immunoglobulins and cytokines (Sakkas *et al.*, 2013).

Tryptophan in particular is an essential amino acid with recognized roles in both neuroendocrine and immune systems. Stress suppresses fish immunity and emerging

590 evidences (see above) show that dietary tryptophan is required to cope with stress
 591 (Aragao *et al.*, 2008; Costas *et al.*, 2008); also, 5HT, melatonin and N-acetylserotonin,
 592 products of tryptophan catabolism, can enhance host immunity by inhibiting the
 593 production of superoxide, scavenging free radicals and attenuating the production of pro-
 594 inflammatory cytokines (Perianayagam *et al.*, 2005). However, tryptophan nutrition should
 595 be carefully considered since recent evidence showed opposite outcomes. Azeredo *et al.*
 596 (2016) tested the effects of tryptophan on the innate immune response of Senegalese sole
 597 (*Solea senegalensis*) in an attempt to explore the links between immune and
 598 neuroendocrine responses and to evaluate possible beneficial effects on disease resistance.
 599 It was observed that tryptophan supplementation (i.e. a level of 2x above tryptophan
 600 requirements) may promote the immune status and disease resistance in fish reared at
 601 low (12.5 kg/m²) or high (31 kg/m²) densities. However, an even higher increase in
 602 tryptophan supplementation (i.e. a level of 4x above tryptophan requirements) showed to
 603 be beneficial in chronically stressed fish, whereas undisturbed individuals showed a
 604 compromised immune status and lower disease resistance. Likewise, Costas *et al.* (2013)
 605 observed a positive effect of tryptophan supplementation (i.e. a 0.5% increase in a dry
 606 matter basis), which in combination with arginine, threonine and lysine, mitigated the
 607 immunosuppressive effects attributed to chronic stress action by counteracting the
 608 decrease in plasma lysozyme, complement and peroxidase activities observed in stressed
 609 fish fed a non-supplemented diet. Moreover, *L. rohita* juveniles decreased total circulating
 610 leucocytes and serum lysozyme activity following exposure to a sub-lethal concentration
 611 of nitrite-nitrogen for 45 days, which was counteracted by dietary tryptophan (i.e. 0.75
 612 and 1.5% increase in a dry matter basis) surplus (Ciji *et al.*, 2015). Akhtar *et al.*
 613 (2013a) and Akhtar *et al.* (2013b) showed that long-term (60 d) tryptophan
 614 supplementation inhibits or suppresses chronic (15 d) thermal and/or salinity stress-
 615 induced decrement in blood white blood cell count and respiratory burst activity; serum
 616 total protein, albumin, globulin and lysozyme in *L. rohita*. Such effects might be due to
 617 mitigation of stress response (serum cortisol) and suppression of oxidative stress (SOD
 618 and CAT activity). In contrast, *Sander lucioperca* juveniles submitted to emersion stress
 619 and long-term tryptophan treatment decreased both growth performance and plasma
 620 lysozyme activity after 91 days of feeding (Mandiki *et al.*, 2016), highlighting again the

complexity of tryptophan effects in fish. All these studies suggest that immunosuppressive conditions increase dietary tryptophan requirement (Fig. 2).

Thus, dietary tryptophan appears to modulate fish immune responses depending on the species, level of supplementation in the diet and administration time when fish are reared under standard husbandry conditions (i.e. undisturbed fish). *S. senegalensis* fed a tryptophan supplemented diet (i.e. 0.5% increase in a dry matter basis) augmented plasma lysozyme levels after 14 days of feeding whereas no changes were observed following 28 days of tryptophan treatment (Costas *et al.*, 2012). Interestingly, *S. senegalensis* fed a similar diet with the same level of tryptophan supplementation increased plasma peroxidase activity after 18 days of feeding, with no changes in plasma lysozyme or complement activities. *D. labrax* fed a tryptophan supplemented diet (i.e. 0.5% increase in a dry matter basis) for 14 days were unable to improve both cellular (e.g. circulating leucocyte numbers) and humoral (e.g. nitric oxide levels) mediated immune responses (Machado *et al.*, 2015). Similarly, *A. persicus* fed a 0.5% increase of tryptophan (dry matter basis) did not change serum lysozyme and hemolytic activities after 5, 10 and 15 days of feeding (Hoseini *et al.*, 2016). In contrast, several dietary tryptophan levels induced detrimental responses in the young *C. idella* after 8 weeks of feeding (Wen *et al.*, 2014; Jiang *et al.*, 2015). For instance, tryptophan deficiency or surplus negatively affected gills and gut integrity by changing tight-junction proteins transcripts, which were probably related to an increased gene expression of pro-inflammatory cytokines and oxidative damage, compared to fish fed an optimum tryptophan level.

Tryptophan catabolism seems to play a critical role in the functions of both macrophages and lymphocytes during infection. In mammals, while the protein-related pool of tryptophan is kept constantly balanced between the processes of protein catabolism and protein synthesis, about the 99% of tryptophan dietary intake is routed to the kynurenine pathway (KP) of tryptophan catabolism (Macchiarulo *et al.*, 2009). IDO catalyses the first and rate-limiting step of the KP, and tryptophan catabolism by IDO seems to have a major immunomodulatory effect during inflammation in mammals (Le Floc'h *et al.*, 2011). In fact, tryptophan concentrations drop progressively in the plasma of animals with inflammation (Melchior *et al.*, 2004). The activation of IDO present in macrophages

is induced by cytokines released by activated immune cells and leads to tryptophan depletion. IDO activity is also responsible for superoxide anions consumption thus exerting direct antioxidant activity (Le Floc'h *et al.*, 2011). In fish, few studies have explored the role of tryptophan during inflammation and infection. Changes in the relative levels of metabolites such as choline, glycerophosphorylcholine and betaine, which are metabolites involved in tryptophan metabolism, were observed by ^1H nuclear magnetic resonance in the head-kidney of *S. salar* submitted to *Aeromonas salmonicida* infection (Liu *et al.*, 2016). *D. labrax* fed a 0.5% (dry matter basis) tryptophan surplus in the diet for 14 days and submitted to an intraperitoneal injection of *Photobacterium damsela* subsp. *piscicida* decreased cellular responses and dropped plasma nitric oxide and bactericidal activity compared to fish fed a control diet (Machado *et al.*, 2015). These authors hypothesized that a drop in nitric oxide levels could be related to the inhibitory effect of 3OH-anthranilic acid (a tryptophan metabolite) on the expression of inducible nitric oxide synthase, one of the main isoforms responsible for nitric oxide production (Chiarugi *et al.*, 2003). Machado *et al.* (2015) also observed a decreased lymphocyte recruitment to the inflammatory focus at 24 h after injection with the pathogenic agent in fish fed the tryptophan surplus compared to fish fed the control diet. It has been hypothesized that this drop in lymphocyte numbers could be linked to a decrease in T cells proliferation due to high expression of IDO in antigen presenting cells. This enzyme can be expressed not only by macrophages but also in dendritic cells (DCs), and IDO⁺ DCs in mammals can potently and dominantly be suppressive for T cell activation (Sharma *et al.*, 2007). Indeed, the tryptophan degrading enzyme IDO plays an important immunoregulatory role in higher vertebrates, and therefore in depth studies are required to unravel its role in fish immune responses.

Since both stress and tryptophan are known to modulate immune function, an immunomodulatory role for 5HT can be expected. In fact, this particular neurotransmitter has been shown to be involved in both mammalian and teleost immune function (Khan & Deschaux, 1997; Mössner & Lesch, 1998). For instance, scientific evidence showed that 5HT can regulate a variety of immune responses including up-regulation of mitogen-stimulated T- and B-lymphocyte proliferation (Aune *et al.*, 1994; Iken *et al.*, 1995; Young & Matthews, 1995). Moreover, 5HT also appears to act as an immunomodulator

for its ability to stimulate or inhibit inflammation (Cloëz-Tayarani *et al.*, 2003). However, the functional role of 5HT in the fish immune system has been investigated much less. Immunosuppressive effects induced by 5HT have been observed on the LPS- and phytohaemagglutinin-stimulated proliferation of *O. mykiss* lymphocytes (Ferriere *et al.*, 1996). Those same authors also described that 5HT_{1A} receptors are expressed in trout lymphocytes after mitogenic stimulation. Similarly, Duffy-Whritenour and Zelikoff (2008) reported that endogenous (*in vivo*) and exogenous (*in vitro*) alterations in 5HT can suppress splenic T- and B-lymphocyte proliferation in the *Lepomis macrochirus*, and suggested a link between 5HT and the immune system in this particular teleost model. A number of studies have also reported changes in the distribution of neuropeptides and neurotransmitters in the digestive tract of parasitized fishes. For instance, Dezfuli *et al.* (2000) and Dezfuli *et al.* (2008) observed 5HT immunoreactivities in immuno-related cells of the intestine wall of parasitized *S. trutta*. Nevertheless, data proving evidence for the role of 5HT in regulating the fish immune response is scarce and further studies should focus on functional aspects such as cytokines secretion through the activation of 5HT receptors in fish leucocytes.

As commented above, melatonin is synthesized through 5HT and is involved in the regulation of several important physiological and behavioural functions, including the mammalian circadian rhythm, patterns of sleep, thermoregulation, reproduction and also immune function (Carrillo-Vico *et al.*, 2005). Regarding its role in immune function, melatonin is for instance involved in the development of thymus and spleen and also modulates innate and adaptive immune responses (Carrillo-Vico *et al.*, 2005). Still, knowledge regarding the actual fate of melatonin in the teleost immune functioning is scarce. Esteban *et al.* (2006) reported clear effects of photoperiod on the humoral innate immune system in two teleost fish, *Sparus aurata* and *D. labrax*, and provided evidence that in fish, as in mammals, melatonin appears to play an important role in modulating immune responses. Interestingly, seasonal changes in melatonin synthesis correlated with changes in total white blood cell numbers and lysozyme activity in *O. mykiss*, but not with the synthesis of ROS during the respiratory burst (Morgan *et al.*, 2008). *S. aurata* intraperitoneally injected with melatonin (1 or 10 mg melatonin/kg of body weight) increased the activity of peroxidase, phagocytosis and ROS production whereas the

expression of immune-relevant genes such as interleukin-1 β , major histocompatibility complex, virus-related response (interferon-regulatory factor-1 and Mx) and lymphocyte markers (immunoglobulin M and T-cell receptor for B and T lymphocytes, respectively) was up-regulated in the head–kidney of melatonin-injected fish 1 and 3 days post-injection and decreased after 7 days (Cuesta *et al.*, 2008). *In vitro* studies showed that *S. aurata* and *D. labrax* head-kidney leucocytes incubated with low (similar to physiological) doses of melatonin did not change innate immune parameters, whereas very high (pharmacological) doses inhibited peroxidase activity and increased ROS production (Cuesta *et al.*, 2007). The melatonin receptors have been cloned in fish, and partial or full-length sequences for the three high affinity melatonin receptor subtypes, MT1, MT2 and Mel1c, have been obtained for several fish species (Esteban *et al.*, 2013). The presence of MT1 receptors on carp leukocytes as well as significant effects of melatonin on leucocyte migration and apoptosis during zymosan-induced peritonitis were also observed in *C. carpio* (Kepka *et al.*, 2015). Those authors described a potent anti-inflammatory function for this hormone, whereas the observed melatonin induced inhibition of leucocyte apoptosis indicates towards a dual function. Indeed, scientific evidence suggests that neuroendocrine/immune interaction via melatonin is evolutionary conserved but further studies are needed to clarify the role of melatonin in teleost immune function.

The kynurenine and niacin pathway

As mentioned before, the KP is the dominant route for tryptophan degradation, accounting for almost 95 % of the tryptophan metabolized. Furthermore, it is considered to be the pathway through which excess tryptophan is removed from the organism, as the highest enzymatic activities occur in the liver where the portal blood supply is readily absorbed by the hepatocytes (Fernstrom, 2016).

Tryptophan is firstly oxidized by one of two possible enzymes (Fig. 1): TDO (also known as pyrrolase) or IDO. The produced N-formylkynurenine is then converted to kynurenine by arylformamidase, which can be metabolized by kynurenine aminotransferase and thereby be converted into the bioactive kynurenic acid. However, because this enzyme's K_m is very high, this branch of the KP will only occur in the

presence of tryptophan supplementation (Fernstrom, 2016). Instead, kynurenine can be further transformed in 3-hydroxykynurenine and 3-hydroxyanthranilic acid (both are biologically active compounds) which will finally be oxidized into acroleyl aminofumarate (2-amino-3-carboxymuconate semialdehyde). The following metabolic steps highly rely on the organism uptake of dietary niacin. Acroleyl aminofumarate can either be fully oxidized to picolinic acid and acetyl-CoA or it can be spontaneously cyclized to quinolinic acid and subsequently to nicotinamide and nicotinic acid (Fernstrom, 2016; Grohmann *et al.*, 2003; Moffett & Namboodiri, 2003a). In fish, tryptophan conversion to niacin is limited and deficiency is known to impair growth and antioxidant capacity in *C. idella* (Li *et al.*, 2016a; Li *et al.*, 2016b). Also, supplementing *Ictalurus punctatus*, diets devoid of niacin with graded levels of tryptophan did not enhance this vitamin endogenous synthesis (Ng *et al.*, 1997). While these are well-established steps in the mammalian KP, only some of the components have been identified so far in teleost fish (Cortés *et al.*, 2016; Serrano & Nagayama, 1992; Yambe *et al.*, 2006; Yuasa *et al.*, 2015). Therefore, whether the exact sequence of events is present and which mechanisms regulate enzyme activities in lower vertebrates, is a matter of further investigation.

The two leading enzymes (TDO or IDO) differ in tissue distribution, substrate affinity and specificity as well as regulation. TDO, almost exclusive of the liver, is more widely conserved than IDO gene sequences and has already been sequenced in a few fish species [*S. salar*, *Oryzias latipes*, *Danio rerio* and *O. mykiss*; Serrano and Nagayama (1992)]. It is highly specific for tryptophan but its affinity is lower compared to IDO's (Le Floc'h *et al.*, 2011). Therefore, TDO reacts only to elevated hepatic amount of tryptophan, particularly when it exceeds the requirement for protein and serotonin synthesis (Murray, 2003). It is rate-limiting in this pathway and its activity is not only induced by substrate availability, but also by glucocorticoids and glucagon (Badawy, 2002).

TDO activity is suppressed when IDO's is induced (Le Floc'h *et al.*, 2011). IDO's affinity to tryptophan is higher than TDO's, but it is not as specific towards this amino acid. In fact, it is also able to accept other indole substrates such as 5HT, 5-hydroxytryptophan or tryptamine (Yuasa *et al.*, 2015). Although the contribution of IDO

to overall activity of KP is small when compared to that of TDO, it acquires great significance in conditions of immune stress. Indeed, tryptophan increasing levels do not enhance IDO activity per se. Instead, IDO is induced by inflammatory stimulus such as LPS or IFN- γ , which has been observed both in mammals (Grohmann *et al.*, 2003) and fish (Cortés *et al.*, 2016). A growing interest has been laid on the KP, particularly when initiated by IDO, due to important biological effects carried out by kynurenine metabolites on the immune response (Grohmann *et al.*, 2003; Moffett & Namboodiri, 2003b; Munn & Mellor, 2013) which makes this not only a tryptophan degradative pathway but an important biosynthetic pathway, too.

The gene expression of IDO is ubiquitous in the rainbow trout, with the lowest levels being detected in the spleen and kidney (Cortés *et al.*, 2016). Its distribution is also highly associated to its biological importance. Strong expression patterns in mucosal tissues of the *O. mykiss* (skin, gills and gut) (Cortés *et al.*, 2016) and its presence in brain and gut macrophages and dendritic cells (Le Floc'h *et al.*, 2011) reveal the importance of IDO-mediated tryptophan metabolism in immune tolerance-leading mechanisms indispensable in tissues where the host is in close and constant contact with an array of microorganisms. Besides depleting tryptophan from the microenvironment, which prevents microorganisms from utilizing it (Yuasa *et al.*, 2007), immune tolerance is carried out by tryptophan metabolites such as 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid which, among other effects, are able to regulate T cell function and to modulate the oxidative status (Frumento *et al.*, 2001).

Interestingly, a completely different physiological role has been attributed to kynurenine which is that of being a pheromone. (Yambe *et al.* (2006)) observed that L-kynurenine present in the urine of female ovulating *Oncorhynchus masou* is responsible for attracting males, acting as a non-hormonal pheromone.

Conclusions and future perspectives

Tryptophan participates in different metabolic pathways involved in an array of physiological functions. Moreover, tryptophan can be directly transformed into other metabolites with important biological functions, including hormones such as melatonin or

neurotransmitters/neuromodulators as 5HT, contributing even more to the versatility of this compound. Even when the literature available on the effects and requirements of this amino acid is extensive, several aspects of tryptophan physiology remain to be elucidated. Recent research showed that, besides species-dependent differences, the tryptophan dietary requirements may also vary depending on other factors such as the stress suffered by the fish or the water quality, highlighting the need of a proper evaluation of those factors when assessing the dietary requirements of a given species. Available data somehow suggest that the teleosts' tryptophan requirement may increase under stressful conditions because of the beneficial effects of this amino acid to suppress stress and toxicant effects, and to improve antioxidant and immune status (Fig. 2). However, in many cases, mechanistic studies are still needed to know to which extent those effects are directly attributable to tryptophan or to related compounds such as 5HT, melatonin, among others. Furthermore, the effects of tryptophan on those physiological aspects have been shown to be very complex and to depend on species, dose and administration timing, and negative effects associated to tryptophan supplementation have been also reported. Therefore, even when tryptophan supplementation could be a promising strategy to improve fish conditions in aquaculture, specific studies to find proper doses and application time for the different farmed species are still needed to optimize its use as a welfare-promoting supplement.

Acknowledgements

No fund was received for this article. There is no conflict of interest about this manuscript.

References

- Acuna-Castroviejo D, Escames G, Venegas C, Diaz-Casado ME, Lima-Cabello E, Lopez LC, Rosales-Corral S, Tan DX, Reiter RJ (2014) Extrapineal melatonin: sources, regulation, and potential functions. *Cell Mol Life Sci*, **71**, 2997-3025.
- Ahmed I (2012) Dietary amino acid L-tryptophan requirement of fingerling Indian catfish, *Heteropneustes fossilis* (Bloch), estimated by growth and haemato-biochemical parameters. *Fish Physiol. Biochem.*, **38**, 1195-1209.
- Ahmed I, Khan MA (2005) Dietary tryptophan requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquac. Res.*, **36**, 687-695.

- Akhtar M, Pal A, Sahu N, Ciji A, Meena D, Das P (2013a) Physiological responses of dietary tryptophan fed *Labeo rohita* to temperature and salinity stress. *J. Anim. Physiol. Anim. Nutr. (Berl.)*, **97**, 1075-1083.
- Akhtar MS, Pal AK, Sahu NP, Ciji A (2013b) Hemato-immunological responses of *Labeo rohita* juveniles to temperature and salinity stress: Effect of dietary L-tryptophan. *Israeli journal of aquaculture-Bamidgeh*, **65**, 1-8.
- Akiyama T, Arai S, Murai T, Nose T (1985) Tryptophan requirement of chum salmon fry. *Nippon suisan gakkaiishi*, **51**, 1005-1008.
- Aldegunde M, Garcia J, Soengas JL, Rozas G (1998) Uptake of tryptophan into brain of rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Zool.*, **282**, 285-289.
- Anderson C, Campbell G (1988) Immunohistochemical Study of 5-Ht-Containing Neurons in the Teleost Intestine - Relationship to the Presence of Enterochromaffin Cells. *Cell Tissue Res*, **254**, 553-559.
- Andrade T, Afonso A, Pérez-Jiménez A, Oliva-Teles A, de las Heras V, Mancera JM, Serradeiro R, Costas B (2015) Evaluation of different stocking densities in a Senegalese sole (*Solea senegalensis*) farm: implications for growth, humoral immune parameters and oxidative status. *Aquaculture*, **438**, 6-11.
- Appa Rao NV, Raza B, Prasad JK, Razi SS, Gottardo L, Ahmad MF, Nussdorfer GG (2001) Melatonin decreases glucocorticoid blood concentration in the rat and palm squirrel, acting directly on the adrenal gland. *Biomedical research*, **22**, 115-117.
- Aragao C, Corte-Real J, Costas B, Dinis MT, Conceição L (2008) Stress response and changes in amino acid requirements in Senegalese sole (*Solea senegalensis* Kaup 1858). *Amino Acids*, **34**, 143-148.
- Aune TM, Golden HW, McGrath KM (1994) Inhibitors of serotonin synthesis and antagonists of serotonin 1A receptors inhibit T lymphocyte function in vitro and cell-mediated immunity in vivo. *The journal of immunology*, **153**, 489-498.
- Azeredo R, Machado M, Moura J, Martos-Sitcha J, Mancera J, Peres H, Oliva-Teles A, Afonso A, Costas B (2016) Dietary tryptophan supplementation modulates immune status and disease resistance in senegalese sole, *Solea senegalensis*, reared at low or high density. In: *International Meeting on Marine Research*. Frontiers in Marine Science, Peniche, Portugal.
- Azpeleta C, Martínez-Álvarez RM, Delgado MJ, Isorna E, De Pedro N (2010) Melatonin reduces locomotor activity and circulating cortisol in goldfish. *Horm. Behav.*, **57**, 323-329.
- Badawy AA (2017) Kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. *International journal of tryptophan research*, **10**.
- Badawy AAB (2002) Tryptophan metabolism in alcoholism. *Nutr Res Rev*, **15**, 123-152.
- Barlow-Walden L, Reiter R, Abe M, Pablos M, Menendez-Pelaez A, Chen L-D, Poeggeler B (1995) Melatonin stimulates brain glutathione peroxidase activity. *Neurochem. Int.*, **26**, 497-502.
- Basic D, Kroghdahl Å, Schjolden J, Winberg S, Vindas MA, Hillestad M, Mayer I, Skjerve E, Höglund E (2013a) Short-and long-term effects of dietary l-tryptophan supplementation on the neuroendocrine stress response in seawater-reared Atlantic salmon (*Salmo salar*). *Aquaculture*, **388**, 8-13.
- Basic D, Schjolden J, Kroghdahl Å, von Krogh K, Hillestad M, Winberg S, Mayer I, Skjerve E, Höglund E (2013b) Changes in regional brain monoaminergic activity

- and temporary down-regulation in stress response from dietary supplementation with L-tryptophan in Atlantic cod (*Gadus morhua*). *Br. J. Nutr.*, **109**, 2166-2174.
- Batabyal D, Yeh S-R (2007) Human tryptophan dioxygenase: a comparison to indoleamine 2, 3-dioxygenase. *J. Am. Chem. Soc.*, **129**, 15690-15701.
- Benakappa S, Varghese T (2003) Dietary requirement of tryptophan for growth and survival of the Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan) fry. *Indian J. Exp. Biol.*, **41**, 1342-1345.
- Benedict CR, Anderson GH, Sole MJ (1983) The influence of oral tyrosine and tryptophan feeding on plasma catecholamines in man. *The American journal of clinical nutrition*, **38**, 429-435.
- Berger M, Gray JA, Roth BL (2009) The Expanded Biology of Serotonin. *Annu Rev Med*, **60**, 355-366.
- Bertrand PP, Bertrand RL (2010) Serotonin release and uptake in the gastrointestinal tract. *Auton Neurosci-Basic*, **153**, 47-57.
- Britan A, Maffre V, Tone S, Drevet JR (2006) Quantitative and spatial differences in the expression of tryptophan-metabolizing enzymes in mouse epididymis. *Cell Tissue Res.*, **324**, 301-310.
- Caamano-Tubio RI, Perez J, Ferreira S, Aldegunde M (2007) Peripheral serotonin dynamics in the rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, **145**, 245-255.
- Cabeza J, Motilva V, Martín MaJ, de la Lastra CA (2001) Mechanisms involved in gastric protection of melatonin against oxidant stress by ischemia-reperfusion in rats. *Life Sci.*, **68**, 1405-1415.
- Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ (2005) A review of the multiple actions of melatonin on the immune system. *Endocrine*, **27**, 189-200.
- Castro C, Pérez-Jiménez A, Guerreiro I, Peres H, Castro-Cunha M, Oliva-Teles A (2012) Effects of temperature and dietary protein level on hepatic oxidative status of Senegalese sole juveniles (*Solea senegalensis*). *Comparative biochemistry and physiology Part A: molecular & integrative physiology*, **163**, 372-378.
- Chiarugi A, Rovida E, Sbarba PD, Moroni F (2003) Tryptophan availability selectively limits NO-synthase induction in macrophages. *J. Leukoc. Biol.*, **73**, 172-177.
- Christen S, Peterhans E, Stocker R (1990) Antioxidant activities of some tryptophan metabolites: possible implication for inflammatory diseases. *Proceedings of the national academy of sciences*, **87**, 2506-2510.
- Ciji A, Sahu N, Pal A, Akhtar M (2013a) Nitrite-induced alterations in sex steroids and thyroid hormones of *Labeo rohita* juveniles: effects of dietary vitamin E and L-tryptophan. *Fish Physiol. Biochem.*, **39**, 1297-1307.
- Ciji A, Sahu N, Pal A, Akhtar M, Meena D (2013b) Haemato-immunological responses of nitrite exposed *Labeo rohita* fed diets supplemented with vitamin E and L-tryptophan, challenged by *Aeromonas hydrophila*. *Israeli journal of aquaculture*, **65**, 889-897.
- Ciji A, Sahu N, Pal A, Dasgupta S, Akhtar M (2012) Alterations in serum electrolytes, antioxidative enzymes and haematological parameters of *Labeo rohita* on short-term exposure to sublethal dose of nitrite. *Fish Physiol. Biochem.*, **38**, 1355-1365.

- Ciji A, Sahu NP, Pal AK, Akhtar MS (2015) Dietary L-tryptophan modulates growth and immuno-metabolic status of *Labeo rohita* juveniles exposed to nitrite. *Aquac. Res.*, **46**, 2013-2024.
- Cloëz-Tayarani I, Petit-Bertron AF, Venters HD, Cavaillon JM (2003) Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells: involvement of 5-hydroxytryptamine_{2A} receptors. *Int. Immunol.*, **15**, 233-240.
- Coloso R, Murillo-Gurrea D, Borlongan I, Catacutan M (2004) Tryptophan requirement of juvenile Asian sea bass *Lates calcarifer*. *Journal of applied ichthyology*, **20**, 43-47.
- Coloso RM, Tiro LB, Benitez LV (1992) Requirement for tryptophan by milkfish (*Chanos chanos* Forsskal) juveniles. *Fish Physiol. Biochem.*, **10**, 35-41.
- Conceição LE, Aragão C, Dias J, Costas B, Terova G, Martins C, Tort L (2012) Dietary nitrogen and fish welfare. *Fish Physiol. Biochem.*, **38**, 119-141.
- Conde-Sieira M, Muñoz J, López-Patiño M, Gesto M, Soengas J, Míguez J (2014) Oral administration of melatonin counteracts several of the effects of chronic stress in rainbow trout. *Domest. Anim. Endocrinol.*, **46**, 26-36.
- Cortés J, Alvarez C, Santana P, Torres E, Mercado L (2016) Indoleamine 2, 3-dioxygenase: First evidence of expression in rainbow trout (*Oncorhynchus mykiss*). *Dev. Comp. Immunol.*, **65**, 73-78.
- Costas B, Aragão C, Dias J, Afonso A, Conceição LE (2013) Interactive effects of a high-quality protein diet and high stocking density on the stress response and some innate immune parameters of Senegalese sole *Solea senegalensis*. *Fish Physiol. Biochem.*, **39**, 1141-1151.
- Costas B, Aragão C, Mancera JM, Dinis MT, Conceição LE (2008) High stocking density induces crowding stress and affects amino acid metabolism in Senegalese sole *Solea senegalensis* (Kaup 1858) juveniles. *Aquac. Res.*, **39**, 1-9.
- Costas B, Aragão C, Soengas JL, Míguez JM, Rema P, Dias J, Afonso A, Conceição LE (2012) Effects of dietary amino acids and repeated handling on stress response and brain monoaminergic neurotransmitters in Senegalese sole (*Solea senegalensis*) juveniles. *Comparative biochemistry and physiology Part A: Molecular & integrative physiology*, **161**, 18-26.
- Coutinho F, Castro C, Rufino-Palomares E, Ordóñez-Grande B, Gallardo M, Oliva-Teles A, Peres H (2016) Dietary glutamine supplementation effects on amino acid metabolism, intestinal nutrient absorption capacity and antioxidant response of gilthead sea bream (*Sparus aurata*) juveniles. *Comparative biochemistry and physiology Part A: molecular & integrative physiology*, **191**, 9-17.
- Cuesta A, Cerezuela R, Esteban M, Meseguer J (2008) In vivo actions of melatonin on the innate immune parameters in the teleost fish gilthead seabream. *J. Pineal Res.*, **45**, 70-78.
- Cuesta A, Rodríguez A, Calderón MV, Meseguer J, Esteban MÁ (2007) Effect of the pineal hormone melatonin on teleost fish phagocyte innate immune responses after in vitro treatment. *Journal of experimental zoology Part A: ecological genetics and physiology*, **307**, 509-515.
- Dabrowski K (1981) Tryptophan requirement of common carp (*Cyprinus carpio* L.) fry. *Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde*, **46**, 64-71.

- Daniels W, Van Rensburg S, Van Zyl J, Van Der Walt B, Taljaard J (1996) Free radical scavenging effects of melatonin and serotonin: possible mechanism. *Neuroreport*, **7**, 1593-1596.
- Dantzer R, Moermede P (1979) Effects of lithium on aggressive behaviour in domestic pigs. *J. Vet. Pharmacol. Ther.*, **2**, 299-303.
- De Boeck G, Nilsson GE, Elofsson U, Vlaeminck A, Blust R (1995) Brain monoamine levels and energy status in common carp (*Cyprinus carpio*) after exposure to sublethal levels of copper. *Aquat. Toxicol.*, **33**, 265-277.
- Del Angel-Meza A, Dávalos-Marín A, Ontiveros-Martínez L, Ortiz G, Beas-Zarate C, Chaparro-Huerta V, Torres-Mendoza B, Bitzer-Quintero O (2011) Protective effects of tryptophan on neuro-inflammation in rats after administering lipopolysaccharide. *Biomed. Pharmacother.*, **65**, 215-219.
- Denbow D, Hobbs F, Hulet R, Graham P, Potter L (1993) Supplemental dietary L-tryptophan effects on growth, meat quality, and brain catecholamine and indoleamine concentrations in Turkeys. *Br. Poult. Sci.*, **34**, 715-724.
- Deng Y-P, Jiang W-D, Liu Y, Jiang J, Kuang S-Y, Tang L, Wu P, Zhang Y-A, Feng L, Zhou X-Q (2014) Differential growth performance, intestinal antioxidant status and relative expression of Nrf2 and its target genes in young grass carp (*Ctenopharyngodon idella*) fed with graded levels of leucine. *Aquaculture*, **434**, 66-73.
- Dezfuli B, Arrighi S, Domeneghini C, Bosi G (2000) Immunohistochemical detection of neuromodulators in the intestine of *Salmo trutta* L. naturally infected with *Cyathocephalus truncatus* Pallas (Cestoda). *J. Fish Dis.*, **23**, 265-273.
- Dezfuli BS, Giovinazzo G, Lui A, Giari L (2008) Inflammatory response to *Dentitruncus truttae* (Acanthocephala) in the intestine of brown trout. *Fish Shellfish Immunol.*, **24**, 726-733.
- Dhabhar FS (2009) A hassle a day may keep the pathogens away: the fight-or-flight stress response and the augmentation of immune function. *Integr. Comp. Biol.*, **49**, 215-236.
- Duerschmied D, Suidan GL, Demers M, Herr N, Carbo C, Brill A, Cifuni SM, Mauler M, Cicko S, Bader M, Idzko M, Bode C, Wagner DD (2013) Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood*, **121**, 1008-1015.
- Duffy-Whritenour J, Zelikoff J (2008) Relationship between serotonin and the immune system in a teleost model. *Brain. Behav. Immun.*, **22**, 257-264.
- Esteban MA, Cuesta A, Chaves-Pozo E, Meseguer J (2013) Influence of melatonin on the immune system of fish: a review. *International journal of molecular sciences*, **14**, 7979-7999.
- Esteban MA, Cuesta A, Rodríguez A, Meseguer J (2006) Effect of photoperiod on the fish innate immune system: a link between fish pineal gland and the immune system. *J. Pineal Res.*, **41**, 261-266.
- Fagbenro O, Nwanna L (1999) Dietary tryptophan requirement of the African catfish, *Clarias gariepinus*. *Journal of applied aquaculture*, **9**, 65-72.
- Falcón J, Besseau L, Magnanou E, Herrero M-J, Nagai M, Boeuf G (2011) Melatonin, the time keeper: biosynthesis and effects in fish. *Cybium*, **35**, 3-18.

1014 Falcón J, Besseau L, Sauzet S, Boeuf G (2007) Melatonin effects on the hypothalamo-
1015 pituitary axis in fish. *Trends Endocrinol. Metab.*, **18**, 81-88.

1016 Falcón J, Migaud H, Munoz-Cueto JA, Carrillo M (2010) Current knowledge on the
1017 melatonin system in teleost fish. *Gen. Comp. Endocrinol.*, **165**, 469-482.

1018 Falcón J, Thibault C, Martin C, Brun-Marmillon J, Claustrat B, Collin JP (1991)
1019 Regulation of melatonin production by catecholamines and adenosine in a
1020 photoreceptive pineal organ. An in vitro study in the pike and the trout. *J. Pineal*
1021 *Res.*, **11**, 123-134.

1022 Fatahi S, Hoseini SM (2013) Effect of dietary tryptophan and betaine on tolerance of
1023 Caspian roach (*Rutilus rutilus caspicus*) to copper toxicity. *International journal of*
1024 *aquatic biology*, **1**, 76-81.

1025 Fatma Abidi S, Khan MA (2010) Dietary tryptophan requirement of fingerling rohu,
1026 *Labeo rohita* (Hamilton), based on growth and body composition. *J. World.*
1027 *Aquac. Soc.*, **41**, 700-709.

1028 Fernstrom JD (2016) A perspective on the safety of supplemental tryptophan based on its
1029 metabolic fates. *The journal of nutrition*, **146**, 2601S-2608S.

1030 Ferrari R, Pugini SMP, Arce AIC, Costa EJX, Melo MP (2014) Metabolite of tryptophan
1031 promoting changes in EEG signal and the oxidative status of the brain. *Cell*
1032 *Biochem. Funct.*, **32**, 496-501.

1033 Ferriere F, Khan N, Troutaud D, Deschaux P (1996) Serotonin modulation of lymphocyte
1034 proliferation via 5-HT1A receptors in rainbow trout (*Oncorhynchus mykiss*). *Dev.*
1035 *Comp. Immunol.*, **20**, 273-283.

1036 Fjaerli O, Lund T, Østerud B (1999) The effect of melatonin on cellular activation
1037 processes in human blood. *J. Pineal Res.*, **26**, 50-55.

1038 Fritsche R, Reid SG, Thomas S, Perry SF (1993) Serotonin-mediated release of
1039 catecholamines in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.*, **178**, 191-
1040 204.

1041 Frumento G, Rotondo R, Tonetti M, Ferrara GB (2001) T cell proliferation is blocked
1042 by indoleamine 2,3-dioxygenase. *Transplantation Proceedings*, **33**, 428-430.

1043 Gaylord T, Rawles S, Davis K (2005) Dietary tryptophan requirement of hybrid striped
1044 bass (*Morone chrysops* × *M. saxatilis*). *Aquacult Nutr*, **11**, 367-374.

1045 Gershon MD (2013) 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr*
1046 *Opin Endocrinol*, **20**, 14-21.

1047 Gesto M, Álvarez-Otero R, Conde-Sieira M, Otero-Rodiño C, Usandizaga S, Soengas JL,
1048 Míguez JM, López-Patiño MA (2016) A simple melatonin treatment protocol
1049 attenuates the response to acute stress in the sole *Solea senegalensis*. *Aquaculture*,
1050 **452**, 272-282.

1051 Gesto M, Lopez-Patino MA, Hernandez J, Soengas JL, Miguez JM (2013) The response
1052 of brain serotonergic and dopaminergic systems to an acute stressor in rainbow
1053 trout: a time course study. *J Exp Biol*, **216**, 4435-4442.

1054 Giri SS, Sen SS, Chi C, Kim HJ, Yun S, Park SC, Sukumaran V (2015) Effect of
1055 dietary leucine on the growth parameters and expression of antioxidant, immune,
1056 and inflammatory genes in the head kidney of *Labeo rohita* fingerlings. *Vet.*
1057 *Immunol. Immunopathol.*, **167**, 36-43.

- Gopal K, Ram M (1995) Alteration in the neurotransmitter levels in the brain of the freshwater snakehead fish (*Channa punctatus*) exposed to carbofuran. *Ecotoxicology*, **4**, 1-4.
- Grohmann U, Fallarino F, Puccetti P (2003) Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol.*, **24**, 242-248.
- Guchhait P, Haldar C (2001) A reproductive phase-dependent effect of dietary L-tryptophan on pineal gland and gonad of a nocturnal bird, Indian spotted owl *Athene brama*. *Acta Biol. Hung.*, **52**, 1-7.
- Gülçin I (2008) Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent. *J. Enzyme Inhib. Med. Chem.*, **23**, 871-876.
- Hajak G, Huether G, Blanke J, Blömer M, Freyer C, Poeggeler B, Reimer A, Rodenbeck A, Schulz-Varzegi M, Rüther E (1991) The influence of intravenous L-tryptophan on plasma melatonin and sleep in men. *Pharmacopsychiatry*, **24**, 17-20.
- Halliwell B, Gutteridge JM (2015) *Free radicals in biology and medicine*, Oxford University Press, USA, Oxford, UK.
- Halver JE (1965) Tryptophan requirements of chinook, sockeye and silver salmon. *FASEB*, **24**, 299.
- Hayaishi O (1996) Utilization of superoxide anion by indoleamine oxygenase-catalyzed tryptophan and indoleamine oxidation. In: *Recent advances in tryptophan research*. Springer, pp. 285-289.
- Herrero M, Martínez F, Míguez J, Madrid J (2007) Response of plasma and gastrointestinal melatonin, plasma cortisol and activity rhythms of European sea bass (*Dicentrarchus labrax*) to dietary supplementation with tryptophan and melatonin. *Journal of comparative physiology B*, **177**, 319-326.
- Höglund E, Bakke MJ, Øverli Ø, Winberg S, Nilsson GE (2005) Suppression of aggressive behaviour in juvenile Atlantic cod (*Gadus morhua*) by l-tryptophan supplementation. *Aquaculture*, **249**, 525-531.
- Höglund E, Øverli Ø, Andersson MÅ, Silva P, Laursen DC, Moltesen MM, Krogdahl Å, Schjolden J, Winberg S, Vindas MA (2017) Dietary L-tryptophan leaves a lasting impression on the brain and the stress response. *Br. J. Nutr.*, **117**, 1351-1357.
- Höglund E, Sørensen C, Bakke MJ, Nilsson GE, Øverli Ø (2007) Attenuation of stress-induced anorexia in brown trout (*Salmo trutta*) by pre-treatment with dietary L-tryptophan. *Br. J. Nutr.*, **97**, 786-789.
- Hoseini SM, Hosseini SA (2010) Effect of dietary l-tryptophan on osmotic stress tolerance in common carp, *Cyprinus carpio*, juveniles. *Fish Physiol. Biochem.*, **36**, 1061-1067.
- Hoseini SM, Hosseini SA, Soudagar M (2012) Dietary tryptophan changes serum stress markers, enzyme activity, and ions concentration of wild common carp *Cyprinus carpio* exposed to ambient copper. *Fish Physiol. Biochem.*, **38**, 1419-1426.
- Hoseini SM, Mirghaed AT, Mazandarani M, Zoheiri F (2016) Serum cortisol, glucose, thyroid hormones' and non-specific immune responses of Persian sturgeon, *Acipenser persicus* to exogenous tryptophan and acute stress. *Aquaculture*, **462**, 17-23.

- Hosseini SA, Hoseini SM (2013) Effect of dietary tryptophan on stress response of wild common carp *Cyprinus carpio* L. *World journal of fish and marine sciences*, **5**, 49-55.
- Hseu JR, Lu FI, Su HM, Wang LS, Tsai CL, Hwang PP (2003) Effect of exogenous tryptophan on cannibalism, survival and growth in juvenile grouper, *Epinephelus coioides*. *Aquaculture*, **218**, 251-263.
- Huether G, Poeggeler B, Reimer A, George A (1992) Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. *Life Sci.*, **51**, 945-953.
- Iken K, Chheng S, Fargin A, Goulet A-C, Kouassi E (1995) Serotonin upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT1A receptors. *Cell. Immunol.*, **163**, 1-9.
- Jia R, Han C, Lei J-L, Liu B-L, Huang B, Huo H-H, Yin S-T (2015) Effects of nitrite exposure on haematological parameters, oxidative stress and apoptosis in juvenile turbot (*Scophthalmus maximus*). *Aquat. Toxicol.*, **169**, 1-9.
- Jiang W-D, Feng L, Qu B, Wu P, Kuang S-Y, Jiang J, Tang L, Tang W-N, Zhang Y-A, Zhou X-Q (2016a) Changes in integrity of the gill during histidine deficiency or excess due to depression of cellular anti-oxidative ability, induction of apoptosis, inflammation and impair of cell-cell tight junctions related to Nrf2, TOR and NF- κ B signaling in fish. *Fish Shellfish Immunol.*, **56**, 111-122.
- Jiang W-D, Wen H-L, Liu Y, Jiang J, Kuang S-Y, Wu P, Zhao J, Tang L, Tang W-N, Zhang Y-A (2015) The tight junction protein transcript abundance changes and oxidative damage by tryptophan deficiency or excess are related to the modulation of the signalling molecules, NF- κ B p65, TOR, caspase-(3, 8, 9) and Nrf2 mRNA levels, in the gill of young grass carp (*Ctenopharyngodon idellus*). *Fish Shellfish Immunol.*, **46**, 168-180.
- Jiang W-D, Wen H-L, Liu Y, Jiang J, Wu P, Zhao J, Kuang S-Y, Tang L, Tang W-N, Zhang Y-A (2016b) Enhanced muscle nutrient content and flesh quality, resulting from tryptophan, is associated with anti-oxidative damage referred to the Nrf2 and TOR signalling factors in young grass carp (*Ctenopharyngodon idella*): Avoid tryptophan deficiency or excess. *Food Chem.*, **199**, 210-219.
- Johnston WL, Atkinson JL, Hilton JW, Were KE (1990) Effect of dietary tryptophan on plasma and brain tryptophan, brain serotonin, and brain 5-hydroxyindoleacetic acid in rainbow trout. *The journal of nutritional biochemistry*, **1**, 49-54.
- Kaushik SJ (1998) Whole body amino acid composition of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. *Aquat. Living Resour.*, **11**, 355-358.
- Kepka M, Szejser E, Pijanowski L, Verburg-van Kemenade BL, Chadzinska M (2015) A role for melatonin in maintaining the pro-and anti-inflammatory balance by influencing leukocyte migration and apoptosis in carp. *Dev. Comp. Immunol.*, **53**, 179-190.
- Khan N, Deschaux P (1997) Role of serotonin in fish immunomodulation. *J. Exp. Biol.*, **200**, 1833-1838.
- Kim K-i, Kayes TB, Amundson CH (1987) Effects of dietary tryptophan levels on growth, feed/gain, carcass composition and liver glutamate dehydrogenase activity

- in rainbow trout (*Salmo gairdneri*). *Comparative biochemistry and physiology Part B: Comparative biochemistry*, **88**, 737-741.
- Konakchieva R, Mitev Y, Almeida OF, Patchev VK (1997) Chronic melatonin treatment and the hypothalamo-pituitary-adrenal axis in the rat: attenuation of the secretory response to stress and effects on hypothalamic neuropeptide content and release. *Biology of the cell*, **89**, 587-596.
- Kulczykowska E, Kalamarz H, Warne JM, Balment RJ (2006) Role of melatonin in fish osmoregulation: Towards a new action of the hormone. *J Exp Zool Part A*, **305a**, 145-145.
- Kumar P, Saurabh S, Pal A, Sahu N, Arasu A (2014) Stress mitigating and growth enhancing effect of dietary tryptophan in rohu (*Labeo rohita*, Hamilton, 1822) fingerlings. *Fish Physiol. Biochem.*, **40**, 1325-1338.
- Le Floc'h N, Otten W, Merlot E (2011) Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids*, **41**, 1195-1205.
- Lepage O, Larson ET, Mayer I, Winberg S (2005a) Serotonin, but not melatonin, plays a role in shaping dominant-subordinate relationships and aggression in rainbow trout. *Horm. Behav.*, **48**, 233-242.
- Lepage O, Larson ET, Mayer I, Winberg S (2005b) Tryptophan affects both gastrointestinal melatonin production and interrenal activity in stressed and nonstressed rainbow trout. *J. Pineal Res.*, **38**, 264-271.
- Lepage O, Tottmar O, Winberg S (2002) Elevated dietary intake of L-tryptophan counteracts the stress-induced elevation of plasma cortisol in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.*, **205**, 3679-3687.
- Lepage O, Vilchez IM, Pottinger TG, Winberg S (2003) Time-course of the effect of dietary L-tryptophan on plasma cortisol levels in rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.*, **206**, 3589-3599.
- Lezoualc'h F, Sparapani M, Behl C (1998) N-acetyl-serotonin (normelatonin) and melatonin protect neurons against oxidative challenges and suppress the activity of the transcription factor NF- κ B. *J. Pineal Res.*, **24**, 168-178.
- Li P, Mai KS, Trushenski J, Wu GY (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, **37**, 43-53.
- Li SQ, Feng L, Jiang WD, Liu Y, Jiang J, Wu P, Kuang SY, Tang L, Tang WN, Zhang YA, Zhou XQ (2016a) Deficiency of dietary niacin impaired gill immunity and antioxidant capacity, and changes its tight junction proteins via regulating NF- κ B, TOR, Nrf2 and MLCK signaling pathways in young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immun.*, **55**, 212-222.
- Li SQ, Feng L, Jiang WD, Liu Y, Wu P, Zhao J, Kuang SY, Jiang J, Tang L, Tang WN, Zhang YA, Zhou XQ (2016b) Deficiency of dietary niacin decreases digestion and absorption capacities via declining the digestive and brush border enzyme activities and downregulating those enzyme gene transcription related to TOR pathway of the hepatopancreas and intestine in young grass carp (*Ctenopharyngodon idella*). *Aquacult Nutr.*, **22**, 1267-1282.
- Lim JE, Porteus CS, Bernier NJ (2013) Serotonin directly stimulates cortisol secretion from the interrenals in goldfish. *General and Comparative Endocrinology*, **192**, 246-255.

- Liu P-f, Du Y, Meng L, Li X, Liu Y (2016) Metabolic profiling in kidneys of Atlantic salmon infected with *Aeromonas salmonicida* based on ¹H NMR. *Fish Shellfish Immunol.*, **58**, 292-301.
- Liu Y, Yuan J, Zhang L, Zhang Y, Cai S, Yu J, Xia Z (2015) Effects of tryptophan supplementation on growth performance, antioxidative activity, and meat quality of ducks under high stocking density. *Poult. Sci.*, **94**, 1894-1901.
- López-Olmeda J, Madrid J, Sánchez-Vázquez F (2006) Melatonin effects on food intake and activity rhythms in two fish species with different activity patterns: Diurnal (goldfish) and nocturnal (tench). *Comparative biochemistry and physiology Part A: molecular & integrative physiology*, **144**, 180-187.
- López-Patiño MA, Conde-Sieira M, Gesto M, Librán-Pérez M, Soengas JL, Míguez JM (2013) Melatonin partially minimizes the adverse stress effects in Senegalese sole (*Solea senegalensis*). *Aquaculture*, **388**, 165-172.
- Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. *Aquat. Toxicol.*, **101**, 13-30.
- Macchiarulo A, Camaioni E, Nuti R, Pellicciari R (2009) Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2, 3-dioxygenase (IDO), a novel target in cancer disease. *Amino Acids*, **37**, 219-229.
- Machado M, Azeredo R, Díaz-Rosales P, Afonso A, Peres H, Oliva-Teles A, Costas B (2015) Dietary tryptophan and methionine as modulators of European seabass (*Dicentrarchus labrax*) immune status and inflammatory response. *Fish Shellfish Immunol.*, **42**, 353-362.
- Maitra SK, Hasan KN (2016) The Role of Melatonin as a Hormone and an Antioxidant in the Control of Fish Reproduction. *Frontiers in Endocrinology*, **7**, 38.
- Mambrini M, Kaushik S (1995) Indispensable amino acid requirements of fish: correspondence between quantitative data and amino acid profiles of tissue proteins. *Journal of applied ichthyology*, **11**, 240-247.
- Mandiki SNM, Redivo B, Baekelandt S, Douxfils J, Lund I, Höglund E, Kestemont P (2016) Long-term tryptophan supplementation decreased the welfare and innate immune status of pikeperch juveniles. *Fish Shellfish Immunol.*, **53**, 113-114.
- Mao X, Lv M, Yu B, He J, Zheng P, Yu J, Wang Q, Chen D (2014) The effect of dietary tryptophan levels on oxidative stress of liver induced by diquat in weaned piglets. *Journal of animal science and biotechnology*, **5**, 49.
- Markus CR, Olivier B, de Haan EH (2002) Whey protein rich in α -lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. *The American journal of clinical nutrition*, **75**, 1051-1056.
- Martínez-Álvarez RM, Morales AE, Sanz A (2005) Antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish Biol. Fish.*, **15**, 75-88.
- Martins CI, Silva PI, Costas B, Larsen BK, Santos GA, Conceição LE, Dias J, Øverli Ø, Höglund E, Schrama JW (2013) The effect of tryptophan supplemented diets on brain serotonergic activity and plasma cortisol under undisturbed and stressed conditions in grouped-housed Nile tilapia *Oreochromis niloticus*. *Aquaculture*, **400**, 129-134.

1240 Matuszak Z, Reszka KJ, Chignell CF (1997) Reaction of melatonin and related indoles
1241 with hydroxyl radicals: EPR and spin trapping investigations. *Free Radic. Biol.*
1242 *Med.*, **23**, 367-372.

1243 Melchior D, Sève B, Floc'h L (2004) Chronic lung inflammation affects plasma amino
1244 acid concentrations in pigs. *J. Anim. Sci.*, **82**, 1091-1099.

1245 Moffett JR, Namboodiri M (2003a) Tryptophan and the immune response. *Immunol. Cell*
1246 *Biol.*, **81**, 247-265.

1247 Moffett JR, Namboodiri MA (2003b) Tryptophan and the immune response. *Immunol*
1248 *Cell Biol*, **81**, 247-265.

1249 Moosmann B, Uhr M, Behl C (1997) Neuroprotective potential of aromatic alcohols
1250 against oxidative cell death. *FEBS Lett.*, **413**, 467-472.

1251 Morales AE, Pérez-Jiménez A, Hidalgo MC, Abellán E, Cardenete G (2004) Oxidative
1252 stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver.
1253 *Comparative biochemistry and physiology Part C: toxicology & pharmacology*,
1254 **139**, 153-161.

1255 Morandini L, Ramallo MR, Moreira RG, Höcht C, Somoza GM, Silva A, Pandolfi M
1256 (2015) Serotonergic outcome, stress and sexual steroid hormones, and growth in a
1257 South American cichlid fish fed with an L-tryptophan enriched diet. *Gen. Comp.*
1258 *Endocrinol.*, **223**, 27-37.

1259 Morgan AL, Thompson K, Auchinachie N, Migaud H (2008) The effect of seasonality
1260 on normal haematological and innate immune parameters of rainbow trout
1261 *Oncorhynchus mykiss* L. *Fish Shellfish Immunol.*, **25**, 791-799.

1262 Mössner R, Lesch K-P (1998) Role of serotonin in the immune system and in
1263 neuroimmune interactions. *Brain. Behav. Immun.*, **12**, 249-271.

1264 Munn DH, Mellor AL (2013) Indoleamine 2,3 dioxygenase and metabolic control of
1265 immune responses. *Trends Immunol*, **34**, 137-143.

1266 Muñoz-Pérez JL, López-Patiño MA, Álvarez-Otero R, Gesto M, Soengas JL, Míguez JM
1267 (2016) Characterization of melatonin synthesis in the gastrointestinal tract of
1268 rainbow trout (*Oncorhynchus mykiss*): distribution, relation with serotonin, daily
1269 rhythms and photoperiod regulation. *Journal of comparative physiology B*, **186**,
1270 471-484.

1271 Munro A (1986) Effects of melatonin, serotonin, and naloxone on aggression in isolated
1272 cichlid fish (*Aequidens pulcher*). *J. Pineal Res.*, **3**, 257-262.

1273 Murray MF (2003) Tryptophan depletion and HIV infection: a metabolic link to
1274 pathogenesis. *Lancet Infect Dis*, **3**, 644-652.

1275 Murthy HS, Varghese T (1997) Dietary tryptophan requirement for the growth of rohu,
1276 *Labeo rohita*. *Journal of applied aquaculture*, **7**, 71-79.

1277 Nagai T, Hamada M, Kai N, Tanoue Y, Nagayama F (1997) Organ distribution of
1278 tryptophan hydroxylase activity in several fish. *Fisheries Sci*, **63**, 652-653.

1279 Narin F, Narin N, BAŞARSLAN F, Baykan A, Sezer S, Akgün H, Akin A, AKÇAKUŞ
1280 M, Ceyran H (2010) The effect of L-tryptophan on the heart in rabbits via
1281 chronic hypoxia. *Turkish journal of medical Sciences*, **40**, 257-263.

1282 Ng W-K, Serrini G, Zhang Z, Wilson RP (1997) Niacin requirement and inability of
1283 tryptophan to act as a precursor of NAD⁺ in channel catfish, *Ictalurus punctatus*.
1284 *Aquaculture*, **152**, 273-285.

1285 NRC (2011) *Nutrient requirement of fish and shrimp*, The National Academies Press,
1286 Washington DC.

1287 Oliva-Teles A (2012) Nutrition and health of aquaculture fish. *J. Fish Dis.*, **35**, 83-108.

1288 Øverli Ø, Winberg S, Damsård B, Jobling M (1998) Food intake and spontaneous
1289 swimming activity in Arctic char (*Salvelinus alpinus*): role of brain serotonergic
1290 activity and social interactions. *Can. J. Zool.*, **76**, 1366-1370.

1291 Paredes SD, Terrón MP, Cubero J, Valero V, Barriga C, Reiter RJ, Rodríguez AB
1292 (2007a) Tryptophan increases nocturnal rest and affects melatonin and serotonin
1293 serum levels in old ringdove. *Physiol. Behav.*, **90**, 576-582.

1294 Paredes SD, Terrón MP, Marchena AM, Barriga C, Pariente JA, Reiter RJ, Rodríguez
1295 AB (2007b) Tryptophan modulates cell viability, phagocytosis and oxidative
1296 metabolism in old ringdoves. *Basic Clin. Pharmacol. Toxicol.*, **101**, 56-62.

1297 Park JW, Youn YC, Kwon OS, Jang YY, Han ES, Lee CS (2002) Protective effect of
1298 serotonin on 6-hydroxydopamine- and dopamine-induced oxidative damage of brain
1299 mitochondria and synaptosomes and PC12 cells. *Neurochem. Int.*, **40**, 223-233.

1300 Pérez-González A, Alvarez-Idaboy JR, Galano A (2015) Free-radical scavenging by
1301 tryptophan and its metabolites through electron transfer based processes. *Journal of*
1302 *molecular modeling*, **21**, 213.

1303 Pérez-González A, Muñoz-Rugeles L, Alvarez-Idaboy J (2014) Tryptophan: antioxidant or
1304 target of oxidative stress? A quantum chemistry elucidation. *RSC Advances*, **4**,
1305 56128-56131.

1306 Pérez-Jiménez A, Peres H, Rubio VC, Oliva-Teles A (2012) The effect of hypoxia on
1307 intermediary metabolism and oxidative status in gilthead sea bream (*Sparus*
1308 *aurata*) fed on diets supplemented with methionine and white tea. *Comparative*
1309 *biochemistry and physiology Part C: toxicology & pharmacology*, **155**, 506-516.

1310 Pérez Jiménez A, Peres H, Cruz Rubio V, Oliva-Teles A (2012) The effect of dietary
1311 methionine and white tea on oxidative status of gilthead sea bream (*Sparus*
1312 *aurata*). *Br. J. Nutr.*, **108**, 1202-1209.

1313 Perianayagam M, Oxenkrug G, Jaber B (2005) Immune-modulating effects of melatonin,
1314 N-acetylserotonin, and N-acetyldopamine. *Ann. N. Y. Acad. Sci.*, **1053**, 386-393.

1315 Pewitt E, Castillo S, Velásquez A, Gatlin DM (2016) The dietary tryptophan requirement
1316 of juvenile red drum, *Sciaenops ocellatus*. *Aquaculture*.

1317 Peyrot F, Ducrocq C (2008) Potential role of tryptophan derivatives in stress responses
1318 characterized by the generation of reactive oxygen and nitrogen species. *J. Pineal*
1319 *Res.*, **45**, 235-246.

1320 Pinillos M, De Pedro N, Alonso-Gómez A, Alonso-Bedate M, Delgado M (2001) Food
1321 intake inhibition by melatonin in goldfish (*Carassius auratus*). *Physiol. Behav.*, **72**,
1322 629-634.

1323 Poston HA, Rumsey GL (1983) Factors affecting dietary requirement and deficiency signs
1324 of L-tryptophan in rainbow trout. *The journal of nutrition*, **113**, 2568-2577.

1325 Rasmussen D, Ishizuka B, Quigley M, Yen S (1983) Effects of tyrosine and tryptophan
1326 ingestion on plasma catecholamine and 3, 4-dihydroxyphenylacetic acid
1327 concentrations. *The journal of clinical endocrinology & metabolism*, **57**, 760-763.

1328 Ravi J, Devaraj K (1991) Quantitative essential amino acid requirements for growth of
1329 catla, *Catla catla* (Hamilton). *Aquaculture*, **96**, 281-291.

- Reiter R, King T, Steinlechner S, Steger R, Richardson B (1990) Tryptophan administration inhibits nocturnal N-acetyltransferase activity and melatonin content in the rat pineal gland. *Neuroendocrinology*, **52**, 291-296.
- Reyes-Gonzales M, Fuentes-Broto L, Martínez-Ballarín E, Miana-Mena F, Berzosa C, García-Gil F, Aranda M, García J (2009) Effects of tryptophan and 5-hydroxytryptophan on the hepatic cell membrane rigidity due to oxidative stress. *J. Membr. Biol.*, **231**, 93-99.
- Roméo M, Bennani N, Gnassia-Barelli M, Lafaurie M, Girard JP (2000) Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. *Aquat. Toxicol.*, **48**, 185-194.
- Rubio V, Sánchez-Vázquez F, Madrid J (2006) Oral serotonin administration affects the quantity and the quality of macronutrients selection in European sea bass *Dicentrarchus labrax* L. *Physiol. Behav.*, **87**, 7-15.
- Sadeghnia HR, Kamkar M, Assadpour E, Boroushaki MT, Ghorbani A (2013) Protective effect of safranal, a constituent of *Crocus sativus*, on quinolinic acid-induced oxidative damage in rat hippocampus. *Iranian journal of basic medical sciences*, **16**, 73-82.
- Saito S, Tachibana T, Choi Y-H, Denbow DM, Furuse M (2005) ICV melatonin reduces acute stress responses in neonatal chicks. *Behav. Brain Res.*, **165**, 197-203.
- Sakkas P, Jones LA, Houdijk JG, Athanasiadou S, Knox DP, Kyriazakis I (2013) Leucine and methionine deficiency impairs immunity to gastrointestinal parasites during lactation. *Br. J. Nutr.*, **109**, 273-282.
- Sanchez S, Sanchez CL, Paredes SD, Rodriguez AB, Barriga C (2008) The effect of tryptophan administration on the circadian rhythms of melatonin in plasma and the pineal gland of rats. *Journal of applied biomedicine*, **6**, 177-186.
- Sanchez W, Palluel O, Meunier L, Coquery M, Porcher J-M, Ait-Aissa S (2005) Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. *Environ. Toxicol. Pharmacol.*, **19**, 177-183.
- Santamaría A, Galván-Arzate S, Lisý V, Ali SF, Duhart HM, Osorio-Rico L, Ríos C, Sut'astný F (2001) Quinolinic acid induces oxidative stress in rat brain synaptosomes. *Neuroreport*, **12**, 871-874.
- Santiago CB, Lovell RT (1988) Amino acid requirements for growth of Nile tilapia. *The journal of nutrition*, **118**, 1540-1546.
- Sarikaya S, Gulcin I (2013) Radical scavenging and antioxidant capacity of serotonin. *Current bioactive compounds*, **9**, 143-152.
- Serrano AE, Nagayama F (1992) Activity and partial purification of liver tryptophan pyrrolase in rainbow trout. *Nippon suisan gakkaiishi*, **58**, 2367-2371.
- Shanks WE, Gahimer GD, Halver JE (1962) The indispensable amino acids for rainbow trout. *The progressive fish-culturist*, **24**, 68-73.
- Sharma MD, Baban B, Chandler P, Hou D-Y, Singh N, Yagita H, Azuma M, Blazar BR, Mellor AL, Munn DH (2007) Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2, 3-dioxygenase. *The journal of clinical investigation*, **117**, 2570-2582.
- Stolte EH, Nabuurs SB, Bury NR, Sturm A, Flik G, Savelkoul HF, Verburg-van Kemenade BL (2008) Stress and innate immunity in carp: corticosteroid receptors and pro-inflammatory cytokines. *Mol. Immunol.*, **46**, 70-79.

- Suárez M, Martínez T, Abellán E, Arizcun M, Pérez-Jiménez A, Hidalgo M, Cardenete G (2009) The effects of the diet on flesh quality of farmed dentex (*Dentex dentex*). *Aquaculture*, **288**, 106-113.
- Tan D-X, Manchester LC, Esteban-Zubero E, Zhou Z, Reiter RJ (2015) Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism. *Molecules*, **20**, 18886-18906.
- Tang L, Feng L, Sun C-Y, Chen G-F, Jiang W-D, Hu K, Liu Y, Jiang J, Li S-H, Kuang S-Y (2013) Effect of tryptophan on growth, intestinal enzyme activities and TOR gene expression in juvenile Jian carp (*Cyprinus carpio* var. Jian): Studies in vivo and in vitro. *Aquaculture*, **412**, 23-33.
- Tejpal CS, Pal AK, Sahu NP, Ashish Kumar J, Muthappa NA, Vidya S, Rajan MG (2009) Dietary supplementation of l-tryptophan mitigates crowding stress and augments the growth in *Cirrhinus mrigala* fingerlings. *Aquaculture*, **293**, 272-277.
- Torres-Farfan C, Richter HG, Rojas-García P, Vergara M, Forcelledo ML, Valladares LE, Torrealba F, Valenzuela GJ, Serón-Ferré M (2003) mt1 Melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin. *The journal of clinical endocrinology & metabolism*, **88**, 450-458.
- Tsai C-L, Jang T-H, Wang L-H (1995) Effects of mercury on serotonin concentration in the brain of tilapia, *Oreochromis mossambicus*. *Neurosci. Lett.*, **184**, 208-211.
- Velarde E, Delgado M, Alonso-Gómez A (2010) Serotonin-induced contraction in isolated intestine from a teleost fish (*Carassius auratus*): characterization and interactions with melatonin. *Neurogastroenterol. Motil.*, **22**, e364-e373.
- Verburg-van Kemenade L, Ribeiro C, Chadzinska M (2011) Neuroendocrine-immune interaction in fish: Differential regulation of phagocyte activity by neuroendocrine factors. *Gen. Comp. Endocrinol.*, **172**, 31-38.
- Verburg-Van Kemenade BL, Stolte EH, Metz JR, Chadzinska M (2009) Neuroendocrine-immune interactions in teleost fish. In: *Fish physiology*, pp. 313-364.
- Vutukuru SS, Chintada S, Madhavi KR, Rao JV, Anjaneyulu Y (2006) Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus danricus*. *Fish Physiol. Biochem.*, **32**, 221-229.
- Walton M, Coloso RM, Cowey C, Adron J, Knox D (1984) The effects of dietary tryptophan levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.*, **51**, 279-287.
- Walton M, Cowey C, Coloso RM, Adron J (1986) Dietary requirements of rainbow trout for tryptophan, lysine and arginine determined by growth and biochemical measurements. *Fish Physiol. Biochem.*, **2**, 161-169.
- Wei K, Yang J (2016) Copper-induced oxidative damage to the prophenoloxidase-activating system in the freshwater crayfish *Procambarus clarkii*. *Fish Shellfish Immunol.*, **52**, 221-229.
- Wen H, Feng L, Jiang W, Liu Y, Jiang J, Li S, Tang L, Zhang Y, Kuang S, Zhou X (2014) Dietary tryptophan modulates intestinal immune response, barrier function, antioxidant status and gene expression of TOR and Nrf2 in young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol.*, **40**, 275-287.
- Wilson R, Cowey C (1985) Amino acid composition of whole body tissue of rainbow trout and Atlantic salmon. *Aquaculture*, **48**, 373-376.

1422 Wilson RP (2002) Amino acids and proteins. In: *Fish nutrition* (ed. by Halver J, Hardy
1423 RW). Academic Press, New York, USA, pp. 143-179.

1424 Wilson RP, Allen Jr OW, Robinson EH, Poe WE (1978) Tryptophan and threonine
1425 requirements of fingerling channel catfish. *The journal of nutrition*, **108**, 1595-
1426 1599.

1427 Winberg S, Lepage O (1998) Elevation of brain 5-HT activity, POMC expression, and
1428 plasma cortisol in socially subordinate rainbow trout. *American journal of*
1429 *physiology-regulatory, integrative and comparative physiology*, **274**, R645-R654.

1430 Winberg S, Nilsson GE (1993) Time course of changes in brain serotonergic activity and
1431 brain tryptophan levels in dominant and subordinate juvenile Arctic charr. *J. Exp.*
1432 *Biol.*, **179**, 181-195.

1433 Winberg S, Øverli Ø, Lepage O (2001) Suppression of aggression in rainbow trout
1434 (*Oncorhynchus mykiss*) by dietary L-tryptophan. *J. Exp. Biol.*, **204**, 3867-3876.

1435 Winberg S, Winberg Y, Fernald RD (1997) Effect of social rank on brain
1436 monoaminergic activity in a cichlid fish. *Brain. Behav. Evol.*, **49**, 230-236.

1437 Wolkers CPB, Serra M, Hoshiba MA, Urbinati EC (2012) Dietary L-tryptophan alters
1438 aggression in juvenile matrinxá *Brycon amazonicus*. *Fish Physiol. Biochem.*, **38**,
1439 819-827.

1440 Wright N, Preslock JP (1975) Melatonin Inhibition of Pineal Enzymes in Coturnix Quail.
1441 *Neuroendocrinology*, **19**, 177-184.

1442 Wu G (2009) Amino acids: metabolism, functions, and nutrition. *Amino Acids*, **37**, 1-17.

1443 Wu G (2013) Functional amino acids in nutrition and health. *Amino Acids*, **45**, 407-411.

1444 Wu H, Aoki A, Arimoto T, Nakano T, Ohnuki H, Murata M, Ren H, Endo H (2015)
1445 Fish stress become visible: A new attempt to use biosensor for real-time
1446 monitoring fish stress. *Biosens. Bioelectron.*, **67**, 503-510.

1447 Xu F, Li J, Ma K, Wang M (1996) Effects of melatonin on hypothalamic γ -
1448 Aminobutyric acid, aspartic acid, glutamic acid, β -Endorphin and serotonin levels
1449 in male mice. *Neurosignals*, **4**, 225-231.

1450 Yambe H, Kitamura S, Kamio M, Yamada M, Matsunaga S, Fusetani N, Yamazaki F
1451 (2006) L-Kynurenine, an amino acid identified as a sex pheromone in the urine
1452 of ovulated female masu salmon. In: *Proceedings of the National Academy of*
1453 *Sciences*, pp. 15370-15374.

1454 Yanez J, Meissl H (1995) Secretion of Methoxyindoles from Trout Pineal Organs in-
1455 Vitro - Indication for a Paracrine Melatonin Feedback. *Neurochem Int*, **27**, 195-
1456 200.

1457 Young M, Matthews J (1995) Serotonin regulation of T-cell subpopulations and of
1458 macrophage accessory function. *Immunology*, **84**, 148.

1459 Yuasa HJ, Mizuno K, Ball HJ (2015) Low efficiency IDO2 enzymes are conserved in
1460 lower vertebrates, whereas higher efficiency IDO1 enzymes are dispensable. *FEBS*
1461 *journal*, **282**, 2735-2745.

1462 Yuasa HJ, Takubo M, Takahashi A, Hasegawa T, Noma H, Suzuki T (2007) Evolution
1463 of vertebrate indoleamine 2,3-dioxygenases. *J Mol Evol*, **65**, 705-714.

1464 Yui R, Nagata Y, Fujita T (1988) Immunocytochemical Studies on the Islet and the Gut
1465 of the Arctic Lamprey, *Lampetra Japonica*. *Arch Histol Cytol*, **51**, 109-119.

1466 Zaminhan M, Boscolo WR, Neu DH, Feiden A, Furuya VRB, MassamituFuruya W
1467 (2017) Dietary tryptophan requirements of juvenile Nile tilapia fed corn-soybean
1468 meal-based diets. *Animal feed science and technology*.

1469

1470

1471 Tables

1472 Table 1: Dietary tryptophan requirement (per cent of dietary protein level) in different
1473 teleosts

Species	Requirement	Protein source ¹	Reference
<i>Catla catla</i>	0.95	AA	(Ravi & Devaraj, 1991)
<i>Ictalurus punctatus</i>	0.50	CS, GL, AA	(Wilson <i>et al.</i> , 1978)
<i>Clarias gariepinus</i>	1.10	CS, GL, AA	(Fagbenro & Nwanna, 1999)
<i>Heteropneustes fossilis</i>	0.80	CS, GL, AA	(Ahmed, 2012)
<i>Lates calcarifer</i>	0.41	FM, SM, GL, AA	(Coloso <i>et al.</i> , 2004)
<i>Oncorhynchus mykiss</i>	0.45	FM, GL, AA	(Walton <i>et al.</i> , 1984)
	0.40	FM, GL	(Walton <i>et al.</i> , 1986)
	0.57-0.71	CS, GL, AA	(Kim <i>et al.</i> , 1987)
<i>Oncorhynchus tshawytscha</i>	0.50	UN	(Wilson, 2002)
<i>Oncorhynchus kisutch</i>	0.50	UN	(Wilson, 2002)
<i>Oncorhynchus keta</i>	0.70	CS, AA	(Akiyama <i>et al.</i> , 1985)
<i>Oncorhynchus nerka</i>	0.50	UN	(Wilson, 2002)
<i>Cyprinus carpio</i>	0.30	ZN, AA	(Dabrowski, 1981)
	1.10	CS, GL, AA	(Tang <i>et al.</i> , 2013)
<i>Chanos chanos</i>	0.60	FM, GL, AA	(Coloso <i>et al.</i> , 1992)
<i>Morone chrysops</i> × <i>M. saxatilis</i>	0.60-0.70	FM, AA	(Gaylord <i>et al.</i> , 2005)
<i>Oreochromis niloticus</i>	1.00	CS, GL, AA	(Santiago & Lovell, 1988)
	1.03	PD, AA	(Zaminhan <i>et al.</i> , 2017)
<i>Labeo rohita</i>	0.90-1.13	CS, GL, AA	(Fatma Abidi & Khan, 2010; Murthy & Varghese, 1997)
<i>Cirrhinus mrigala</i>	0.95-1.20	CS, GL, AA	(Ahmed & Khan, 2005; Benakappa & Varghese, 2003)
<i>Ctenopharyngodon idella</i>	1.27-1.30	FM, CS, GL, AA	(Wen <i>et al.</i> , 2014)
<i>Sciaenops ocellatus</i>	0.80	DMM, AA	(Pewitt <i>et al.</i> , 2016)

1474 ¹ AA: amino acid mixture; CS: casein; GL: gelatine; FM: fishmeal; SM: squid meal;
1475 ZN: zein; PD: practical diet containing corn meal, soybean meal, poultry by-product,
1476 corn gluten; DMM: red drum muscle meal; UN: unknown.

A **B**

1479 Fig. 1. General L-tryptophan metabolic routes (besides its role as a component for
 1480 protein synthesis) in vertebrates. A) Serotonin and melatonin biosynthetic pathways from

1481 L-tryptophan and B) Main catabolic route for L-tryptophan (kynurenine pathway)
 1482 (Badawy, 2017; Falcón *et al.*, 2010; Fernstrom, 2016; Macchiarulo *et al.*, 2009). In fish,
 1483 the routes leading to the production of serotonin and melatonin are well established and
 1484 known to be similar to those of other vertebrates. However, further research is still
 1485 needed regarding the functionality of the kynurenin-niacin pathway, especially in what
 1486 concerns the branch leading to the production of niacin (separated by a red line and
 1487 arrow in the picture), which is well established in mammals but has not been confirmed
 1488 in fish as yet (see the main text for further details). *TpOH*: tryptophan hydroxylase;
 1489 *AAAD*: aromatic amino acid decarboxylase; *AANAT*: aryl-alkylamine-N-acetyltransferase;
 1490 *HIOMT*: hydroxyindole-*O*-methyltransferase; *TDO*: tryptophan 2,3-dioxygenase; *IDO*:
 1491 indoleamine 2,3-dioxygenase; *AF*: arylformamidase; *KAT*: kynurenine aminotransferase;
 1492 *KMO*: kynurenine 3-monooxygenase; *3-HAAO*: 3-hydroxyanthranilic acid 3,4-dioxygenase.
 1493

Fig. 2: Factors affecting teleosts' dietary tryptophan requirement