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Research Article

Molecular characterization and expression analysis of the IFN-gamma and NOD1 gene in tropical gar *Atractosteus tropicus*

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ABSTRACT. The tropical gar (*Atractosteus tropicus*), a freshwater species mostly found in swamps, has been used for human consumption for several generations. In this study, the expression of interferon-gamma (*ifn-γ*) and domain nucleotide-binding oligomerization (*nod1*) involved in the immune system was evaluated in different tissues such as the brain, heart, spleen, liver, pancreas, kidney, intestine, and gonad in wild and captive adult organisms and during early ontogeny. Specific quantitative polymerase chain reaction (qPCR) primers were used to compare the expression between tissues and during larviculture, starting with embryos (0 days after hatching, [DAH]) and at 5, 10, 15, 20, 25, and 30 DAH. The intestine, pancreas, and liver showed maximum *ifn-γ* and *nod1* expression. Additionally, the expression of genes *ifn-γ* and *nod1* was detected in embryos (0 DAH), decreasing between 5 and 10, then increasing at 25 and 30 DAH. Based on these results, it can be concluded that genes encoding *ifn-γ* and *nod1* are expressed differentially across tissues of tropical gar adults and are regulated during larval development.

Keywords: *Atractosteus tropicus*; *ifn-γ*; *nod1*; adults; larvae

INTRODUCTION

The tropical gar (*Atractosteus tropicus*) is a carnivorous freshwater fish native to southeast Mexico, of great commercial importance and considered ideal for sustainable aquaculture due to its rapid growth and its adaptation to the consumption of balanced feed, in addition to surviving low oxygen dissolved in the environment water (Jiménez-Martínez et al. 2019). Due to this, the scientific production generated for this species has increased considerably being focused on the areas of larviculture, reproduction, physiology, nutrition and molecular biology (López et al. 2005, Márquez-Couturier et al. 2006, Jésus-Contreras 2008, Huerta et al. 2009, Frías-Quintana et al. 2010, 2016, 2017, Jiménez-Martínez et al. 2019, 2020, 2021, Martínez-Burguete et al. 2021, 2022, 2024, Pérez-Jiménez et al. 2022, Arellano-Carrasco et al. 2023).

In this regard, one of the less explored aspects of this species is the functioning of the immune system

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(Mokhtar et al. 2023). In this way, fish's immune system is similar to other vertebrates, divided into innate and adaptive (Harris & Bird 2000). Innate immunity is the first line of immune defense and plays a key role in disease resistance. It consists mainly of barriers such as mucosal surfaces and the skin with a wide range of associated substances that prevent harmful materials from entering the body (Secombes & Wang 2012). While acquired immunity is the second line of defense divided into cellular and humoral and depends largely on T and B lymphocytes, respectively, it is considered a key factor for the success of vaccination (Penagos et al. 2009).

Within the innate, natural, or non-specific defense system, we find two important genes: interferon-gamma (*ifn-γ*) is a cytokine capable of modulating innate and adaptive immune responses in teleost fish, participating in multiple processes and at different points of activation of the immune system and therefore is essential in response to pathogens, and the gene that encodes protein 1 that contains the domain nucleotide-binding oligomerization (*nod1*) where it identifies bacterial molecules and stimulates an immune reaction, it is widely expressed in a variety of cell types, such as epithelial cells, stromal cells and endothelial cells where it induces chemokine production and recruitment of acute inflammatory cells *in vivo* (Pereiro et al. 2019, Yin et al. 2021).

In this study, our objective was the cloning, characterization, and expression analysis of the *ifn-γ* and *nod1* genes in *A. tropicus* organisms to observe the variation of these genes from initial ontogeny and wild and captive adults for their subsequent application in digestive physiology and digestive nutrition assays that can be used as markers of the health status of the organisms as a profitable aquaculture strategy.

MATERIALS AND METHODS

Fish acquisition

In carrying out this research, a total of 20 *A. tropicus* organisms were obtained, 10 wild individuals (400-520 g and 32 to 34 cm in total length), which were captured in the El Horizonte lagoon in the community of Espino, 32 km from the city of Villahermosa, Tabasco, Mexico, with geographical coordinates 18º14'50"N and 92º49'58"W and 10 captive individuals (550-580 g and 30 to 35 cm in total length), from the facilities of the Aquatic Resources Physiology Laboratory of DACBiol-UJAT. The captive organisms were cultured in polyethylene tanks of 1.94 m in diameter by 0.70 m in height and fed with a diet for rainbow trout *Oncorhynchus mykiss* (45% protein and 16% fat, El Pedregal®, Toluca, Mexico) with particle diameters that ranged between 5.5 and 9.0 mm).

Four hundred and fifty *A. tropicus* embryos were acquired from an induced broodstock (one 3.5 kg female and three males with an average weight of 1.5 kg) using LHRHa induction (35 μg $fish^{-1}$) and were maintained in a 2,000 L tank. After absorption of the yolk sac (two days after hatching, DAH), larvae were fed five times a day (at 08:00, 11:00, 13:00, 15:00, and 18:00 h) starting with *Artemia* nauplii (AN, 2-5 nauplii mL^{-1}) when opening the mouth until 17 DAH. Trout feed (TD, Silver Cup® 46% proteins and 16% lipids) was provided until 31 DAH. In this sense, the size of the food particles was adjusted according to the larvae growth (from 250 to 500, 500-750, and >750 μm). During larviculture, sampling was carried out on different DAH: at 0 (embryos), 5, and 10 DAH with 48 organisms (16 per tank), and 15, 20, 25, and 30 DAH (10 per tank) in triplicate. Larvae were killed by cold water shock (-4°C) and collected before the first feeding, and each replicate was rinsed with distilled water, transferred to a 1.5 mL tube containing RNAlater (Life Technologies, Carlsbad, CA, USA), and stored at -80°C until analysis.

RNA extraction and cDNA synthesis

Adult individuals of *A. tropicus*, both wild and captive, were sacrificed by the heat shock method (-4ºC) following the steps of the methodology of Matthews & Vargas (2012) to obtain the tissues: brain, heart, spleen, liver, pancreas, kidney, intestine and ovary in the case of females and testicle in the case of males. Larvae were sampled on different DAH (10 larvae per tank) before the first feeding, starting from the embryo (considered as 0 DAH) and 5, 10, 15, 20, 25, and 30 DAH. The larvae were removed from each tank and rinsed for subsequent analysis. RNA extraction was performed from a tissue pool and 10 larvae per replicate using the TRIzol technique (Río et al. 2010, El-Ashram et al. 2016). The product obtained was rehydrated in ultrapure distilled water and was subsequently quantified at 260/280 to identify the purity and concentration of the samples. cDNA synthesis was performed using one microgram of RNA and random primer using the iScriptTM Select kit 170-8896 (Bio-Rad, Hercules, California, USA) following the manufacturer's instructions.

Partial cDNA isolation and characterization in PCR endpoint

To perform the endpoint PCR, complementary DNA from the liver tissue, since it plays an important role in

all metabolic processes, was chosen using Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) with the following reaction mixture: 2 µL of cDNA, 2 µL of 10x Buffer, 1 µL of 50 mmol L^{-1} MgCl2, 2 µL of 10 mmol L^{-1} dNTPs, 0.2 µL of each oligonucleotide previously determined from the alignment (using Clustal-W software, Infobiogen) of the corresponding sequences available in the library of different species of fish (Table 1). This process was carried out under the following conditions: 10 min at 95°C, followed by 40 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 50 s with an extension of 5 min at 75°C in a thermocycler, for the endpoint of 96 wells.

The amplification products were analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide using a 1,000 bp molecular weight marker (Promega, Madison, WI, USA) and observed with translucent UV (UVP, Canada). The single product was cut from the gel and purified using a PureLink® PCR Purification Kit (Invitrogen, Carlsbad, CA, USA). The purified product was sequenced at the Synthesis and Sequencing Unit of the Biotechnology Institute of the National Autonomous University of Mexico. The liver was chosen as the reference organ for this process and sent for sequencing. Sequences were submitted to GenBank (*nod1* accession number: PQ156528 and *ifn-γ* accession number: PQ156529). The partial sequences obtained were edited and analyzed using ExPASy translation software to search for the open reading frame (ORF). Then, they were translated into AA sequences using standard genetic codes. Nucleotide sequences were compared to DNA sequences in NCBI's GenBank database network service (https://blast.ncb i.nlm.nih.gov/). The phylogenetic tree was generated using neighbor-joining (NJ) methods based on the AA sequence using MEGA 7.0 software.

Real-time PCR

RNA isolation and reverse transcription in *A. tropicus* adults were performed in the brain, heart, spleen, liver, pancreas, kidney, intestine, and ovary in the case of females and testis in the case of males. The resulting cDNA from adult and larval tissues was diluted in 200 µL of distilled water. The quantitative polymerase chain reaction (qPCR) was performed on a 96-well CFX96 Real-Time System Thermal Cycle Thermocycler (Model C1000, CA). The reaction mixture included 10 µL of Eva Green, 2 µL of cDNA, and 0.2 µM of each primer. The specific primers used in this analysis are provided in Table 1. Each gene of interest was analyzed using synthetic products obtained from intestine dissection in triplicate. The real-time PCR procedure was carried out with the oligonucleotides designed for the IFN-gamma and NOD1 transcripts under the conditions of 2 min at 95°C followed by 38 cycles at 95°C for 10 s, 60°C for 30 s and extension at 70°C for 5 s (Mullis et al. 1986).

The Cq value, the expression level of repeated fluorescence units, was captured from each run to detect each transcript. Each reaction was normalized by amplifying elongation factor 1alpha (*ef1*) as a housekeeping gene to determine partial expression (Pfaffl 2001).

Statistical analysis

The relative expression of *nod1* and *ifn-γ* between different adult tissues and different DAH of larvae was analyzed using the Kruskal-Wallis test. *Post-hoc* Nemenyi tests were performed to determine significant differences between activities based on age (significance value of $P < 0.05$). All statistical analyses were performed using STATISTICATM software v.7.0 (Statsoft, Tulsa, OK).

RESULTS

PCR amplification and sequencing analysis

PCR amplification using primers was visualized on an agarose gel (Fig. 1), where partial sequences were produced for *ifn-γ* 762 nucleotides encoding 254 amino acids (Fig. 2) and *nod1* with a size of 348 nucleotides encoding 116 amino acids (Fig. 3). The AA sequence of *ifn-γ* in *A. tropicus*, according to Bootstrap analysis, establishes a value of 98% and for *nod1*, a value of 100% with *Lepisosteus oculatus* compared to the other species of fish and mammals (Fig. 4).

Relative expression in adult fish tissues

The assay performed showed an expression of *ifn-γ* in all organs. However, the proportion of the transcript was different in all tissues (Fig. 5). In the case of captive females, the highest expression of *ifn-γ* was detected in the ovary, followed by the pancreas and intestine. In contrast, low expressions were detected in the liver, heart, brain, kidney, and spleen. In wild females, the liver had the highest expression, followed by the intestine, pancreas, heart, kidney, ovary, brain, and spleen. For captive males, the highest expression of *ifn-γ* was detected in the intestine, followed by the liver and pancreas, while the lowest expressions were present in the heart, followed by the brain, kidney, testicle, and spleen. In wild males, the highest expres-

Table 1. Oligonucleotides used for sequencing and real-time polymerase chain reaction in *Atractosteus tropicus*.

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Size, pb	Step
ifn-γ	gatttgaaacacgttttaacgctg	tgaatatgatttggaacctccca	809	RT-PCR
	caacttcacgtctgccatca	gcacgttttccagggatgag	162	qPCR
nod1	ccctgaccaacgtgttcatgctc	tttctatattcgcattcagtaaa	394	RT-PCR
	cgatactttcaggaccggga	aaggttgacaggttcccact	189	qPCR
ef1	cctgcaggacgtctacaagatcg	gacctcagtggtcacgttgga	120	qPCR

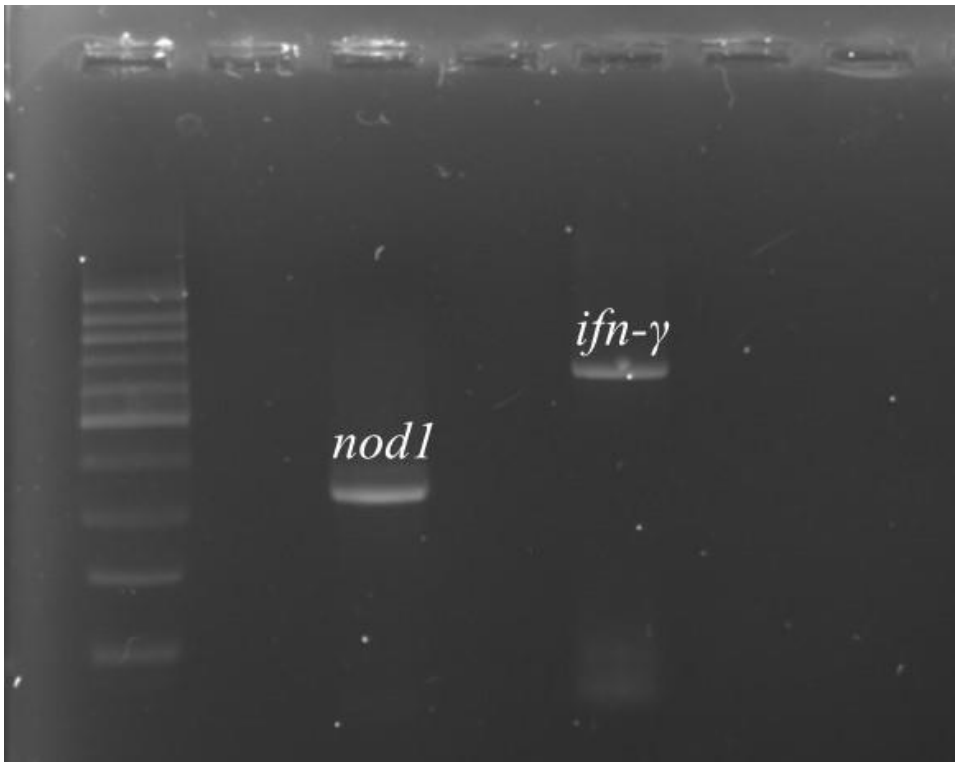

Figure 1. Agarose electrophoresis showing the fragments amplified *nod1* and *ifn-γ* from *Atractosteus tropicus* by PCR for sequencing.

sion was in the liver and intestine, and the lowest was in the kidney, testicle, spleen, brain, pancreas, and heart. In males, significant expression was found only in the intestine of the captive species; in contrast, in females, the significance was found in the captive species' pancreas, intestine, and ovary ($P < 0.05$).

As with the gamma interferon assay, *nod1* expression was quantified in the captive and wild species, separating them by sex (Fig. 6). In captive females, the highest *nod1* expression was observed in the liver, followed by the intestine, pancreas, and ovary. At the same time, the lowest levels were found in the heart, brain, spleen, and kidney. In wild females, the highest expression was detected in the liver, and the lowest levels were found similarly in the intestine, ovary, kidney, pancreas, spleen, heart, and brain. In captive males, the highest *nod1* expression was found in the intestine, followed by the pancreas and liver, while the lowest levels were found in the spleen, testicle, heart, kidney, and brain. In wild males, the highest *nod1* expression was found in the liver, followed by the intestine and pancreas, while the lowest levels were found in the kidney, testicle, spleen, heart, and brain. Expression in organs was significant only in the intestine of the captive male species ($P < 0.05$). In the case of females, no significance was found.

Figure 2. Partial sequence of nucleotides and amino acids (AA) encoding *ifn-γ* from *Atractosteus tropicus* taken from Gen Bank to design specific oligonucleotides for qPCR.

Relative expression in larvae

In the final assay, the expression of *ifn-γ* and *nod1* during the initial ontogeny in *A. tropicus* was quantified. It was first detected in the embryo (0 DAH); subsequently, the expression decreases, increasing on day 20, presenting its maximum expression on day 25, and decreasing on day 30, showing significant differences between the days of the initial ontogeny ($P < 0.05$) (Figs. 7-8).

DISCUSSION

Characterization and expression of *ifn-γ* and *nod1* in *A. tropicus* tissues

In the present work, the expression levels of two genes involved in inflammatory processes, *ifn-γ*, and *nod1*, were identified and quantified in the freshwater species *A. tropicus*. Like our work, various studies have been reported on the characterization and differential expres-

PQ156529.1 *Atractosteus tropicus nod1* mRNA, partial cds

atg gcg gag gtg ttc ctc agt cgc ccc tcc agc ccc agc ttg ctg aag aaa agc agc agg 60

M A E V F L S R P S S P S L L K K S S R 20

tgt caa gc**c gat act ttc agg acc ggg a**gg gag act ctg atg gct ttc ggc aag ctg gcc 120

>>>>>>>>>>>>>>>>>>>>>

C Q A D T F R T G R E T L M A F G K L A 40

aat ctt ggc atg gag aaa act ggc ttc atg ttc aat cac gat gag gtg tcg tcc tgc ggc 180

N L G M E K T G F M F N H D E V S S C G 60

ctg acg gag aag gag ctg cag ctg gga ttt ctg agg cct gcc agt cac tac gat ggc **agt** 240

<<<

L T E K E L Q L G F L R P A S H Y D G S 80

ggg aac ctg tca acc ttt gag ttt ctt cat gtc acc ctt cag tct ttt ttt gca gct ttt 300

<<<<<<<<<<<<<<<<<<<

G N L S T F E F L H V T L Q S F F A A F 100

ttg ctg gtg cag gat gaa aac ata ggc tct gtg ggt att ctg aaa ttc 348

L L V Q D E N I G S V G I L K F 116

Figure 3. Partial sequence of nucleotides and amino acids (AA) encoding *nod1* from *Atractosteus tropicus* taken from Gen Bank to design specific oligonucleotides for qPCR.

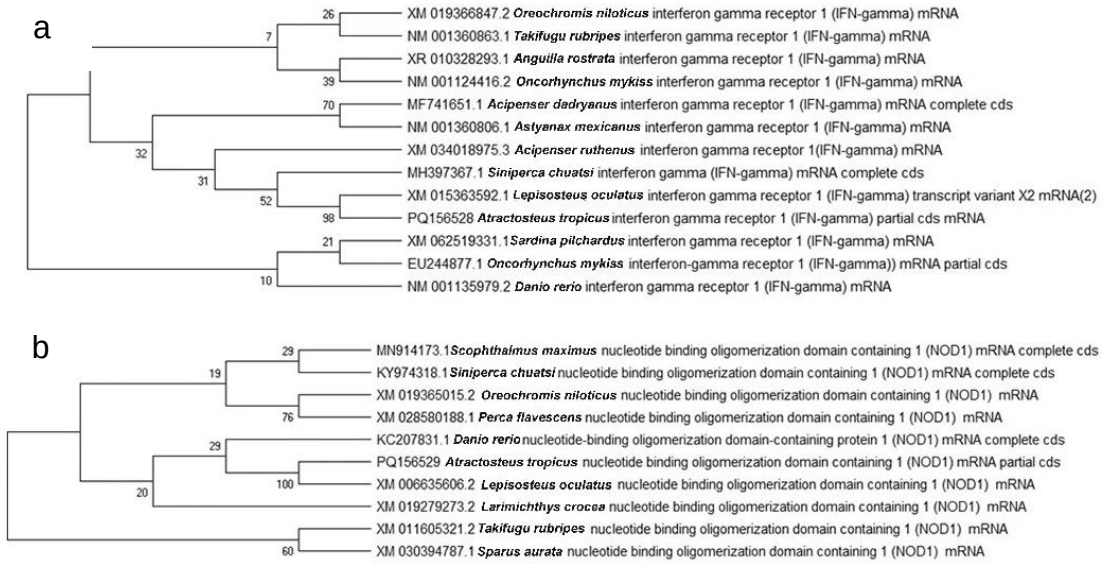

Figure 4. Phylogenetic tree based on the sequence of a) *ifn-γ* and b) *nod1* and from *Atractosteus tropicus* and other fi using the neighbor-joining (NJ) method. Values at branch points represent percentage frequencies for tree topology after 1,000 iterations.

sion of these genes in various fish species, in the case of *ifn-γ*, highlighting species such as *Danio rerio* (Yoon et al. 2016), *O. mykiss* (Hu et al. 2021), *Takifugu rubripes* (Zou et al. 2004), *Tetraodon nigroviridis* (Igawa et al. 2006), *Salmo salar* (Robertsen 2006), and *Cyprinus carpio* (Stolte et al. 2008). The *nod1* has been identified in *Siniperca chuatsi* (Gu et al. 2018), *Cirrhinus mrigala* (Swain et al. 2013a), *Miichthys miiuy* (Bi et al. 2017), *Catla catla* (Swain et al. 2013b), *Ictalurus punctatus* (Sha et al. 2009), among others.

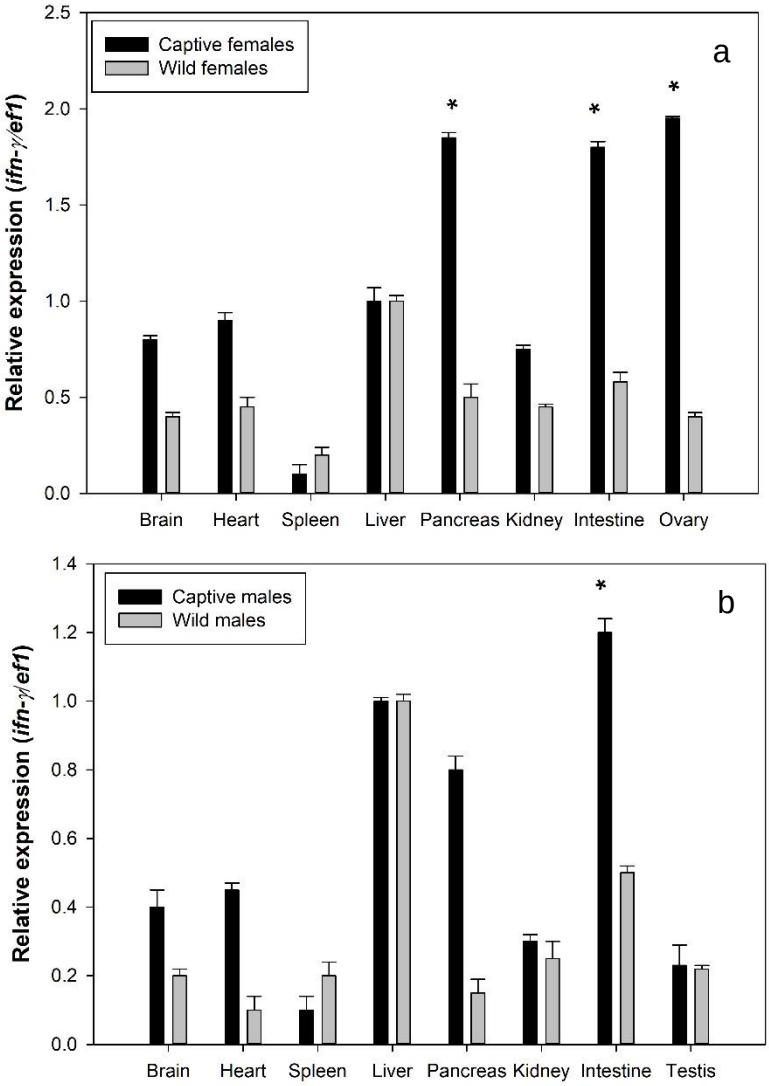

Figure 5. Relative expression of *ifn-γ* in different tissues of wild and captive a) female and b) male adults of *Atractosteus tropicus* (mean ± standard error of the mean; n = 3). *Indicate significant differences between the tissue expression levels ($P < 0.05$).

According to our results, *ifn-γ* was expressed in all the tissues analyzed, showing high levels of expression in the liver, pancreas, intestine, and ovaries in the case of females, mainly in captive organisms. Probably the expression of *ifn-γ* in these tissues shows an immune response and the detection of possible inflammatory diseases as reported in fish and mammals (Toyonaga et al. 1994, Sun et al. 2011, Qi et al. 2013). Similarly, the liver is considered a control center of the immune system. The expression of *ifn-γ* in this tissue may be indicative of an infection such as *Nocardia seriousolae* reported in black bass (*Micropterus salmoides*) (Danese & Gasbarrini 2005, León et al. 2006, Dios et al. 2010, Li et al. 2018, Poggi et al. 2019, Sánchez-Velázquez et al. 2024, Yu et al. 2024). It is important to mention that there is much similarity in the exons and introns of *ifn-γ* in teleosts and mammals, so it is important to relate that the high expression of *ifn-γ* in mammals is indicative of fatty liver, hepatitis, and cellular responses in macrophages, T lymphocytes, and cancer cells (Chavez-Pozo et al. 2010, Oehlers et al. 2011, Askari et al. 2012, Chettri et al. 2012, Coskun et al. 2013, Heinecke et al. 2014, Salinas 2015, Klosterhoff et al. 2015, Mukherjee et al. 2019, Salas et al. 2020, Mokhtar et al. 2023, Sayyaf-Dezfuli et al. 2023, Buchmann et al. 2024). Likewise, the expression of *ifn-γ* in the pancreas shown in our study in *A. tropicus* may be because this gene plays a role in protecting cells

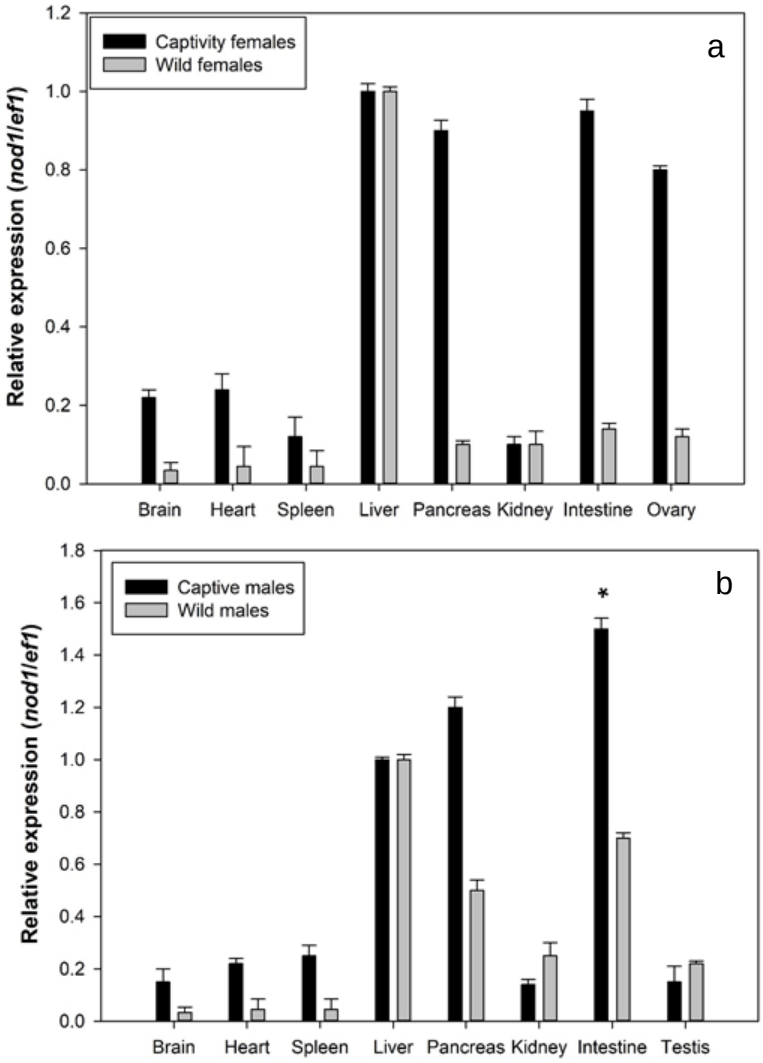

Figure 6. Relative expression of *nod1* in different tissues of wild and captive a) female and b) male adults of *Atractosteus tropicus* (mean ± standard error of the mean; n = 3). *Indicate significant differences between the tissue expression levels ($P < 0.05$).

from the infectious pancreatic necrosis virus as reported in fish such as *Ctenopharyngodon idella*, *Siniperca chuatsi*, and *O. mykiss* (Che et al. 2010, Li et al. 2019, Hu et al. 2021). While the expression of *ifn-γ* in the intestine in *A. tropicus* can tell us about the function that it presents in the intestinal immune system by helping to defend against bacterial infections within the intestinal tract, there are also studies where they mention that the high expression of this gene occurs when the fish is exposed to pathogens, which allows an immune response (Zou et al. 2005, Mulder et al. 2007). In addition, the intestine is considered a key part of the immune system and contains 80% of the body's defenses (Dawood 2021). On the other hand, it has been reported that *ifn-γ* is produced by CD4+ and CD8+ T lymphocytes, Tγ/δ cells, and NK cells in response to some immune or inflammatory stimulus (He et al. 2023). The *ifn-γ* regulates the expression of cytokines related to Th1-type immunity, including *il-1β*, *il-6*, *il-12*, and *tnf-α* involved in immunomodulatory, antiviral, and antiproliferative activities (Hu et al. 2021). The *ifn-γ* gene is produced largely under certain pathological circumstances such as chronic inflammation, infection, and autoimmunity (Yoon et al. 2016).

In the case of *nod1* gene expression, the expression in both wild and captive females and males of *A. tropicus* was detected in all tissues. Likewise, the highest expression levels were detected in the liver, pancreas, and intestine, showing the same behavior as *ifn-γ*, with a large difference in expression in captive

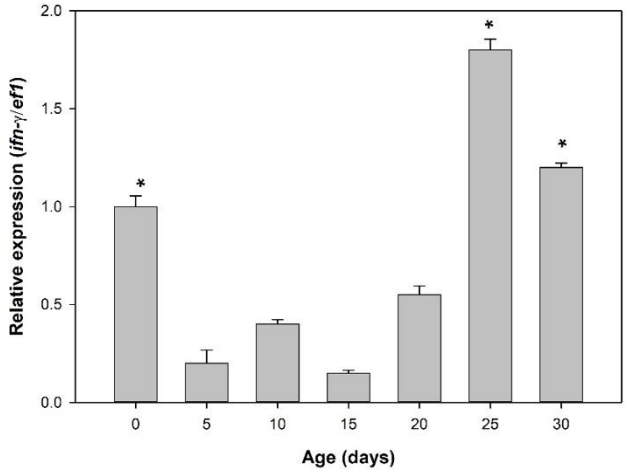

Figure 7. Relative expression of *ifn-γ* during the early ontogeny of *Atractosteus tropicus*. *Indicates significant differences in function of developmental time ($P < 0.05$).

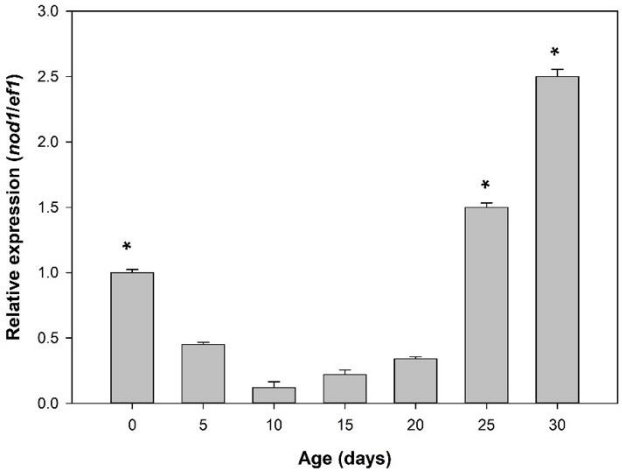

Figure 8. Relative expression of *nod1* during the early ontogeny of *Atractosteus tropicus.* *Indicates significant differences in function of developmental time ($P < 0.05$).

organisms. The high expression of this gene in the liver shown in *A. tropicus* agrees with what was found in species such as *Miichthys miiuy* and *Siniperca chuatsi* where it is mentioned that *nod1* is an important key modulator of immunometabolic processes, mainly in the liver, where multiple processes take place (Li et al. 2015, Bi et al. 2017, Gu et al. 2018). Likewise, the expression of *nod1* in the intestine is indicative of the immunological and metabolic adaptations of the gastrointestinal system, as mentioned in *O. niloticus*, where this gene is highly expressed in epithelial cells or macrophages associated with the intestine (Nayak 2010, Swain et al. 2013a, Gao et al. 2018). In the case of the pancreas, the expression of *nod1* in the case of teleost fish is mentioned that it functions as a receptor for microbial signals within the pancreatic islets, which helps in the efficient secretion of glucose-stimulated insulin (Watanabe et al. 2017, Bi et al. 2018, Qiu et al. 2022, Xia et al. 2023).

Likewise, the *ifn-γ* and *nod1* genes participate in response to viral and bacterial infections such as Gram-positive *Streptococcus*, *Lactococcus*, *Enterococcus*, and *Vagococcus*, causing high mortality in aquaculture crops; this is due to effects on the immune system due to factors such as poor water quality, stress, and nutritional deficiencies (Shtrichman & Samuel 2001, Hou et al. 2012, Park et al. 2012, Qi et al. 2013, Swain et al. 2013a, Bhat et al. 2018, Paria et al. 2018, Dawood 2021, Hu et al. 2021). Taking into account the point of nutritional deficiencies, in our study, the diet used for *A. tropicus* captive organisms is a commercial trout feed that has 16% lipids, a very high percentage for this tropical species causing a possible effect on the immune system, due to this the nutrition of the fish must be optimized including the appropriate nutrients, vitamins, minerals and the addition of probiotics that improve the immune system (Penagos et al. 2009, Triana-García et al. 2013, Jiménez-Martinez et al. 2019).

Expression of larvae of *ifn-γ* and *nod1* in *A. tropicus*

According to the expression of *ifn-γ* and *nod1* during larval development in *A. tropicus*, these genes were detected from day 0 DAH; this coincides with what has been reported in species such as *Danio rerio*, Atlantic cod (*Gadus morhua*), tilapia *O. niloticus* and *Ctenopharyngodon idella* where they mention that this expression is due to the presence of maternal mRNA transmitted by the mother that encodes protective factors present at the egg stage that drive the development of the embryo until the zygote can transcribe its RNA. The developing larva can express immune genes shortly after fertilization (Dios et al. 2010, Kleppe et al. 2012, Gao et al. 2018, Buchmann & Secombes 2022, Mahapatra et al. 2024). The highest gene expression levels were presented in the last days of larval development. In the case of *ifn-γ* it occurred on day 25. For *nod1*, it occurred on day 30 DAH, which may be due to two factors: the first is that these days, the species *A. tropicus* is considered a juvenile with all its developed organs involved in the expression of *ifn-γ* and *nod1* (Frías-Quintana et al. 2017, Jiménez-Martínez et al. 2019). According to the above, in teleost fish, the most important role in the modulation of inflammation is played by the lymphoid tissues associated with mucosa in the intestine, skin, gills,

kidney, and spleen (De Filippo et al. 2010, Swain et al. 2013b, Salinas 2015, Prabu et al. 2016, Costantini et al. 2017, Herrera et al. 2019, Parada-Venegas et al. 2019, Akhtar et al. 2021).

The second point is probably the effect of the diet administered to the adult organisms of *A. tropicus* in captivity. The percentage of lipids is very high, mainly for the larval stage, which can also cause degenerative lesions in various organs, such as the liver, presenting hepatic steatosis and influencing the immune response. These can cause autoimmune diseases (Shtrichman & Samuel 2001, Penagos et al. 2009, Chaves-Pozo et al. 2010, Triana-García et al. 2013, Bhat et al. 2018).

Based on our results, it can be concluded that *ifn-γ* and *nod1* are differentially expressed in all the tissues analyzed, mainly in the intestine, pancreas, and liver, showing the highest expression in captive organisms and in the larval stage they are expressed in the last days of development 25 and 30 DAH indicating that the organisms present some alteration in their immune system due to the culture conditions, mainly due to the effect of the diet used.

This study is a starting point for future research on nutrition, digestive physiology, and toxicology at different stages of the life cycle of *A. tropicus*, evaluating the expression of these genes to obtain fish with strong and healthy immune systems for aquaculture purposes.

Credit author contribution

L.D. Jiménez-Martínez: conceptualization, investigation, methodology, project administration, resources, supervision, validation, visualization, writing-original draft, writing-review, and editing; G. Asencio-Alcudia: data curation, formal analysis, methodology, supervision, validation, writing-original draft, writing-review, and editing; C.A. Álvarez-González: conceptualization, investigation, methodology, project administration, resources, supervision, validation, visualization, writing-original draft, writing-review and editing; A. Castillo-Collado: data curation, formal analysis, investigation, methodology, supervision, validation, visualization, writing-original draft, writing-review and editing; V. Morales-García: advice and data analysis, investigation, methodology, validation, writing-original draft; C.S. Alvarez-Villagomez: conceptualization, formal analysis, investigation, supervision, validation, writing-original draft, writing-review, and editing; C. Rodríguez-Pérez: data curation, formal analysis, methodology, supervision, validation, writing-original draft, writing-review, and editing; C. Sepúlveda-Quiroz: formal analysis, investigation, methodology, supervision, validation, visualization, writing-original draft, writing-review and editing; R. Martínez-García: writing, reviewing and editing draft and final document; G. Pérez Jiménez: data curation, formal analysis, methodology, supervision, validation, writing-original draft, writing-review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

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REFERENCES

Akhtar, M., Chen, Y., Ma, Z., et al. 2021. Gut microbiota-derived short-chain fatty acids are potential mediators in gut inflammation. Animal Nutrition, 8: 350-360. doi: 10.1016/j.aninu.2021.11.005

Arellano-Carrasco, J.G., Martínez-García, R., Asiain-Hoyos, A., et al. 2023. Effects of dietary sodium propionate on growth, digestive enzyme activity, and expression of immune system genes in juveniles of tropical gar (*Atractosteus tropicus*). Aquaculture Journal, 3: 227-237. doi: 10.3390/aquacj3040018

Askari, N., Correa, R.G., Zhai, D., et al. 2012. Expression, purification, and characterization of recombinant NOD1 (NLRC1): A NLR family member. Journal of Biotechnology, 157: 75-81. doi: 10.1016/j.jbiotec.2011.10.007

Bhat, M.Y., Solanki, H.S., Advani, J., et al. 2018. Comprehensive network map of interferon gamma signaling. Journal of Cell Communication and Signaling, 12: 745-751. doi: 10.1007/s12079-018-0486-y

Bi, D., Gao, Y., Chu, Q., et al. 2017. NOD1 is the innate immune receptor for iE-DAP and can activate NF-κB pathway in teleost fish. Developmental & Comparative Immunology, 76: 238-246. doi: 10.1016/j.dci.2017.06.0

Bi, D., Wang, Y., Gao, Y., et al. 2018. Recognition of lipopolysaccharide and activation of NF-κB by cytosolic sensor NOD1 in teleost fish. Frontiers in Immunology, 9: 1413. doi: 10.3389/fimmu.2018.01413

Buchmann, K. & Secombes, C. 2022. Principles of fish immunology. Springer, Cham, pp. 495-510.

Buchmann, K., Karami, A.M. & Duan, Y. 2024. The early ontogenetic development of immune cells and organs in teleosts. Fish & Shellfish Immunology, 146: 109371. doi: 10.1016/j.fsi.2024.109371

Chaves-Pozo, E., Zou, J., Secombes, C.J., et al. 2010. The rainbow trout (*Oncorhynchus mykiss*) interferon response in the ovary. Molecular Immunology, 47: 1757-1764. doi: 10.1016/j.molimm.2010.02.030

Chen, W.Q., Xu, Q.Q., Chang, M.X., et al. 2010. Molecular characterization and expression analysis of the IFN-gamma related gene (IFN-γrel) in grass carp *Ctenopharyngodon idella*. Veterinary Immunology and Immunopathology, 134: 199-207. doi: 10.1016/j.vetimm.2009.09.007

Chettri, J.K., Raida, M.K., Kania, P.W., et al. 2012. Differential immune response of rainbow trout (*Oncorhynchus mykiss*) at early developmental stages (larvae and fry) against the bacterial pathogen *Yersinia ruckeri*. Developmental & Comparative Immunology, 36: 463-474. doi: 10.1016/j.dci.2011.08.014

Coskun, M., Salem, M., Pedersen, J., et al. 2013. Involvement of JAK/STAT signaling in the pathogenesis of inflammatory bowel disease. Pharmacological Research, 76: 1-8. doi: 10.1016/j.phrs.2013.06.007

Costantini, L., Molinari, R., Farinon, B., et al. 2017. Impact of omega-3 fatty acids on the gut microbiota. International Journal of Molecular Sciences, 18: 2645. doi: 10.3390/ijms18122645

Danese, S. & Gasbarrini, A. 2005. Chemokines in inflammatory bowel disease. Journal of Clinical Pathology, 58: 1025-1027. doi: 10.1136/jcp.2005.030916

Dawood, M.A. 2021. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Reviews in Aquaculture, 13: 642-663. doi: 10.1111/raq.12492

De Filippo, C., Cavalieri, D., Di Paola, M., et al. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America, 107: 14691-14696. doi: 10.1073/pnas.1005963107

Dios, S., Romero, A., Chamorro, R., et al. 2010. Effect of the temperature during antiviral immune response ontogeny in teleosts. Fish & Shellfish Immunology, 29: 1019-1027. doi: 10.1016/j.fsi.2010.08.006

El-Ashram, S., Al Nasr, I. & Suo, X. 2016. Nucleic acid protocols: Extraction and optimization. Biotechnology Reports, 12: 33-39. doi: 10.1016/j.btre.2016.10.001

Frías-Quintana, C., Álvarez-González, C. & Márquez-Couturier, G. 2010. Diseño de microdietas para el cultivo de pejelagarto *Atractosteus tropicus*, Gill, 1863. Universidad y Ciencia, 26: 265-282.

Frías-Quintana, C., Álvarez-González, C., Tovar-Ramírez, D., et al. 2017. Use of potato starch in diets of tropical gar (*Atractosteus tropicus*, Gill, 1863) larvae. Fishes, 2: 3. doi: 10.3390/fishes2010003

Frías-Quintana, C.A., Domínguez-Lorenzo, J., Álvarez-González, C.A., et al. 2016. Using cornstarch in microparticulate diets for larvicultured tropical gar (*Atractosteus tropicus*). Fish Physiology and Biochemistry, 42: 517-528.

Gao, F.Y., Pang, J.C., Lu, M.X., et al. 2018. Molecular characterization, expression, and functional analysis of NOD1, NOD2, and NLRC3 in Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 73: 207-219. doi: 10.1016/j.fsi.2017.12.012

Gu, T., Lu, L., Wang, J., et al. 2018. The NOD1 and NOD2 in mandarin fish (*Siniperca chuatsi*): Molecular characterization, tissue distribution, and expression analysis. BMC Genetics, 19: 61. doi: 10.1186/s12863-018-0667-y

Harris, J. & Bird, D.J. 2000. Modulation of the fish immune system by hormones. Veterinary Immunology and Immunopathology, 77: 163-176. doi: 10.1016/s0165-2427(00)00235-x

He, Z., Tian, H., Xing, J., et al. 2023. Full-length transcriptome sequencing of lymphocytes respond to IFN-γ reveals a Th1-skewed immune response in flounder (*Paralichthys olivaceus*). Fish & Shellfish Immunology, 134: 108636. doi: 10.1016/j.fsi.2023.108636

Heinecke, R.D., Chettri, J.K. & Buchmann, K. 2014. Adaptive and innate immune molecules in developing rainbow trout, *Oncorhynchus mykiss* eggs and larvae: Expression of genes and occurrence of effector molecules. Fish & Shellfish Immunology, 38: 25-33. doi: 10.1016/j.fsi.2014.02.010

Herrera, M., Mancera, J.M. & Costas, B. 2019. The use of dietary additives in fish stress mitigation: Comparative endocrine and physiological responses. Frontiers in Endocrinology, 10: 447. doi: 10.3389/fendo.2019.00447

Hou, Q.H., Yi, S.B., Ding, X., et al. 2012. Differential expression analysis of nuclear oligomerization domain proteins NOD1 and NOD2 in orange-spotted grouper (*Epinephelus coioides*). Fish & Shellfish Immunology, 33: 1102-1111. doi: 10.1016/j.fsi.2012.08.015

Hu, Y., Alnabulsi, A., Alnabulsi, A., et al. 2021. Characterization and analysis of IFN-gamma producing cells in rainbow trout *Oncorhynchus mykiss*. Fish & Shellfish Immunology, 117: 328-338. doi: 10.1016/j.fsi.2021.07.022

Hu, X., Li, J., Fu, M., et al. 2021. The JAK/STAT signaling pathway: from bench to clinic. Signal

Transduction and Targeted Therapy, 6: 402. doi: 10.1038/s41392-021-00791-1

Huerta-Ortiz, M., González, C.A.Á., Couturier, G.M., et al. 2009. Sustitución total de aceite de pescado con aceite vegetal en larvas de pejelagarto *Atractosteus tropicus*. Kuxulkab', 28: 51-58.

Igawa, D., Sakai, M. & Savan, R. 2006. An unexpected discovery of two interferon gamma-like genes along with interleukin (IL)-22 and -26 from teleost: IL-22 and -26 genes have been described for the first time outside mammals. Molecular Immunology, 43: 999-1009.

Jésus-Contreras, R. 2008. Protein/energy ratio in juvenile alligator gar (*Atractosteus tropicus*) using semi-purified diets. Dissertation, Universidad Juárez Autónoma de Tabasco, Villahermosa.

Jiménez-Martínez, L.D., Álvarez-González, C.A., De la Cruz-Hernández, E., et al. 2019. Partial sequence characterization and ontogenetic expression of genes involved in lipid metabolism in the tropical gar (*Atractosteus tropicus*). Aquaculture Research, 50: 162-72. doi: 10.1111/are.13879

Jiménez-Martínez, L.D., Morales-Garcia, V., Frias-Quintana, C.A., et al. 2021. Quality evaluation of reference gene expression on different tissues in adults of tropical gar *Atractosteus tropicus*. Pakistan Journal of Zoology, 54: 1-10. doi: 10.17582/journal.pjz/20200913180928

Jiménez-Martínez, L.D., Tovar-Ramírez, D., Álvarez-González, C.A., et al. 2020. Assessment of dietary lipid sources in tropical gar, *Atractosteus tropicus* larvae: Growth parameters and intermediary lipogenic gene expression. Aquaculture Research, 51: 2629-2640. doi: 10.1111/are.14603

Kleppe, L., Edvardsen, R.B., Kuhl, H., et al. 2012. Maternal 3'UTRs: from egg to onset of zygotic transcription in Atlantic cod. BMC Genomics, 13: 1-14. doi: 10.1186/1471-2164-13-443

Klosterhoff, M.C., Pereira-Júnior, J., Rodrigues, R.V., et al. 2015. Ontogenic development of kidney, thymus and spleen and phenotypic expression of CD3 and CD4 receptors on the lymphocytes of cobia (*Rachycentron canadum*). Anais da Academia Brasileira de Ciências, 87: 2111-2121. doi: 10.1590/0001-3765201520140623

León, A.J., Garrote, A.J. & Arranz, E. 2006. Citocinas en la patogenia de la enfermedad inflamatoria intestinal. Medicina Clínica, 127: 145-152. doi: 10.1157/13090382

Li, L., Chen, S.N., Laghari, Z.A., et al. 2019. Receptor complex and signaling pathway of the two type II IFNs, IFN-γ and IFN-γrel in mandarin fish or the socalled Chinese perch *Siniperca chuatsi*. Developmental & Comparative Immunology, 97: 98-112. doi: 10.1016/j.dci.2019.03.016

Li, J., Gao, Y. & Xu, T. 2015. Comparative genomic and evolution of vertebrate NOD1 and NOD2 genes and their immune response in miiuy croaker. Fish & Shellfish Immunology, 46: 387-397. doi: 10.1016/j.fsi.2015.06.026

Li, T., Shan, S., Wang, L., et al. 2018. Identification of a fish-specific NOD-like receptor subfamily C (NLRC) gene from common carp (*Cyprinus carpio* L.): Characterization, ontogeny and expression analysis in response to immune stimulation. Fish & Shellfish Immunology, 82: 371-377. doi: 10.1016/j.fsi.2018.08.045

López, S.D., Márquez, G., Contreras, W., et al. 2005. Evaluation of commercial diets on growth and survival of tropical gar *Atractosteus tropicus* juveniles in captivity. Memories of Aquaculture America, New Orleans.

Mahapatra, S., Ganguly, B., Pani, S., et al. 2024. Unveiling the dynamics of embryogenesis and immune genes expression pattern in the Amur common carp (*Cyprinus carpio haematopterus*). Gene Expression Patterns, 52: 119367. doi: 10.1016/j.gep.2024.119367

Martínez-Bautista, G., Martínez-Burguete, T., Peña-Marín, E.S., et al. 2022. Hypoxia-and hyperoxia-related gene expression dynamics during developmental critical windows of the tropical gar *Atractosteus tropicus.* Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 263: 111093.

Martínez-Burguete, T., Peña-Marín, E.S., García-Gasca, A., et al. 2021. Nutrigenomic marker discovery by de novo transcriptomic sequencing during early development of the tropical gar (*Atractosteus tropicus*). Aquaculture Research, 52: 3829-3842.

Martínez-Burguete, T., Peña-Marín, E.S., Llera-Herrera, R.A., et al. 2023. Identification and expression analysis of transcripts involved in taurine biosynthesis during early ontogeny of tropical gar *Atractosteus tropicus*. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 285: 111501.

Márquez-Couturier, G., Álvarez-González, C.A., Contreras-Sánchez, W.M., et al. 2006. Avances en la alimentación y nutrición del pejelagarto *Atractosteus tropicus*. Memorias del Octavo Simposium Internacional de Nutrición Acuícola, Noviembre 15-17, 2006, Mazatlán, Sinaloa, México.

Matthews, M. & Vargas, Z. 2012. Anesthesia and euthanasia in zebrafish. ILAR Journal, 53: 192-204. doi: 10.1093/ilar.53.2.192

Mokhtar, D.M., Zaccone, G., Alesci, A., et al. 2023. Main components of fish immunity: An overview of the fish immune system. Fishes, 8: 93. doi: 10.3390/fishes8020093

Mukherjee, T., Hovingh, E.S., Foerster, E.G., et al. 2019. NOD1 and NOD2 in inflammation, immunity and disease. Archives of Biochemistry and Biophysics, 670: 69-81. doi: 10.1016/j.abb.2018.12.022

Mulder, I.E., Wadsworth, S. & Secombes, C.J. 2007. Cytokine expression in the intestine of rainbow trout (*Oncorhynchus mykiss*) during infection with *Aeromonas salmonicida*. Fish & Shellfish Immunology, 23: 747-759. doi: 10.1016/j.fsi.2007.02.002

Mullis, K., Faloona, F., Scharf, S., et al. 1986. Specific enzymatic amplification of DNA *in vitro*: The polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology, 51: 263-273. doi: 10.1101/sqb.1986.051.01.032

Nayak, S.K. 2010. Role of gastrointestinal microbiota in fish. Aquaculture Research, 41: 1553-1573. doi: 10.1111/j.1365-2109.2010.02546.x

Oehlers, S.H., Flores, M.V., Hall, C.J., et al. 2011. The inflammatory bowel disease (IBD) susceptibility genes NOD1 and NOD2 have conserved anti-bacterial roles in zebrafish. Disease Models & Mechanisms, 4: 832-841. doi: 10.1242/dmm.006122

Parada-Venegas, D., De la Fuente, M.K., Landskron, G., et al. 2019. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Frontiers in Immunology, 10: 277. doi: 10.3389/fimmu.2019.00277

Paria, A., Makesh, M., Chaudhari, A., et al. 2018. Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) in Asian seabass, *Lates calcarifer*: Cloning, ontogeny and expression analysis following bacterial infection or ligand stimulation. Fish & Shellfish Immunology, 79: 153-162. doi: 10.1016/j.fsi.2018.04.061

Park, S.B., Hikima, J., Suzuki, Y., et al. 2012. Molecular cloning and functional analysis of nucleotide-binding oligomerization domain 1 (NOD1) in olive flounder, *Paralichthys olivaceus*. Developmental & Comparative Immunology, 36: 680-687. doi: 10.1016/j.dci.2011.11.007

Penagos, G., Barato, P. & Iregui, C. 2009. Sistema inmune y vacunación de peces. Acta Biológica Colombiana, 14: 3-26. doi:10.15453/10226

Pereiro, P., Figueras, A. & Novoa, B. 2019. Insights into teleost interferon-gamma biology: An update. Fish & Shellfish Immunology, 90: 150-164. doi: 10.1016/j.fsi.2019.04.002

Pérez-Jiménez, G.M., Peña-Marín, E.S., Maytorena-Verdugo, C.I., et al. 2022. Incorporation of fructooligosaccharides in diets influences growth performance, digestive enzyme activity, and expression of intestinal barrier function genes in tropical gar (*Atractosteus tropicus*) larvae. Fishes, 7: 137. doi: 10.3390/fishes7030137

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research, 29: e45. doi: 10.1093/nar/29.9.e45

Poggi, A., Benelli, R., Venè, R., et al. 2019. Human gut-associated natural killer cells in health and disease. Frontiers in Immunology, 10: 961. doi: 10.3389/fimmu.2019.00961

Prabu, D.L., Sahu, N.P., Pal, A.K., et al. 2016. Immunomodulation and interferon-gamma gene expression in sutchi catfish, *Pangasianodon hypophthalmus*: effect of dietary fucoidan rich seaweed extract (FRSE) on pre and post-challenge period. Aquaculture Research, 47: 199-218. doi: 10.1111/are.12482

Qi, Y.F., Huang, Y.X., Wang, H.Y., et al. 2013. Elucidating the crosstalk mechanism between IFN-gamma and IL-6 via mathematical modelling. BMC Bioinformatics, 14: 41. doi: 10.1186/1471-2105-14-41

Qiu, P., Ishimoto, T., Fu, L., et al. 2022. The gut microbiota in inflammatory bowel disease. Frontiers in Cellular and Infection Microbiology, 12: 733992. doi: 10.3389/fcimb.2022.733992

Rio, D.C., Ares, M., Hannon, G.J., et al. 2010. Purification of RNA using TRIzol (TRI Reagent). Cold Spring Harbor Protocols, 10: prot5439. doi: 10.1101/pdb.prot5439

Robertsen, B. 2006. The interferon system of teleost fish. Fish & Shellfish Immunology, 20: 172-191. doi: 10.1016/j.fsi.2005.01.010

Salas, A., Hernandez-Rocha, C., Duijvestein, M., et al. 2020. JAK-STAT pathway targeting for the treatment of inflammatory bowel disease. Nature Reviews: Gastroenterology & Hepatology, 17: 323-337. doi: 10.1038/s41575-020-0273-0

Salinas, I. 2015. The mucosal immune system of teleost fish. Biology, 4: 525-539. doi: 10.3390/biology4030525

Sánchez-Velázquez, J., Peña-Herrejón, G.A. & Aguirre-Becerra, H. 2024. Fish responses to alternative feeding ingredients under abiotic chronic stress. Animals, 14: 765. doi: 10.3390/ani14050765

Sayyaf-Dezfuli, B., Lorenzoni, M., Carosi, A., et al. 2023. Teleost innate immunity, an intricate game between immune cells and parasites of fish organs: who wins, who loses. Frontiers in Immunology, 14: 1250835. doi: 10.3389/fimmu.2023.1250835

Secombes, C.J. & Wang, T. 2012. The innate and adaptive immune system of fish. Infectious disease in aquaculture. Woodhead Publishing, Swaston, pp. 3-68.

Sha, Z., Abernathy, J.W., Wang, S., et al. 2009. NOD-like subfamily of the nucleotide-binding domain and leucine-rich repeat containing family receptors and their expression in channel catfish. Developmental & Comparative Immunology, 9: 991-999. doi: 10.1016/j.dci.2009.04.004

Shtrichman, R. & Samuel, C.E. 2001. The role of gamma interferon in antimicrobial immunity. Current Opinion in Microbiology, 4: 251-259. doi: 10.1016/s1369-5274(00)00199-5

Stolte, E.H., Savelkoul, H.F., Wiegertjes, G., et al. 2008. Differential expression of two interferon-gamma genes in common carp (*Cyprinus carpio* L.). Developmental & Comparative Immunology, 32: 1467-1481. doi: 10.1016/j.dci.2008.06.012

Sun, B., Skjæveland, I., Svingerud, T., et al. 2011. Antiviral activity of salmonid gamma interferon against infectious pancreatic necrosis virus and salmonid alphavirus and its dependency on type *i* interferon. Journal of Virology, 85: 9188-9198. doi: 10.1128/jvi.00319-11

Swain, B., Basu, M. & Samanta, M. 2013a. NOD1 and NOD2 receptors in mrigal (*Cirrhinus mrigala*): inductive expression and downstream signalling in ligand stimulation and bacterial infections. Journal of Biosciences, 38: 533-548. doi: 10.1007/s12038-013-9330-y

Swain, B., Maiti, N.K. & Samanta, M. 2013b. Nucleotide binding and oligomerization domain 1 (NOD1) receptor in catla (*Catla catla*): Inductive expression and downstream signaling in ligand stimulation and bacterial infections. International Research Journal of Biological Sciences, 2: 55-61.

Toyonaga, T., Hino, O., Sugai, S., et al. 1994. Chronic active hepatitis in transgenic mice expressing interferon-gamma in the liver. Proceedings of the National Academy of Sciences, 91: 614-618. doi: 10.1073/pnas.91.2.614

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Triana-García, P.A., Gutierrez-Espinosa, M.C. & Eslava-Mocha, P.R. 2013. Rendimiento productivo e hígado graso en tilapia híbrida (*Oreochromis* spp.): Influencia de dos fuentes de lípidos. Orinoquia, 17: 183-196.

Watanabe, T., Asano, N., Kudo, M., et al. 2017. Nucleotide-binding oligomerization domain 1 and gastrointestinal disorders. Proceedings of the Japan Academy - Series B: Physical and Biological Sciences, 93: 578-599. doi: 10.2183/pjab.93.037

Xia, B., Liu, X., Li, Z., et al. 2023. The effects of microbiota-targeted approaches in inflammatory bowel disease: probiotics, probiotic foods, and prebiotics. Current Opinion in Food Science, 49: 100956. doi: 10.1016/j.cofs.2022.100956

Yin, L., Lv, M., Qiu, X., et al. 2021. IFN-γ manipulates NOD1-mediated interaction of autophagy and *Edwardsiella piscicida* to augment intracellular clearance in fish. Journal of Immunology, 207: 1087-1098. doi: 10.4049/jimmunol.2100151

Yoon, S., Alnabulsi, A., Wang, T.Y., et al. 2016. Analysis of interferon gamma protein expression in zebrafish (*Danio rerio*). Fish & Shellfish Immunology, 57: 79-86. doi: 10.1016/j.fsi.2016.08.023

Yu, R., Zhang, W., Yu, P., et al. 2024. IFN-γ enhances protective efficacy against *Nocardia seriolae* infection in largemouth bass (*Micropterus salmoides*). Frontiers in Immunology, 15: 1361231. doi: 10.3389/fimmu.2024.1361231

Zou, J., Carrington, A., Collet, B., et al. 2005. Identification and bioactivities of IFN-γ in rainbow trout *Oncorhynchus mykiss*: the first Th1-type cytokine characterized functionally in fish. Journal of Immunology, 175: 2484-2494. doi: 10.4049/jimmunol.175.4.2484

Zou, J., Yoshiura, Y., Dijkstra, J.M., et al. 2004. Identification of an interferon gamma homologue in Fugu, *Takifugu rubripes*. Fish & Shellfish Immunology, 17: 403-409. doi: 10.1016/j.fsi.2004.04.015