UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO
División Académica de Ciencias Biológicas
"Estudio en la duda. Acción en la fe"

"ANÁLISIS DE LOS MECANISMOS INVOLUCRADOS EN LA RUTA DE BIOSÍNTESIS DE TAURINA EN EL PEJELAGARTO *(Atractosteus tropicus, Gill 1863)"*

Trabajo recepcional, en la modalidad de:

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Para obtener el grado de:

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de Sistemas Tropicales

Presenta:

MCA. Talhia Martínez Burguete

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Villahermosa, Tabasco, México **Diciembre, 2023**

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DEDICATORIA

A la memoria de mis abuelitos Colacha, Beto, Elena y Cheo.

See you in the stars sweet little Sam.

La muerte deja un dolor que nadie puede curar; el amor deja recuerdos que nadie puede borrar.

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RESUMEN

La taurina, es un ácido amino sulfónico que existe de forma natural en mamíferos, aves, peces e invertebrados y está involucrado en una amplia variedad de funciones fisiológicas y metabólicas. En peces, la disponibilidad de taurina es regulada durante el desarrollo ontogenético, en donde su capacidad de síntesis endógena depende de la especie. Está capacidad de síntesis endógena ha sido descrita en tres diferentes rutas. Estas rutas y los transcritos/genes que participan en ellas son: I) Ruta del ácido cisteíno sulfínico, cisteína dioxigenasa (*cdo1*), ácido sulfinico descarboxilasa (*csad*); II) Ruta del ácido cisteíno, *cdo1*, ácido glutámico descarboxilasa (*gad*); III) Ruta de la cisteamina, 2-aminoetanetiol dioxigenasa (*ado*). Por su parte, el encargado de distribuir esta taurina producida durante la biosíntesis, es el transportador de taurina (*taut*), ya que juega un papel importante durante la regulación de la taurina disponible en el organismo.

En el presente trabajo de doctorado se utilizó al pejelagarto *Atractosteus tropicus* como modelo para estudiar los diferentes mecanismos de biosíntesis de taurina a partir del estudio de los genes involucrados en su biosíntesis y su transporte desde el punto de vista bioinformático, molecular y nutricional. Estos resultados permitirán tener una perspectiva más amplia para comprender mejor estos procesos moleculares evolutivamente primitivos en un pez ancestral.

En la primera parte de esta tesis (Capítulo 2) se realizó una búsqueda referenciada de los principales procesos fisiológicos en los que participan los diferentes genes involucrados en la biosíntesis (*cdo*, *csad*, *gad* y *ado*) y el transportador (*taut*) de taurina. En esta revisión se observó que diferentes procesos fisiológicos como la reproducción, la digestión, los sistemas olfativo, visual, circulatorio y muscular se ven afectados cuando alguno de los genes no es sintetizado por el organismo y/o existe alguna eliminación o silenciamiento por técnicas moleculares. Así también, se realizó la inferencia de los sitios de unión de factores de transcripción (TFBS) y los factores de transcripción involucrados en la regulación de la biosíntesis y transporte de taurina desde un enfoque bioinformático. Se utilizaron como referencia las regiones promotoras de diferentes especies de peces. Como resultado se encontró que Homeobox protein BarH-like 1 (BARX1), Brain-specific homeobox protein homolog (BSX), Helicase-like Transcription Factor (HLTF), Homeobox protein Hox-A7 (HOXA7), Homeobox protein Hox-B3 (HOXB3), Homeobox protein Hox-B6 (HOXB6), Homeobox protein Meis1 (MEIS1), Homeobox protein Meis3 (MEIS3), Nuclear factor of activated T cells 1 (NFATC1), y Homeobox protein Nkx-6.2 (NKX6-2) se

encontraban en las regiones promotoras de todos los genes implicados en la biosíntesis y transporte de taurina de las diferentes especies estudiadas. Estos resultados permiten sentar un antecedente para futuras investigaciones relacionadas con la identificación de factores de transcripción en genes relacionados con la biosíntesis de taurina.

En la segunda parte (Capítulo 3), se identificó si *A. tropicus* tiene la capacidad de síntesis de taurina durante su desarrollo ontogenético y en diferentes órganos de juveniles tempranos. Como resultado se reportan las secuencias correspondientes a *cdo*, *csad*, *gad*, *ado* y *taut* de *A. tropicus*. Estas secuencias fueron obtenidas de su transcriptoma utilizando como referencia los ortólogos de *L. oculatus*. Las secuencias analizadas se utilizaron para conocer su similitud y filogenia con la de otras especies, así como para la identificación de sus patrones de expresión en embriones, larvas y órganos de juveniles tempranos. Los resultados mostraron una expresión fluctuante de todos los transcritos involucrados en la biosíntesis y transporte de taurina en las diferentes etapas analizadas. La expresión de todos los transcritos está presente desde la eclosión. La expresión de todos los transcritos a excepción de *csad*, disminuye 23 días después de la eclosión (dde), cuando el organismo ha finalizado su período larval. En juveniles tempranos (31 dde), se observó que la expresión de *cdo*, *ado* y *taut* está presente en todos los órganos examinados (hígado, cerebro, ojo, branquias, piel, músculo, intestino y estómago). Los resultados sugieren que embriones, larvas y juveniles pueden sintetizar taurina utilizando las tres diferentes rutas. Se puede sugerir que la ruta de la cisteamina (ruta III) es la ruta principal en las diferentes etapas estudiadas en el pejelagarto. Sin embargo, se considera que es órgano dependiente, ya que la ruta del ácido cisteíno sulfínico es predominante en el hígado, mientras que la del ácido cisteíno solo ocurre en ojo, cerebro y piel. Si bien, los resultados aportan una idea del mecanismo de biosíntesis de taurina en embriones, larvas y juveniles tempranos de pejelagarto, es necesario realizar estudios adicionales, para entender mejor el efecto de la taurina a diferentes niveles fisiológicos y nutricionales.

En la tercera parte del presente trabajo (Capítulo 4), se realizó un experimento para analizar el efecto que tiene la suplementación de taurina en el crecimiento, sobrevivencia, la caracterización morfológica de la mucosa intestinal y la expresión relativa de genes implicados en la biosíntesis y el transporte de taurina en larvas de pejelagarto. Se probaron cinco dietas las cuales consistieron en una dieta basal (0% taurina), más cuatro con diferentes porcentajes de inclusión de taurina (0.5, 1.0, 1.5, y 2.0%). Las harinas de pescado, ave y cerdo fueron lavadas con etanol para remover el

contenido de taurina en estas fuentes de proteína. Los tratamientos se realizaron por triplicado utilizando 120 organismos por unidad experimental y distribuidos aleatoriamente en 15 tanques. Los organismos fueron alimentados cinco veces al día (08:00, 11:00, 13:00, 15:00, and 18:00 h) durante un período de 22 días. Los resultados mostraron diferencias significativas entre los tratamientos con taurina suplementada y la dieta sin taurina (0% taurina; $P < 0.05$). La suplementación con taurina también mostró un aumento en la supervivencia a medida que se incrementaba el nivel de taurina, siendo 2.0% taurina la dieta que mostró mejores porcentajes de supervivencia (41.11 ± 2.55 %). La descripción morfológica del moco intestinal mostró que está constituido por pliegues que forman vellosidades distribuidas a través del intestino a medida que aumenta el nivel de taurina suplementada. Así mismo, la altura del epitelio intestinal también presentó los valores más altos en taurina al 2.0% (17.176 ± 2.99 µm), mientras que los más bajos se observaron en taurina al 0% (16.832 ± 5.06 µm). Los resultados de expresión relativa de los transcritos involucrados en la biosíntesis de taurina mostraron que todos los genes son sobre expresados cuando se agrega taurina, a excepción de los tratamientos 0.5% en *cdo,* y 1.5% en *csad* y *gad*. Este experimento dosis-respuesta, es el primero en evaluar el efecto de la suplementación de taurina en la familia de los Lepisosteidos y contribuye al conocimiento del metabolismo de la taurina en peces ancestrales.

Los resultados de la presente investigación de doctorado contribuyen al conocimiento del metabolismo de la taurina en *A. tropicus* y determinan si el organismo en etapas larvarias y prejuveniles tiene la capacidad de síntesis *de novo*. Así mismo, el uso de la taurina como suplemento en las dietas de larvas contribuye al uso de un nuevo aditivo, a partir del cual se podrán diseñar nuevas dietas que puedan ser exploradas en etapas juveniles y adultas en el organismo buscando mejorar sus condiciones de cultivo.

ABSTRACT

Taurine is an amino sulfonic acid that occurs naturally in mammals, birds, fish, and invertebrates and is involved in various physiological and metabolic functions. In fishes, the availability of taurine is regulated during ontogenetic development, where its endogenous synthesis capacity is species-dependent. This capacity to synthesize taurine has been describe in three different pathways. These pathways and transcripts/genes are I) Cysteine sulfinate-dependent pathway, cysteine dioxygenase type 1 (*cdo1*) and cysteine sulfinic acid decarboxylase (*csad*); II) Cysteine acid pathway, *cdo1* and glutamic acid decarboxylase (*gad*); and III) Cysteamine pathway, 2-aminoethanethiol dioxygenase (*ado*). On the other hand, the taurine transporter (*taut*) is responsible for the distribution of the taurine produced during biosynthesis since it plays an important role in regulating taurine availability in the organism.

In the present doctoral work, tropical gar *Atractosteus tropicus* was used as a model to determine the different mechanisms of taurine biosynthesis by studying the genes involved in its biosynthesis and transportation from a bioinformatic, molecular and nutritional perspective. Thus, allowing a broader perspective to understand this evolutionary primitive molecular process in an ancestral fish.

Hence, to cover the first part of this thesis (Chapter 2), the main physiological processes in fish in which the different genes involved in taurine biosynthesis (*cdo, csad, gad*, and *ado*) and taurine transporter (*taut)* participate are reported. As a result, it was found that different physiological processes such as reproduction, digestion, and the olfactory, visual, circulatory, and muscular systems are affected when the organism does not synthesize any of the genes or there is some elimination or silencing by molecular techniques. To expand the knowledge, we aim to infer and find the transcription factor binding sites (TFBS) and the transcription factors involved in the regulation of taurine biosynthesis and transportation from a bioinformatic approach. The promoter regions of different fish species were used as reference. Binding sites for Homeobox protein BarH-like 1 (BARX1), Brain-specific homeobox protein homolog (BSX), Helicase-like Transcription Factor (HLTF), Homeobox protein Hox-A7 (HOXA7), Homeobox protein Hox-B3 (HOXB3), Homeobox protein Hox-B6 (HOXB6), Homeobox protein Meis1 (MEIS1), Homeobox protein Meis3 (MEIS3), Nuclear factor of activated T cells 1 (NFATC1), and Homeobox protein Nkx-6.2 (NKX6-2) are found in the promoter regions of all the genes involved in taurine biosynthesis.

These results, help us provide a precedent for future research related to identifying transcription factors in genes associated with taurine biosynthesis and transportation.

For the second part (Chapter 3), it was identified whether *A. tropicus* has the capacity to synthesize taurine during its ontogenetic development and in different organs of early juveniles. As a result, the corresponding sequences of *cdo*, *csad*, *gad*, *ado*, and *taut* are reported. These sequences were retrieved from *A. tropicus* transcriptome using the orthologs of *L. oculatus* as reference. Sequences were analyzed to determine their similarity and phylogeny with other species and to identify their expression patterns in embryos, larvae, and organs of early juveniles. The results showed a fluctuating expression of all the transcripts involved in taurine transportation and biosynthesis in the analyzed stages. The expression levels of all transcripts are present from hatching. The expression levels of all transcripts but *csad* decreases at 23 days after hatch (dah) when the organism has finished its larval period. In early juveniles (31 dah), *cdo*, *csad*, and *taut* expression levels are present in all examined organs (liver, brain, eye, gills, skin, muscle, intestine, and stomach). The results suggest that embryos, larvae, and juveniles can synthesize taurine using the three different pathways. In addition, it can also be suggested that the cysteamine pathway (pathway III) is the main pathway in the different stages studied in tropical gar. However, it is organ-dependent since the cysteine sulfinate-dependent pathway is predominant in the liver, whereas the cysteine acid pathway only occurs in the brain, eye, and skin. Although the results give us an idea of the mechanism of taurine biosynthesis in embryos, larvae and early juveniles of tropical gar, additional studies are necessary to understand better the effect of taurine at different physiological and nutritional levels.

In the third part of this work (Chapter 4), we evaluated the effect of taurine supplementation on growth, survival, morphological characterization of the intestinal mucosa, and the relative expression of genes involved in taurine transportation and biosynthesis in tropical gar larvae. Five diets were tested, consisting of a basal diet (0% taurine) and four diets with different percentages of taurine inclusion (0.5, 1.0, 1.5, and 2.0%). Fish, poultry, and pork meals were washed with ethanol to remove the taurine content in these protein sources. The treatments were carried out in triplicate using 120 organisms per experimental unit and randomly distributed in 15 tanks. The organisms were fed five times a day (08:00, 11:00, 13:00, 15:00, and 18:00 h) for 22 days. The results showed significant differences between the treatments with supplemented taurine and the

diet to which no taurine was added (0% taurine). Taurine supplementation also showed an increase in survival as the taurine level increased, with 2.0% taurine showing the best survival percentage (41.11 ± 2.55%). The morphological description of the intestinal mucus showed that it is constituted by folds that form villi distributed throughout the intestine as the level of supplemented taurine increases. Likewise, the height of the intestinal epithelium also presented the highest values in 2.0% taurine (17.176 ± 2.99 µm), while the lowest values were observed in 0% taurine (16.832 ± 5.06 µm). The relative expression results of the transcripts involved in taurine biosynthesis showed that all genes are overexpressed when taurine is added, except for the 0.5% *cdo* and 1.5% *csad* and *gad* treatments. This experiment is the first to evaluate the effect of taurine supplementation in the Lepisosteidae family and contributes to the knowledge of taurine metabolism in ancestral fishes.

This research contributes to the knowledge of taurine metabolism in *A. tropicus* and determine if the organism in larvae and pre-juvenile stages has the capacity for *de novo* synthesis. Likewise, using taurine as a supplement in larvae diets contribute to the use of a new additive, from which new diets can be designed and explored in juvenile and adult stages in the organism that could improve their culture conditions.

ÍNDICE

Capítulo 1

Introducción general

Introducción

La taurina, es una pequeña molécula que, si bien en la literatura es considerado como un aminoácido debido a que esta no se incorpora a las proteínas, no puede considerarse como tal, ya que contiene un grupo tiol y es el único ácido sulfónico natural conocido que se encuentra de forma libre en el organismo (Huxtable & Sebring, 1986; Sirdah, 2015). Se sabe que existe de forma natural en diferentes tejidos y órganos de mamíferos, aves, peces e invertebrados acuáticos como las ostras y mejillones, principalmente en corazón, retina, músculo esquelético, cerebro, intestino, así como en el plasma, glóbulos y leucocitos (El-Sayed, 2014; Hosoi, 2005; Huxtable, 1992; Jacobsen & Smith, 1968) por lo que está involucrado en diferentes funciones, entre las que destacan la formación de la bilis, la osmorregulación, la estabilización de la membrana celular, efecto antioxidante, señalización de calcio, contractilidad miocárdica, el desarrollo de la retina, del cerebro y del músculo esquelético (Goodman et al., 2009; Mezzomo et al., 2018; Yang et al., 2015; Zhao et al., 2017). Sin embargo, los mecanismos exactos por los cuales estas acciones son posibles aún no son del todo entendidos.

En peces, diferentes estudios han demostrado que la taurina es regulada durante su desarrollo (Chang et al., 2013; Kozlowski et al., 2008; Pinto et al., 2010), mientras que en etapas juveniles y adultas su requerimiento es específico dependiendo de la especie, esto debido a que puede ser sintetizada de forma *de novo* o puede ser adquirida a través del alimento (Kim et al., 2005a; Salze et al., 2018; Yokoyama et al., 2001). En especies marinas se ha observado que la implementación de taurina tiene efectos positivos en el crecimiento, sobrevivencia y desarrollo morfológico, en especial si son especies carnívoras (Kim et al., 2008; Salze et al., 2012) mientras que en especies dulceacuícolas se ha demostrado que, a pesar de tener una mejor habilidad para sintetizarla, su implementación en la dieta podría ser poco favorable o no tener ninguna inferencia en su crecimiento y sobrevivencia (Espe et al., 2008). No obstante, algunas de estas especies tienen una habilidad limitada de síntesis, por lo que requieren una fuente exógena de taurina, particularmente en aquellos casos donde la proteína animal es sustituida por vegetal (Al-Feky et al., 2016; Hoseini et al., 2018).

Estudios previos han recalcado la importancia de la familia Lepisosteidae como un grupo basal que divergió del resto de los peces teleósteos antes de la duplicación de su genoma al hacer estudios puntuales para determinar la posición filogenética que algunos genes ocupaban (Eames et al., 2012;

Grone & Maruska, 2015; Song et al., 2013). Esto ha podido ser comprobado por completo con los avances de las tecnologías de secuenciación, con los cuales se ha logrado la anotación del genoma de *Lepisosteus oculatus* (Braasch et al., 2016) y el transcriptoma de larvas y adultos de *Atractosteus tropicus* (Cribbin et al., 2017; Martínez-Burguete et al., 2021). Esto permite conectar a los teleósteos a la biología humana, ya que su linaje representa el grupo hermano de teleósteos no duplicados (Braasch et al., 2016). Por lo que, tomando en cuenta que los Lepisosteiformes son un grupo particularmente valioso para comprender los mecanismos de la evolución molecular de los vertebrados, gracias a su proximidad ecológica y evolutiva a los teleósteos, el pejelagarto al ser una especie perteneciente a este grupo es un candidato ideal para poder estudiar a nivel molecular los mecanismos de biosíntesis de taurina.

Actualmente no existen trabajos relacionados con el mecanismo de la biosíntesis de taurina en los Lepisosteiformes o con la implementación de taurina en su dieta. De esta forma en el presente estudio se aborda el análisis de los genes asociados a la biosíntesis y transporte de taurina en el pejelagarto a través de un enfoque bioinformático, molecular y nutricional que permitirá ampliar el conocimiento de su biología básica y del uso de un nuevo aditivo para futuras investigaciones.

Antecedentes

Biosíntesis de taurina en peces

En animales, existen cinco diferentes rutas de biosíntesis de taurina (Huxtable, 1992; Salze & Davis, 2015; Stipanuk, 2004) de las cuales, tres han sido descritas en peces (Sampath et al., 2020; Haga et al., 2015). Las rutas a partir de las cuales la taurina es sintetizada a partir de metionina a través del catabolismo de la cisteína se pueden observar en la Figura 1. En breve, 1) Ruta del ácido cisteíno sulfínico, en donde la cisteína es oxidada por acción de la cisteína dioxigenasa (CDO) a ácido cisteíno sulfinico y se descarboxila por la cisteína ácido sulfinico descarboxilasa (CSAD) a hipotaurina que es oxidado a taurina por hipotaurina deshidrogenasa (HP-DH); 2) Ruta del ácido cisteíno: al igual que en la ruta anterior la cisteína es oxidada a ácido cisteíno sulfínico por acción de CDO1, sin embargo, en lugar de ser descarboxilado, este se metaboliza a ácido cisteíco que a su vez es descarboxilado por el ácido glutámico descarboxilasa (GAD) para convertirse en taurina; 3) Ruta de la cisteamina: en esta ruta cisteína es convertida en cisteamina a través de coenzima A, posteriormente cisteamina es oxidada por 2-aminoetanetiol dioxigenasa (ADO) y convertida a hipotaurina, la cual por acción de HP-DH se convierte en taurina.

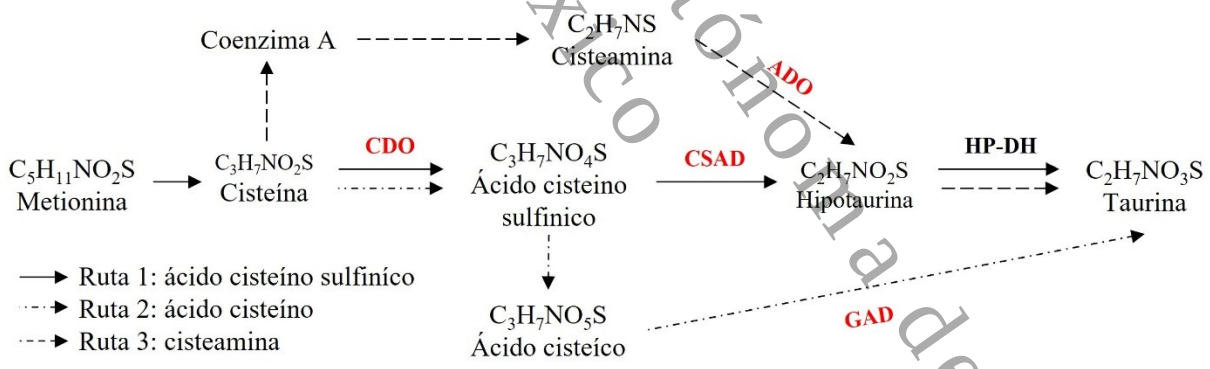

Figura 1. Rutas de biosíntesis de taurina. Adaptada de Salze et al. (2015). CDO: cisteína dioxigenasa; CSAD: ácido sulfinico descarboxilasa; GAD: ácido glutámico descarboxilasa; ADO: 2-aminoetanetiol dioxigenasa; HP-DH: hipotaurina deshidrogenasa.

Está reportado que la enzima limitante en la biosíntesis de taurina es CSAD, es decir a mayor actividad de CSAD mayor cantidad de taurina disponible en el sistema (Chang et al., 2013; Haga et al., 2015) por lo que, la biosíntesis y transporte de taurina podría estar limitada por la actividad de dicha enzima. Sin embargo, la ruta predominante depende de la especie, y se debe considerar

el tipo de tejido, el estadio de desarrollo, los hábitos alimenticios y el ambiente en el que viven (El-Sayed, 2014). De esta forma, la síntesis de taurina es regulada mayormente por la biosíntesis de las enzimas anteriormente mencionadas (Sampath et al., 2020). Además, se debe considerar al transportador de taurina (TAUT), ya que es el principal transportador de la taurina producida durante la biosíntesis *de novo* principalmente en el hígado y el cerebro (Baliou et al., 2020; Han et al., 2006; Kozlowski et al., 2008).

La mayor parte de los trabajos realizados en peces teleósteos se han enfocado en la respuesta de los organismos en la suplementación de taurina en la dieta, particularmente cuando se hace una sustitución de harina animal por harina vegetal, por lo que si bien no es el fin del presente trabajo, sería interesante realizar estudios de mutagénesis dirigida (CRISPR/CAS9) que permitan entender la importancia de las rutas de síntesis de taurina y su papel en el crecimiento, supervivencia y estado fisiológico del pejelagarto que permitan comprender la posible condición basal del metabolismo de taurina en peces óseos a través de la evolución.

Estudios acerca de los genes involucrados en la biosíntesis de taurina

Los principales genes que codifican para proteínas que participan en las diferentes rutas de biosíntesis de taurina son *cdo1, csad, gad* y *ado*. Por su parte, el transportador de taurina (*taut*) juega un papel importante ya que tiene una respuesta adaptativa a los cambios en la disponibilidad de taurina en el organismo.

Cisteína dioxigenasa tipo 1 (*cdo1*)

El gen de la cisteína dioxigenasa tipo 1 (*cdo1*), es un gen que codifica para la proteína CDO (EC 1.13.11.20) y es una de las principales enzimas en la formación de taurina y sulfato (relación 2:1) que se encuentra en altos niveles en el hígado, mientras que, en el riñón, cerebro y pulmón, son niveles bajos los que predominan (Stipanuk et al., 2006). Este gen ha sido ampliamente estudiado en mamíferos (Dawson et al., 2020; Driggers et al., 2015; Stipanuk et al., 2015) y estos trabajos han demostrado que la eliminación o silenciamiento de CDO1 puede producir anomalías en los organismos.

En peces *cdo1* ha sido reportado en diferentes especies como trucha arcoíris (*Oncorhynchus mykiss*) (Wang et al., 2015), pez cebra (*Danio rerio*) (Liu et al., 2017), carpa dorada (*Carassius*

auratus) (Luo et al., 2019) y atún rojo (*Thunnus thynnus*) (Betancor et al., 2019). Dentro de los trabajos que se han realizado, se encuentra el de Wang et al. (2016) en donde secuenciaron la región que codifica para la proteína Cdo1 de *O. mykiss* y *Paralichthys olivaceus* y encontraron que mientras la primera tiene un ORF de 600 nucleótidos que traducen para una proteína de 200 aminoácidos (AA), la segunda cuenta con un ORF de 603 nucleótidos que se traduce en 201 AA y comparten una similitud del 84%. En el caso del estudio de los factores de transcripción que regulan la expresión del gen se ha demostrado que existe una conservación espacial entre especies en donde los factores de transcripción KLF1 (Kruppel like factor); MZF1 (myeloid zinc finger); ZNF300 (zinc finger protein 300); ZNF239 (zinc finger protein 239); DLX1 (distal-less homeobox 1); y CEBPB (CCAAT enhancer binding protein beta) podían unirse (Dawson et al., 2020).

Cisteína ácido sulfínico descarboxilasa (*csad*)

El gen cisteína ácido sulfínico descarboxilasa (*csad*), es un gen que codifica para la proteína CSAD (EC 4.1.1.29) y está considerada como la enzima limitante en la producción de taurina en el hígado (de la Rosa & Stipanuk, 1985). Su expresión ha sido medida en órganos como el cerebro, branquias, corazón, riñón, bazo, hígado, intestino, músculo, tejido adiposo, ovario y testículo (Betancor et al., 2019; Haga et al., 2015; Poppi et al., 2019). Se sabe que la falta de actividad de la enzima en gatos puede provocar ceguera por lo que se considera que la taurina es un nutriente esencial en su alimentación (Hayes et al., 1975). En peces se sabe que especies de la familia Labridae, Scombridae, Soleidae y Rajidae se ha reportado que carecen de la actividad de Csad (Salze & Davis, 2015). Algunos estudios en el silenciamiento o eliminación del gen sugieren que la mortalidad y anormalidades cardiacas pueden ser los efectos que sufren los organismos cuando esto ocurre (Chang et al., 2013; Park et al., 2014) y que a su vez pueden ser recuperados a través de la suplementación de taurina en la dieta (Park et al., 2017).

Por su parte Haga et al. (2015) han caracterizado el tamaño de la secuencia en pargo japonés (*Pagrus major*) y medregal japonés (*Seriola quinqueradiata*) y encontraron que el tamaño de la secuencia en la primera fue de 1882 nucleótidos y el de la segunda 1821 mientras que la secuencia de aminoácidos en ambas es de 508 AA. En *D. rerio* Chang et al. (2013) encontraron que esta secuencia tiene1449 pares de bases y su secuencia de aminoácidos es de 482 AA. Además, con los avances de las tecnologías de secuenciación y la cantidad de genomas disponibles en las diferentes

bases de datos, la mayor parte de las secuencias han sido inferidas haciendo uso de los avances de estas tecnologías.

Ácido glutámico descarboxilasa (*gad*)

El ácido glutámico descarboxilasa (*gad*) o glutamato descarboxilasa, es un gen que codifica para la proteína GAD (EC 4.1.1.15) y se sintetiza y expresa principalmente en el cerebro (Wu et al., 1979). Esta enzima participa en una ruta alterna a la principal para la formación de taurina y ha sido estudiado principalmente en gatos, bovinos y ratas (Hayes et al., 1975; Wu, 1982; Zhao et al., 2018) y se sabe que existen tres diferentes isoformas del gen en vertebrados (Grone & Maruska, 2016).

En peces el gen ha sido estudiado en carpa dorada (*C. auratus*) (Lariviere et al., 2002), pez cebra (*D. rerio*) (Mueller & Guo, 2009), el pez abisal *Coryphaenoides (Nematonurus) armatus* (Trudeau et al., 2000) y detectado por RNAseq en *Syngnathus scovelli* (Beal et al., 2018). Por su parte Cocco et al. (2017) en el pez cebra estudiaron su localización en diferentes regiones del cerebro, y encontraron tres diferentes isoformas, de las cuales dos se asemejan a la isoforma gad1 y gad2 de mamíferos.

2-aminoetanetiol dioxigenasa (*ado*)

El gen 2-aminoetanetiol dioxigenasa (*ado*), también conocido como cisteamina dioxigenasa (cao) codifica para la proteína ADO (EC 1.13.11.19) y participa en la ruta alterna de biosíntesis de taurina al oxidar a la cisteamina para formar hipotaurina que posteriormente por acción de la hipotaurina deshidrogenasa produce taurina (Salze & Davis, 2015; Stipanuk & Ueki, 2011).

La expresión de este gen ha sido estudiada en diferentes tejidos y órganos de peces como hepatopáncreas, cerebro, branquias, intestino, músculos, ojo, corazón, bazo, riñón y vesícula biliar (Betancor et al., 2019; Watson et al., 2014). Por su parte, Gonzales-Plasus et al. (2019) realizaron la caracterización de la secuencia de nucleótidos y aminoácidos en la carpa común (*Cyprinius carpio*). Ellos encontraron una secuencia de 790 nucleótidos con una secuencia deducida de 260 aminoácidos.

Transportador de taurina (*taut*)

El transportador de taurina (*taut*) también conocido como transportador de taurina dependiente de cloruro y sodio (*slc6a6*) es un gen de vital importancia que controla la acumulación de taurina intracelular, contribuyendo al transporte de taurina en las células y en las mitocondrias de los peces (Sampath et al., 2020). Esta regulación de *taut* puede ser observada en la distribución de diferentes tejidos de peces como el rodaballo (*Psetta máxima*) (Wang et al., 2017), atún rojo (*T. thynnus)* (Betancor et al., 2019) y la carpa china *Ctenopharyngodon idella* (Yan et al., 2019). Por ejemplo, en el pez cebra, Kozlowski et al. (2008) observó la regulación de este gen durante la embriogénesis a partir de la técnica de knockdown. En este estudio, encontraron que el gen está presente desde etapas tempranas del desarrollo embrionario en todos los tejidos donde se sabe que la taurina juega un papel importante. Además, observaron que el knockdown causa baja expresión del gen y resulta un fenotipo que implica apoptosis celular en el cerebro y la médula espinal durante la embriogénesis.

De esta forma, conocer cómo se comportan estos genes durante el desarrollo ontogenético y en los diferentes tejidos, permitirá determinar no solo los patrones de expresión de estos genes, si no también determinar si las diferentes rutas son parecidas o diferentes al resto de peces teleósteos.

Suplementación de taurina en peces

Para el funcionamiento adecuado de cualquier organismo es necesario que los nutrientes que componen su dieta estén considerados para cumplir funciones fisiológicamente importantes en su metabolismo celular y animal que permitan un crecimiento, reproducción y salud óptima. En el caso de la taurina, se considera que su requerimiento es condicional debido a que diferentes factores como enfermedades, estadio de vida y ciclo reproductivo podrían afectar sus requerimientos nutricionales (El-Sayed, 2014; Salze & Davis, 2015; Sampath et al., 2020). Los peces pueden adquirir la taurina de dos formas: a) a través de la absorción de la taurina en la dieta y b) a partir de la síntesis *de novo* a partir de sus precursores metionina/cisteína (Kuzmina et al., 2010).

Como se mencionó anteriormente, el requerimiento es específico para cada especie ya que mientras en especies marinas se ha observado que la implementación de taurina tiene efectos

positivos en el crecimiento, sobrevivencia y desarrollo morfológico (Partridge & Woolley, 2017; Salze et al., 2012), en algunas especies dulceacuícolas como el pez cebra se ha demostrado que a pesar de tener una mejor habilidad para sintetizarla, su implementación en la dieta podría no tener ninguna inferencia en su crecimiento y reproducción (Guimarães et al., 2018). Sin embargo, se debe considerar en ambos casos, cuando la especie tiene una capacidad limitada, sobre todo cuando la fuente de proteína en su alimentación es de origen vegetal (Wu et al., 2021).

De esta forma, los requerimientos de taurina en peces se han estudiado mayormente a través de estudios de dosis-respuesta en donde se ha demostrado que la suplementación en la dieta tiene efectos en la fisiología, el metabolismo, el cultivo y la nutrición de peces dulceacuícolas y marinos (Martins et al., 2021; Matsunari et al., 2013; Salze et al., 2012). Estos estudios dosis-respuesta en larvas se han enfocado en enriquecer el alimento vivo o adicionar taurina en el alimento. En el caso del alimento vivo una de las principales presas naturales durante la alimentación son los copépodos, quienes tienen alrededor de entre 5-19 g/kg de taurina, mientras que en el caso de los rotíferos y la *Artemia* su contenido de taurina oscila entre 0-0.5 g/kg y 7.2-8.2 g/kg respectivamente (van der Meeren et al., 2008). Los rotíferos y la *Artemia* son generalmente utilizados para la alimentación de larvas recién eclosionadas en cautiverio, y uno de los principales problemas a los que se enfrenta la acuacultura es la baja sobrevivencia de organismos durante su cultivo. De esta forma, diferentes estudios se han enfocado en utilizar a la taurina en el enriquecimiento de alimento vivo y han observado diferentes beneficios (Hawkyard et al., 2016; Matsunari et al., 2013; Partridge & Woolley, 2017). Esto ha sido observado en larvas del pez marino *Rachycentron canadum*, en donde se ha demostrado que el enriquecimiento de taurina en *Artemia*, tiene un efecto sobre la morfología, el crecimiento y la actividad enzimática (Salze et al., 2011, 2012). Por su parte, Matsunari et al. (2013) utilizaron el enriquecimiento de rotíferos con distintos niveles de taurina que posteriormente fueron administrados a larvas de *Seriola dumerili*. En ambos trabajos los autores demostraron que la inclusión de taurina ayuda también en la sobrevivencia de los organismos. Por otra parte, Betancor et al. (2019) también utilizando el enriquecimiento de alimento vivo, demostraron el efecto de su incorporación en larvas de *T. thynnus* en su crecimiento, encontrando que si bien, todos los niveles de taurina administrados a los organismos mostraron una respuesta positiva, los que fueron alimentados con una dieta que contenía 1 g de taurina por 106 rotíferos, presentaron un mejor crecimiento, mejor expresión en los genes involucrados en la ruta de biosíntesis de taurina, el transportador de taurina, genes digestivos y antioxidantes. Este

beneficio también ha sido observado en larvas de *Thunnus orientalis* y *T. albacares* por parte de Katagiri et al. (2017) en donde el enriquecimiento de rotíferos con taurina ayuda en el crecimiento de estas especies. Además, los análisis químicos mostraron que el contenido de taurina en los rotíferos, así como en larvas de ambas especies era mucho mayor que en el alimento que no fue enriquecido. También se observó que el momento de preflexión de la notocordia comenzó en el día 7 (*T. orientalis*) y 8 (*T. albacares*) para ambos grupos. Sin embargo, para el día 16 había más larvas en estado de post-flexión en las larvas alimentadas con alimento enriquecido para *T. orientalis* mientras que en *T. albacares* la etapa de flexión y la frecuencia estaba presente desde el día 9 hasta el día 14 en ambos tratamientos, mientras que el contenido de taurina fue mayor en las larvas que tomaron el alimento enriquecido.

Por otra parte, tanto las dietas comerciales como la harina de pescado contienen cierta cantidad de taurina (Kim et al., 2005a; 2005b). Por lo que se han realizado diferentes aproximaciones para que al incluir la taurina en la dieta esta contenga la menos cantidad posible de taurina exógena. Dentro de estas aproximaciones se encuentran las de lavar la harina tres veces con 70% etanol cuando proviene de una fuente animal (Hu et al., 2018; Kim et al., 2007). Un ejemplo de este es el trabajo realizado en juveniles de *P. olivaceus* y *C. carpio* por Kim et al. (2008). Estos autores observaron que la capacidad de biosíntesis de taurina entre una especie marina y una dulceacuícola es diferente. Mientras en *P. olivacious* la inclusión ayuda en el crecimiento de los organismos, en *C. carpio* no existe ninguna diferencia en el crecimiento de los diferentes tratamientos que utilizaron. Lo que significa que *P. olivacious* tiene una capacidad limitada para sintetizar taurina, por lo que es necesario que sea incluida en la dieta.

En especies de agua dulce, Al-Feky et al. (2016) evaluaron el efecto de la suplementación de taurina en *Oreochromis niloticus* cuando la base de la dieta está basada en harina de soya y encontraron que cuando los organismos son alimentados con 1.0% de taurina en la dieta, las hembras tienen un mejor rendimiento en el número de desoves que obtienen. En especies marinas como *Seriola dumerili* se ha reportado que la alimentación de reproductores con dietas que contienen cierto porcentaje de taurina tiene un efecto positivo sobre la fecundidad y fertilización de estos organismos (Sarih et al., 2019). Por ejemplo, Salze et al. (2019) reportaron que en *Seriola dorsalis* esta suplementación de taurina en reproductores también tiene un efecto sobre las larvas recién eclosionadas al mejorar el volumen del saco vitelino, así como las probabilidades de

sobrevivir a la primera alimentación; cosa que no ocurrió con las larvas del grupo control. De esta forma, ellos diseñaron un nuevo experimento en donde estos investigadores optaron también por alimentar con rotíferos y *Artemia* enriquecida con taurina para observar los efectos que podría tener en las larvas provenientes de las reproductoras del control. En este nuevo estudio, todas las larvas de los reproductores control y alimentados con la dieta control (sin enriquecer) murieron a los 15 días después de la eclosión, lo que permite observar lo importante de la taurina en la alimentación.

Contrario a lo que pasa con las especies marinas anteriormente mencionadas, Guimarães et al. (2018) quienes investigaron el efecto de suplementar taurina con dietas vegetales en el pez cebra, hallaron que, si bien la implementación no afecta en el crecimiento del organismo ni en el desempeño reproductivo, si tiene un efecto en la utilización de lípidos y estado redox. También es importante resaltar que es probable que la cantidad de taurina en las dietas suplementadas no haya sido la adecuada, ya que el control comercial que utilizaron era la dieta que contenía mayor cantidad de taurina.

La suplementación de taurina en larvas, juveniles o adultos de especies pertenecientes a la familia Lepisosteidae no ha sido reportada anteriormente. De esta manera el análisis de la expresión de los genes involucrados en la biosíntesis de taurina y los parámetros de crecimiento al final del estadio larval de *A. tropicus*, cuando los organismos fueron alimentados con diferentes niveles de inclusión de taurina, podrán utilizarse para determinar si el organismo tiene la capacidad de síntesis y si se obtiene algún beneficio de esta para su crecimiento y sobrevivencia.

Estudios realizados en *Atractosteus tropicus*

El pejelagarto *A. tropicus* es un pez carnívoro de agua dulce que pertenece al grupo basal de los Lepisosteidos, los cuales divergieron del resto de los teleósteos antes de la duplicación de su genoma (Amores et al., 2011; Barrientos-Villalobos & Espinosa De Los Monteros, 2008; Braasch et al., 2016). El grupo de los Lepisosteidos ha sido sugerido como modelo para sugerir procesos moleculares evolutivamente primitivos para los diferentes grupos de peces y poder compararlo con humanos (Braasch et al., 2014, 2016; Martin & Holland, 2017; Wcisel et al., 2017). Sin embargo, no existen trabajos que hablen sobre el metabolismo de la taurina en los Lepisosteidos, únicamente

hay trabajos reportados en otras especies donde utilizan las secuencias de *L. oculatus* para análisis filogenéticos (Betancor et al., 2019; Gonzales-Plasus et al., 2019; Grone & Maruska, 2016).

El pejelagarto *A. tropicus*, es una especie de gran importancia en el Sureste mexicano y durante los últimos años, las investigaciones realizadas se han enfocado en diferentes aspectos que permitan determinar los requerimientos nutricionales durante diferentes etapas de desarrollo para lograr su producción (Frías-Quintana et al., 2016; Guerrero-Zárate et al., 2014; Márquez-Couturier et al., 2006; Palma-Cancino et al., 2019). Esto, ha permitido el diseño de diferentes dietas para su uso durante su larvicultivo (Frías-Quintana et al., 2010, 2016, 2017), y en etapas juveniles (Huerta et al., 2018; Nieves-Rodríguez et al., 2018; Sepúlveda-Quiroz et al., 2020); así como esquemas de alimentación (Escalera-Vázquez et al., 2018; Saenz De Rodrigáñez et al., 2018). En la Tabla 1 se presentan los trabajos más recientes del uso de fuentes alternativas o de suplementos en diferentes etapas del pejelagarto.

Tabla 1. Fuentes alternativas o suplementos utilizadas en la dieta de larvas y juveniles de pejelagarto *A. tropicus*.

Referencia	Fuente o suplemento	Estadio de desarrollo
Nájera-Arzola et al. (2018)	Manano oligosacáridos	Juvenil
Guerrero-Zárate et al. (2019)	Carbohidratos/Lípidos	Juvenil
Sepúlveda-Quiroz et al. (2020)	Fructooligosacáridos	Juvenil
Pérez-Jiménez et al. (2022)	Fructooligosacáridos	Larva
Maytorena-Verdugo et al. (2022)	Manano oligosacáridos	Larva
De La Cruz-Marín et al. (2023)	Inulina	Larva
Cigarroa-Ruiz et al. (2023)	β-glucanos	Larva

Sin embargo, a pesar de los esfuerzos que se han realizado, aún existen diferentes problemas relacionados al momento de hacer la sustitución total del alimento vivo al alimento artificial durante el larvicultivo, debido a la falta de aceptación al nuevo alimento y al canibalismo que por naturaleza presenta la especie. Dentro de los trabajos realizados en los que se ha observado que las dietas utilizadas pueden ayudar a prevenir el canibalismo se encuentra el de Jiménez-Martínez et al. (2020) en larvas utilizando diferentes fuentes de lípidos. Los autores encontraron que las larvas

alimentadas con aceite de pescado o lecitina de soya mostraban una mejor respuesta al haber menos canibalismo y tener mejor sobrevivencia y presentar mayor crecimiento que aquellas alimentadas con aceite de maíz, aceite de canola o aceite de oliva. Estos autores sugieren que el uso de la lecitina de soya podría ser una mejor opción ya que su uso proporciona una composición de fosfolípidos más digeribles que los lípidos neutros. Recientemente Sepúlveda-Quiroz et al. (2023) realizaron el estudio de los diferentes comportamientos de ataque que conducen al canibalismo en larvas de pejelagarto y describe diferentes estrategias que pueden ser utilizadas para disminuir este tipo de comportamiento en la especie.

En cuanto al estudio de genes, se han caracterizado las secuencias parciales y los patrones de expresión de genes del metabolismo lipogénico (Jiménez-Martínez et al., 2018), el gen de tripsina (Jesús-De la Cruz et al., 2020), genes de la hormona de crecimiento e insulina (Zamora-Solís et al., 2021) y genes involucrados en la biosíntesis de ácidos grasos poliinsaturados de cadena larga durante el desarrollo larvario (De la Cruz-Alvarado et al., 2021). También han sido estudiados genes de referencia en adultos de pejelagarto (Jiménez Martínez et al., 2022).

Por su parte Martínez-Bautista et al. (2022) estudiaron la expresión de genes relacionados con hipoxia e hiperoxia durante períodos llamados "ventanas críticas de desarrollo" en función de estresores como la temperatura, la disponibilidad de oxígeno y la salinidad. Mientras que Aranda-Morales et al. (2021) determinaron los niveles totales de nitrógeno y amonio desionizado en juveniles de pejelagarto y evaluaron los efectos transcripcionales asociados a proteínas Rhesus y transportadores de iones.

Actualmente, está disponible la secuenciación masiva del gen 16sRNA de las bacterias residentes en el intestino de juveniles y adultos de la especie (Méndez-Pérez et al., 2020), así como el transcriptoma gonadal, muscular y cerebral de hembras y machos (Cribbin et al., 2017) y durante el desarrollo larvario (Martínez-Burguete et al., 2021).

Justificación

El pejelagarto (*A. tropicus*) es una especie de gran importancia en el estado de Tabasco, por lo que la mayor parte de los trabajos que se han desarrollado se han enfocado en desarrollar paquetes tecnológicos que permitan su cultivo para posteriormente ser transferibles a los productores. Sin embargo, es necesario aprovechar la posición basal en la que se encuentra para comprender aspectos biológicos que nos permitan contestar preguntas más puntuales con respecto a los diferentes metabolismos basales.

Por su parte, los estudios que se han desarrollado con respecto a la taurina en mamíferos y peces han logrado determinar diferentes funciones fisiológicas y farmacológicas en las que este aminoácido tiene alguna participación o influencia. Sin embargo, no existe ningún trabajo reportado hasta la fecha en la familia de los Lepisosteidos, donde se hayan estudiado los mecanismos de biosíntesis de taurina, ni mucho menos lo que pasa cuando se da una suplementación de ésta en diferentes etapas de desarrollo. Por lo que, a través del estudio de los genes que participan en los diferentes mecanismos de biosíntesis que han sido reportados en otras especies y tomando en cuenta que ya se tiene el transcriptoma del pejelagarto en el presente estudio nos enfocamos en determinar si el organismo tiene la capacidad de síntesis en etapas larvarias, sí las rutas son tejido-específico en juveniles tempranos, y qué pasa cuando existe una suplementación de taurina en larvas.

Estos resultados contribuirán al conocimiento de la biología básica y molecular durante etapas larvarias y en prejuveniles de *A. tropicus* y permitirá también determinar si suplementar taurina en las dietas tiene un beneficio para el organismo.

Objetivos

Objetivo general

Determinar si *Atractosteus tropicus* en la etapa larval y juvenil tiene la capacidad de síntesis de taurina por medio del estudio de los genes que participan en su mecanismo metabólico.

Objetivos particulares

- Conocer el papel fisiológico e identificar los factores de transcripción de los transcritos que participan en el transporte y la biosíntesis de taurina de diferentes especies de peces.
- Identificar los transcritos codificantes para proteínas que participan en las diferentes rutas de biosíntesis de taurina, así como su transporte durante el desarrollo larval y en órganos de juveniles tempranos de *A. tropicus*
- Analizar el efecto de la suplementación de taurina en la expresión de genes involucrados en el transporte y biosíntesis de taurina (*taut, cdo, csad, gad,* y *ado*) de larvas de *A. tropicus.*

Hipótesis

Si *A. tropicus* tiene la capacidad de sintetizar taurina esta podrá ser detectada a través de la expresión de los transcritos que codifican para proteínas que participan en la ruta de biosíntesis de taurina durante las diferentes etapas de desarrollo.

La detección de los transcritos que participan en las diferentes rutas de biosíntesis de taurina permitirá determinar si la ruta es tejido-especifica en juveniles tempranos de *A. tropicus*.

La suplementación de taurina en la dieta de larvas de pejelagarto tendrá un efecto en la variación de los transcritos que participan en su síntesis *de novo*.

Estructura general del documento

La estructura general del presente documento está dividida en 6 capítulos y Anexos. Estos capítulos se encuentran preparados de acuerdo con las revistas indizadas en las cuales fueron o serán sometidos y cubren los objetivos particulares de esta investigación. En esta estructura general del documento está incluida la introducción general (Capítulo 1), seguida por tres capítulos elaborados en formato de publicación (Capítulo 2, 3 y 4), una discusión general (Capítulo 5), conclusiones y recomendaciones (Capítulo 6) y Anexos. Los capítulos 2, 3 y 4 se encuentran escritos de acuerdo con los formatos e idioma en el cual fueron sometidos o que serán sometidos.

Una breve descripción general de cada capítulo integrado en formato de publicación se presenta a continuación.

Capítulo 2: Se realizó una revisión bibliográfica del rol fisiológico en peces de los genes involucrados en las diferentes rutas de biosíntesis de taurina y el transporte de taurina. Así mismo, la identificación de factores de transcripción de forma *in silico* en los diferentes genes en estudio fue realizada para complementar y enriquecer este capítulo.

Capítulo 3: Se identificaron las rutas metabólicas y patrones de expresión de genes involucrados en la biosíntesis y transporte de taurina durante el desarrollo larvario y en diferentes órganos de juveniles tempranos de pejelagarto.

Capítulo 4: Se evaluó el efecto de la suplementación de taurina en los índices de crecimiento, la supervivencia y la expresión de genes de larvas completas de pejelagarto, así como la caracterización morfológica de la mucosa intestinal de larvas.

Las figuras y el material suplementario de la presente tesis pueden ser consultados en el siguiente QR

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Capítulo 2

Physiological role of genes involved in taurine biosynthesis in fishes and in silico approach to determined transcription factors in their promoters' zone

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Review

Physiological role of genes involved in taurine biosynthesis in fishes and in silico approach to determine transcription factors in their promoters' zone

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ABSTRACT. Taurine, an amino sulfonic acid that is sometimes referred to as an amino acid, is endogenously synthesized by the action of the key genes/enzymes: cysteine dioxygenase (*cdo*), cysteine sulfonate decarboxylase (*csad*), glutamate decarboxylase (*gad*), and 2-amino ethanethiol dioxygenase (*ado*). The taurine transporter (*taut*) also distributes this taurine formation across the plasma. These genes have been identified as important in different physiological processes such as reproduction, digestion, olfactory, visual, circulatory, and muscular systems. Thus, a literature review of these genes in fish has been described in the present work. Moreover, there is null information regarding the study of regulatory elements such as transcription factors (TFs) in taurine biosynthesis and transportation genes of fishes. In this interest and taking advantage of the availability of different sequence databases, bioinformatics can be applied as a first approach for an in silico identification of the putative TFs and transcription factor binding sites (TFBS) that might play an important role in regulating these genes. The results showed that some are commonly shared, whereas TFs and TFBs vary among fish species. Hence, binding sites for homeobox protein BarH-like 1 (BARX1), brain-specific homeobox protein homolog (BSX), helicase-like transcription factor (HLTF), homeobox protein Hox-A7 (HOXA7), homeobox protein Hox-B3 (HOXB3), homeobox protein Hox-B6 (HOXB6), homeobox protein Meis1 (MEIS1), homeobox protein Meis3 (MEIS3), nuclear factor of activated T cells 1 (NFATC1), and homeobox protein Nkx-6.2 (NKX6-2) were commonly found in the promoter regions of genes involved in taurine transportation and biosynthesis. Additionally, our results suggested that the frequency of HOXB3, a transcription factor involved in development, has repetitive TFBS sites in the promoter region of all species analyzed in the present study. Although bioinformatics gives us an approach to determine putative TFs and TFBS, further work is needed to verify how the found regulatory elements play a key role in taurine biosynthesis and transportation.

Keywords: ß-aminosulfonic acid; taurine biosynthesis; taurine transporter; transcription factor

INTRODUCTION

Taurine, also known as 2-aminoethanesulfonic acid, is a simple molecule first isolated from the bile acid of the ox (*Bos taurus*) in 1827 by Leopold Gmelin and Friedrich Tiedemann (Tiedemann & Gmelin, 1827). It can be found in free form in most animals and is often called an amino acid (Huxtable & Sebring 1986). However, due to its structure, it is not part of the α-amino acids that synthesize proteins since it lacks the fundamental and less acid carboxyl group, what gives it its zwitterionic nature similarity to neutral membrane

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phospholipids, phosphatidylcholine, and phosphatidylethanolamine (Huxtable 1992); hence its high-water solubility and low lipophilicity, explaining its impermeability of biological membranes (Lambert et al. 2015). Moreover, some small peptides are known to exist naturally; nonetheless, there is no evidence of transfer RNA (tRNA) that codes for taurine, and its sulfonate group replaces the carboxyl group necessary for the formation of a peptide bond, therefore, cannot be part of translated peptide chains (Bittner et al. 2005).

The taurine pool can be found in vertebrate species' brains, retina, liver, kidney, heart, and muscle (Jacobsen & Smith 1968, Huxtable 1992). Furthermore, in mammals, the physiological role of taurine has been extensively investigated (Huxtable & Sebring 1986, Pion et al. 1987, Huxtable 1992, Militante et al. 2000, Parsons et al. 2001, Goodman et al. 2009, Ueki et al. 2012, Han et al. 2015) while in fishes different studies have demonstrated its relevance in the physiology of marine and freshwater fishes (El-Sayed 2014, Salze & Davis 2015, Mezzomo et al. 2018, Zhang et al. 2019, Sampath et al. 2020). As in mammals, different physiological roles of taurine have been observed in fishes that are involved in respiration, circulation, digestion, osmoregulation, sensorial system, muscular system, central nervous system (CNS), and reproduction (Higuchi et al. 2012, Mezzomo et al. 2018, Brill et al. 2019, Ceccotti et al. 2019, Salze et al. 2019). Most species can acquire taurine through food absorption in the intestine or endogenous synthesis from its precursor's methionine/cysteine (Kuzmina et al. 2010).

Most studies in taurine have been focused on the use of supplemented taurine. Moreover, transcriptomics has been applied to identify the effects on visual and olfactory system (Hu et al. 2018, 2020). Hu et al. (2018) studied the effects of taurine as a feed attractant in plant protein-based diets for large yellow croaker. Thus, the sequencing of the olfactory epithelium was conducted to identify genes differentially expressed in the taurine group (TAU) *vs*. controls. They detected 77 olfactory receptor genes, including 37 up-regulated unigenes, validating the expression of eight genes (52N4 (olfactory receptor family 52 subfamily N member 4), 10C1 (olfactory receptor family 10 subfamily C member 1), 2D3 (olfactory receptor family 2 subfamily D member 3), 13C2 (olfactory receptor family 13 subfamily C member 2), 4C11 (olfactory receptor family 4 subfamily C member 11), 2A12 (olfactory receptor family 2 subfamily A member 12) and 1361) using quantitative reverse transcription-PCR (RT-qPCR). The function of the differentially expressed genes was defined by the gene ontology (GO) and the Kyoto encyclopedia of genes and genomes (KEGG) and found that they are mainly involved in signaling and cell communication (GO) and olfactory transduction (KEGG), allowing further insights into the detailed mechanism of the olfactory system of fishes.

Consequently, this study demonstrates that using transcriptomics might be a better approach to understanding taurine roles. Another example of the role of taurine in the sensory system has been the work realized by Brill et al. (2019) in seabass *Dicentrarchus labrax* also fed protein diets. Although no effect on retinal anatomy or functional properties of luminous sensitivity were found in this work, authors found that the spectral sensitivity peak of individuals fed a 5% taurine diet was rightward shifted (i.e. towards longer wavelengths) relative to that of fish fed a 0 or 1.5% taurine diet.

Although taurine supplementation has widely been studied in fish species (Salze & Davis 2015, Sampath et al. 2020, Li et al. 2022) including the identification of genes involved in taurine biosynthesis of different species such as zebrafish *Danio rerio* (Liu et al. 2017), goldfish *Carassius auratus* (Luo et al. 2019), and tropical gar *Atractosteus tropicus* (Martínez-Burguete et al. 2023), there is no information regarding regulatory elements in those genes involved in the endogenous synthesis of taurine. Gene regulatory elements include promoters, enhancers, silencers, and insulators, where the promoter includes a core promoter and a proximal promoter region (Chatterjee & Ahituy 2017). In eukaryotes, the core promoter represents the minimal elements required to initiate transcription (Taher et al. 2015). Thus, these elements required to initiate transcription in the promoter region contain the general transcription machinery and transcription factors (Riethoven 2010). Transcription factors are proteins that can bind to specific DNA sequences of promoter regions (Mitsis et al. 2020). TFs and their binding sites in these promoter regions can be predicted using bioinformatic tools. Large data sets can be analyzed using bioinformatics to find patterns among organisms, whether model or non-model species.

Hence, in the present work, we have summarized the main physiological roles played by taurine biosynthesis and transport genes in different fish species based on a literature review. Moreover, due to the lack of information regarding putative transcription factors (TFs) and transcription factors binding sites (TFBS) that could be involved in regulating taurine biosynthesis and transportation genes in fishes, a bioinformatic approach was applied to investigate them.

Taurine biosynthesis and its transporters

In fishes, the biosynthesis of taurine occurs through three different pathways (Fig. 1), and the predominant pathway will depend on the species, type of tissue, stage of development, feeding habits, and the environment in which they live (Haga et al. 2015, Salze & Davis 2015, Sampath et al. 2020). One different pathway is pathway I (cysteine sulfinate-dependent pathway), where cysteine is oxidized by cysteine dioxygenase (CDO) and converted into cysteine sulfinic acid, which is then decarboxylated by cysteine sulfonate decarboxylase (CSAD) to form hypotaurine which is converted in taurine by hypotaurine dehydrogenase (HP-DH), CDO regulates the cysteine concentration, and CSAD enzyme is the rate-limiting step in taurine biosynthesis; pathway II (cysteine acid pathway), is also regulated by CDO. However, the product is metabolized to cysteic acid and converted to taurine by decarboxylation through glutamate decarboxylase (GAD). In pathway III (cysteamine pathway), taurine is obtained by converting cysteine into coenzyme A and then converted to cysteamine, which is oxidized by 2-aminoethanethiol dioxygenase (ADO) to form hypotaurine and form taurine by HP-DH. However, in the case of hypotaurine to taurine conversion by the action of HP-DH in pathways I and III, it is known that this reaction can also occur spontaneously, in addition to the fact that HP-DH has not been characterized (Roysommuti & Wyss 2015). The distribution of taurine endogenously synthesized or acquired from food is mainly regulated by the taurine transporter (*taut*; Wang et al. 2017, Xiong et al. 2020)

Cysteine dioxygenase (*cdo*)

Cysteine dioxygenase (*cdo*) is a gene that codes for the CDO protein (EC 1.13.11.20) and is one of the main enzymes in the formation of taurine and sulfate (2:1 ratio). It is found at high levels in the liver, while low levels predominate in the kidney, brain, and lungs (Stipanuk 2004). This iron metalloenzyme catalyzes the addition of molecular oxygen to the thiol group of cysteine, producing cysteine sulfinic acid, thus playing an important role in the catabolism of cysteine (Stipanuk et al. 2006). In mammals, it has extensively been studied (Driggers et al. 2015, Stipanuk et al. 2015, Dawson et al. 2020), and among these works, it has been shown that the deletion or silencing of *cdo* can produce abnormalities in organisms. For example, in rodents, Ueki et al. (2011) observed the differences in the physiological function of CDO and what happened after the loss of its activity. To achieve this, they crossed mice carrying a CDO null allele ($CDO^{+/-}$) to generate $CDO^{-/-}$, $CDO^{+/-}$ and $CDO^{+/+}$ mice and observed that $CDO^{-/-}$ mice had higher postnatal mortality, deficit growth and pathology of the connective tissue in the elastic fibers of the foot, as well as low levels of taurine and high levels of cysteine, which is due to the lack of flow of the CDO dependent catabolic pathways. However, in fishes, the depletion through knockout models of CDO has not been explored. The reported works in fishes regarding CDO are focused on the identification, isolation, and regulation of the gene in some species like rainbow trout *Oncorhynchus mykiss* (Wang et al. 2015), zebrafish *D. rerio* (Liu et al. 2017) and goldfish *C. auratus* (Luo et al. 2019).

Cysteine sulfonate decarboxylase (*csad*)

The cysteine sulfonate decarboxylase (*csad*) gene is a gene that codes for the CSAD protein (EC 4.1.1.29) and is considered the limiting enzyme in the production of taurine, which is produced mainly in the liver (De la Rosa & Stipanuk 1985). CSAD catalyzes a decarboxylation reaction to cysteine sulfinic acid to form hypotaurine, which is then converted to taurine (Yokoyama et al. 2001). It is known that the lack of enzyme activity in cats can cause blindness, which is why taurine is considered an essential nutrient in their diet (Hayes et al. 1975). Its expression has been measured in organs such as the brain, gills, heart, kidney, spleen, liver, intestine, muscle, adipose tissue, ovary, and testis (Haga et al. 2015, Betancor et al. 2019, Poppi et al. 2019). In fish, species of the family Labridae, Scombridae, Soleidae, and Rajidae have been reported to lack the activity of CSAD (Salze & Davis 2015). Some studies on the silencing or elimination of the gene suggest that mortality and cardiac abnormalities may be the effects that organisms suffer when this occurs (Chang et al. 2013) and that, in turn, they can be recovered through taurine supplementation in the diet (Park et al. 2014). Among the works carried out in fish, which allow us to observe what happens when one of the genes involved in the biosynthesis of taurine is silenced or eliminated, is that carried out by Chang et al. (2013) in zebrafish. In this work, the researchers mutated a part of the *csad* gene, presumed to be the limiting enzyme in endogenous taurine production. In this work, they found that by mutating the gene and therefore not producing the CSAD protein, there was a reduction in taurine levels in embryos, early mortality increased, and cardiac abnormalities were found (pericardial edema, cardiac tube malformation). Therefore, to test whether taurine supplementation could influence embryos with the phenotype of cardiac abnormalities, they supplemented taurine in the environ-

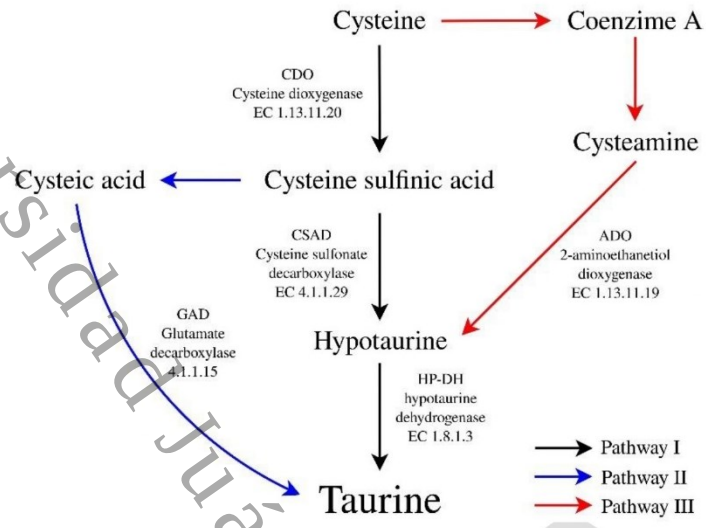

Figure 1. Pathways of taurine biosynthesis (adapted from Salze & Davis 2015). Pathway I, cysteine sulfinate-dependent pathway; Pathway II, cysteine acid; Pathway III, cysteamine.

ment in which the embryos were and managed to rescue these embryos. Nevertheless, most works on fish species have been conducted to identify their punctual identification and expression (Huang et al. 2014, Haga et al. 2015, Betancor et al. 2019, Poppi et al. 2020, Ma et al. 2021).

Glutamate decarboxylase (*gad*)

Glutamate decarboxylase (*gad*), also known as cysteic acid decarboxylase (*cad*), is a gene that codes for the GAD/CAD protein (EC 4.1.1.15) and is synthesized and expressed mainly in the brain (Wu et al. 1979). This enzyme participates in an alternative route to the main one for the formation of taurine, where cysteine is oxidized to cysteine sulfinic acid by the action of CDO, and later, instead of being decarboxylated, cysteine sulfinic acid is metabolized to cysteic acid which GAD/CAD decarboxylates to become taurine (Salze & Davis 2015).

It is known that there are three different isoforms of the gene in vertebrates (Grone & Maruska 2016). In fishes, some studies have been directed at the deep sea armed grenadier *Coryphaenoides* (*Nematonurus*) *armatus* (Trudeau et al. 2000), goldfish (Lariviere et al. 2002), zebrafish (Mueller & Guo 2009), and detected by RNAseq in gulf pipefish *Syngnathus scovelli* (Beal et al. 2018). Hence, in zebrafish, Cocco et al. (2017) studied *gad* localization in different brain regions and found three different paralogs, of which two resemble the *gad1* paralog of mammals and the third one the *gad2*. However, it has yet to be studied in fish in recent years. Therefore, with the advances in sequencing technology and bioinformatics, it would be interesting to study the regulation of the gene and its mechanism in fish.

2-aminoethanethiol dioxygenase (*ado*)

The 2-aminoethanethiol dioxygenase (*ado*) gene, also known as cysteamine dioxygenase (*cao*), encodes the ADO protein (EC 1.13.11.19) and participates in the alternate route of taurine biosynthesis by oxidizing cysteamine to form hypotaurine, which subsequently action of hypotaurine dehydrogenase to produce taurine (Stipanuk et al. 2015). Although it is the least studied gene of those involved in taurine biosynthesis, its expression in fishes may have been studied in different tissues and organs such as the hepatopancreas, brain, gills, intestine, muscles, eye, heart, spleen, kidney, and gallbladder of Atlantic bluefin tuna *Thunnus thynnus* (Watson et al. 2014, Betancor et al. 2019). Hence, Gonzales-Plasus et al. (2019) characterized the nucleotide and amino acid sequence in common carp *Cyprinus carpio* and quantified its expression in different tissues.

Taurine transporter

Taurine is distributed across the plasma membrane by taurine transporters (Mezzomo et al. 2018). These taurine transporters are the proton/amino acid symporter (PAT1) encoded by the solute carrier family 36 member 1 (*slc36a1*) and taurine transporter (TAUT) encoded by solute carrier family 6 membrane 6 (*slc6a6*)

being this last one the most studied (Lambert et al. 2015, Chen et al. 2019, Seidel et al. 2019). Hence, it is known that the adaptive response of *taut* to changes in the availability of taurine, when there are reduced levels of dietary taurine, elevated the expression of the gene (Han et al. 2006). In fishes, this regulation of *taut* availability can be observed in the distribution of different tissues of fishes like turbot *Scophthalmus maximus* (Wang et al. 2017, Wei et al. 2019), Atlantic bluefin tuna (Betancor et al. 2019), grass carp *Ctenopharyngodon idella* (Yan et al. 2019) and goldfish (Xiong et al. 2020). Kozlowski et al. (2008) characterized *taut* in zebrafish and observed its expression during embryogenesis.

Additionally, to understand the role of *taut*, they investigated the effect that a knockdown of the gene could have on these embryos. The results they found were a) the expression of *taut* is present from the very early stages of embryonic development in all tissues where taurine is known to be essential (retina, heart, brain, kidney, and blood vessels) and b) *taut* knockdown caused its low expression and results in a phenotype that involves cell apoptosis in the brain and spinal cord. These results determine the importance of *taut* during embryogenesis.

Therefore, further work regarding the use of different tools in molecular biology, such as site-directed mutagenesis or bioinformatics, to identify transcription factors that regulate taurine biosynthesis genes and the transportation of taurine should be accomplished for a better understanding of the physiological roles played by taurine.

METHODOLOGY

Retrieval of promoters, nucleotide, and amino acid sequences of organisms can be obtained from different public databases such as Ensembl, NCBI, and Uniprot (Yates et al. 2020, Madeira et al. 2022 Martin et al. 2023). For the present study, the Ensembl database (https://www.ensembl.org/index.html) was accessed to retrieve sequences for promoter analysis.

Promoter analysis of TFBS involved in taurine biosynthesis and transportation

Promoter regions spanning +2000 to -1 bp for *cdo*, *csad*, *gad*, *ado*, and *taut* of different species were retrieved from Ensembl (https://www.ensembl.org/) and converted to FASTA files. Species and their gene identifiers used for the analysis can be found in Table 1. The retrieved sequences were used to identify the TFs and TFBS using the software CiiiDER, version 0.9 (Gearing et al. 2019) using the Jaspar 2020 core vertebrate sequences. Because TFBS varies and rarely matches the model perfectly, a default deficit score of 0.05 was used, where a deficit score of 0 represents a perfect match. Once the software was run, results were obtained in .csv format, converted to .xls format, and used to calculate TFBS frequency to construct heatmaps using GraphPad Prism version 9.3.0 (San Diego, CA, USA).

Identification of TFs share among the promoter region of genes in the study was obtained using the http://bioinformatics.psb.ugent.be/webtools/Venn/ webtool of the Van de Peer Lab.

RESULTS

Complete raw results from the program CiiiDER can be found in Supplementary Material 1. The CiiiDER program predicted the presence of several TFs and TFBS on the promoter regions of all species in the study (Table 2). Identification of putative TFs exclusive of the promoter zone of the genes in each species is shown (Table 3).

Cysteine dioxygenase (*cdo*)

The promoter sequences of different fish species were used to identify the putative TFs and TFBS in *cdo*. Our finding shows that Arid3a, Arid3b, BARX1, BSX, Foxf, FOXL1, GATA3, GATA5, GSX1, GSX2, HLTF, HOXA7, HOXB3, HOXB6, LHX1, MEIS1, MEIS3, NFATC1, NFATC3, NFATC4, NKX6-2, RHOXF1, SOX15, Sox17, ZNF354C were present in all species while Alx3, Dlx1, Dlx2, Dlx3, DRGX, HOXB2 and HOXB8 were present in all except zebrafish. A representation of the comparison among the promoter zone of some of the species in the study can be observed (Fig. 2). The frequency of the TFBS of the TFs present in all species was also identified (Fig. 3). Moreover, the frequency of the TFBS for BSX, HLTF, and HOXB3 is constant among species. In addition, it can also be observed that LHX1 frequency in zebrafish is higher than in the rest of the species.

Cysteine sulfonate decarboxylase (*csad*)

Four hundred eighty-six putative TFs were found in the promoter region of *csad* of different fish species (Suppl. Mat. 1). We also found that Arid3b, BARX1, BSX, EVX1, EVX2, GSX1, HLTF, HOXA7, HOXB2, HOXB3, HOXB6, MEIS1, MEIS3, NFATC1, NFATC3, NKX6-2, PDX1, and RHOXF1 are present in all the species in study. The frequency of the TFBS

Table 1. Species and accession number of the genes in Ensembl used for the in silico approach to determine transcription factors (TFs) and transcription factor binding sites (TFBS) using CiiiDER software version 0.9 (Gearing et al. 2019).

Species	Common name	*cdo*	*csad*	*gad*	*ado*	*taut*
Poecilia formosa	Amazon molly	ENSPFOG00000002497	ENSPFOG00000001389	ENSPFOG00000011193	ENSPFOG00000020791	ENSPFOG00000008415
Salmo salar	Atlantic salmon	ENSSSAG00000045718	ENSSSAG00000077542	ENSSSAG00000081646	ENSSSAG00000071248	ENSSSAG00000068263
Salmo trutta	Brown trout	ENSSTUG00000030331	ENSSTUG00000049081	ENSSTUG00000030337	ENSSTUG00000021119	ENSSTUG00000013971
Ictalurus punctatus	Channel catfish	ENSIPUG00000025116	ENSIPUG00000022545	ENSIPUG00000020814	ENSIPUG00000023869	ENSIPUG00000009147
Cyprinus carpio	Common carp	ENSCCRG00000019916	ENSCCRG00000050160	ENSCCRG00000009363	ENSCCRG00000009651	ENSCCRG00000028865
Electrophorus electricus	Electric eel	ENSEEEG00000007791	ENSEEEG00000020397	ENSEEEG00000004961	ENSEEEG00000006255	ENSEEEG00000023351
Dicentrarchus labrax	European seabass	ENSDLAG00005030923	ENSDLAG00005001675	ENSDLAG00005000274	ENSDLAG00005020461	ENSDLAG00005010961
Takifugu rubripes	Fugu	ENSTRUG00000020607	ENSTRUG00000001632	ENSTRUG00000017805	ENSTRUG00000012216	ENSTRUG00000009909
Sparus aurata	Gilthead seabream	ENSSAUG00010005231	ENSSAUG00010018857	ENSSAUG00010026343	ENSSAUG00010014882	ENSSAUG00010000496
Carassius auratus	Goldfish	ENSCARG00000068233	ENSCARG00000017734	ENSCARG00000023792	ENSCARG00000047487	ENSCARG00000058589
Poecilia reticulata	Guppy	ENSPREG00000013161	ENSPREG00000020183	ENSPREG00000004050	ENSPREG00000012002	ENSPREG00000017002
Oryzias latipes	Japanese medaka	ENSORLG00000003390	ENSORLG00000008058	ENSORLG00000017268	ENSORLG00000027492	ENSORLG00000010119
Cyclopterus lumpus	Lumpfish	ENSCLMG00005007809	ENSCLMG00005009685	ENSCLMG00005021952	ENSCLMG00005021103	ENSCLMG00005002451
Oreochromis niloticus	Nile tilapia	ENSONIG00000014479	ENSONIG00000012260	ENSONIG00000008762	ENSONIG00000021007	ENSONIG00000000371
Esox lucius	Northern pike	ENSELUG00000018836	ENSELUG00000018621	ENSELUG00000021349	ENSELUG00000005239	ENSELUG00000018892
Oncorhynchus mykiss	Rainbow trout	ENSOMYG00000029549	ENSOMYG00000024325	ENSOMYG00000008187	ENSOMYG00000041907	ENSOMYG00000033005
Pygocentrus nattereri	Red-bellied piranha	ENSPNAG00000002994	ENSPNAG00000021775	ENSPNAG00000019728	ENSPNAG00000014619	ENSPNAG00000024574
Lepisosteus oculatus	Spotted gar	ENSLOCG00000008927	ENSLOCG00000006576	ENSLOCG00000007840	ENSLOCG00000018234	ENSLOCG00000013551
Seriola lalandi dorsalis	Yellowtail amberjack	ENSSLDG00000009729	ENSSLDG00000003612	ENSSLDG00000002119	ENSSLDG00000024277	ENSSLDG00000006439
Danio rerio	Zebrafish	ENSDARG00000099389	ENSDARG00000026348	ENSDARG00000093411	ENSDARG00000053571	ENSDARG00000098438

Table 2. Total transcription factor (TF) and transcription factor binding sites (TFBS) found in the fish under study.

Species	Common name	*cdo*		*csad*		*gad*		*ado*		*taut*	
		TF	TFBS	TF	TFBS	TF	TFBS	TF	TFBS	TF	TFBS
Poecilia formosa	Amazon molly	222	743	188	1135	233	919	137	404	179	472
Salmo salar	Atlantic salmon	184	965	198	533	173	526	201	746	189	441
Salmo trutta	Brown trout	201	649	191	502	189	606	216	643	190	541
Ictalurus punctatus	Channel catfish	164	385	214	795	211	685	228	723	219	1052
Cyprinus carpio	Common carp	262	968	224	783	198	618	213	937	213	545
Electrophorus electricus	Electric eel	227	963	179	778	132	309	203	624	212	729
Dicentrarchus labrax	European seabass	198	658	147	385	201	797	187	526	210	678
Takifugu rubripes	Fugu	222	652	183	544	223	494	259	956	222	506
Sparus aurata	Gilthead seabream	233	651	220	840	225	725	244	919	195	605
Carassius auratus	Goldfish	242	717	177	525	192	442	206	586	210	1064
Poecilia reticulata	Guppy	221	720	186	704	170	809	135	408	180	516
Oryzias latipes	Japanese medaka	211	1526	197	567	160	554	184	474	203	718
Cyclopterus lumpus	Lumpfish	189	458	204	754	223	854	251	1366	222	921
Oreochromis niloticus	Nile tilapia	226	953	220	472	198	736	285	688	202	675
Esox lucius	Northern pike	155	374	199	571	205	987	184	781	193	485
Oncorhynchus mykiss	Rainbow trout	186	557	241	951	197	631	217	651	199	508
Pygocentrus nattereri	Red-bellied piranha	189	494	230	680	211	904	206	505	225	652
Lepisosteus oculatus	Spotted gar	185	828	88	125	196	978	246	1048	189	543
Seriola lalandi dorsalis	Yellowtail amberjack	245	709	223	774	203	755	238	740	216	585
Danio rerio	Zebrafish	150	490	184	648	199	1064	196	866	219	815

of the TFs present in all species was also identified (Fig. 4). It can be observed that the frequencies of HLTF and HOXB3 in most of the species are higher than in the rest of the TFs. However, the frequency of the TFBS for spotted gar is really low.

Glutamate decarboxylase (*gad*)

The promoter region of *gad* was revised among 20 fish species, and 488 putative TFBS were found (Suppl. Mat. 1). The search retrieved that ALX3, Arid3a, BARHL2, BARX1, BSX, Dlx1, Dlx2, DRGX, EVX1, EVX2, GATA3, GATA5, HESX1, HLTF, HOXA6, HOXA7, HOXB3, HOXB6, HOXB8, LHX1, MEIS1, MEIS3, MIXL1, NFATC1, NFATC3, NFIX, NKX6-2, PRRX1, RAX, RAX2, RHOXF1, SHOX, Shox2, SOX15, Sox17, SRY, TLX2, UNCX, and ZNF354C were present in all studied species. Moreover, the TFBS frequency of these TFs is described in Figure 5, where it can be observed that HOXB3 in the studied promoter regions in the different fish species appears more frequently. In addition, Nile tilapia *Oreochromis niloticus* also presents high frequencies for EVX1, EVX2, and NKX6-2.

2-aminoethanethiol dioxygenase (*ado*)

In silico search for TFBS among 20 fish species, we retrieved 508 putative TFs (Suppl. Mat. 1). Our search found the following TFs to be present in all species: ALX3, Arid3a, ARNT::HIF1A, BARHL2, BARX1, BSX, Dlx1, Dlx2, Dlx3, DLX6, DRGX, ESX1, EVX1, EVX2, GATA3, GSX1, HESX1, HLTF, HOXA7, HOXB3, HOXB6, LHX1, MEIS1, MEIS3, MIXL1, NFATC1, NFIX, NKX6-2, OTX1, PRRX1, RAX, RAX2, SHOX, Shox2, SOX15, Sox17, TBX3, TLX2, UNCX, ZNF384. The frequency of the TFs can be observed in Figure 6. The frequency of HOXB3 in the promoter region of all species in the study was considerably higher than in the rest of the TFs. However, in species like seabass *Dicentrarchus labrax*, Japanese medaka *Oryzias latipes*, and red-bellied piranha *Pygocentrus nattereri*, the frequency of HLTF was higher than in HOXB3. Meanwhile, the frequency of ZNF384 TFBS for electric eel was higher than in the rest of the TFs.

Taurine transporter

The identification of TFBS among promoters' sequences of the gene *taut* for fish species gave back a total of 506 putative TFs (Suppl. Mat. 1). A total of at least 45 TFs are present in all fish species. These TFs are: ALX3, Arid3a, BARX1, BSX, Dlx1, Dlx2, Dlx3, DLX6, DRGX, ESX1, GATA3, GATA5, GSX1, GSX2, HESX1, HLTF, HOXA7, HOXB2, HOXB3, HOXB6, ISX, LBX2, LHX1, MEIS1, MEIS3, MIXL1,

Table 3. Putative transcription factors identified to appear only in each species of fish for the promoter sequence of cysteine dioxygenase (*cdo*), cysteine sulfonate decarboxylase (*csad*), glutamate decarboxylase (*gad*), 2-amino ethanethiol dioxygenase (*ado*), and taurine transporter (*taut*).

Species	Common name	*cdo*	*csad*	*gad*	*ado*	*taut*
Poecilia formosa	Amazon molly	ZBTB7C		SMAD3, SMAD5, TGIF2LY, Zic2, JUND(var.2)	POU4F3	ATF4, IRF2, JUN, SOX14, STAT1::STAT2, TAL1::TCF3
Salmo salar	Atlantic salmon	DUX4	HNF4A, HNF4G, HOXA13, MEF2A, OSR1, Znf423			CEBPA, MAX::MYC
Salmo trutta	Brown trout	ELK1, ELK3, ERF, ERG, ETS1, ETV3, FEV, FLI1, ZBTB33	ATF6, CREB3, CREB3L1, KLF15, XBP1, ZBTB18	RORA(var.2), RORC		CREB1, Hmx1, HSF1, HSF4, Klf1
Ictalurus punctatus	Channel catfish		FOXH1, HNF1A, HNF1B, MEF2C, Sox3, ZBTB6, ZNF148	Prdm15, ZNF148	TFAP2B(var.2), TFAP2E, YY1	CEBPG, Ebf2, JDP2, JUNB, Mecom, PHOX2B, TBX2, TBX4, TBX5
Cyprinus carpio	Common carp	ATOH7, BHLHE23, OLIG1, PBX3, Zic2	POU4F1, Pou5f1::Sox2	TBX1, TBX15, TBX18, TBX20	HOXA13, HOXD10, ZBTB18	ESR2, ESRRA, ESRRB, Nr5a2, POU3F3, RORA(var.2)
Electrophorus electricus	Electric eel	SREBF1	Crx, NFIC(var.2), NFIX(var.2), PBX1	NHLH1, NR1I2	DPRX, NR6A1, Plagl1, SOX21, ZNF740	NRF1, OSR1, TEAD1, TEAD4, ZFP57
Dicentrarchus labrax	European seabass	ESRRA, ESRRB, Esrrg, Nr5a2	DBP, FOXE1	Ebf2, EBF3, EGR1, HOXA13, HOXD13, PBX3, PKNOX1, SP1, SP2, SP4, SP9	Gfi1b, PROX1, SREBF1(var.2)	ZNF274
Takifugu rubripes	Fugu	MAFF, NR4A1, NRL, POU4F1, POU4F3, Rhox11, SOX12, ZFP57	USF1, USF2	CTCFL, ELF1, ELF5, ETV1, ETV5,,FOXC2, FOXD2, GABPA, HEY2, IKZF1, POU2F1, POU3F2, POU3F3, SCRT1, SCRT2, SPIB, SREBF1(var.2), TFEC	ETS2, FOXN3, HES2, NR2C2, PBX3, TFAP2A, TFAP2C(var.2), THRB	Creb3l2, ELK1, ELK4, ETS2, ETV3, HES2, HEY2, KLF11, SPDEF, TFEB, TFEC, ZBTB7A
Sparus aurata	Gilthead seabream	CTCFL, IRF2, NR1I2, SOHLH2	ETS2, RXRA::VDR, SPDEF, TFAP2B, TFAP2C, TFAP2C(var.2), TFAP2E, TFCP2	CEBPG, GCM1, GCM2, Wt1	CREM, MYBL1, ZBTB7C	RFX1
Carassius auratus	Goldfish	ATF7, CEBPG, Creb5, FOS::JUN(var.2), FOSB::JUN, FOSL1::JUN(var.2), JDP2(var.2), JUNB(var.2), STAT1, Stat4, Stat5a::Stat5b	ETV5, ETV6, ONECUT3	Crx, CUX2, ESR1, ESR2, PHOX2A, PHOX2B, PROP1, RELA, TFDP1	CEBPG(var.2), PROP1, SMAD3, SMAD5	MEF2A, RELB
Poecilia reticulata	Guppy		E2F6, E2F7, E2F8, GRHL2	SOX9	POU3F2	PBX3, PKNOX1, TCF7L1
Oryzias latipes	Japanese medaka	GFI1, Gfi1b, Hmx2, Hmx3, NR3C1, NR3C2, SP8	KLF4, Sox1	CEBPD, CEBPG(var.2), Sox3, TEF		SP1, TFAP2A(var.2), Wt1

Continuation

Species	Common name	*cdo*	*csad*	*gad*	*ado*	*taut*
Cyclopterus lumpus	Lumpfish	SMAD2::SMAD3::SMAD4	PKNOX1, POU3F1, POU4F3, Pparg::Rxra	Dmrt1, MYC, TFAP2B(var.2), TFAP2E, TFCP2	Lhx3, Nr2f6(var.2), ONECUT1, ONECUT2, PAX5, Rarg, RFX1, ZBTB6	HOXD10, KLF4, NR2C2, NR2F1(var.2),Pparg::Rxra, SP8, THRB
Oreochromis niloticus	Nile tilapia	GATA1::TAL1, POU3F1, Pou5f1::Sox2, POU5F1B, TEAD1, TEAD4	EHF, ELF1, ETV1, FOXC2, GABPA, GCM2, GFI1, KLF9, MEF2B, MEF2D, NKX2-2, SP8, STAT1, Stat4, Stat5a::Stat5b, Stat5b	GFI1, KLF4	BACH2(var.2), Crx, Mecom, TBX1, TBX15, TBX18, TBX20, TBX21, TCFL5, USF2, ZNF143	RELA, ZNF341
Esox lucius	Northern pike	CTCF, DMRTC2, Klf1	PAX3	DUXA, FOXN3		ONECUT2, ONECUT3, PBX1, TCF21(var.2)
Oncorhynchus mykiss	Rainbow trout	FOS::JUN, FOS::JUNB, FOS::JUND, FOSL1, FOSL1::JUN, IRF7, JUNB, JUND, TFAP2B(var.2)	BACH2, RFX1, RFX3, ZBTB7C	JUN		ATOH1(var.2)
Pygocentrus nattereri	Red-bellied piranha	HOXB9, HOXC12, HOXC9, HOXD12, KLF15, KLF16	STAT1::STAT2	FOSL1::JUND(var.2), TEAD1, TEAD4, TFAP2A	BACH2, BHLHE22, ELF1, ETV1, GABPA, IKZF1, OLIG3, SIX1	DBP
Lepisosteus oculatus	Spotted gar	RUNX2, ZBTB32	CTCF, CTCFL, Plagl1	OVOL2, TFAP2B(var.3)	CEBPA, CEBPB, CEBPD, CEBPE, CEBPG, FOXB1, MAX::MYC, ORA(var.2), RORC, SCRT1, SCRT2, ZNF449	FOSB::JUN, FOSB::JUNB(var.2), FOSL1::JUN(var.2), FOSL1::JUND(var.2), FOSL2::JUN(var.2), FOSL2::JUND(var.2), JUND(var.2)
Seriola lalandi dorsalis	Yellowtail amberjack	IRF6, KLF11, KLF6, SCRT1, TCF21(var.2), ZNF449		ONECUT2, ONECUT3, PLAGL2	HOXD13, MAFF, MAFG, NKX2-2, PLAGL2, Stat5a	
Danio rerio	Zebrafish	CREM, HES2, MXI1, TEF, TFEC	PROP1, YY2	BHLHE23, GATA1, OLIG1, OLIG2, SMAD2::SMAD3::SMAD4, TCF21(var.2)	POU2F2, POU2F3, Prdm15	

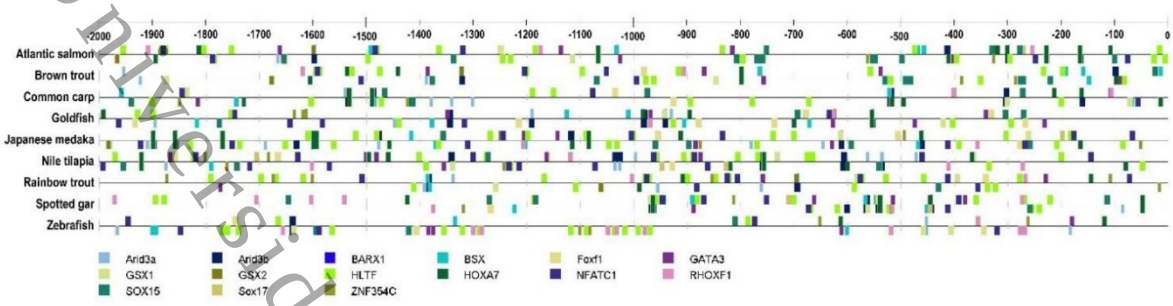

Figure 2. Top 15 putative transcription factors found in the promoter regions of *cdo* among some of the fish studied in the present review.

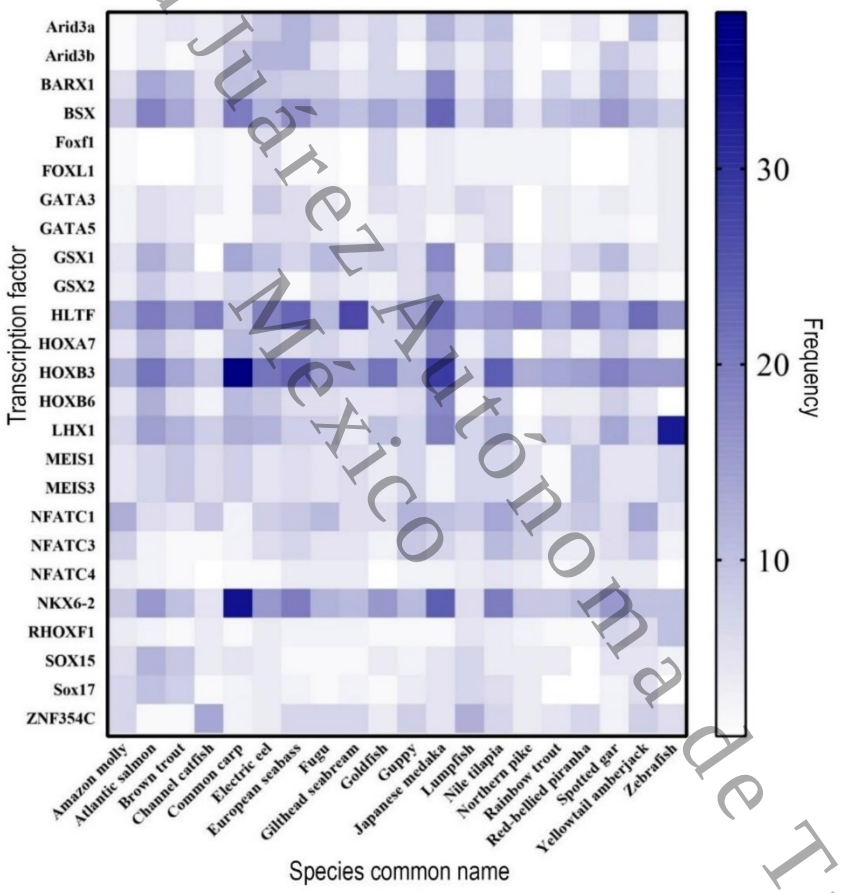

Figure 3. Frequency of transcription factors binding sites (TFBS) of transcription factors (TFs) found in the promoter region of *cdo* from fish species examined in the present study. Each column represents one species in study. The color of each cell is determined by the frequency of the TFBS in the promoter zone. Darker blue represents higher frequency within the promoter region of a TFBS of each TF and lighter blue represents low to absence of the TFBS.

NFATC1, NFATC3, NFATC4, NFIX, NKX6-2, NR2C1, PDX1, PRRX1, RAX, RAX2, SHOX, Shox2, Sox17, SRY, TLX2, UNCX, ZNF354C, ZNF384. The frequency of these TFs is graphed (Fig. 7). It can be observed that TFBS for HOXB3 is the most frequent for almost all species. However, ZNF384 in fugu presents higher frequencies in its promoter region.

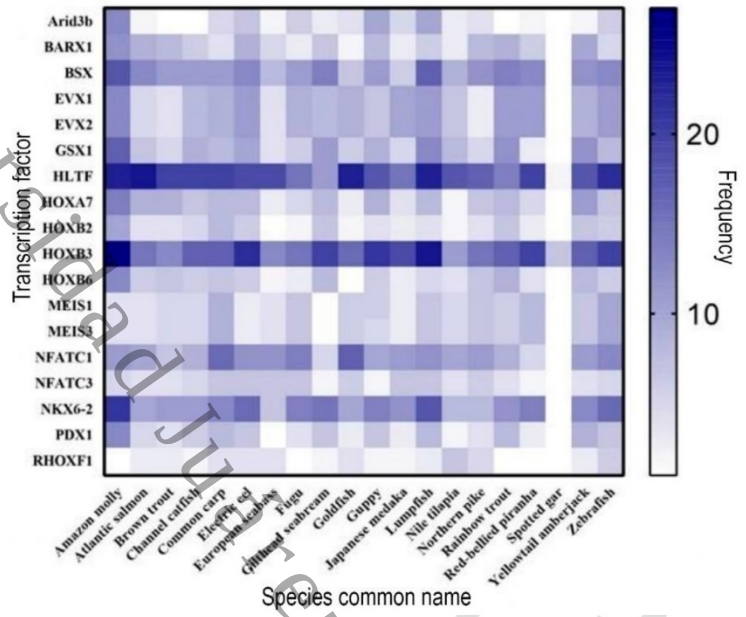

Figure 4. Frequency of transcription factors binding sites (TFBS) of transcription factors (TFs) found in the promoter region of *csad* from fish species examined in the present study. Each column represents one species in study. The color of each cell is determined by the frequency of the TFBS in the promoter zone. Darker blue represents higher frequency within the promoter region of a TFBS of each TF and lighter blue represents low to absence of the TFBS.

Identification of transcription factors among taurine biosynthesis and transport

In addition to identifying TFBS among species, the identification of putative transcription factors was compared among the genes involved in taurine biosynthesis. We found that binding sites for homeobox protein BarH-like 1 (BARX1), brain-specific homeobox protein homolog (BSX), helicase-like transcription factor (HLTF), homeobox protein Hox-A7 (HOXA7), homeobox protein Hox-B3 (HOXB3), homeobox protein Hox-B6 (HOXB6), homeobox protein Meis1 (MEIS1), homeobox protein Meis3 (MEIS3), nuclear factor of activated T cells 1 (NFATC1), and homeobox protein Nkx-6.2 (NKX6-2) were commonly found in the promoter regions of genes involved in taurine transportation and biosynthesis.

DISCUSSION

In the present study, a literature review was conducted to identify the major physiological roles played by genes involved in taurine transportation and biosynthesis in fishes. Taurine biosynthesis depends on each species' production capacity (Sampath et al. 2020). Thus, it has been reported that physiological damage can occur when this capacity is limited, or taurine is not being acquired from food sources (Li et al. 2022). This limited capacity has been identified in some species of the family Labridae, Scombridae, Soleidae, and Rajidae because of CSAD's lack of activity (Salze & Davis 2015). Meanwhile, the disruption of *csad* and *taut* has identified cardiac anomalies in zebrafish (Kozlowski et al. 2008, Chang et al. 2013, Park et al. 2014) as has been observed in other vertebrates such as mice, when elimination of *cdo* has been accomplished (Ueki et al. 2011).

Additionally, the identification of TFs and TFBS in the promoter regions of different fish species was also investigated to have a first look into the identification of putative TFs that might be regulating genes involved in the biosynthesis and transport of taurine due to the lack of information regarding this issue. Regulatory elements, such as TFs, play a key role in the regulation of genes, inducing or repressing their expression (Tellechea-Luzardo et al. 2023), while the combination of the TFBS with the promoter determines the condition of the gene expression (Lu & Rogan 2018). Therefore, our results showed that a wide distribution of TFs and TFBS are present in the promoter zone of the different fish studied. It was observed that a set of

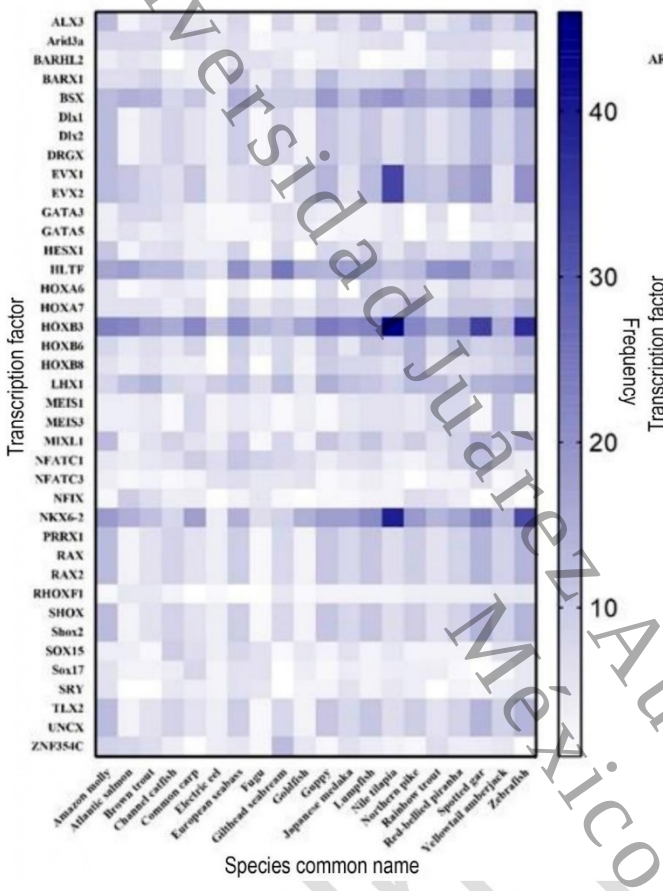

Figure 5. Frequency of transcription factors binding sites (TFBS) of transcription factors (TFs) found in the promoter region of *gad* from fish species examined in the present study. Each column represents one species in study. The color of each cell is determined by the frequency of the TFBS in the promoter zone. Darker blue represents higher frequency within the promoter region of a TFBS of each TF and lighter blue represents low to absence of the TFBS.

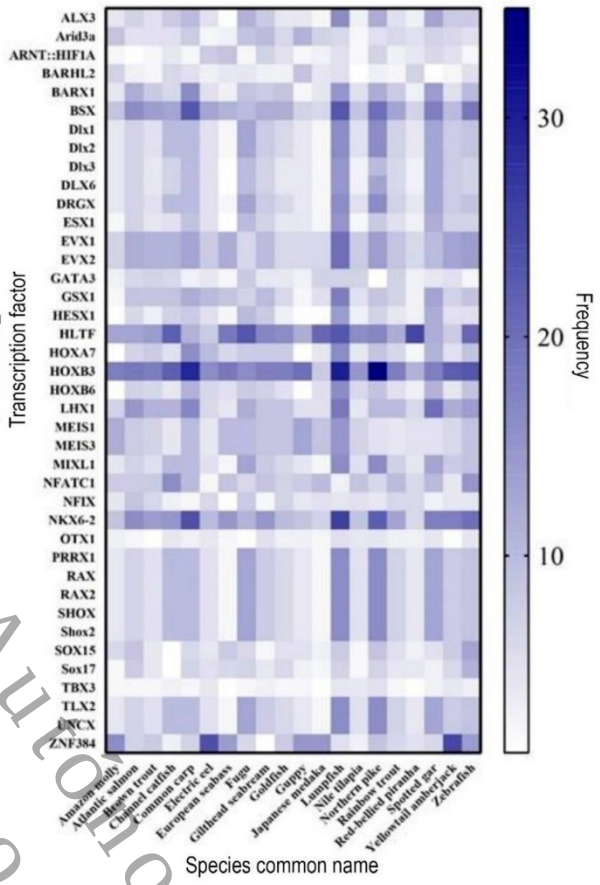

Figure 6. Frequency of transcription factors binding sites (TFBS) of transcription factors (TFs) found in the promoter region of *ado* from fish species examined in the present study. Each column represents one species in study. The color of each cell is determined by the frequency of the TFBS in the promoter zone. Darker blue represents higher frequency within the promoter region of a TFBS of each TF and lighter blue represents low to absence of the TFBS.

TFs is shared among species depending on the gene in the study. Nonetheless, it was also observed that in the case of zebrafish, some TFs are not shared with the other species for the promoter region of *cdo*. It was also noted that the promoter region of spotted gar for the gene *csad* was not completed as the rest of the species in the database Ensembl, limiting the recognition of TFs for this species.

There were the following shared set of TFs present in the promoter zone of the genes *cdo*, *csad*, *gad*, *ado*, and *taut* of all the fish analyzed: homeobox protein BarH-like 1 (BARX1), brain-specific homeobox protein homolog (BSX), helicase-like transcription factor (HLTF), homeobox protein Hox-A7 (HOXA7), homeob ox protein Hox-B3 (HOXB3), homeobox protein Hox-B6 (HOXB6), homeobox protein Meis1 (MEIS1), homeobox protein Meis3 (MEIS3), nuclear factor of activated T cells 1 (NFATC1), and homeobox

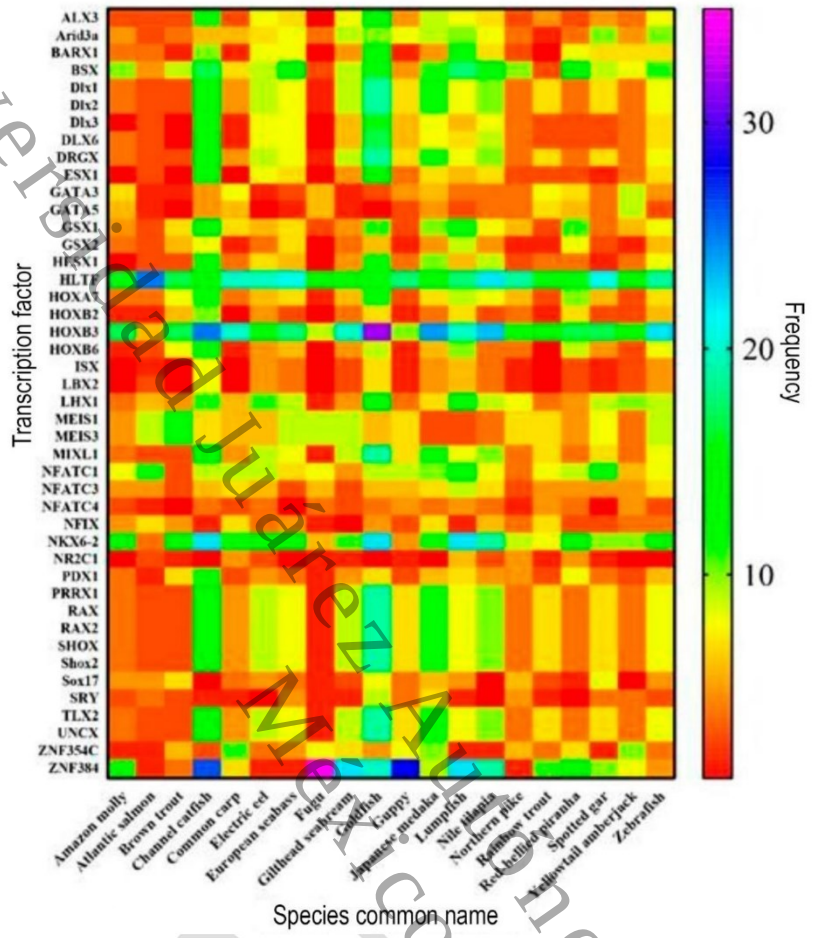

Figure 7. Frequency of transcription factors binding sites (TFBS) of transcription factors (TFs) found in the promoter region of *taut* from fish species examined in the present study. Each column represents one species in study. The color of each cell is determined by the frequency of the TFBS in the promoter zone. Purple represents higher frequency within the promoter region of a TFBS of each TF and red represents low to absence of the TFBS.

protein Nkx-6.2 (NKX6-2). These TFs have been described to play an important role in normal development, immunity, and the regulation of transcription by RNA polymerase II (Guner & Karlstrom 2007, Lyon et al. 2013, Uribe & Bronner 2015, Lu et al. 2019, Rittgers et al. 2021, Torres et al. 2023, Wan et al. 2023)

As mentioned in the introduction, other factors can affect the biosynthesis of taurine in fishes, such as species, type of tissue, stage of development, eating habits, and the environment in which they live (Haga et al. 2015, Salze & Davis 2015, Sampath et al. 2020), thus also affecting the regulation of the TFs that bind to the DNA sequence of different promoters zone. In this sense, the recent advantages in the sequencing of genomes and transcriptomes, as well as the availability of resources for the free use of different databases (i.e. NCBI, Ensembl), seems to be the following path to finding more about the mechanism and regulation of taurine biosynthesis. Furthermore, using bioinformatic and molecular tools to measure and analyze the expression levels of RNA should also be applied.

CONCLUSIONS

Taurine biosynthesis and taurine transportation genes regulate endogenous taurine availability in fish species. Our literature review suggests that the lack or null expression of the main key genes in the synthesis or transportation of taurine could affect fish physiologically, particularly when a disruption, silencing, or elimination of *cdo* or *taut* occurs. Additionally, our in

silico approach to determining putative TFs suggests that the following are shared among the promoter region of all study species in genes implicated in taurine transportation and biosynthesis: homeobox protein BarH-like 1 (BARX1), brain-specific homeobox protein homolog (BSX), helicase-like transcription factor (HLTF), homeobox protein Hox-A7 (HOXA7), homeobox protein Hox-B3 (HOXB3), homeobox protein Hox-B6 (HOXB6), homeobox protein Meis1 (MEIS1), homeobox protein Meis3 (MEIS3), nuclear factor of activated T cells 1 (NFATC1), and homeobox protein Nkx-6.2 (NKX6-2). However, it is necessary to recall that it has also been found that each species has its regulatory elements and that further work regarding how these TFs regulated taurine synthesis needs to be further investigated.

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SUPPLEMENTARY MATERIAL

Supplementary Material 1. Complete CiiiDER analysis results of transcription factors and transcription factor binding sites in promoter region of the genes cysteine dioxygenase (*cdo*), cysteine sulfonate decarboxylase (*csad*) promoters, glutamate decarboxylase (*gad*), 2-amino ethanethiol dioxygenase (*ado*), taurine transporter (*taut*) of different fish species.

Capítulo 3

Identification and expression analysis of transcripts involved in taurine biosynthesis during early ontogeny of Tropical gar *Atractosteus tropicus*

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Identification and expression analysis of transcripts involved in taurine biosynthesis during early ontogeny of tropical gar *Atractosteus tropicus*

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ABSTRACT

In fishes, the availability of taurine is regulated during ontogenetic development, where its endogenous synthesis capacity is species dependent. Thus, different pathways and involved enzymes have been described: pathway I (cysteine sulfinate-dependent pathway), cysteine dioxygenase type 1 (*cdo1*) and cysteine sulfinic acid decarboxylase (*csad*); pathway II (cysteic acid pathway), *cdo1* and glutamic acid decarboxylase (*gad*); and pathway III (cysteamine pathway), 2-aminoethanethiol dioxygenase (*ado*); whereas taurine transporter (*taut*) is responsible for taurine entry into cells on the cell membrane and the mitochondria. This study determined if the tropical gar (*Atractosteus tropicus*), an ancient holostean fish model, has the molecular mechanism to synthesize taurine through the identification and analysis expression of transcripts coding for proteins involved in its biosynthesis and transportation, at different embryo-larvae stages and in different organs of juveniles (31 dah). We observed a fluctuating expression of all transcripts involved in the three pathways at all analyzed stages. All transcripts are expressed during the beginning of larval development; however, *ado* and *taut* show a peak expression at 9 dah, and all transcripts but *csad* decreased at 23 dah, when the organism ended the larval period. Furthermore, at 31 dah, we observed *taut* expression in all examined organs. The transcripts involved in pathways I and III are expressed differently across all organs, whereas pathway II was only observed in the brain, eye, and skin. The results suggested that taurine biosynthesis in tropical gar is regulated during its early development before first feeding, and the pathway might also be organ-type dependent.

1. Introduction

Taurine is an amino sulfonic acid that exists naturally in mammals, birds, fish, and invertebrates, and it can be found in different tissues and organs such as the heart, retina, skeletal muscle, brain, intestine, as well as in plasma and blood cells. This amino acid is involved in a wide variety of functions, such as the formation of bile salts, in the skeletal muscle development, the formation of the retina and the brain (El-Sayed, 2014; Huxtable and Sebring, 1986; Schaffer et al., 2010). The principal roles of taurine are osmoregulation, cell membrane stabilization, and myocardial contractility (Huxtable, 1992; Lambert et al., 2015; Schaffer et al., 2000).

In fishes, taurine requirement is specific for each species and can be taken exogenously from feed or synthesized endogenously (Chang et al.,

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2013; Salze and Davis, 2015). In marine species, taurine supplementation positively affects growth, survival, and morphological development, especially if they are carnivorous species (Matsunari et al., 2013; Salze et al., 2012). Freshwater species as zebrafish *Danio rerio* have a better ability to synthesize taurine and have shown that its supplementation in the diet could not be required for better performance (Guimarães et al., 2018). However, most of these studies have focused on diet supplementation and have not described how biosynthesis is achieved during early development. Hence, further work on describing how taurine biosynthesis plays a key role during ontogenetic development in fishes needs to be accomplished.

Three different pathways of taurine biosynthesis have been described in fishes (Fig. 1). The transcripts involved in these different pathways are 1) The pathway I (cysteine sulfinate-dependent pathway), cysteine dioxygenase type 1 (*cdo1*) and cysteine sulfinic acid decarboxylase (*csad*), 2) the pathway II (cysteic acid pathway), *cdo1*, and glutamic acid decarboxylase (*gad*), and 3) the pathway III (cysteamine pathway), 2-aminoethanethiol dioxygenase (*ado*). Taurine transporter (*taut*), also known as Sodium- and chloride-dependent taurine transporter (slc6a6), is the leading transporter of produced taurine during biosynthesis mainly in the liver and brain (Baliou et al., 2020; Han et al., 2006; Kozlowski et al., 2008). Different studies report that cysteine sulfinic acid decarboxylase (CSAD) is the limiting enzyme in taurine biosynthesis, which means the higher CSAD activity, the greater the amount of taurine available in the system (Chang et al., 2013; Haga et al., 2015). The predominant pathway will depend on the species, type of tissue, stage of development, feeding habits, and the environment in which they live (El-Sayed, 2014; Salze and Davis, 2015; Sampath et al., 2020; Wang et al., 2016), such as in Japanese yellowtail *Seriola quinqueradiata* were hepatic CSAD activity in juveniles was not present (Yokoyama et al., 2001) or Atlantic bluefin tuna *Thunnus thynnus* (Betancor et al., 2019) were the expression of genes involved in taurine biosynthesis has been proved to increase during development. Nonetheless, there is no information regarding the different pathways or transcripts involved in taurine synthesis in the Lepisosteiformes order.

Previous studies had emphasized the importance of the Lepisosteidae family as a basal group that diverged from the rest of teleost fishes before the duplication of its genome when making specific studies to understand genes in molecular mechanisms of vertebrates during evolution (Eames et al., 2012; Grone and Maruska, 2015; Song et al., 2013). These mechanisms have been fully verified with advances in sequencing technologies, including the annotation of the genome of *Lepisosteus oculatus* (Braasch et al., 2016) and the transcriptome of adults *Atractosteus tropicus* (Cribbin et al., 2017). This study allows teleosts to connect to human biology since their lineage represents the sister group of unduplicated teleosts (Braasch et al., 2016). Thus, considering that lepisosteiformes are a precious group to understand the mechanisms of molecular evolution of vertebrates, thanks to their ecological and evolutionary proximity to teleosts, *A. tropicus* being a species belonging to this group, is an ideal candidate to study in a molecular level the mechanisms of taurine biosynthesis. Therefore, the present study aimed to investigate if the expression of transcripts coding for proteins involved in taurine biosynthesis and its transportation is present during *A. tropicus* early ontogeny and in different organ types of juveniles to better understand the taurine molecular mechanism in this ancestral fish.

2. Methods

2.1. Ethics statement

All procedures of fish manipulation, including euthanasia methods, followed the Official Mexican Standards NOM-062-ZOO-1999, and the ethical rules for zebrafish and other tropical finfish by the American Veterinary Medical Association (2013).

2.2. Fish rearing

A total of 600 fertilized eggs of *A. tropicus* were obtained from an induced breeding spawning at the Laboratory of Physiology in Aquatic Resources (LAFIRA) of División Académica de Ciencias Biológicas (DACBiol) of the Universidad Juárez Autónoma de Tabasco (UJAT), México. The maintenance and feeding conditions of broodstock were according to Márquez-Couturier et al. (2006). The spawning was induced with an intraperitoneal injection of LHRHa hormone (35 μg kg^{-1}) using a single female (3.5 kg) kept with three males (1.5 kg) for mating in a 2000 L circular tank. After spawning (16 h post-hormonal induction), the eggs adhered to plastic yarn material deployed as an artificial substrate, and the adults were removed from the tank; hatching was reached 2 days after spawning. Larvae were placed in three 70 L circular plastic tanks (200 larvae per tank) coupled to a 1500 L recirculation system equipped with a solids depositor, a biological filter, a silica sand filter (Hayward S166T, Delavan, WI), and two thermostatic heaters (Eheim Jäger 300 W, 3619010, Deizisau, Germany). Water quality was monitored until organisms reached 31 days after hatch (dah), where temperature (28.0 ± 0.6 °C), dissolved oxygen (5.9 ± 0.6 mg/L), and pH (7.2 ± 0.2) were recorded by an oximeter (YSI 85, Yellow Springs, OH) and a pHmeter (HANNA HI 991001, Woonsocket, RI). A photoperiod time of 12 h light / 12 h darkness was maintained. The organisms were fed five times a day (8:00, 11:00, 13:00, 15:00, and 18:00 h) since the absorption of the yolk (5 dah) with a combination of *Artemia* nauplii. (Biogrow, ProAqua, México) and trout feed (TF, 52% protein and 16% lipids, El Pedregal® SilverCup, Toluca, México) during the first 10 days, *Artemia* nauplii were provided daily starting with a density of 50 individuals per larvae at 5 dah and increasing in accordance to the consumption per day until the withdrawal. The gradual

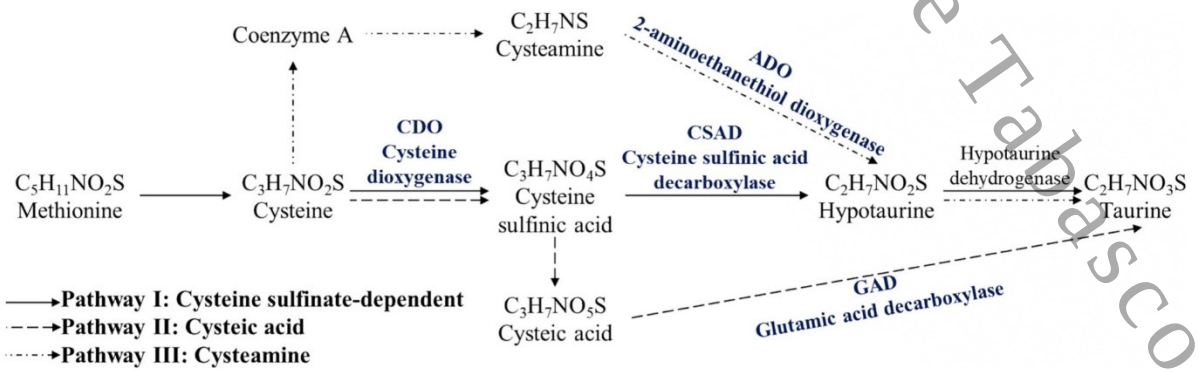

Fig. 1. Pathways of taurine biosynthesis. Adapted from Salze and Davis, 2015.

withdrawal of *Artemia* started from 12 to 14 dah, and from 15 to 30 dah, the organisms were fed only with trout feed. Particle sizes used for feeding were 250–500, 500–750, and > 750 μm in accordance with the mouth size. Organisms were fed to apparent satiation, which was stablished as the point where organism stop approaching pellets after a second round of food consumption in each meal.

2.3. Ontogenetic development and organs distribution of taurine biosynthesis transcripts

Whole organisms of *A. tropicus* were sampled and collected at 0 (embryo), 1 (eleuteroembryo), 3 (mouth opening), 5 (first feeding), 7, 9, 15, 17, and 23 dah. Developmental stages corresponding to sampling points were selected in accordance with Martínez-Burguete et al. (2021). Furthermore, samples collected at 17 and 23 days were added to complement the larval stage and observe the fluctuation of taurine biosynthesis transcripts when the larvae were fed only with TF. Ten organisms (embryos or lavae) collected from each experimental unit were rinsed with distilled water and then transferred to microtubes that contained 1.0–1.5 mL (0, 1, and 3 dah), 2.5–3.0 mL (5, 7, and 9 dah), and 4.0–4.5 mL (15, 17 and 23 dah) of RNALater (Life Technologies, Carlsbad, CA, USA). The samples were maintained at room temperature for 24 h before placing at −80 °C, according to the supplier's instructions, until analysis.

A total of three organisms per replicate (average weight 554.4 ± 68.3 mg and 57.5 ± 2.9 mm total length) at 31 dah (juveniles) were dissected. Individual organs (liver, brain, eye, gills, skin, muscle, intestine, and stomach) were collected and maintained as mentioned above for larvae. A period of 18 h of fasting was considered before the collection of samples from 7 to 31 dah. All larvae and juvenile were euthanized by a cold thermal shock until loss of orientation and subsequent operculum inactivity before preservation.

2.4. Sequence analysis

The nucleotide and amino acid (AA) sequences of *cdo1, csad, gad, ado*, and *taut*, as well as the reference genes ribosomal protein L8 (*rpl8*) and elongation factor 1 alpha 1 (*ef1a1*), were retrieved from a comprehensive transcriptome assembly (Martínez-Burguete et al., 2021) using as reference the proteins reported for *L. oculatus* in NCBI (https://www.ncbi.nlm.nih.gov/). The obtained AA sequences from the *A. tropicus* transcriptome were compared using MatGAT2.02 software (Campanella et al., 2003) to know the similarity and identity with orthologs sequences from other species. Phylogenetic and molecular evolutionary analyses were conducted using the amino acid sequences of different orthologs using neighbor-joining (NJ) methods, based on the AA sequence using MEGA version 10.2.5 through 1000 bootstrap iterations. In the construction of trees, cephalochordate orthologues from the genus Branchiostoma were used as outgroups since they represent the closest ancestor of vertebrates (Huang et al., 2014). Sequence orthologs were retrieved from the Ensembl (https://www.ensembl.org/) and NCBI databases.

2.5. RNA extraction and cDNA synthesis

The total RNA of three organisms of each of the samples (pool samples per experimental unit) were isolated using TRIzol (Invitrogen, Life Technologies California, USA). Each RNA was treated with DNAse I (Promega) to eliminate contamination by genomic DNA. The concentration and purity of the RNA were measured by the 260/280 ratio in a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). Additionally, each RNA was inspected on a 1.2% agarose gel using an aliquot of 1 μL of sample mixed with 1 μL of run buffer for each of the samples. The reverse transcription into cDNA was performed using the Maxima First Strand cDNA Synthesis Kit (BioRad, Irvine, CA, USA) from 1 μg of total RNA according to the manufacturer's protocol. The cDNA generated was stored in aliquots at −20 °C until use.

2.6. Quantitative polymerase chain reaction analysis

From the sequences in Section 2.4, the specific primer pairs for each transcript were designed using Primer3web version 4.1.0 (Untergasser et al., 2012). The specific primers of each transcript are given in Table 1. The corresponding quantitative polymerase chain reaction (qPCR) analysis of each of the transcripts was carried out following the recommendations of Hellemans et al. (2007), and Bustin et al. (2009). The qPCR reactions, standardization of the alignment temperature, and the standard curves of each pair of primer were made in a CFX96 TouchTM Real-Time Detection System (BioRad). The qPCR reaction mixture contained 5 μL of qMix, 2 μL cDNA, and 0.46 μM of each primer and adjusted to 10 μL using DEPC-treated water. A pool of cDNA corresponding to a mixture of all samples was used as a template for the standardization of temperature and standard curves. The thermal program for standardization included 3 min at 95 °C of denaturalization, followed by 39 cycles at 95 °C for 10 s and 72 °C for 1 min with four different temperatures for amplification (60, 58.7, 55.2, and 52.5 °C), and extension at 72 °C for 5 s. Duplicated standard curves of each pair of primers were generated to determine the efficiency. These curves were carried out by five serial dilutions analysis corresponding to pool cDNA from all samples. The thermal program for standard curves and qPCR analysis was the same as above but with only one temperature alignment (60 °C). The efficiency value and calculation of relative expression were calculated according to Escalante-Rojas et al. (2018) using $RQ = (1 + E)^{(Cqmin - Cq)}$ and $RE = RQ_t/RQ_{rf}$ equations. Where: RQ = relative quantity of each gene; E = specific efficiency of each gene; Cqmin – Cq = absolute difference for each Cq sample against the lowest Cq in the data for each gene; RE = Relative expression; *t* = target gene; *rf* = reference gene. All samples were carried out in triplicate. Primers design for reference gene *ef1a1* were not suitable in the present study and only *rpl8* was used as a reference.

Table 1
Sequences and other features of primers used for this study.

Primer name	Primer sequence (5′-3′)	Product size	Efficiency %	R^2
CDO	F: AGGTCATGGCAGCAGTATCC R: CTGTGCAGGCCAATGGAATC	189	89.7	0.9999
CSAD	F: TTCCTGGTGAGTGCTACCTC R: TGAGTCTGCCCTCTCAATCC	180	92.5	0.986
GAD1	F: TCACTGAAGGAACGCCAGTC R: TGCATGGTCGGTTCTTCTCC	149	88.2	1
ADO	F: ACGCAATCTCACGCAACTG R: TCCCGAGGCATTATCTGTGG	77	103.9	0.994
TAUT	F: AGAGCGGATTAAGGCAGTGG R: CAGGCCGTTGTTCCCATTTG	121	108.6	0.991
RPL8	F: TGTGCTGCCTGGAAGAGAAG R: TTTCGGGGTTGTGGGAGATG	90	99.82	0.9999
EF1a1	F: CCGACACTGTGGCTTTTGTG R: TCCAGCCCTTGAACCATGTC	90	–	–

2.7. Statistical analysis

The relative expression of *cdo1, csad, gad, ado* and *taut* among different dah and organ type of *A. tropicus* were analyzed by one-way ANOVA with a Tukey's test for post hoc comparisons (significance value of $P < 0.05$) after validating the normality (Kolmogorov-Smirnov test) and homoscedasticity (Levine test). All tests were performed using the software GraphPad Prism version 9.3.0 (San Diego, CA, USA).

3. Results

3.1. Sequence analysis

For identifying homologous proteins involved in taurine biosynthesis and reference genes used in this study, sequences belonging *to* L. *oculatus* were aligned (BLASTP) against the transcriptome reported by Martínez-Burguete et al. (2021). Using contigs identifiers of the resulting proteins, nucleotides sequences were also searched. The resulting sequences of the BLAST analysis for proteins and nucleotides are shown in Supplementary_file_1. Sequences presenting possible different isoforms were aligned using MultAlin (Corpet, 1988) to observe if protein sequences were different (see Supplementary_file_1). Full length nucleotide and deduced amino acid sequence of transcripts involved in taurine biosynthesis and reference genes found by bioinformatic search corresponding to *A. tropicus* transcriptome were registered in the NCBI GenBank (Table 2; https://submit.ncbi.nlm.nih.gov/about/bankit/).

The different amino acid sequence comparison against other species using MatGAT showed a high similarity to L. *oculatus* and *A. tropicus*. However, similarities with other species were found depending on the analyzed sequence (Table 3; see Supplementary_file_2 for complete results). The sequence of *gad* showed a high similarity to *Gallus gallus*. However, *Branchiostoma belcheri* has no similarity because *gad* and *taut* are not present in its genome. Therefore, we used *Branchiostoma floridae* as an outgroup for tree construction of *gad*. Unusually, any taurine biosynthesis transcripts were identified in *Oreochromis mossambicus*. On the other hand, *Anguilla japonica* has not *gad* and *ado* in the available databases.

3.2. Phylogenetic analysis

Phylogenetic trees of *cdo1*, *csad*, *gad*, *ado*, and *taut* were constructed to evaluate the molecular evolutionary relationship against orthologs sequences of other species (see Supplementary_file_3). By using the phylogenetic analysis, we found that in all sequences a separation between mammals and fish occurs. Hence, as an ancestral fish, *A. tropicus* forms a clade with L. *oculatus* in all sequences. Nevertheless, as a member of the Lepisosteidae family, this also occurs with *Atractosteus spatula* for *cdo1* and *ado* sequences. We also found that *A. tropicus*

Table 2
Characteristics of retrieve sequences from *A. tropicus* transcriptome.

Transcript name	Accession number	Sequence size	
		transcript (bp)	ORF (AA)
Cystein dioxygenase type 1 (*cdo1*)	MN389565	1930	201
Cystein sulfinic acid decarboxylase (*csad*)	MN389567	1944	513
Glutamic acid decarboxylase (*gad*)	MN389568	1900	506
	MN389569	3372	592
		3502	565
		3356	
2-aminoethanethiol dioxygenase (*ado*)	MN389566	1520	255
Taurine transporter (*taut*)	MN389570	6117	625
		6011	
Ribosomal protein L8 (*rpl8*)	MN389571	848	257
Elongation factor (*ef1a1*)	MN389572	2036	463

Table 3
Top 5 amino acid comparison of sequences participating in taurine biosynthesis and taurine transportation of *Atractosteus tropicus* and other species. Cysteine dioxigenase type 1 (*cdo*), cysteine sulfinic acid decarboxylase (*csad*), glutamic acid decarboxylase (*gad*), 2-aminoethanethiol dioxygenase (*ado*) and taurine transporter (*taut*). Similarity (%) = S (%); Identity (%) = I (%). Complete comparison of similarity and identity can be found in Supplementary_file_2.

	Cdo			Csad			Gad			Ado			TauT	
Species	S (%)	I (%)	Species	S (%)	I (%)	Species	S (%)	I (%)	Species	S (%)	I (%)	Species	S (%)	I (%)
Lepisosteus oculatus (XP_006626919.1)	100	99.5	*Lepisosteus oculatus* (XP_006629342.2)	92.4	91.5	*Lepisosteus oculatus* (XP_006636584.1)	99.8	99.7	*Atractosteus spatula* (MBN33231671)	99.6	99.6	*Lepisosteus oculatus* (XP_015203627.1)	100	100
Atractosteus spatula (MBN33234731)	97	96	*Anguilla japonica* (BAL222277.1)	87.1	79.4	*Latimeria chalumnae* (XP_006603035.1)	97.5	89.2	*Lepisosteus oculatus* (XP_006630446.1)	99.2	99.2	*Lepisosteus oculatus* (XP_006631061.1)	99.4	99.4
Anguilla japonica (BAL222276.1)	94	84.1	*Astyanax mexicanus* (XP_022532784.1)	84.4	72.8	*Gallus gallus* (NP_990244.1)	96.5	90	*Salmo salar* (XP_014060776.2)	83.1	69.9	*Anguilla japonica* (BAM11116.1)	96.8	93.8
Astyanax mexicanus (XP_007260847.1)	92	81.6	*Salmo salar* (XP_014009644.1)	84.2	72.6	*Chelonia mydas* (XP_007056648.1)	95.9	89.9	*Danio rerio* (NP_998358.1)	81.6	67.1	*Cyprinus carpio* (BAA89537.1)	95.8	92.8
Oreochromis niloticus (XP_003451108.4)	92	84.6	*Oncorhynchus mykiss* (XP_021463770.2)	83.6	72.6	*Mus musculus* (NP_032103.2)	94.9	88.4	*Latimeria chalumnae* (XP_005997771.1)	80	66.4	*Salmo salar* (NP_001133272.1)	95.8	92.3

orthologs groups are closely related with *Anguilla japonica* and primitive fish *Latimeria chalumnae* (see Supplementary_file_3).

3.3. Relative expression of taurine biosynthesis transcripts during early stages

Relative expression of transcripts involved in taurine biosynthesis (*cdo1*, *csad*, *gad*, and *ado*) was performed in whole fish during early ontogeny of *A. tropicus* from 0 to 23 dah ($P < 0.05$) (Fig. 2). The relative expression of all transcripts was detected at hatch (0 dah) and was broadly expressed in all ages ($P < 0.05$) (Fig. 2). Transcript expression levels of *cdo1* were barely detected at the embryo stage and showed a significant difference with the rest of the developmental stages. ($P < 0.05$) (Fig. 2A). The highest expression level of *cdo1* is observed in 3 dah and then decreased and a second expression peak is observed at 9 dah before decreasing until 23 dah ($P < 0.05$) (Fig. 2A). Meanwhile, *csad* has increasing expression levels from 0 to 5 dah where the highest expression occurs, and then fluctuates from 7 to 23 dah ($P < 0.05$) (Fig. 2B). Two expression peaks were also identified for *gad* at 3 and 17 dah ($P < 0.05$) (Fig. 2C). High expression levels of *ado* were detected at the beginning of the ontogeny at the embryo stage (0 dah) followed by a decreased until 5 dah ($P < 0.05$) (Fig. 2D). However, an increased was notably observed at 9 dah, where the highest expression peak was detected, and then expression levels drops again at 15 dah, followed by an increased at 17 dah ($P < 0.05$) (Fig. 2D).

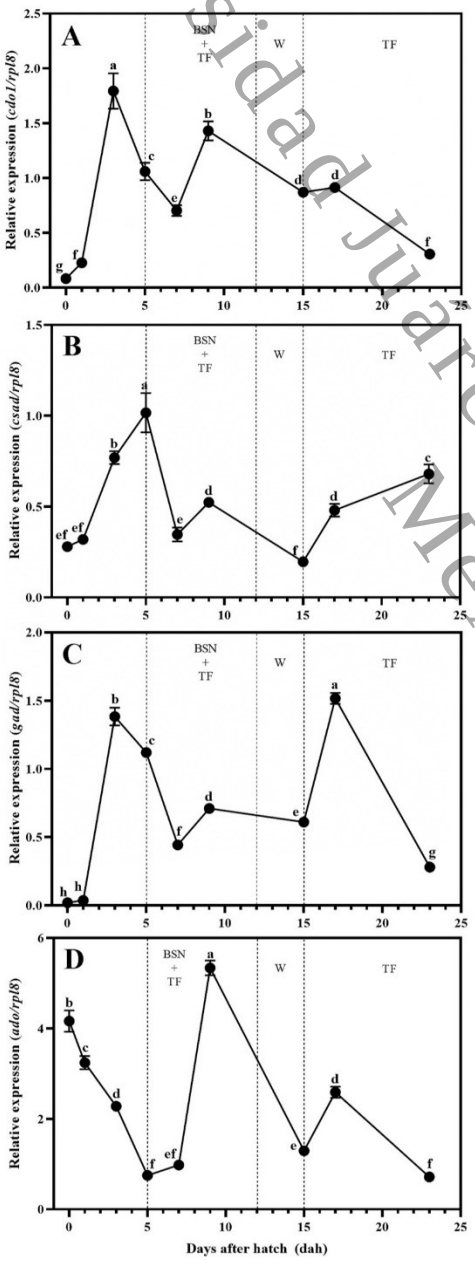

Fig. 2. Relative expression profiles of A) *cdo*, B) *csad*, C) *gad*, and D) *ado* (normalized with the expression of *rpl8*) during embryo and larvae stages of *A. tropicus*. Values in graph are represented by means ± SD (n = 3). Different letters indicate a significant difference ($P < 0.05$). BSN, brine shrimp nauplii; TF, Trout food; W, withdrawal.

3.4. Relative expression of transcripts involved in taurine biosynthesis in different organ types

Relative expression was carried out in different organ types of 31 dah *A. tropicus* juveniles. As shown in Fig. 3, transcripts involved in the different taurine pathways showed a variability organ distribution depending on the tested transcript. The highest relative expression of *cdo1* was observed in the intestine, followed by the liver, muscle, eye, stomach, gill, and skin, meanwhile, the lowest expression was found in the brain ($P < 0.05$) (Fig. 3A). In contrast, the main *csad* expression level was detected in the liver, followed by the skin, muscle, and intestine, with very low expression levels in the gill and eye, whereas expression in the brain and stomach were not detected at all (Fig. 3B). Moreover, the highest expression levels of *gad* were detected in the eye followed by brain; very low expression was observed in the skin (Fig. 3C). The highest expression levels of *ado* were detected in the eye, followed by the liver and skin, while lower expression levels were detected in the brain, intestine, gill, muscle, and stomach (Fig. 3D).

3.5. Relative expression of taurine transport during early stages and in different organ types

The qPCR results showed that *taut* is ubiquitously present during the early age of *A. tropicus*. The transcript abundance level of *taut* is first detected at embryo (0 dah), and has a first expression peak at 1 dah, followed by low expressions at 3, 5 and 7 dah (Fig. 4A). The highest transcript abundance of *taut* occurs at 9 dah and then drops at 15 dah and has a third expression peak at 17 dah, finalizing with a decrease at 23 dah ($P < 0.05$) (Fig. 4A).

The distribution of *taut* in different organ types was broadly distributed. The highest expression levels of *taut* were found in the eye followed by skin, gill, and intestine, and were barely detected in the liver, muscle, brain, and stomach ($P < 0.05$) (Fig. 4B).

3.6. Taurine pathway prediction according to age and organ type

Based on the heat map, fluctuation of the different transcripts involved in the three taurine pathways occurs at all developmental stages and in most organ types in *A. tropicus* (Fig. 5). According to the results, transcription levels of *csad*, which participates in the cysteine-sulfinate-dependent pathway mainly occur at 5 dah, while the transcription levels of the *gad* (cysteic acid pathway) is predominate at 3 and 17 dah. However, the transcript expression levels of *ado*, which participates in the cysteamine pathway, seems to be more modulated than the rest of the transcripts (Fig. 5A). Moreover, in Fig. 5B, we can visualize that the principal transcript involved in the different pathways appears to be organ dependent. This is the case of high levels of *csad* of the cysteine-sulfinate-dependent pathway majorly occurring in the liver,

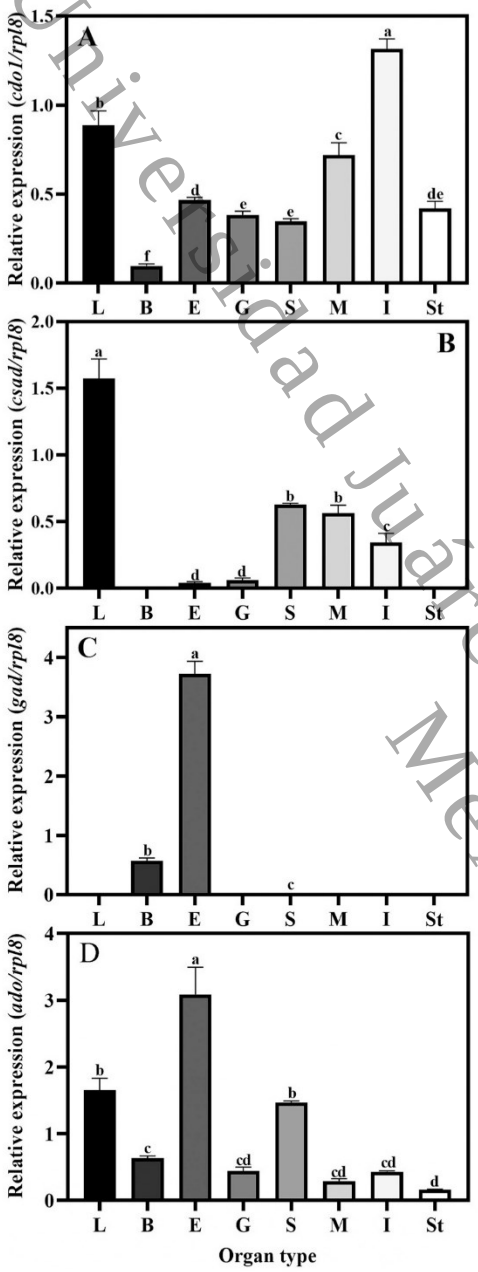

Fig. 3. Relative expression profiles of A) *cdo*, B) *csad*, C) *gad*, and D) *ado* (normalized with the expression of *rpl8*) in different organs of *A. tropicus* juveniles (31 dah). L (liver), B (brain), E (eye), G (gills), S (skin), M (muscle), I (intestine) and St (Stomach). Values in graph are represented by means ± SD ($n = 3$). Different letters indicate a significant difference ($P < 0.05$).

followed by skin, muscle, and intestine, whereas levels of transcript expression of *gad* from the cysteic acid pathway only occurs in the eye, brain and it is almost no present in the skin. However, high expression levels of *ado* participating in the cysteamine pathway occur in most organs, mainly in the eye, liver, and skin.

4. Discussion

The present study determines if *A. tropicus* has the principal key transcripts coding for proteins involved in taurine biosynthesis and its transport during its early ontogeny and different organ types. Hence, complete sequences involved in the three different taurine biosynthesis pathways (Haga et al., 2015; Salze and Davis, 2015) and transport were successfully obtained and registered in the NCBI database (Table 2). The amino acid comparison and phylogenetic analyses distinguished differences between teleost fish, mammals, reptiles, and bird sequences. All examined sequences were found closely related to *A. spatula*. Both species are part of the Lepisostei family; hence they have a strong relationship (Braasch et al., 2016; Wright et al., 2012). However, there is no information regarding the characterization or expression profile of any of the transcripts involved in taurine metabolism in Lepisosteiformes during early ontogeny or organ distribution.

4.1. Taurine biosynthesis transcripts during early ontogenetic development

The present study detected the expression patterns of the four critical transcripts involved in taurine biosynthesis pathways from 0 to 23 dah. Our results showed that at the embryo stage (0 dah) the transcript abundance of *ado* is more abundant than *cdo1, csad,* and *gad.* Although the expression of *ado* is higher at 1 and 3 dah than in the rest of the transcripts, it decreases while *cdo1, csad* and *gad* increase. Hence, taurine biosynthesis transcripts detection during early ontogenetic stages is consistent with other studies, such as *cdo1* in goldfish *Carassius auratus* (Luo et al., 2019), *csad* and *gad* in zebrafish *Danio rerio* (Chang et al., 2013; Martin et al., 1998), *ado, cdo1* and *csad* in Atlantic bluefin tuna *T. thynnus*, L. (Betancor et al., 2019). These authors inferred the different transcripts are maternally transferred through the egg, and after our findings we propose that the same occurs in *A. tropicus*.

Moreover, our findings show *taut* is also expressed at hatch and fluctuates from 0 to 23 dah, indicating taurine uptake is occurring during all stages of development. It has been corroborated that *taut* expression patterns are present during early cleavage stages and embryogenesis in zebrafish, where the expression of *taut* was demonstrated in the retina, brain, heart, kidney, and blood vessels and found that the knockdown of *taut* leads to cell death in the central nervous system and increase mortalities (Kozlowski et al., 2008). Expression patterns during embryogenesis and different post-hatch days of *taut* have also been described in goldfish *C. auratus* where high levels of mRNA transcripts are found before hatching and then regulated through development (Xiong et al., 2020). Our findings show that taurine biosynthesis transcripts and *taut* might also be maternally derived in some degree, since the liver and brain -the main taurine organ synthesizers- (Huxtable, 1992; Salze and Davis, 2015), are not entirely differentiated in the larval period of *A. tropicus.* These findings suggested that the transcription of genes encoding for enzymes involved in pathways I (cysteine sulfinate-dependent) and II (cysteic acid) are activated during these first days of development to synthesize taurine before first exogenous feeding, while *taut* expression levels decreased after 1 dah, probably due to taurine reduction available in the yolk sac (Wang et al., 2017; Xiong et al., 2020). However, *taut* remains constitutively expressed until exogenous feeding (5 dah) in the present study.

The tropical gar is a carnivorous fish that requires live prey at the beginning of its exogenous feeding (Márquez-Couturier et al., 2006), and the most common live prey used in captivity is *Artemia* spp. (Escalera-Vázquez et al., 2018; Palma-Cancino et al., 2019). It has been reported that taurine content in live prey as rotifers or *Artemia* varies between 0 and 0.5 g/kg and 7.2–8.2 g/kg, respectively (van der Meeren et al., 2008). Hence, the influence in growth indexes during exogenous feeding with enriched *Artemia* for larvae of species like yellowtail kingfish *Seriola lalandi* (Partridge and Woolley, 2017) and Atlantic bluefin tuna *T. thynnus* (Betancor et al., 2019) has been demonstrated.

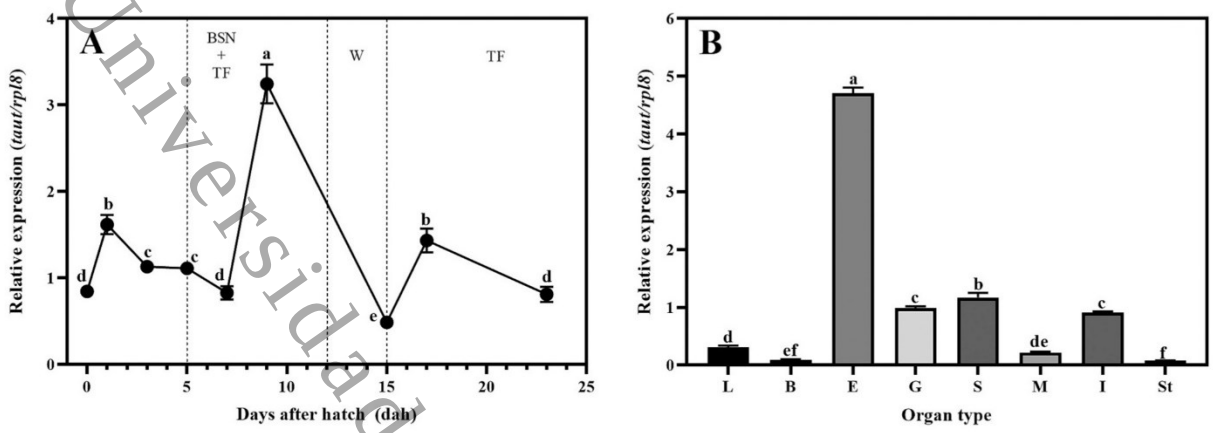

Fig. 4. Relative expression profiles of *taut* (normalized with the expression of *rpl8*) during A) embryo and larvae stages and B) different organs of *A. tropicus* juveniles (31 dah). BSN, brine shrimp nauplii; TF, Trout food; W, withdrawal. L (liver), B (brain), E (eye), G (gills), S (skin), M (muscle), I (intestine) and St (Stomach). Values in graph are represented by means ± SD (n = 3). Different letters indicate a significant difference ($P < 0.05$).

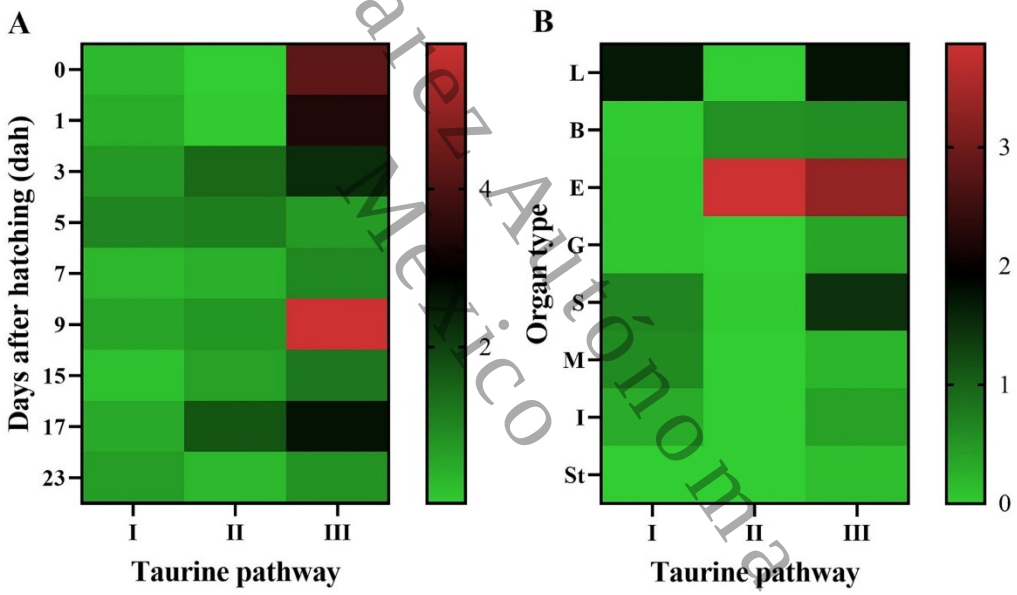

Fig. 5. Heatmaps identifying the predominant transcript involved in taurine biosynthesis pathways during A) early ontogeny and B) different organ types of *A. tropicus*, L (liver), B (brain), E (eye), G (gills), S (skin), M (muscle), I (intestine) and St (Stomach), B) I (cysteine sulfinate-dependent pathway), II (cysteic acid pathway) and III (cysteamine pathway).

Moreover, it has also been observed that fish species fed with rotifers have lower levels of taurine when the last is not supplemented in the prey for larvae of amberjack *Seriola dumerili* (Matsunari et al., 2013). In this regard, some studies have confirmed an inverse relationship between taurine content and expression levels of *taut* in some species, as has been describe in larvae of Atlantic cod *Gadus morhua* (Rise et al., 2015) and germ cells of Japanese eel *Anguilla japonica* (Higuchi et al., 2013). Thence, besides not using an enriched live prey was present in this study, the decreased *taut* expression levels after the first feeding of *A. tropicus* could indicate available taurine for the organism. Nevertheless, further work regarding taurine enrichment in live prey for the organism should be accomplished. Since we used a co-feeding regime with the TF diet from 5 to 15 dah, taurine could also be derived from the TF diet. This effect has been demonstrated with commercial diets containing taurine in ranges from 6 to 7.29 mg/g (Kim et al., 2005a, 2005b).

For *A. tropicus*, Frías-Quintana et al. (2015) reported that the activity of most digestive enzymes increases at 9 dah, mainly acid and alkaline proteases, and lipases, while at 15 dah the maturation of the digestive system is complete. Thence, the present study results suggest that the level of gene expression increased at 9 dah in all analyzed transcripts, particularly in *ado* and *taut* might play a role in the maturation of the digestive system since a decrease of all transcripts occurs at 15 dah. Moreover, another increase in all transcripts occurs with the complete food regime change to the TF diet. However, the increased expression of *ado* is higher than for the rest of the transcripts indicating *A. tropicus* might be relying on the cysteamine pathway to synthesize taurine at 17 dah. We also observed a drop in the level expression of all transcripts but in *csad* at 23 dah when the larval period ended, which might indicate that probably during the juvenile stage, taurine biosynthesis in *A. tropicus* differed from the embryo and larval development.

The ontogenetic development of *A. tropicus* showed a fluctuation of all transcripts involved in taurine. For this reason, further studies regarding nutritional, physiological, or even more targeted studies, such as genome editing using clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9), transcription activator-like effector nucleases (TALENs), or zinc-finger nucleases (ZFNs) should be interesting to apply in the taurine metabolism transcripts (Gaj et al., 2016).

4.2. Taurine biosynthesis transcripts in different organ types

Our results showed that the expression of the different transcripts involved in taurine biosynthesis and its transport varies among organs, which has been observed in other fishes (Betancor et al., 2019; Gonzales-Plasus et al., 2019; Luo et al., 2019; Wang et al., 2016; Xiong et al., 2020). Cysteine sulfinate-dependent and cysteic acid pathways relay in the transformation of precursors methionine/cysteine to cysteine sulfinic acid by the action of *cdo1* before the action of *csad* or *gad* for the synthesis of taurine (Salze and Davis, 2015), indicating the importance of *cdo1* to regulate both pathways. In the present study, the highest relative expression of *cdo1* was observed in the intestine, followed by the liver and muscle, which is similar to what has been observed in other species like freshwater fish goldfish *C. auratus* (Luo et al., 2019), where higher expression levels of *cdo1* are found in the liver followed by the intestine (Betancor et al., 2019). Moreover, in *T. thynnus* the highest expression level of *cdo1* was found in adipose tissue. Hence, this difference between expression levels among species might indicate how the regulation of *cdo1* varies among species. In the liver, the regulation of *cdo1* has also been demonstrated in rainbow trout *Oncorhynchus mykiss* and Japanese flounder *Paralichthys olivaceus*, two species with high and low taurine biosynthesis ability, respectively (Wang et al., 2016). Moreover, our results have shown high expression levels of *csad* in the liver but no presence of the transcript in the brain or stomach, while *gad* was only expressed in the eye, brain, and barely in the skin. Therefore, we suggest that at this juvenile stage in *A. tropicus*, the preferred pathway of taurine synthesis might be organ dependent; while the liver, skin, muscle, and intestine depend on the cysteine sulfinate-dependent pathway, the eye and brain depend on the cysteic acid pathway.

The functional aspects of the cysteine sulfinate-dependent pathway have been well characterized in mammals through the study of *csad* (Hayes et al., 1975; de la Rosa and Stipanuk, 1985; Park et al., 2017; Wu, 1982). For example, it has been proved that the lack of Csad activity produces retinal degeneration in cats. Thus, taurine is considered an essential nutrient in their food (Hayes et al., 1975). Furthermore, in fishes, *csad* expression patterns vary among species (Haga et al., 2015; Poppi et al., 2019). Hence, it has been found high activities of Csad in the liver of freshwater species like red hybrid tilapia *Oreochromis niloticus* and *O. mykiss* (Divakaran et al., 1992; Yokoyama et al., 2001) while marine species of the families Labridae, Scombridae, Soleidae, and Rajidae have a deficiency of Csad (Salze and Davis, 2015). Still, most studies have focused on taurine supplementation in fish (Martins et al., 2021; Sun et al., 2021; Zhang et al., 2021). The present study results showed that *csad* expression patterns among organs are ubiquitous but were not found in the brain and stomach. Nevertheless, studies regarding the use of cysteine, cysteic acid, or cysteamine as precursors should be applied to establish if there is an influence on taurine biosynthesis transcripts. As mentioned before, we observed *gad* transcript involved in pathway II of taurine biosynthesis is only expressed in the brain, eye, and skin, where the expression is most abundant in the eye. This high expression of *gad* in the eye could be explained by the fact that taurine plays an essential role as a neurotransmitter in the central nervous system (Wu and Prentice, 2010). Our results are very consistent with other studies on mammals such as cats, bovines, and rats (Hayes et al., 1975; Wu et al., 2018). Moreover, three different paralogs are known in vertebrates (Grone and Maruska, 2016). Hence, in fish, the gene has been studied in zebrafish *Danio rerio* (Mueller and Guo, 2009), the deep-sea fish *Coryphaenoides* (*Nematonurus*) *armatus* (Trudeau et al., 2000), and detected by RNAseq in *Syngnathus scovelli* (Beal et al., 2018). On the other hand, Cocco et al. (2017) studied its localization in different brain regions of *D. rerio* and found three different paralogs, of which two resemble the mammalian *gad1* and the other the *gad2* paralog.

In fishes, the transcript expression of *ado* has been studied in different tissues of common carp *Cyprinus carpio* (Gonzales-Plasus et al., 2019), finding the highest expression levels in hepatopancreas followed by the brain. On the other hand, in marine fish *T. thynnus* (Betancor et al., 2019), high expression levels were found in the testis and brain. However, in the present study, high expression levels of *ado* were found in the eye, followed by the liver and skin, implying the cysteamine pathway might play an essential role in taurine synthesis in these tissues. Moreover, the activity of Ado has also been measured in the liver of *O. mykiss* and bluegill *Lepomis macrochirus* (Goto et al., 2001) though they exhibit low and high activity, respectively. Besides, there is no more information regarding the cysteamine pathway in fishes.

The absorption of taurine into cells is mainly regulated by the activity of *taut* on the cell membrane (Wang et al., 2017; Xiong et al., 2020). Thus, our results showed *taut* expression levels in the examined organs are widely distributed in *A. tropicus*, and the highest expression levels occur in the eye. Hence, the importance of *taut* has been studied in a mice model by disrupting the expression of the gene, finding decreased taurine levels in different tissues, reducing fertility, and severe retinal degeneration (Heller-Stilb et al., 2002). In fishes, the expression levels of *taut* have shown a difference between freshwater and marine fishes (El-Sayed, 2014; Sampath et al., 2020). The expression levels of *taut* in freshwater fish like goldfish *C. auratus* (Xiong et al., 2020) are highly expressed in the intestine and gill, while during embryogenesis of *D. rerio,* the *taut* expression patterns are visible in the retina, brain, heart, kidney, and blood vessels (Kozlowski et al., 2008). The highest expression levels of *taut* in the tissues of marine water fishes vary among species, whereas turbot *Psetta maxima* (Wang et al., 2016) present high levels in the liver, Senegalese sole *Solea senegalensis* (Pinto et al., 2012) has it in the brain. Furthermore, *taut* regulation has also been studied in euryhaline fishes like Atlantic salmon *Salmo salar* (Zarate and Bradley, 2007). These differences in organ distribution of *taut* expression might be due to the capability to regulate taurine's transportation or to each species' ecological factor.

The use of bulk rt-pcr in embryo and larvae limits the appreciation of precise expression of the different transcripts in study at an organ level in the present work. Hence, the use of other techniques like in situ hybridization, which have been used in zebrafish for the localization of *taut* and *csad* in whole embryos (Chang et al., 2013; Kozlowski et al., 2008), could be used for further studies in *A. tropicus*. The use of in situ hybridization would help to determine if what has been observed in brain an eye at 31 dah using qPCR, also occurs in early stages of the organism.

5. Conclusion

For the first time, complete sequences and expression profiles of taurine metabolism and its transport were identified in the ancestral fish *A. tropicus*. The present results suggest that *A. tropicus* larvae and juveniles could synthesize taurine through the three pathways. Additionally, it can be suggested that the cysteamine pathway is the main taurine pathway at different early ontogenetic stages in *A. tropicus*, mainly at the embryo stage and at 9 dah. Whereas at 5 dah cysteine sulfinate-dependent pathway might be predominant. Results also suggest that taurine biosynthesis might be organ type-dependent, indicating that while the predominant cysteine sulfinate-dependent pathway occurs in the liver, only the cysteic acid pathway occurs in the brain and eye. Additionally, also the cysteamine pathway mainly occurs in the eye. Further research is needed to determine the effect of taurine at different physiological and nutritional levels.

Gene accession numbers

Cystein dioxygenase type 1 *cdo1* (GenBank accession no. **MN389565**); 2-aminoethanethiol dioxygenase *ado* (GenBank accession no. **MN389566**); Cystein sulfinic acid decarboxylase *csad* (GenBank accession no. **MN389567**); Glutamic acid decarboxylase *gad* (GenBank accession no. **MN389568**, GenBank accession no. **MN389569**); Taurine transporter *taut* (GenBank accession no. **MN389570**); Ribosomal protein L8 *rpl8* (GenBank accession no. **MN389571**); Elongation factor *ef1a1* (GenBank accession no. **MN389572**). The data was derived from the resources available from Mendeley Data at http://dx.doi.org/10.17632/jp8d4875nf.1.

Declaration of Competing Interest

The authors declare there is no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2023.111501.

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Capítulo 4

Effects of taurine supplementation on growth performance, morphological characterization of intestinal mucosa, and taurine biosynthesis genes in larvae of tropical gar *Atractosteus tropicus*

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Effects of taurine supplementation on growth performance, intestine morphological structure, and taurine biosynthesis genes in larvae of tropical gar *Atractosteus tropicus*

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Abstract

In the present study, we investigated the effect of dietary taurine supplementation on growth performance, morphological characterization of the intestinal mucosa, and relative expression of genes involved in taurine biosynthesis and taurine transport in larvae of tropical gar at the end of a 22-day feeding trial. Four different taurine-level diets were prepared by including taurine (0.5, 1.0, 1.5, and 2.0%) in the basal control diet (0% taurine). Fish, poultry, and pork meals were ethanol washed to remove taurine content in the protein source. Treatments were carried out in triplicated and randomly distributed in 15 plastic tanks of 50 L coupled to a recirculation system. Experimental units contained 120 fish each and were fed five times a day (8:00, 11:00, 13:00, 15:00, and 18:00 h). The five different diets and *Artemia* nauplii, were supplied from the absorption of the yolk sac (3 days after hatch, DAH) up to 13 DAH. From 10 to 13 DAH, larvae were provided with a co-feeding of biomass, and from 14 DAH to the end of the experiment (24 DAH), only experimental diets were supplied. The results showed taurine inclusion improved growth parameters compared to the basal diet (0% taurine). They showed an increas survival rate as taurine was gradually added, being 2.0% taurine, the diet with better survival results (41.11 ± 2.55 %). Morphological characterization of the intestinal mucosa showed it is constituted of folds forming villi distributed through the intestine as supplemented taurine level raises. The height of intestinal epithelium also presents the highest values in 2.0% taurine (17.176 ± 2.99 µm, whereas the lowest was observed in 0% taurine (16.832 ± 5.06 µm). The relative expression of transcripts involved in taurine biosynthesis was also explored. Results showed *cdo*, ado, and *taut* are upregulated when taurine is added to the basal diet, and this upregulation also occurs for *csad* and *gad* in treatments 0.5, 1.0, and 2.0% taurine. However, 1.5% taurine is downregulated for *csad* and *gad*. The present study is the first to evaluate the effect of taurine supplementation

in a species belonging to the Lepisosteidae family. It contributes to the knowledge of taurine metabolism in *A. tropicus*.

Keywords: taurine, larvae, ancestral, growth performance, transcript expression, *Atractosteus tropicus*

1. Introduction

Taurine, also known as 2-aminoethanesulfonic acid, is a free-form amino acid molecule that exists in limited form in plants such as algae (Tevatia et al., 2015) and naturally in animals such as mammals, birds, aquatic invertebrates (oysters and mussels) and in fish (Salze and Davis, 2015; Sampath et al., 2020). In mammals, the beneficial effect of taurine has been extensively investigated (Goodman et al., 2009; Han et al., 2006; Huxtable, 1992; Huxtable and Sebring, 1986; Militante et al., 2000; Parsons et al., 2001; Pion et al., 1987; Warskulat et al., 2007).

In fishes, the taurine effect has been studied for several years (Goto et al., 2003; Kim et al., 2005; KIM et al., 2003; Kozlowski et al., 2008; Matsunari et al., 2006; Takagi et al., 2011, 2008). However, it is in recent years that it has gained greater relevance through the different studies that have allowed us to observe its effects on the physiology, metabolism, culture, and nutrition of freshwater and marine fishes (Aragão et al., 2023; Cho et al., 2022; El-Sayed, 2014; Martins et al., 2021; Mezzomo et al., 2018; Salze et al., 2012; Shen et al., 2021; Sun et al., 2021; Wu et al., 2021). Although taurine is a non-essential nutrient, its addition could improve fish performance (Davis et al., 2021; Peter et al., 2021). For example, in marine fishes it has observed positive effect on growth, survival and morphological development, especially if they are carnivorous species (Bañuelos-Vargas et al., 2014; Liu et al., 2018; Salze et al., 2012; Satriyo et al., 2017). Dietary taurine supplementation may be indispensable for these fishes, particularly if fed plant-based diets. In freshwater fishes such as common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), it has been reported that they can synthesize taurine and its implementation in the diet could have no inference in its growth and survival; hence, they may not need exogenous supplemental taurine (Espe et al., 2008; Goto et al., 2001; Yokoyama et al., 2001). On the contrary, taurine supplementation in other freshwater

fishes has been found essential for optimal performance in species like grass carp (*Ctenopharyngodon idellus*) (Yan et al., 2019) and Nile tilapia (*Oreochromis niloticus*) (Al-Feky et al., 2016a). Meanwhile, most of these studies have focused on exploring the effects of taurine supplementation, and few explore how genes involved in taurine biosynthesis are affected by taurine supplementation (Poppi et al., 2020; Wang et al., 2016, 2015). Therefore, it is known that *de novo* synthesis is species-specific in fish (Kim et al., 2008; Satriyo et al., 2017). This *de novo* taurine synthesis depends on the species' capability to synthesize those genes/enzymes involved in the different pathways that have been described for fishes (Sampath et al., 2020). These genes are cysteine dioxygenase type 1 (*cdo1*) and cysteine sulfinic acid decarboxylase (*csad*) for pathway I (cysteine sulfinate-dependent pathway); *cdo1*, and glutamic acid decarboxylase (*gad*) for pathway II (cysteine acid pathway); 2-aminoethanethiol dioxygenase (*ado*) for pathway III (cysteamine pathway). Thus, some species might not synthesize enough taurine through any of the pathways and would require acquiring it from exogenous feeding in the diet. Moreover, the taurine transporter (*taut*) is an important membrane transporter that participates in the regulation of the taurine pool produced by these genes and plays a critical role in taurine transportation (Baliou et al., 2020; Tappaz, 2004).

In the present work, tropical gar *Atractosteus tropicus*, an ancestral fish belonging to the Lepisosteidae family, was used to determine if genes involved in the machinery for taurine biosynthesis and taurine transportation are influenced by taurine supplementation. Tropical gar is a very important fish in Southeast Mexico that has been studied in recent years making it possible to understand different physiological processes such as cardiovascular and respiratory (Burggren et al., 2016; Martínez et al., 2021; Martínez-Bautista et al., 2022) and digestive systems (Frías-Quintana et al., 2015; Guerrero-Zárate et al., 2014; Jesús-De la Cruz et al., 2020).

Results in the digestive system studies have allowed the design of different diets during larvae rearing using different sources or additives such as cornstarch (Frías-Quintana et al., 2016), potato starch (Frías-Quintana et al., 2017), lipid sources (Jiménez-Martínez et al., 2020), mannan-oligosaccharides (Maytorena-Verdugo et al., 2022), fructooligosaccharides (Pérez-Jiménez et al., 2022), inulin (De La Cruz-Marín et al., 2023), β-glucans (Cigarroa-Ruiz et al., 2023), and sodium propionate (Arellano-Carrasco et al., 2023). However, there is no information regarding the use of taurine supplementation in the species. Therefore, we hypothesize, as has been described in other carnivorous fishes (Kim et al., 2005; López et al., 2015; Satriyo et al., 2017) that taurine will improve the growth and survival of *A. tropicus* but will regulate taurine biosynthesis and transportation genes. Thus, taking the advantage that tropical gar is an ancestral carnivorous freshwater fish, the present study aimed to investigate the effects of taurine supplementation of *A. tropicu*s larvae on larval growth performance, survival, morphological characterization of the intestinal mucosa, and transcript expression of genes involved in taurine transportation and biosynthesis.

2. Material and methods

2.1 Tropical gar fish rearing and care

A total of 1800 *A. tropicus* recently hatched larvae were obtained from an induced breeding spawning using one female (3.5 kg) and 3 males (1.5 kg average weight) using the hormone LHRHa (35 µg/kg of fish). After spawning (16 hours post-induction), the reproducers were removed from the tank, and only eggs were kept there until 3 days after hatching (DAH). The newly hatched larvae (3 DAH) were placed in 70 L cylindrical-conical plastic tank (150 larvae per tank). These tanks are in an indoor recirculating freshwater system consisting of a main 800

L reservoir supplied with biological filters that functioned as a solids depositor, in addition to a pump 3/4 HP centrifuge (Jacuzzi, JWPA5D-230A, Delavan WI, USA). The water quality of the system was monitored until organisms reached 24 DAH, where temperature (29.1 ± 0.6 °C), dissolved oxygen (5.7 ± 0.7 mg/L) and pH (6.7 ± 0.2) were recorded by an oximeter (YSI 85, Ohio, USA) and a potentiometer (HANNA HI 991001, Romania, Europe). The organisms were kept in a photoperiod of 12h light/12h darkness.

2.2 Experimental diets

Diets were prepared at the department of nutrition of the Laboratory of Physiology in Aquatic Resources (LAFIRA) of División Académica de Ciencias Biológicas (DACBiol) of the Universidad Juárez Autónoma de Tabasco (UJAT), México. The formulation of experimental diets is shown in Table 1. Fish, poultry, and pork meal were washed thrice with 70% ethanol to remove taurine content before diet formulation (Gonzales-Plasus et al., 2019; Satriyo et al., 2017). A basal diet was used as a control (0% taurine) based on the formulation proposed by (Frías-Quintana et al., 2016) (44% protein and 15% lipid) with modifications. Due to the removal of lipids in the washed meals, lipids analysis of meals was determined to ensure the quantity of lipids plus the add oil in the diets were 15% lipid in all diets. Taurine was supplemented in 4 of the five experimental diets with 0.5, 1.0, 1.5, and 2.0 % taurine. Diets were elaborated following the indications of Alvarez-González et al. (2001). In brief, dry ingredients were sieved (except for pulverized taurine) and then weighted using an analytical scale (Ohaus, mod. CS2000, China). Macronutrients were mixed for 15 minutes in a manual mixer, then micronutrients were added and mixed for 15 more minutes. Then, liquid ingredients were weighted and added to the mixture, and finally, 35-40% of water was added to the mixture and

mixed for 15 minutes. The pelletization process was done using a meat grinder (TORREY® Model M-22R1, León, Guanajuato, Mexico) with a screen of 5 mm. Pellets were dried at 60 °C for 12 h in an oven (CORIAT® Model HC-35-D, Ciudad de Mexico, Mexico). After the assigned time and diets cool down, pellets were crushed manually, sieved to obtain different homogenized particle sizes and kept refrigerated until use.

The proximal composition of experimental diets (moisture and ash levels) was analyzed according to the AOAC (2000). The amino acid content, including taurine, was performed in all diets and *Artemia* at Laboratorio de Nutrición Acuícola at Universidad Autónoma de Baja California, Ensenada, Baja California, Mexico (Table 2). The taurine inclusion of the experimental diets analyzed were 0.5 mg g^{-1} (0%), 0.9 mg g^{-1} (0.5%), 1.2 mg g^{-1} (1.0%), 1.7 mg g^{-1} (1.5%), 2.2 mg g^{-1} (2.0%) and 0.8 mg g^{-1} *Artemia.*

2.3 Experimental design

For the present study, a simple factor experiment was designed to evaluate the supplementation of different taurine levels. Diets were designed as 0, 0.5, 1.0, 1.5, and 2.0% taurine. Assignment of treatments was carried out in triplicated and randomly distributed, planting 120 fish (0.03 ± .001g - 1.68 ± 063 cm, average body weight and total length respectively) per experimental unit and distributed in 15 cylindrical-conical plastic tanks of 50 L couple to a recirculation system. Fish were fed five times daily (8:00, 11:00, 13:00, 15:00, and 18:00 h) to satiation for 22 days according to the feeding regime.

2.4 Feeding regime

The feeding regime started at the beginning of the yolk absorption (3 DAH), which was considered the beginning of the experiment. Fish were fed five times daily (8:00, 11:00, 13:00, 15:00, and 18:00 h) starting with a dry diet offered by hand followed by live prey (*Artemia* nauplii) from 3 DAH to 13 DAH. During this time, from 10 to 13 DAH, they were provided with a co-feeding of biomass, where inert food and biomass were provided as a cookie. Only experimental diets were fed from 14 DAH to the end of the experiment (24 DAH). Diets were provided at apparent satiation and particle size was adjusted according to larval growth (250–500, 500–750 and 750 μm). For the remotion of mortality, exceeding food and feces, siphoning was performed one-hour post feeding from the thanks.

2.5 Growth performance and survival

The present trial lasted 22 days from the experiment's beginning (3 DAH) to the end (24 DAH). After 18 h of fasting, fish in each experimental unit were collected for measurements. Wet weight was determined using an analytical balance (OHAUS Explorer 224), and the total length of each fish was determined by photography in a transparent flat container. The total length of the obtained images was measured using the program ImageJ 1.52v freeware software (National Institutes of Health, Bethesda, MD, USA). A final count of organisms for the experimental unit was performed by the end of the experiment. Obtained data was used to calculate specific growth rate (SGR), weight gain rate (WGR), condition factor (CF), and survival rate (SR) according to the following equations: specific growth rate (SGR,%) = [(Ln final weight - Ln initial weight) / number of days] * 100; weight gain rate (WGR,%) = [(final weight - initial weight) / initial weight] * 100; condition factor (CF,%) = (final body weight / final body length^3) * 100; survival (S,%) = (final number of fish / initial number of organisms) * 100.

2.6 Sampling

Samples at the end of the trial were collected after 18 h of fasting for histology and qPCR analysis. Three individuals per replicate (9 fish per treatment) were collected for each technique at the end of the experiment. For histology, samples were preserved in 2.0 ml tubes in Davidson solution at room temperature. Samples for qPCR were preserved in a 2.0 ml tube containing RNAlater (Life Technologies, Carlsbad, CA, USA), conserved at room temperature for one day and maintained at -80 °C until analysis. All organisms were rinsed with distilled water before preservation.

2.7 Histological techniques

Samples previously fixed in Davidson were dehydrated in a graded series of ethanol (50%, 70%, 80%, 96% and 100%), cleared (ethanol-xilol, xilol) and embedded in paraffin. Then, sample blocks were cut with a microtome in sagittal sections to a 5 µm thickness. Paraffin from the cut section was deparaffined, rehydrated and stained with haematoxylin and eosin (H&E). For intestine examination of samples, photographs were taken with an Axiocam ERc 5s attached to a Zeiss Primo Star optical microscope (Jena, Germany) and observations and measurements were taken using digital imaging software (ZEN lite; Carl Zeiss Microscopy GmbH, Jena, Germany).

2.8 Larvae RNA extraction, cDNA synthesis and transcript expression analysis

Total RNA extraction of whole larvae was performed using TRIZOL (Invitrogen, Life Technologies California, USA) following the manufacturer's instructions. Genomic DNA contamination was treated in each RNA using DNAse I (Promega). The concentration and purity

of RNA samples were determined by the 260/280 ratio in a NanoDrop 1000 Spectrophotometer (Jenway GenovaNano, Cole-Parmer, Staffordshire, UK). One µg of total RNA was reverse transcribed into cDNA using the Maxima First Strand cDNA Synthesis Kit (BioRad, Irvine, CA, USA) and was stored in aliquots at -20 °C until use.

For the present study, specific primer pairs for each transcript involved in taurine biosynthesis and reference genes have been tested before (Aranda-Morales et., 2021; Jimenez-Martínez et al., 2020; Martínez-Burguete et al., 2023). The specific pair of primers for each transcript is given in Table 3. The corresponding quantitative polymerase chain reaction (qPCR) analysis of each of the transcripts was carried out following the recommendations of Hellemans et al. (2007) and Bustin et al. (2009) in a 96-well thermocycler CFX96 TouchTM Real-Time Detection System (BioRad, Hercules, CA, USA). The qPCR reaction mixture contained 5 µl of Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific, Hemel Hempstead, UK), 2 µl cDNA (1/10 diluted), and 0.3 µM of each primer and adjusted to 10 µl using DEPC-treated water. The thermal program for qPCR analysis included a UDG pre-treatment at 2 min at 50°C and an initial denaturation step at 95 °C for 10 min, followed by 39 cycles: 10 s at 95 °C, 30 s at 60 s and 5 s at 70 °C. At the end of the qPCR run, a melt curve of 0.5 °C increments from 65 °C to 95 °C was performed, confirming the amplification of a single product in each reaction. All samples contained triplicates for unit for treatment and negative controls (no cDNA) for each study pair of primers. The geometric mean of reference genes elongation factor 1 (ef1) and ribosomal protein L8 (rpl8) were used for relative quantification of the target gene transcripts according to the method described by Vandesompele et al. (2002). The relative quantification was calculated according to Escalante-Rojas et al. (2018).

2.9 Statistical analysis

Results were performed using GraphPad Prism version 9.3.0 (San Diego, CA, USA). The tank was considered as the experimental unit; hence every treatment had n = 3, and it was represented by the average of sampled fish per tank. Normality and homogeneity were tested for all analysis data (Shapiro-Wilk and Levene tests, correspondingly). After postulates were accomplished, data was analyzed by one-way ANOVA with Tukey HSD posteriori test to detect the difference between treatments (significant value of $P < 0.05$).

3. Results

3.1 Growth and survival

Growth results after 22 days trial of feeding experimental diets to tropical gar larvae showed significant differences ($P < 0.05$) in larvae fed with taurine inclusion (0.5, 1.0, 1.5, and 2.0 %) when compared to the taurine unsupplemented diet (0% taurine; Fig. 1). The weight results (Fig. 1A) showed that larvae fed with 2.0% taurine inclusion level have the highest average value (0.154 ± 0.003 g) while the lowest weight was found for those larvae fed with 0% taurine inclusion level (0.115 ± 0.004 g). A similar pattern was found for length results for supplemented diets 0.5, 1.5, and 2,0%, where diets with taurine inclusion showed no difference among them but showed difference with the taurine unsupplemented diet (Fig. 1B; $P < 0.05$). The highest average length obtained by larvae was for 2.0% taurine (3.521 ± 0.02 cm) and the lowest was 0% taurine (3.334 ± 0.027 cm). Growth indexes are shown in Table 4. Differences between fish fed diets with supplemented taurine level where not found for SGR, WG, and CF. The highest SGR, WG, and CF were found in 2.0% taurine. However, these parameters were significantly decreased in the unsupplemented taurine diet (Table 4; $P < 0.05$).

The taurine supplementation showed an increased in survival as the taurine level was increased (Fig. 3; $P < 0.05$). The highest survival rate was shown in the 2.0% taurine diet (41.11 ± 2.55 %) and showed significant differences ($P < 0.05$) with the rest of the treatments. Larvae fed 0% taurine had the lowest survival rate (24.72 ± 1.27 %) and had no significant difference with diet fed 0.5% taurine ($P > 0.05$).

3.2 Histological description

Morphological characterization of intestinal mucus of *A. tropicus* larvae under the investigation of the present work showed it is constituted of folds that form villi distributed through the intestine (Fig. 1A, and Fig. 1B), which are made up of cells arranged in a columnar manner characterized as enterocytes (Fig. 1C, and Fig. 1D). Nonetheless, towards the caudal zone the shortening of these villi can be observed until they are no longer present in the anus zone (Fig. 1A).

Enterocytes are elongated cellular projections (striae) arranged in the form of a brush from the basal zone towards the periphery of the lumen; their cytoplasm is eosinophilic in color with a slightly basophilic nucleus (Fig. 1D, Fig. 1E, and Fig. 1F). Moreover, no microvilli were observed towards the distal area of the epithelium. Among enterocytes arrangements, interspersed zones with goblet cells can be observed and have an elongated pyriform shape with a vesicular structure that rests on the basal membrane with a mucus-secreting activity which pours its content by exocytosis towards the apical surface (Fig. 1E, and Fig. 1F). When contrasting the different levels of supplemented taurine, we observed 2.0% taurine presents the highest values of intestinal epithelium height (17.176 ± 2.99 µm), while the lowest values were observed in 0% taurine (16.832 ± 5.06 µm).

3.3 Taurine biosynthesis transcripts relative expression

The effect of taurine supplementation on the relative expression of genes involved in taurine biosynthesis in larvae of *A. tropicus* are shown in Fig. 4. The results showed the highest relative expression of *cdo* in larvae fed taurine supplementation 1.0 and 1.5% (Fig. 4A; $P < 0.05$). The peak relative expression of *csad* occurs in supplemented taurine 0.5 and 1.0% while 1.5% taurine is downregulated compared to the rest of the treatments (Fig. 4B; $P < 0.05$). The relative expression of *gad* was significantly higher in larvae fed 2.0% taurine compared to the rest of the treatments (Fig. 4C; $P < 0.05$). The highest relative expression of *ado* was present in larvae fed 2.0% taurine and was significantly different to 0 and 1.5% taurine (Fig. 4D; $P < 0.05$).

3.4 Taurine transporter transcript relative expression

To determine the effect of taurine supplementation on taurine transporter (*taut*), the relative expression on whole *A. tropicus* larvae was analyzed by qPCR at the end of the experiment. The dietary taurine supplementation effect on taut relative expression of *A. tropicus* larvae is represented in Fig. 5. The relative expression of *taut* in taurine supplemented diets significantly differs from unsupplemented taurine diet ($P < 0.05$). Results showed that graded taurine increased relative expression of *taut* until 1.0% taurine and is substantially different from the rest of the diets (0, 0.5, 1.5, and 2.0% taurine) ($P < 0.05$).

4. Discussion

The effects of taurine supplementation in freshwater and marine fishes varies among species (Guimarães et al., 2018; Huang et al., 2021; Li et al., 2021; Martins et al., 2021; Ramos-pinto et al., 2021; Salze et al., 2018; Shen et al., 2021). Thus, the requirements of taurine in fish diets to

improve any species' performance depends on its capabilities to synthesize taurine (Li et al., 2022; Sampath et al., 2020). Moreover, it has been described that carnivorous fishes tend to have insufficient endogenous synthesis, and supplementation is needed in their food, particularly in those where plant-based diets are used (Aragão et al., 2014; Gaon et al., 2021; Tong et al., 2020). Hence, previous studies have demonstrated taurine supplementation improves different growth parameters and intestine morphology and regulates taurine transportation and biosynthesis genes (Adeshina and Abdel-Tawwab, 2020; de la Rosa and Stipanuk, 1985; Dehghani et al., 2020; Kim et al., 2008; Liu et al., 2017; Wiriduge et al., 2020; Xu et al., 2020). Nonetheless, there is a general lack of information in the Lepisosteidae family regarding whether species in this family have or do not had the capability to de novo synthesize taurine or if they need to acquire it from the diet. Therefore, the present study in *A. tropicus*, a carnivorous freshwater fish belonging to this family and with a relevant economic importance in Southeast Mexico, was designed to investigate the influence of taurine supplementation in diets where taurine was removed from the protein source. In the present study, our findings show that taurine supplementation on diets for *A. tropicus* larvae has a positive effect on growth indexes, survival, enhanced intestine morphological structure and regulates expression of genes involved in the biosynthesis and transportation of taurine.

4.1 Growth performance and survival

Our study showed a significant difference between taurine-supplemented diets (0.5, 1.0, 1.5, and 2.0%) and the basal diet (0% taurine) for growth parameters and survival. The positive effect of taurine supplementation on growth and survival has also been found in larvae species such as Nile tilapia (*Oreochromis niloticus*) (Al-Feky et al., 2016a), Senegalese sole (*Solea senegalensis*)

(Pinto et al., 2010), red sea bream (*Pagrus major*) (Kim et al., 2016), California yellowtail (*Seriola dorsalis*) (Salze et al., 2019), Atlantic bluefin tuna (*Thunnus thymus*, L.) (Betancor et al., 2019), and rock sole (*Lepidopsetta polyxystra*) (Hawkyard et al., 2014). Although most of the afore mentioned studies have focused on live prey taurine enrichment, our results are in accordance with them, indicating this positive effect in *A. tropicus* larvae growth and survival might be due to taurine assimilation during the larvae period. Likewise, studies using microparticulate diets in larvae have been run on freshwater fish Nile tilapia (*O. niloticus*) (Al-Feky et al., 2016a) or marine fish yellow drum (*Nibea albiflora*) (Xie et al., 2014) finding difference among diets with supplemented taurine. Our results showed that graded taurine has not significant difference in weight, length, SGR, WG, and CF parameters between diets supplemented with taurine which has also been observed in juveniles of rice field eel (*Monopterus albus*) (Hu et al., 2018) and grey mullet (*Mugil cephalus*) (Koven et al., 2023). Results also showed that the basal diet used as control (0% taurine supplemented) presents a low survival rate, and as taurine is incremented in the diets, a better survival rate is shown. Hence, the lack of variability between treatments with supplemented taurine in growth parameters and a higher survival rate at 2.0% taurine supplementation confirms the importance of taurine in *A. tropicus* during larvae rearing. Moreover, poor growth parameters and low survival when compared to the rest of the treatments also discharged the possibility that the number of organisms in the tank could influence growth. These results also might imply that whereas taurine improves growth parameters starting at 0.5% taurine supplementation, it may not satisfy the requirements and therefore, growth parameters starting at 0.5% taurine supplementation may not satisfy the requirements. Therefore, low survival is shown in low supplemented diets. In accordance with our results, it has been explored in *N. albiflora* larvae that fish fed diets with

2.0% taurine had better growth parameters and survival rates than those without taurine (Xie et al., 2014). In contrast to our findings, most studies in larvae stages have suggested taurine supplementation to be between 0.5% and 1.5% taurine (Betancor et al., 2019; Izquierdo et al., 2019; Sampath et al., 2020) and that taurine inclusion yielded larger promoting effects on growth and feed utilization in marine fishes than freshwater fishes (Li et al., 2022). Nevertheless, it has been suggested that optimum taurine level is a species-specific factor for fish (Sampath et al., 2020).

Considering the fact that a specific species' taurine requirement can change with the stage of development (Salze and Davis, 2015; Wang et al., 2015) differences in the level of taurine assessment in fish stages can be observed between larvae, juveniles, and reproduction of the same species (Sampath et al., 2020). In a two-year experimental in marine fish broodstock of California yellowtail (*Seriola dorsalis*) (Salze et al., 2019) where control and a taurine-supplemented (0.28% and 2.67% taurine dry diet) diets were fed to females and males; authors found that taurine supplemented diet improved reproductive yield of females such as a number of spawn and eggs, egg viability, female fertility, and egg morphometrics. Additionally, when a spawn with plenty of eggs for larval rearing was acquired, larvae from each broodstock treatment were reared using either larval diets containing none too little taurine or a taurine-enhanced larval diet. Diets consisted of rotifers and *Artemia*, where the control diet had 0 and 0.04% taurine, respectively, whereas in the taurine diet, rotifers and *Artemia* had 0.07 and 0.12% taurine. Larvae spawned from the control that were fed a control diet. They died by 15 days post-hatch indicating the importance of taurine supplementation for California yellowtail. Additionally, higher mortality was also found in larvae from control broodstock that were fed taurine when compared to larvae from taurine supplemented either fed control or taurine diet

suggesting the important role of taurine during gametogenesis and embryogenesis and that an absence in broodstock diets cannot be eased by supplementation during larval rearing (Salze et al., 2019). On the other hand, taurine supplementation of juvenile California yellowtail (*S. dorsalis*) has been explored alone or in combination with methionine, and a positive effect has been found in those fed alone (1.4%) (Garcia-Organista et al., 2019). In freshwater fish, Nile tilapia (*O. niloticus*) taurine supplementation has also been explored in larvae (Al-Feky et al., 2016b) and in broodstock (Al-Feky et al., 2016a), authors found that while in larvae 9.7 g kg^{-1} are need it, for broodstock 8 g kg^{-1} dietary taurine are enough for optimal reproductive performance. Therefore, further studies are required to explored in tropical gar to determine if there is a difference among ontogenetic development stages in its taurine requirements.

4.2 Intestine morphological structure

Treatments were contrasted to observe the difference between intestinal epithelium height and 2.0% taurine presents the highest values (17.176 ± 2.99 µm), while the lowest values were observed in 0% taurine (16.832 ± 5.06 µm). This higher value in 2.0% taurine could be due to the size of enterocytes, promoting better digestion and absorption of nutrients and lipids. It is important to point out that the treatment with the lowest taurine presented more mucus vesicles, indicating greater goblet cell activity. In fishes, goblet cells are the principal mucus cell type in the intestine epithelium and serve as the primary site for nutrient digestion and mucosal absorption (Salinas and Parra, 2015). Moreover, it can also form a line of defense at the intestinal mucosa and have a common secretory role (Pelaseyed et al., 2014). Therefore, high goblet cell activity could be explained by the role of taurine in controlling the microbiota in the intestine, where a higher dose of taurine reduces the presence of pathogens, which decreases the activity of

goblet cells (Rimoldi et al., 2016). In freshwater fish channel catfish (*Ictalurus punctatus*) (Shi et al., 2021), authors observed the effect of oxidized-fish-oil diets significantly reduced the villi length, goblet cell quantity, and muscular thickness of the intestine.

Moreover, the addition of taurine to oxidized-fish-oil diets reversed this trend, indicating taurine can maintain the structural integrity of the intestine. Intestinal abnormalities have been observed particularly in fish species when plant protein source-based diets are used (Martins et al., 2019; Pervin et al., 2020; Venold et al., 2012; Zhu et al., 2021). Furthermore, taurine supplementation in young grass carp (*Ctenopharyngodon idella*) was observed to positively affect growth, enteritis resistance, intestinal antimicrobial compounds, and attenuated intestinal inflammation and intestinal immune function (Yan et al., 2019).

4.3 Taurine biosynthesis-related genes

The ability of a fish to biosynthesize taurine through any of the different pathways could be reflected in differences in the expression levels/activities of the key enzymes and the taurine transporter (Liu et al., 2017; Wang et al., 2016; Yokoyama et al., 2001; Zarate and Bradley, 2007) and could be influenced by different factors including taurine supplementation (Tappaz, 2004). Of the three pathways described to biosynthesize taurine, the deficiency of Csad in the cysteine sulfinate-dependent pathway, has been generally marked as the rate-limiting enzyme for taurine synthesis (Chang et al., 2013; Park et al., 2017; Seidel et al., 2019). However, in contrast to this, taurine biosynthesis genes (*cdo*, *csad*, *gad*, and *ado*) and taurine transporter (*taut*) have been described for *A. tropicus* larvae early ontogeny and in different organ-types in early juveniles suggesting that at least in larvae, the species seems to be more relatable to the cysteamine pathway and that in early juveniles the pathway is organ-dependent (Martínez et al., 2023, in

revision). Thus, we explored the relative expression of taurine biosynthesis-related genes in the present study to determine if exogenous feeding affects taurine metabolism in *A. tropicus* larvae.

It has been demonstrated that taurine biosynthesis varies between freshwater and marine species such as common carp (*C. carpio*), Japanese flounder (*Paralichthys olivaceus*) and rainbow trout (*O. mykiss*) (Kim et al., 2008; Wang et al., 2016). Besides, it is considered that freshwater fish can synthesize taurine from methionine and cysteine via the cysteine sulfinic acid pathway but cannot be synthesized in carnivorous marine fish, and therefore, must be supplemented in their diets (Nakamura et al., 2021). In the present study, low expression of all analyzed genes in 0% taurine when compared to the rest of the treatments was found, excluding 1.5% taurine for *csad* and *gad*. Taurine supplementation in taurine biosynthesis genes has been observed in larvae of Atlantic bluefin tuna (*T. thynnus*, L.) (Betancor et al., 2019) where authors observed an increment for *cdo* and *csad* for larvae fed 1.0% taurine, which is similar to the upregulation observed in the present study for these genes. This upregulation of *csad* has also been observed in grey mullet (*M. cephalus*) juveniles from the control (0% taurine) to 1.0% taurine (Koven et al., 2023). For *ado*, Betancor et al. (2019) found a downregulation for larvae fed 1.5% taurine when compared to the rest of the treatments, which differs with the upregulation found in taurine add treatments when compared to the control (0% taurine) in our study. This upregulation in *ado* relative expression of *A. tropicus* larvae in diets with added taurine might indicate that the organism also depends on the cysteamine pathway to produce taurine. Low expression of *gad* in the control (0% taurine) and 1.5% taurine in the present study was found, and overexpression was mainly present in 2.0% taurine, indicating that the organism is also relying on the cysteic acid pathway, whereas taurine is supplemented.

The activity of Taut is upregulated when circulating taurine levels are low to increase the reabsorption and recycling of existing taurine and downregulated when plasma taurine levels are high to allow maintenance of appropriate concentrations (Liu et al., 2017). Organisms that can regulate *taut* expression do so rapidly after dietary taurine intake or changes in dietary protein or taurine content (Baliou et al., 2020; Shen et al., 2021; Warskulat et al., 2007). In Atlantic bluefin tuna, the relative expression of *taut* is regulated by dietary taurine in a dose-dependent manner, indicating that when taurine levels are low, *taut* expression is upregulated to promote and enhance the absorption and transport of taurine (Betancor et al., 2019). Contrary to these, in Goldfish, taurine supplementation at 4.0 and 6.0 g/kg significantly increases *taut* mRNA expression level (Xiong et al., 2020). Our results in the present study showed low relative expression levels of *taut* in fish fed 0, 0.5, 1.5, and 2.0% taurine diets and may indicate that taurine maintenance of appropriate concentrations is occurring. Nonetheless, peak *taut* expression occurred for larvae fed 1.0% taurine, indicating a modulation of taut expression. However, further research applying other techniques is needed to expand the knowledge of taurine regulation in tropical gar larvae.

In conclusion, our results demonstrate taurine supplementation in diets for *A. tropicus* larvae has several benefits during its larviculture. Whereas there is no significant difference among treatments with added taurine in growth performance, survival of *A. tropicus* larvae increases with a 2.0% taurine level. We also demonstrate that dietary taurine significantly affected key enzymes' gene expression participating in the three taurine biosynthesis pathways. However, further research is needed to demonstrate the benefit of taurine supplementation not only in growth performance parameters of larvae but also in juveniles and adults helping to improve the production of *A. tropicus* and to use the species as a model for biomedical studies.

Ethics approval

This work was performed under the Official Mexican Standards NOM-062-ZOO-1999, 2001 and the ethical rules for zebrafish and other tropical finfish by the American Veterinary Medical Association (AVMA, 2013).

CRediT author statement

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Declaration of competing interests

None.

Data availability

Data of the present study are available upon request to the authors.

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Tables

Table 1. Formulation of experimental diets for tropical gar *A. tropicus* larvae.

	Taurine diets (%)				
Ingredients (g kg^{-1})	**0**	**0.5**	**1**	**1.5**	**2**
Fish meal[a]	305.4	305.4	305.4	305.4	305.4
Poultry meal[a]	150	150	150	150	150
Pork meal[a]	150	150	150	150	150
Soybean meal[a]	150	150	150	150	150
Cornstarch[b]	77.4	77.4	77.4	77.4	77.4
Soybean oil[c]	101.2	101.2	101.2	101.2	101.2
Taurine[d]	0	5	10	15	20
Cellulose[e]	25	20	15	10	5
Vitamin premix[f]	5	5	5	5	5
Mineral premix[f]	10	10	10	10	10
Grenetin[g]	20	20	20	20	20
Vitamin C[h]	5	5	5	5	5
Vitamin E[h]	1	1	1	1	1
Proximal composition g 100 g^{-1} dry matter					
Moisture	4.85	4.22	4.52	4.7	4.77
Ash	14.39	14.19	14.18	14.02	14.41
Taurine	0.48	0.85	1.25	1.71	2.22

[a]Marine and agricultural proteins S.A. de C.V., Guadalajara, Jalisco; [b]MSA Corn Industrializer S.A de C.V. Guadalajara, Jalisco, México; [c]Ragasa Industries, S.A. de C.V., Guadalupe, Nuevo León, México; [d]Sigma-Aldrich, catalogue no. T0625; [e]Sigma-Aldrich, catalogue no. C8002; [f]Vitamin premix composition g/mg or International Units per kg of diet: Vitamin A, 10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 IU; Vitamin K3, 4.0 g; Thiamine B1, 8.0 g; Riboflavin B2, 8.7 g; Pyridoxine B6, 7.3 g; Vitamin B12, 20.0 mg; Niacin, 50.0 g; Pantothenic acid, 22.2 g; Inositol, 0.15 mg; Nicotinic Acid, 0.16 mg; Folic Acid, 4.0 g; Biotin, 500 mg; Vitamin C, 10.0 g; Choline 0.3 mg, Excipient q.s. 2 g; Manganese, 10 g; Magnesium, 4.5 g; Zinc, 1.6 g; Iron, 0.2 g; Copper, 0.2 g; Iodine, 0.5 g; Selenium, 40 mg; Cobalt 60 mg. Excipient q.s. 1.5 g; [g]D'gari Productos alimenticios y dietéticos Relámpago, Tlalpan, Edomex, Mexico; [h]Rovimix Stay-C 35 DSM, Jalisco.

Table 2. Total amino acid content (mg g^{-1} dry mass) in experimental diets and *Artemia* used for tropical gar *A. tropicus* larvae.

	Taurine diets (%)					
Amino acid	0	0.5	1.0	1.5	2.0	*Artemia*
Essential amino acids						
Histidine	1.19	1.19	1.05	1.05	1.02	1.00
Arginine	3.37	3.37	3.32	3.32	3.26	5.14
Threonine	1.54	1.49	1.47	1.47	1.45	2.91
Valine	2.62	2.58	2.58	2.56	2.54	3.55
Methionine	0.65	0.88	0.86	0.81	0.81	1.50
Lysine	2.53	2.48	2.64	2.70	2.62	1.74
Isoleucine	1.84	1.83	1.78	1.81	1.80	2.80
Leucine	3.53	3.45	3.49	3.50	3.46	4.54
Phenylalanine	1.99	2.00	1.97	1.98	1.98	2.88
Non-essential amino acids						
Aspartic acid	4.49	4.41	4.34	4.34	4.31	5.99
Serine	2.64	2.58	2.54	2.46	2.48	4.33
Glutamic acid	8.35	8.19	8.03	7.98	7.92	9.52
Glycine	4.72	4.66	4.66	4.26	4.23	3.20
Alanine	3.46	3.41	3.42	3.37	3.28	4.13
Tyrosine	1.61	1.63	1.61	1.69	1.63	2.18
Others						
TAU	0.48	0.85	1.25	1.71	2.22	0.79

Table 3. Primer used for this study.

Gene	Primer sequence (5'-3')	Product size (bp)	Efficiency (%)	R^2	Reference
Cysteine dioxygenase type 1 (*cdo1*)	F: AGGTCATGGCAGCAGTATCC R: CTGTGCAGGCCAATGGAATC	189	89.7	0.9999	Martínez-Burguete et al. (2023)
Cysteine sulfinic acid decarboxylase (*csad*)	F: TTCCTGGTGAGTGCTACCTC R: TGAGTCTGCCCTCTCAATCC	180	92.5	0.986	Martínez-Burguete et al. (2023)
Glutamic acid decarboxylase (*gad*)	F: TCACTGAAGGAACGCCAGTC R: TGCATGGTCGGTTCTTCTCC	149	88.2	1	Martínez-Burguete et al. (2023)
2-aminoethanethiol dioxygenase (*ado*)	F: ACGCAATCTCACGCAACTG R: TCCCGAGGCATTATCTGTGG	77	103.9	0.994	Martínez-Burguete et al. (2023)
Taurine transporter (*taut*)	F: AGAGCGGATTAAGGCAGTGG R: CAGGCCGTTGTTCCCATTTG	121	108.6	0.991	Martínez-Burguete et al. (2023)
Ribosomal protein L8 (*rpl8*)	F: TGTGCTGCCTGGAAGAGAAG R: TTTCGGGGTTGTGGGAGATG	90	90.6	0.9999	Aranda-Morales et al. (2021)
Elongation factor 1 (*ef1*)	F: CCTGCAGGACGTCTACAAGATCG R: GACCTCAGTGGTCACGTTGGA	121	99.82	0.9891	Jiménez-Martínez et al. (2018)

Table 4. Growth indexes in larvae of *A. tropicus* fed experimental diets with different taurine levels for 22 days trial. Values are presents as mean ± SD, n = 3. Significant difference between the experimental diets in the same row are indicated by different letters ($P < 0.05$).

	Taurine diet (%)				
Index	0.0	0.5	1.0	1.5	2.0
SGR (% day^{-1})	6.11±0.15^{b}	7.25±0.27^{a}	7.19±0.12^{a}	7.26±0.34^{a}	7.44±0.08^{a}
WG (%)	283.34±12.98^{b}	393.06±29.73^{a}	386.04±12.98^{a}	394.71±37.74^{a}	413.4±8.72^{a}
CF (%)	0.311±0.016^{b}	0.344±0.003^{a}	0.347±0.01^{a}	0.346±0.006^{a}	0.353±0.01^{a}

Figure captions

Figure 1. Growth of *A. tropicus* larvae, A) weight (g), B) length (cm). Values are presents as mean ± SD, n = 3. Different letters showed a significant difference as determined by one way ANOVA ($P < 0.05$).

Figure 2. Survival of *A. tropicus* larvae at the end of the experimental trial (22 days). Values are presents as mean ± SD, n = 3. Different letters showed a significant difference as determined by one way ANOVA ($P < 0.05$).

Figure 3. Intestine description of *A. tropicus* larvae. Haematoxylin and eosin (H & E) a) intestine towards the caudal zone "4x", b) intestine towards the cephalic zone "4x", c) small intestine villi "10x", d) small intestine villi "40x", e) villi near the anus "40x", f) enterocytes details "100x": * (villi), muscle (m), lumen (l), liver (L), enterocytes (e), food debris in the lumen (fd), mucus vesicles (➞), length of a villus (dotted line).

Figure 4. Relative expression of transcripts: A) cysteine dioxygenase type 1 (*cdo1*), B) cysteine sulfinic acid decarboxylase (*csad*), C) glutamic acid decarboxylase (*gad*), and D) 2-aminoethanethiol dioxygenase (*ado*) of *A. tropicus* fed different levels of taurine. Values are presented as normalized expression ratios means using elongation factor 1 (*ef1*) and ribosomal protein L8 (*rpl8*) geometric mean. Values are presents as mean ± SD, n = 3. Different letters showed a significant difference as determined by one way ANOVA ($P < 0.05$).

Figure 5. Relative expression of taurine transporter (taut) of *A. tropicus* fed different levels of taurine. Values are presented as normalized expression ratios means (n = 3) using elongation factor 1 (*ef1*) and ribosomal protein L8 (*rpl8*) geometric mean. Different letters showed a significant difference as determined by one way ANOVA ($P < 0.05$).

Figure 1.

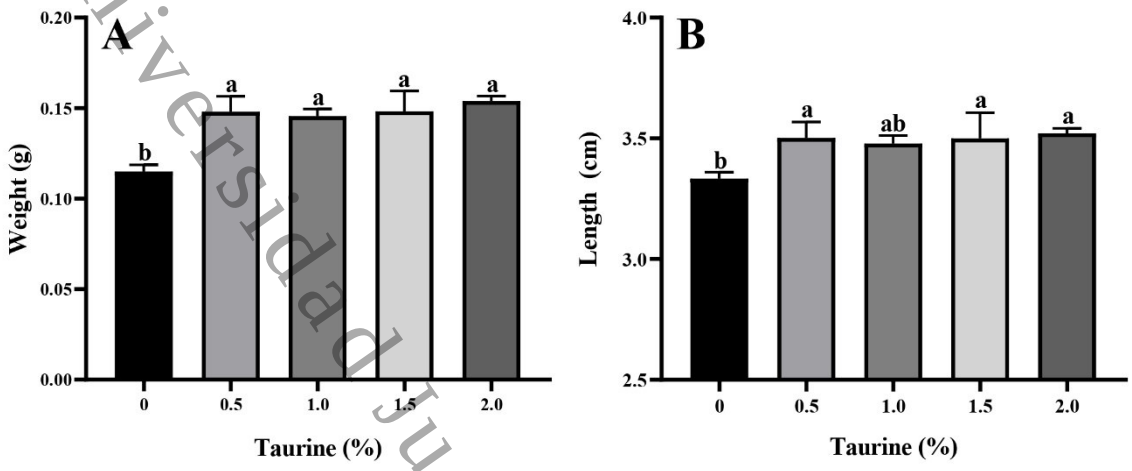


Figure 2.

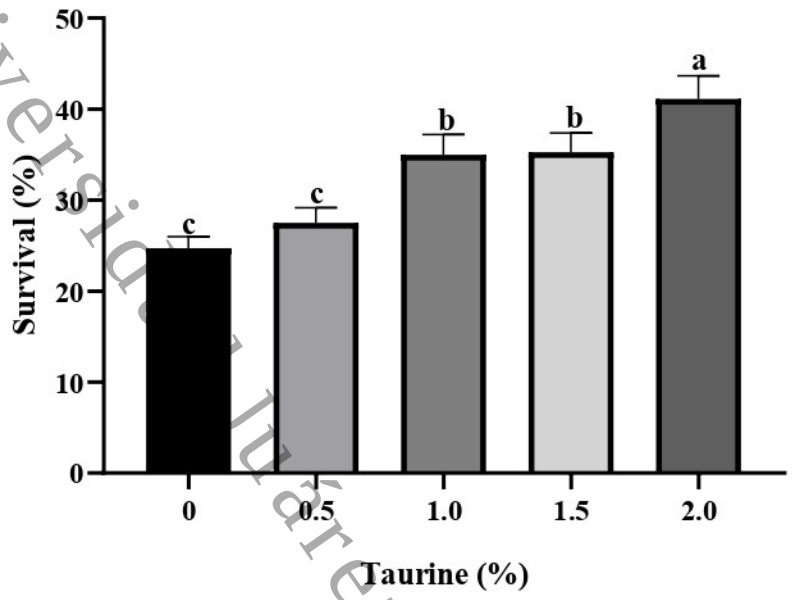


Figure 3.

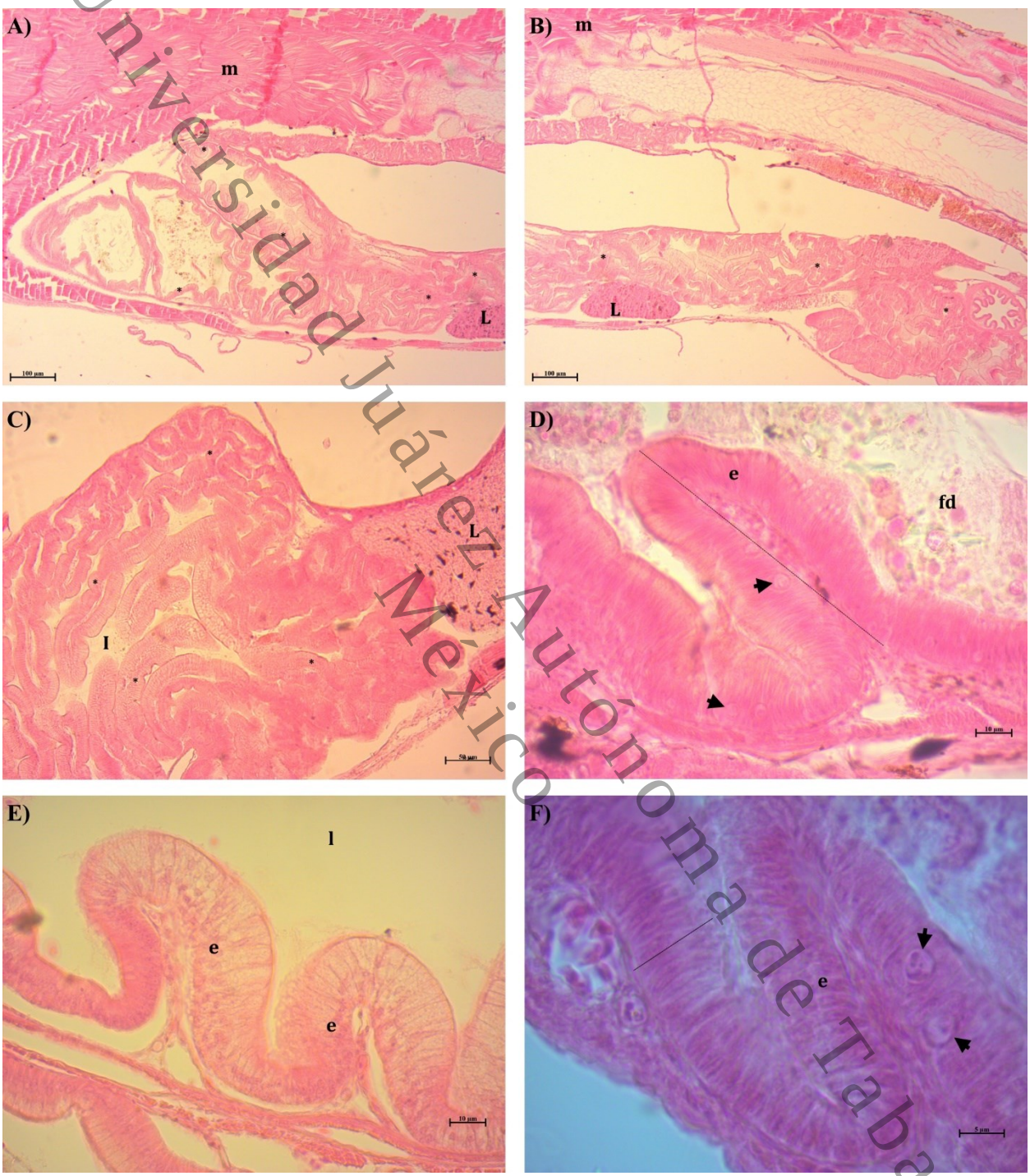


Figure 4.

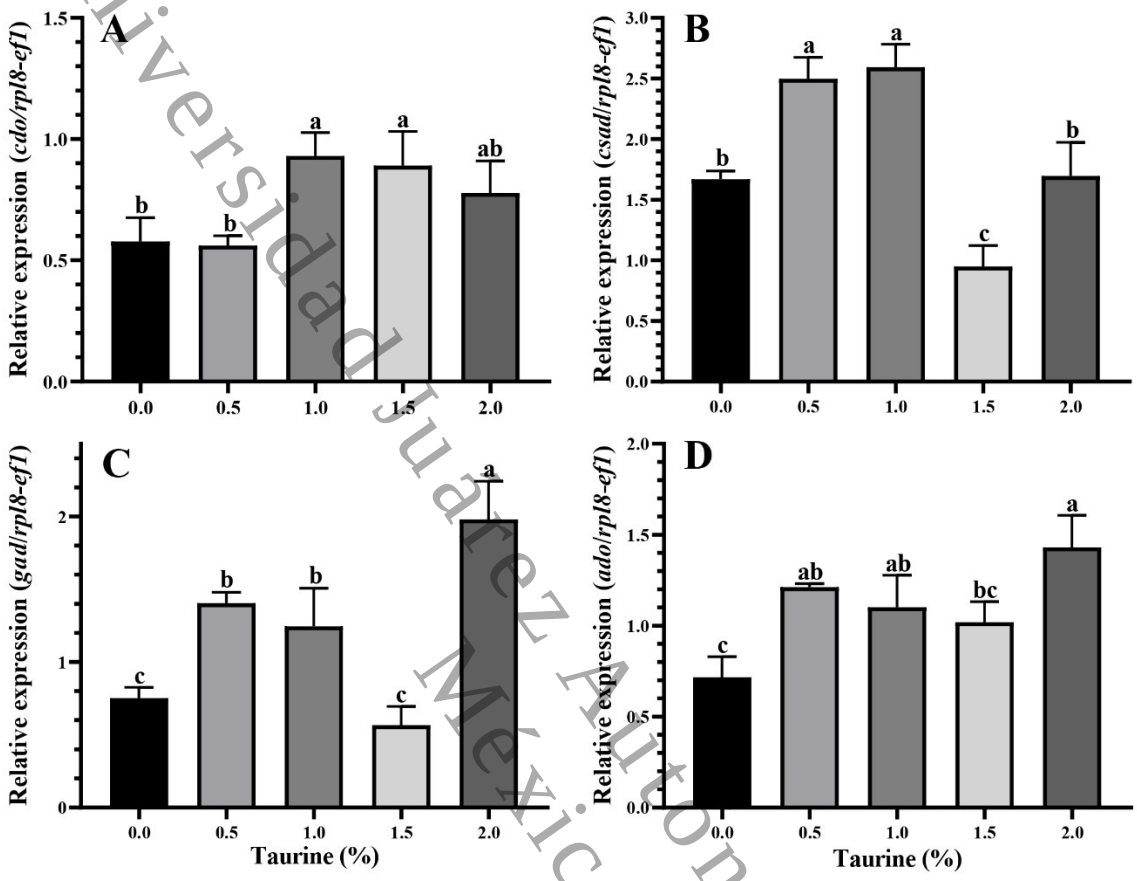
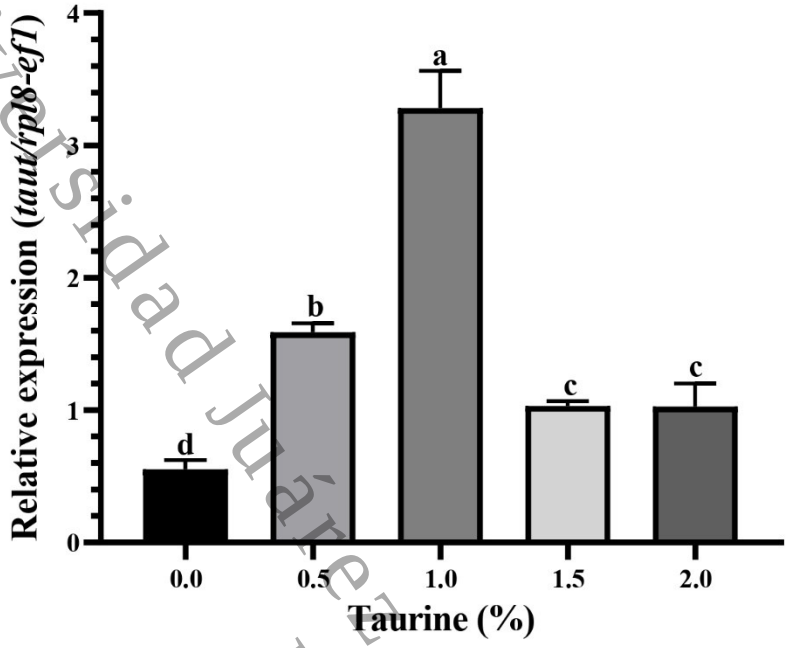


Figure 5.



Capítulo 5
Discusión general

La ruta predominante en la biosíntesis de taurina depende de diferentes factores como el estadio de desarrollo, el tipo de tejido, los hábitos alimenticios y el ambiente en el que vive una especie (El-Sayed, 2014). Estas rutas pueden ser determinadas a través del estudio de los genes que participan a la síntesis *de* novo y el transporte de taurina (Baliou et al., 2020; Kim, Matsunari, Takeuchi, et al., 2008; Liu et al., 2017). Por esta razón, utilizando diferentes enfoques que permitieron ampliar el conocimiento y entendimiento de estos genes en estudio, en la presente tesis se realizó una búsqueda referenciada de sus principales roles fisiológicos, se detectaron elementos reguladores en las zonas promotoras, se determinaron los patrones de expresión durante el desarrollo ontogenético en larvas completas y en órganos de juveniles tempranos, y se analizó el efecto de la suplementación de taurina en larvas completas de pejelagarto.

Roles fisiológicos y factores de transcripción

En el capítulo 2 de la presente tesis, se realizó una búsqueda referenciada de los principales roles fisiológicos en los que los genes que participan en el transporte y biosíntesis de taurina se ven involucrados. Así mismo, se realizó la inferencia de los principales factores de transcripción y sus sitios de unión de estos genes.

La taurina es un ácido sulfónico natural que generalmente es considerado un aminoácido (Huxtable & Sebring, 1986). Sin embargo, debido a su estructura y que no se incorpora a las proteínas, no puede considerarse como tal, ya que carece del grupo carboxilo fundamental, lo que le confiere una similitud con los fosfolípidos de membrana neutra, la fosfatidilcolina y la fosfatidiletanolamina (Huxtable, 1992) lo que les da su alta solubilidad en agua y baja lipofilia explicando así su impermeabilidad a las membranas biológicas (Lambert et al., 2015). Además, aunque se sabe que existen algunos péptidos de forma natural como la glutaurina, no existe evidencia de un ARN de transferencia que codifique para taurina y su grupo sulfonato reemplace al grupo carboxil necesario para la formación de un enlace peptídico (Bittner et al., 2005). Entender esto nos permite establecer la importancia de la taurina en los organismos y el porqué de su participación en diversos procesos fisiológicos.

Está taurina que utilizan los organismos para los diferentes procesos fisiológicos en los que participa puede ser adquirida a través de la absorción de la comida en el intestino o de forma *de novo* por medio los precursores metionina/cisteína (Kuzmina et al., 2010). Como se describió en

el Capítulo 1, estás rutas y genes para sintetizar taurina endógenamente son: I) Ruta del ácido cisteíno sulfínico, cisteína dioxigenasa (*cdo1*), ácido sulfinico descarboxilasa (*csad*); II) Ruta del ácido cisteíno, *cdo1*, ácido glutámico descarboxilasa (*gad*); III) Ruta de la cisteamina, 2-aminoetanetiol dioxigenasa (*ado*). En donde, la primera es considerada como la ruta primaria y las otras dos se consideran rutas secundarias para la síntesis de taurina. Además, está el transportador de taurina (*taut*), que juega un papel importante durante la regulación de la taurina disponible en el organismo. También, cabe destacar que la síntesis de taurina se realiza principalmente en el hígado y el cerebro (de la Rosa & Stipanuk, 1985; Goto et al., 2003; Huxtable & Sebring, 1986).

El enfoque para estudiar el papel que desempeña cada uno de estos genes en la síntesis de taurina se ha basado principalmente en medir los niveles de expresión o las actividades enzimáticas de las proteínas que codifican después de ensayos de suplementación (Kim, Matsunari, Takeuchi, et al., 2008; Wang et al., 2016; Yokoyama et al., 2001; Zhao et al., 2017). Entonces, dependiendo del gen en estudio son los resultados que se han observado en los efectos fisiológicos de diferentes especies de peces.

En el caso de los estudios realizados en *cdo*, se sabe que su actividad/expresión regula la síntesis de taurina de dos rutas (ruta I - del ácido cisteíno sulfínico; ruta II del ácido cisteíno) (Wang et al., 2016). En el caso de mamíferos, se ha observado que en ratones el bloqueo o inactivación de la expresión de *cdo* desestabiliza la expresión de *csad* y no altera la expresión del gen *ado* el cual participa en la ruta III – cisteamina. Además, esta alteración provoca mayor mortalidad postnatal, poco crecimiento y patologías del tejido conectivo en las fibras elásticas del pie, así como bajos niveles de taurina y altos niveles de cisteína; indicando la falta del flujo de las rutas dependientes de *cdo* ya que la taurina que se sintetiza a partir de la ruta de la cisteamina, es insuficiente para mantener los niveles de taurina en la ausencia de taurina dietaria (Ueki et al., 2011). En peces, este tipo de estudios no han sido dirigidos en peces para *cdo* o *ado,* pero si para *csad*. Así mismo, diferentes estudios realizados en mamíferos muestran que la deficiencia de taurina está marcada por la baja o nula actividad/expresión de Csad/*csad* (Hayes et al., 1975; Miyazaki et al., 2019; Pion et al., 1987; Wu, 1982). Esto ha sido corroborado en peces marinos principalmente en aquellos pertenecientes a las familias Labridae, Scombridae, Soleidae and Rajidae (Salze & Davis, 2015; Yokoyama et al., 2001). Si bien, no es un pez marino, en el pez cebra, se ha demostrado que la mutación del gen *csad* para interrumpir la síntesis de taurina reduce los niveles de este en embriones, aumenta su mortalidad y hace que presenten anomalías cardiacas

como edema pericardial y malformación del tubo cardíaco (Chang et al., 2013). Asimismo, los autores demostraron que la suplementación de taurina en el agua donde estaban estos organismos que presentaban anomalías, ayudaba a recuperar algunos de los organismos con fenotipos cardiacos. La mayor parte de los trabajos reportados en peces para la ruta de la cisteamina se basan en identificar los niveles de expresión en diferentes tejidos y órganos (Betancor et al., 2019; Gonzales-Plasus et al., 2019).

La segunda ruta secundaria donde se sintetiza taurina, la ruta del ácido cisteíno, donde el gen en estudio fue *gad*, se sintetiza principalmente en el cerebro (Wu et al., 1979) y se sabe que pueden existir tres paralógos diferentes (Cocco et al., 2017; Grone & Maruska, 2016; Lariviere et al., 2002). En peces, dentro de los trabajos relevantes se ha demostrado su expresión utilizando herramientas transcriptómicas en el pez pipa del Golfo (*Syngnathus scovelli*) (Beal et al., 2018), clonación en pez dorado (Lariviere et al., 2002) y la identificación por hibridación *in situ* en el pez cebra (Mueller & Guo, 2009). Sin embargo, la mayor parte de la información disponible sobre este gen se basa en los resultados de los ensambles genómicos disponibles en las bases de datos del NCBI.

La taurina sintetizada endógenamente o adquirida de forma exógena es distribuida a través de la membrana plasmática por los transportadores *taut* y *pat1* (proton/amino acid symporter - transportador 1 de aminoácidos acoplados a protones), en donde *taut* es el mayor responsable de la distribución (Lambert et al., 2015; Seidel et al., 2019). Se sabe que *taut* tiene una respuesta adaptativa a los niveles de taurina disponibles, y que cuando hay niveles altos de taurina dietaria, los niveles de *taut* disminuyen (Flaherty et al., 1997; Han et al., 2006). En peces, se ha demostrado que el silenciamiento de este gen en embriones de pez cebra causa daños fenotípicos en la apoptosis celular del cerebro y la espina dorsal (Kozlowski et al., 2008).

Si bien, esta revisión nos permitió observar un panorama general de los procesos fisiológicos en los que están implicados los diferentes genes en estudio, hacen falta más trabajos utilizando herramientas moleculares, como lo son mutagénesis dirigida, hibridación *in situ*, o el uso de herramientas bioinformáticas para entender el papel que juegan en estos roles fisiológicos de la taurina.

De esta forma, para ampliar el conocimiento a partir de datos genómicos ya disponibles en las bases de datos NCBI y Ensembl, se realizó una búsqueda *in silico* en las zonas promotoras utilizando un programa bioinformático (Ciiider versión 0.9; Gearing et al., 2019) de aquellos

factores de transcripción y sus sitios de unión que podrían estar regulando la transcripción de los genes en estudio.

Los resultados mostraron que, si bien cada gen tiene sus propios sitios de unión y factores de transcripción dependiendo de la especie, existen aquellos que pueden ser encontrados en todas las especies (ver Supplementary file 1 del capítulo 2) que podrían estar regulando la expresión de estos genes. También existen aquellos factores de transcripción que cuando son comparados entre las diferentes secuencias promotoras nos permitió identificar que están presentes en todos los genes estudiados y en todas las especies cuando son comparados entre sí. El estudio de los factores de transcripción en peces *in vivo* aún es muy limitado, por lo que este enfoque da un primer acercamiento a futuros estudios que permitan entender mejor su rol en la síntesis endógena de taurina.

Transcritos involucrados en la biosíntesis de taurina durante el desarrollo ontogenético y en diferentes órganos de juveniles tempranos

En el capítulo 3 se determinó si embriones y larvas completas durante el desarrollo ontogenético (0 a 23 dde) y diferentes tipos de órganos de juveniles tempranos de *A. tropicus* (31 dde) tienen los transcritos que codifican para proteínas que participan en la biosíntesis y transporte de taurina. Es por ello por lo que las secuencias completas de los diferentes genes que participan en las tres diferentes rutas de biosíntesis (Haga et al., 2015; Salze & Davis, 2015) y el transporte de taurina fueron obtenidos y registrados en la base de datos del NCBI: cisteína dioxigenasa tipo 1 *cdo1* (MN389565); ácido sulfínico descarboxilasa *csad* (MN389567); 2-aminoetanetiol dioxigenasa *ado* (MN389566); ácido glutámico descarboxilasa *gad* (MN389568, MN389569); transportador de taurina *taut* (MN389570). La comparación de aminoácidos y el análisis filogenético de las secuencias hicieron una distinción entre peces teleósteos, mamíferos, réptiles y secuencias de peces y se encontró una estrecha relación con las secuencias de *L.* oculatus y *Atractosteus spatula*, ambas especies pertenecen a la familia Lepisostei (Braasch et al., 2016; Wright et al., 2012). Sin embargo, no existe información acerca de la caracterización o el perfil de expresión de ninguno de los transcritos involucrados en el metabolismo de taurina de los Lepisosteiformes.

Además, en el presente estudio se detectaron los patrones de expresión de los cuatro transcritos principales involucrados en las tres diferentes rutas de biosíntesis de taurina de 0 a 23 dde. Los resultados muestran que en la etapa embrionaria (0 dde) la expresión de transcritos de *ado* es mayor que *cdo1*, *csad* y *gad*. Mientras que a partir de 5 dde la expresión de *ado* disminuye y los niveles aumentan en *cdo1*, *csad* y *gad*. Por lo tanto, la detección de estos transcritos involucrados en la biosíntesis de taurina durante las primeras etapas ontogenéticas es consistente con otros estudios, como *cdo1* en el pez dorado *Carassius auratus* (Luo et al., 2019) *csad* y *gad* en el pez cebra *Danio rerio* (Chang et al., 2013; Martin et al., 1998), *ado*, *cdo1* y *csad* en atún rojo del Atlántico *T. thynnus,* L. (Betancor et al., 2019). Estos autores infieren que las diferentes transcritos son transferidos de forma materna a través del huevo, y tras nuestros hallazgos proponemos que lo mismo ocurre en *A. tropicus*.

Por su parte, la expresión de *taut* es fluctuante de 0 a 23 dde, lo que indica que la absorción de taurina ocurre durante todas las etapas de desarrollo. Se ha corroborado que los patrones de expresión de *taut* están presentes durante las primeras etapas de división y embriogénesis en el pez cebra, donde se demostró la expresión de *taut* en la retina, el cerebro, el corazón, los riñones y los vasos sanguíneos y se encontró que el silenciamiento génico de *taut* conduce a la muerte celular en el sistema nervioso central y aumenta la mortalidad (Kozlowski et al., 2008). Patrones de expresión durante la embriogénesis y días después de la eclosión de *taut* también han sido descritos en el pez dorado *C. auratus* en donde niveles altos del transcrito fueron encontrados antes de la eclosión y posteriormente regulado durante el desarrollo (Xiong et al., 2020). Al igual que los otros genes en estudio, *taut* también podría ser de origen materno en algún grado, ya que el hígado y el cerebro -los principales órganos sintetizadores de taurina- (Huxtable, 1992; Salze & Davis, 2015), no están completamente diferenciados en el período larval de *A. tropicus*. Estos resultados sugieren que la transcripción de los genes que codifican para enzimas involucradas en la ruta I y II son activadas durante los primeros días de desarrollo para sintetizar taurina antes de la primera alimentación exógena, mientras que la expresión de *taut* disminuye después de 1 dde debido a la reducción de la disponibilidad de taurina en el saco vitelino (Wang et al., 2017; Xiong et al., 2020). Sin embargo, *taut* permanece expresada hasta la alimentación exógena en este estudio.

El pejelagarto en un pez carnívoro que requiere de presas vivas al inicio de su alimentación exógena (Márquez-Couturier et al., 2006), siendo *Artemia* spp. la presa viva más utilizada en la especie (Escalera-Vázquez et al., 2018; Palma-Cancino et al., 2019). El contenido de taurina en

alimento vivo como rotíferos o *Artemia* varía entre 0-0.5 g/kg y 7.2-8.2 g/kg, respectivamente (van der Meeren et al., 2008). La influencia del alimento vivo en el crecimiento durante la alimentación exógena ha sido probada utilizando el enriquecimiento de *Artemia* en especies como *Seriola lalandi* (Partridge & Woolley, 2017) y *T. thynnus* (Betancor et al., 2019). Además, se ha demostrado que larvas alimentadas con rotíferos tienen menores niveles de taurina cuando no es suplementado en las presas de *Seriola dumerili* (Matsunari et al., 2013). En este sentido, algunos estudios han confirmado una relación inversa entre el contenido de taurina y los niveles de expresión de *taut* en algunas especies, como se ha descrito en larvas de *Gadus morhua* (Rise et al., 2015) y células germinales de *Anguilla japónica* (Higuchi et al., 2013). Por lo tanto, aunque no se utilizaron presas vivas enriquecidas, la disminución de los niveles de expresión de *taut* después de la primera alimentación de *A. tropicus* podría indicar la disponibilidad de taurina para el organismo. No obstante, se sugieren futuros trabajos con el enriquecimiento de taurina en el alimento vivo de pejelagarto. Debido a que se utilizó una co-alimentación entre alimento vivo y alimento microparticulado de 5 a 15 dde, la taurina también podría estar presente en la dieta ya que ha sido demostrado que el contenido de taurina en las dietas comerciales varía entre 6 a 7.29 mg/g (Kim et al., 2005a; 2005b).

En *A. tropicus* se ha reportado que enzimas digestivas como las proteasas ácidas y alcalinas, y lipasas incrementan a los 9 dde y que la maduración del sistema digestivo esta completo a los 15 dde (Frías-Quintana et al., 2015). Los resultados de niveles de expresión a los 9 dde en todos los transcritos analizados, particularmente en *ado* y *taut* podrían estar jugando un rol importante en la maduración del sistema digestivo ya que ocurre una disminución de todos los transcritos en el día 15 dde. Además, se produce otro pico de los niveles de expresión en todos los transcritos cuando ocurre el cambio completo del régimen alimentario a la dieta particulada. Por otra parte, la expresión relativa de *ado* es mayor que el resto de los transcritos a los 17 dde, indicando que *A. tropicus* podría estar dependiendo de la ruta de la cisteamina. También se observó una disminución en la expresión de todos los transcritos a los 23 dde a excepción de *csad*, cuando el período larval ha terminado, lo que podría sugerir que probablemente la ruta de biosíntesis de taurina en etapas juveniles de *A. tropicus* difiere de las etapas embrionarias y larvarias.

El desarrollo ontogenético de *A. tropicus* mostró una fluctuación de todos los transcritos involucrados en la biosíntesis de taurina. Es así como estudios adicionales relacionados con cuestiones, nutricionales, fisiológicas, o el uso de herramientas de edición genómica utilizando

CRISPR/Cas9, TALENs o ZFNs (Gaj et al., 2016) deberían ser interesantes para aplicar en el metabolismo de la taurina utilizando como modelo el pejelagarto.

Por otra parte, los resultados de esta tesis muestran que la expresión de los diferentes transcritos involucrados en el transporte y biosíntesis de taurina varía dependiendo el órgano, lo cual ha sido también observado en otras especies (Betancor et al., 2019; Gonzales-Plasus et al., 2019; Luo et al., 2019; Wang et al., 2016; Xiong et al., 2020). Las rutas del ácido cisteíno sulfínico y del ácido cisteíno dependen de *cdo* en la transformación de los precursores metionina/cisteína antes de la acción de *csad* o *gad* para la síntesis de taurina (Salze & Davis, 2015), indicando su importancia para regular ambas rutas. En este estudio, encontramos los picos más altos de la expresión relativa de *cdo* en el intestino, seguido del hígado y el musculo, lo cual ha sido observado en otras especies de peces de agua dulce como *C. auratus* (Luo et al., 2019) donde también las expresiones más altas son encontradas en el hígado seguida del intestino. Por otra parte, en la especie marina *T, thynnus* los niveles de expresión más altos ocurren en el tejido adiposo (Betancor et al., 2019). De esta forma, la diferencia entre los niveles de expresión entre especies podría indicar la variación de *cdo1* entre especies. La regulación de *cdo1* en el hígado también ha sido demostrada entre *o. mykiss* y *P. olivaceus* dos especies con alta y baja capacidad de biosíntesis respectivamente (Wang et al., 2016). Además, los resultados mostraron altos niveles de expresión de *csad* en el hígado, pero no se encontró expresado en el cerebro o estómago, mientras que *gad* solo se expresó en el ojo, cerebro y escasamente en la piel. De esta forma, se podría inferir que en esta etapa del pejelagarto la ruta de síntesis es órgano dependiente; mientras el hígado, la piel, el musculo, y el intestino dependen en la ruta del ácido cisteíno sulfínico, el ojo y el cerebro dependen de la ruta del ácido cisteíno.

Los aspectos funcionales de la ruta de ácido cisteíno sulfínico han sido bien caracterizados en mamíferos como el gato, en donde se ha probado que la deficiencia de la actividad de Csad produce degeneración en la retina y se considera que es un nutriente esencial en su alimentación (Hayes et al., 1975). En peces, los patrones de expresión son variados y se han encontrado altas actividades de Csad en especies dulceacuícolas como *O. niloticus* y *O. mykiss* (Divakaran et al., 1992; Yokoyama et al., 2001) mientras que especies marinas de las familias Labridae, Scombridae, Soleidae, y Rajidae tienen una deficiencia (Salze & Davis, 2015). En el presente estudio, los patrones de expresión de *csad* en los órganos son ubicuos, pero no se presentó la expresión en el estómago y el cerebro. Asimismo, el aplicar estudios sobre el uso de cisteína, ácido cistéico o

cisteamina como precursores podrían establecer si hay una influencia en los transcritos involucrados en la biosíntesis de taurina.

Los altos niveles de expresión de *gad* encontrados en el ojo podrían estar explicados por el hecho de que la taurina juega un papel esencial como neurotransmisor en el sistema nervioso central (Wu & Prentice, 2010). Estos altos niveles de expresión son consistentes con lo encontrado en otros estudios en mamíferos como gatos, bovinos y ratas (Hayes et al., 1975; Wu, 1982; Wu et al., 2018). Además, se sabe que existen tres diferentes páralogos en vertebrados (Grone & Maruska, 2016). En peces, el gen ha sido estudiado en *Danio rerio* (Mueller & Guo, 2009), el pez de aguas profundas *Coryphaenoides (Nematonurus) armatus* (Trudeau et al., 2000) y ha sido detectado por RNAseq en *Syngnathus scovelli* (Beal et al., 2018). También ha sido estudiado en diferentes regiones del cerebro de *D. rerio* y se encontró que cuenta con tres diferentes páralogos, de los cuales dos son parecidos al *gad1* de mamíferos y el otro a *gad2*.

La expresión de *ado* en peces ha sido estudiada en tejidos de *Cyprinus carpio* (Gonzales-Plasus et al., 2019), en donde los niveles más altos de expresión son encontrados en el hepatopáncreas seguidos del cerebro. Por su parte, en el pez marino *T. thynnus* (Betancor et al., 2019) se encontraron altos niveles de expresión en el cerebro y las gonadas. En el presente estudio, los altos niveles de expresión de *ado* fueron encontrados en el ojo, seguido del hígado y la piel, por lo que suponemos que la ruta de la cisteamina podría jugar un rol esencial en la síntesis de taurina en estos órganos. La actividad de Ado también ha sido estudiada en el hígado de *O. mykiss* y *Lepomis macrochirus* (Goto et al., 2001) exhibiendo baja y alta actividad respectivamente.

La absorción de taurina en las células está principalmente regulada por la actividad de *taut* en la membrana celular (Wang et al., 2017; Xiong et al., 2020). Nuestros resultados, muestran que los niveles de *taut* están ampliamente distribuidos en *A. tropicus* y que los niveles más altos ocurren en el ojo. La importancia de *taut* se ha estudiado en ratones, en donde la disrupción de la expresión del gen utilizando silenciamiento génico, demostró reducidos niveles en los tejidos, reduciendo la fertilidad y degeneración severa de la retina (Heller-Stilb et al., 2002). En peces, existen diferencia en los niveles de expresión de peces dulceacuícolas y marinos (El-Sayed, 2014; Sampath et al., 2020). Los niveles de expresión en *C. auratus* presenta altos niveles en el intestino y branquias (Xiong et al., 2020). En el caso de *D. rerio* durante la embriogénesis los patrones de expresión de *taut* son visibles en la retina, el cerebro, el corazón, el riñón y los vasos sanguíneos (Kozlowski et

al., 2008). En especies marinas los niveles varían dependiendo la especie, *Psetta máxima* (Wang et al., 2017), presenta los niveles más altos en el hígado y *Solea senegalensis* (Pinto et al., 2012) los presenta en el cerebro. Estás diferencias en la distribución de la expresión de *taut* podría ser debido a la capacidad del organismo para regular el transporte de taurina o debidos factores ecológicos de cada especie.

En nuestro trabajo, el uso de bulk qPCR en embriones y larvas limita la apreciación precisa de los diferentes niveles de expresión a nivel órgano. Sin embargo, nos permite tener un panorama general de los mecanismos de biosíntesis de taurina durante estas etapas y en los órganos de juveniles tempranos. Por lo que, el uso de otras técnicas como hibridación *in situ*, la cual ha sido utilizada en el pez cebra para la localización de *taut* y *csad* en embriones completos (Chang et al., 2013; Kozlowski et al., 2008), podría ser utilizado para futuros trabajos.

Efecto de la suplementación de taurina en parámetros de crecimiento, sobrevivencia, caracterización morfológica de la mucosa intestinal y expresión de genes involucrados en la biosíntesis de taurina

Los efectos de la suplementación de taurina en peces dulceacuícolas y marinos varían entre las especies (Aragão et al., 2023; Guimarães et al., 2018; Huang et al., 2021; Koven et al., 2023). De tal forma que los requerimientos de taurina en las dietas para mejorar su rendimiento dependen de las capacidades de la especie para sintetizar taurina de forma *de novo* (Li et al., 2022; Sampath et al., 2020). Además, se ha descrito que peces carnívoros tienden a tener una insuficiencia en la síntesis endógena por lo que la suplementación es necesaria, particularmente en etapas larvarias o cuando se busca sustituir la dieta animal por vegetal (Allon et al., 2016; Aragão et al., 2023; Gaon et al., 2021; Tong et al., 2020). Por lo tanto, estudios previos han demostrado que la suplementación con taurina mejora diferentes parámetros de crecimiento, la morfología del intestino y regula el transporte de taurina y los genes de biosíntesis (Adeshina & Abdel-Tawwab, 2020; de la Rosa & Stipanuk, 1985; Dehghani et al., 2020; Kim, Matsunari, Nomura, et al., 2008; Liu et al., 2017; Wijerath et al., 2020; Xu et al., 2020). No obstante, existe una falta general de información en la familia Lepisosteidae con respecto a si las especies de esta familia tienen o no la capacidad de sintetizar taurina *de novo* o si necesitan adquirirla de la dieta. Por lo tanto, el presente estudio en *A. tropicus*, un pez carnívoro dulceacuícola perteneciente a esta familia y con una importancia

económica relevante en el sureste de México, fue diseñado para investigar la influencia de la suplementación con taurina en dietas donde se eliminó la taurina de la fuente de proteína. En el presente estudio, nuestros hallazgos mostraron que la suplementación con taurina en dietas para larvas de *A. tropicus* tiene un efecto positivo en los índices de crecimiento, supervivencia, mejora las características morfológicas de la mucosa intestinal y regula la expresión de genes involucrados en la biosíntesis y transporte de taurina.

En el presente trabajo se encontraron diferencias en los parámetros de crecimiento y la sobrevivencia entre las dietas suplementadas con taurina (0.5, 1.0, 1.5, and 2.0%) y la dieta basal (0% taurina). Esto ha sido observado en especies de larvas como el lenguado senegalés (*Solea senegalensis*) (Pinto et al., 2010), el pargo japonés (*Pagrus major*) (Kim et al., 2016), la aleta amarilla de California (*Seriola dorsalis*) (Salze et al., 2019), el atún de aleta azul (*Thunnus thymus*, L.) (Betancor et al., 2019) y el lenguado de roca del norte (*Lepidopsetta polyxystra*) (Hawkyard et al., 2014), los cuales se enfocan principalmente en el enriquecimiento de presas vivas. Por otra parte, estudios utilizando alimentos microparticulados han sido empleados en el pez dulceacuícola *O. niloticus* (Al-Feky et al., 2016a) y el pez marino corvina amarilla (*Nibea albiflora*) (Xie et al., 2014). Nuestros resultados están en concordancia con estos, ya que el efecto positivo en el crecimiento y la supervivencia de las larvas de *A. tropicus* podría deberse a la asimilación de taurina durante el periodo larvario. Nuestros resultados mostraron que la taurina suplementada no presenta diferencias significativas entre tratamientos para el peso, la talla, la tasa de crecimiento específica, el peso ganado y el factor de condición, lo cual ha sido también observado en juveniles de múJol (*Mugil cephalus*) (Koven et al., 2023). Los resultados del presente estudio también mostraron baja sobrevivencia en las larvas alimentadas con la dieta basal (0% taurina) y se observó que entre mayor fue el porcentaje de taurina agregada, mayor fue la sobrevivencia. De esta forma, la no variabilidad entre tratamientos con taurina y la mayor sobrevivencia en los organismos alimentados con 2.0% taurina confirman su importancia durante el larvicultivo de *A. tropicus*. Además, el bajo crecimiento y baja sobrevivencia de las larvas alimentadas con 0% taurina también descartan que el número de organismos en las unidades experimentales influya en los parámetros de crecimiento. Estos resultados también podrían implicar que, si bien la taurina mejora los parámetros de crecimiento a partir de la suplementación con 0.5% taurina, es posible que no satisfaga los requerimientos del organismo, por lo tanto, se observe una baja sobrevivencia en dietas con baja suplementación. De acuerdo con nuestros resultados, estos concuerdan con los

resultados de *N. albiflora* donde peces alimentados con dietas al 2.0% mostraron mejores parámetros de crecimiento y tasas de supervivencia que aquellos sin taurina (Xie et al., 2014). Sin embargo, la mayoría de los resultados en etapas larvarias sugieren que la suplementación está entre 0.5% y 1.5% de taurina y también que la inclusión de taurina produce mayores efectos en el crecimiento y la utilización de alimentos en peces marinos que en peces de agua dulce (Betancor et al., 2019; Izquierdo et al., 2019; Li et al., 2022; Sampath et al., 2020). No obstante, se ha sugerido que la suplementación de taurina adecuada es más un factor específico de cada especie (El-Sayed, 2014).

Tomando en cuenta que el requerimiento de taurina es un factor especifico de cada especie, se ha estudiado además que este requerimiento puede cambiar con el estadio desarrollo de una especie (Salze & Davis, 2015; Wang et al., 2015). Por lo tanto, se pueden observar estas diferencias entre larvas, juveniles y reproductores de una misma especie. Por ejemplo, en un estudio realizado en *S. dorsalis* en donde reproductores fueron alimentadas con una dieta control (0.28% taurina) y una dieta suplementada (2.67% taurina) se observó las hembras alimentadas con taurina tuvieron un mejor desempeño en el número de desoves y la cantidad, viabilidad y características de los huevos. Asimismo, a partir de desoves con suficientes huevos en ambos tratamientos para llevar a cabo el larvicultivo, estás fueron utilizadas para un segundo experimento. En este experimento, las larvas fueron criadas utilizando dietas que consistieron en rotíferos y *Artemia* donde la dieta control tenía 0 y 0.04% de taurina respectivamente, mientras que en la dieta con taurina los rotíferos y *Artemia* tenían 0,07 y 0.12% de taurina. Los resultados mostraron que las larvas alimentadas con el alimento control que provenían de una hembra que también recibió un alimento control murieron en el día 15 después de la eclosión indicando la importancia de la taurina en *S. dorsalis*. Además, también se encontró una mayor mortalidad en las larvas de los reproductores del control que recibieron taurina en comparación con las larvas alimentadas con el alimento control o taurina suplementada provenientes de reproductores alimentados con taurina. Los autores sugieren el importante rol de la taurina durante la gametogénesis y la embriogénesis, y que la ausencia de está en las dietas de los reproductores no puede ser mitigada por la suplementación en la cría de las larvas (Salze et al., 2019). Por otra parte, en juveniles de *S. dorsalis* se ha estudiado la suplementación de taurina sola o en combinación con metionina, y los resultados demostraron que los mejores efectos son en aquellas alimentadas con taurina (1.4% taurina). En el caso de peces dulceacuícolas, la suplementación en etapas larvarias (Al-Feky et al., 2016a) y reproductores (Al-

Feky et al., 2016b) de *O. niloticus* ha permitido determinar que mientras larvas utilizan 0.97% taurina, los reproductores utilizan 0.8% taurina. Esto nos permite observar que en las tres diferentes etapas estudiadas en *S. dorsalis* los porcentajes utilizados de taurina son diferentes y que en el caso de *O. niloticus* no varía tanto posiblemente debido a su capacidad de síntesis *de novo*. Por lo tanto, se sugiere que estudios enfocados en determinar si existe una diferencia en sus requerimientos de taurina entre las diferentes etapas del pejelagarto también sean aplicados.

Así mismo, para determinar si existían diferencias entre tratamientos se realizó la caracterización morfológica de la mucosa intestinal. Al contrastar los tratamientos, se pudieron observar diferencias en la altura del epitelio intestinal entre los tratamientos, en donde los valores más altos fueron encontrados en 2.0% taurina (17.176 ± 2.99 µm), mientras que los valores más bajos se observaron en 0% taurina (16.832 ± 5.06 µm). Esto podría sugerir que 2.0% taurina estimula el tamaño de los enterocitos, favoreciendo una mejor digestión y absorción de nutrientes y lípidos. Es importante señalar que el tratamiento con menor cantidad de taurina presentó un mayor número de vesículas de muco, lo que indica una mayor actividad de las células caliciformes. En los peces, las células caliciformes son el principal tipo de célula mucosa en el epitelio intestinal y sirven como sitio principal para la digestión de nutrientes y la absorción de la mucosa (Salinas y Parra, 2015). Además, también pueden formar una línea de defensa en la mucosa intestinal y tener un papel secretor común (Pelaseyed et al., 2014). Por lo tanto, la alta actividad de las células caliciformes podría explicarse por el papel de la taurina en el control del microbiota en el intestino, donde una dosis más alta de taurina reduce la presencia de patógenos, disminuyendo la actividad de las células caliciformes (Rimoldi et al., 2016). En la especie dulceacuícola, pez gato americano (*Ictalurus* punctatus), los autores observaron que el efecto de las dietas de aceite de pescado oxidado reducía significativamente la longitud de las vellosidades, la cantidad de células caliciformes y el grosor muscular del intestino (Shi et al., 2021). Asimismo, la adición de taurina a las dietas de aceite de pescado oxidado revirtió esta tendencia, indicando que la taurina puede mantener la integridad estructural del intestino. Se han observado anormalidades intestinales particularmente en especies en donde se utilizan dietas basadas en fuentes de proteínas vegetales (Martins et al., 2019; Pervin et al., 2020; Venold et al., 2012; Zhu et al., 2021). Además, se observó que la suplementación con taurina en la carpa china (*Ctenopharyngodon idella*) tiene un efecto positivo en el crecimiento, la resistencia a la enteritis, los compuestos antimicrobianos intestinales y la inflamación intestinal atenuada y la función inmune intestinal (Yan et al., 2019).

La capacidad de un pez para biosintetizar taurina a través de cualquiera de las diferentes rutas podría reflejarse en las diferencias de los niveles de expresión/actividades de las enzimas clave y el transportador de taurina (Liu et al., 2017; Wang et al., 2016; Yokoyama et al., 2001; Zarate & Bradley, 2007) y puede ser influenciado por diferentes factores incluyendo la suplementación de taurina (Tappaz, 2004). Por otra parte, de las diferentes rutas que se han descrito para biosintetizar taurina se ha encontrado que la deficiencia de Csad en la ruta del ácido cisteíno sulfínico, es la limitante para la síntesis (Chang et al., 2013; Park et al., 2017; Seidel et al., 2019). Sin embargo, de acuerdo con los resultados obtenidos en el Capítulo 3 de la presente tesis, se sugiere que para larvas de *A. tropicus*, en esta etapa el organismo depende más de la ruta de la cisteamina, mientras que en juveniles temprano la ruta es órgano-dependiente.

Diferentes estudios han mostrado que la biosíntesis de taurina varía entre especies dulceacuícolas y marinas como la carpa común (*C. carpio*), el lenguado japonés (*Paralichthys olivaceus*) y la trucha arcoíris (*O. mykiss*) (Kim, Matsunari, Takeuchi, et al., 2008; Wang et al., 2016). Además, se ha encontrado que peces dulceacuícolas pueden sintetizar taurina a partir de metionina o cisteína a través de la ruta del ácido cisteíno sulfínico pero que, en el caso de los peces marinos, estos no pueden sintetizarlo o su síntesis no es suficiente y por eso deben adquirirlo a través de la dieta (Nakamura et al., 2021). En esta tesis los resultados mostraron que las larvas alimentadas con 0% taurina tenían una baja expresión de todos los genes involucrados en la biosíntesis y transporte de taurina en comparación al resto de los tratamientos, excluyendo al tratamiento 1.5% para los genes *csad* y *gad*. Nuestros resultados coinciden con lo observado en el atún rojo (*T. thynnus*, L.) para los genes *cdo* y *csad* en donde larvas suplementadas con 1.0% taurina están sobre expresadas del resto de los tratamientos (Betancor et al., 2019). Esta sobre expresión de *csad* con 1.0% taurina también ha sido encontrada en juveniles de mújol (*M. cephalus*) (Koven et al., 2023). En el caso de la expresión de *ado* encontramos que existe una sobrexpresión en larvas alimentadas con taurina suplementada en comparación con los organismos en la dieta 0% taurina lo cual difiere de lo encontrado en el atún rojo (*T. thynnus*, L.) (Betancor et al., 2019), en donde existe una subexpresión en 1.5% taurina en comparación con el resto de los tratamientos. Esto, sugiere que posiblemente en nuestro estudio, las larvas en los tratamientos sobreexpresados también están dependiendo de la ruta de la cisteamina para sintetizar taurina. Por su parte, se encontró baja expresión de *gad* en 0% y 1.5% taurina en comparación al resto de los tratamientos y una sobreexpresión fue encontrada principalmente en 2.0% taurina lo que podría indicar que la

especie también está dependiendo de la ruta del ácido cisteíno independientemente de la suplementación.

La actividad del transportador de taurina es sobreexpresada cuando los niveles de taurina son bajos, para incrementar la reabsorción y el reciclado de la taurina existente, y subexprexada cuando los niveles de taurina en el plasma son altos, permitiendo así el mantenimiento apropiado de las concentraciones (Liu et al., 2017). Además, aquellos organismos que son capaces de regular la expresión de *taut* lo hacen de manera rápida después de la ingesta dietética de taurina o cambios en la proteína dietética o el contenido de taurina (Baliou et al., 2020; Warskulat et al., 2007). En el atún rojo la expresión relativa de *taut* es regulada por la taurina dietaria de forma que es dependiente de la dosis, es decir cuando los niveles de expresión son bajos, la expresión sobre regulada para promover y potenciar la absorción y transporte de taurina (Betancor et al., 2019). Contrario a esto, en el pez dorado, la suplementación de 0.4% y 0.6% incrementan significativamente los niveles de expresión de *taut* (Xiong et al., 2020). En el caso de la presente investigación, los resultados mostraron una baja expresión de *taut* en los tratamientos 0, 0.5, 1.5, y 2.0% taurina lo que podría indicar que se está produciendo el mantenimiento de concentraciones adecuada de taurina. La sobreexpresión de *taut* en larvas alimentadas al 1.0% indican la modulación de la expresión de *taut*. Sin embargo, futuros trabajos son necesarios aplicando otras técnicas son necesarios para expandir el conocimiento de la regulación de taurina en larvas de pejelagarto.

Capítulo 6
Conclusiones y Recomendaciones

Conclusiones

En el presente trabajo de doctorado se abordó el estudio del análisis de los mecanismos de los genes que participan en las diferentes rutas de biosíntesis de taurina, así como su transportador utilizando enfoques bioinformáticos, moleculares y nutricionales utilizando principalmente como modelo de estudio al pejelagarto *Atractosteus tropicus*.

Los diferentes procesos fisiológicos en los que participan los genes que participan en las diferentes rutas de biosíntesis de taurina (*cdo*, *csad*, *gad*, *ado*) y el transporte de taurina (*taut*) son la reproducción, la digestión, los sistemas olfativo, visual, circulatorio y muscular. Estos procesos han sido identificados en diferentes especies a partir de estudios que permiten determinar si estos genes pueden ser o no sintetizados por la especie, así como a través del uso de diferentes herramientas moleculares como silenciamiento génico. Estos genes son importantes ya que regulan la taurina endógena y exógena presentes en las diferentes especies de peces.

El uso de herramientas bioinformáticas permitió la identificación de elementos reguladores en las zonas promotoras de *cdo*, *csad*, *gad*, *ado* y *taut*. Sitios de unión para los factores de transcripción Homeobox protein BarH-like 1 (BARX1), Brain-specific homeobox protein homolog (BSX), Helicase-like Transcription Factor (HLTF), Homeobox protein Hox-A7 (HOXA7), Homeobox protein Hox-B3 (HOXB3), Homeobox protein Hox-B6 (HOXB6), Homeobox protein Meis1 (MEIS1), Homeobox protein Meis3 (MEIS3), Nuclear factor of activated T cells 1 (NFATC1), y Homeobox protein Nkx-6.2 (NKX6-2) fueron encontrados en todos los genes que participan en la biosíntesis y transporte de taurina. Además, se observó que la frecuencia de HOX-B3 tiene sitios de unión múltiple en todas las zonas promotoras de todos los genes de las diferentes especies de peces analizadas.

Secuencias completas de nucleótidos y aminoácidos correspondientes a *cdo*, *csad*, *gad*, *ado* y *taut*, fueron identificadas a partir del transcriptoma disponible de pejelagarto. Los niveles de expresión de estos genes aparecen desde la eclosión de *A. tropicus* y en diferentes órganos de juveniles tempranos indicando que estos organismos tienen la maquinaria para sintetizar taurina *de novo* utilizando cualquiera de las tres rutas de biosíntesis. La ruta de biosíntesis principal en *A. tropicus* es la ruta de la cisteamina, particularmente en embriones y 9 dde, mientras que a los 5 y 9 dde predomina la ruta del ácido cisteíno sulfínico. La ruta de biosíntesis es órgano dependiente, ya que la ruta predominante en hígado es la ruta del ácido cisteíno sulfínico, en cerebro, ojo y

levemente en piel, únicamente ocurre la ruta del ácido cisteíco. Mientras que la ruta de la cisteamina si bien ocurre en todos los órganos, esta principalmente ocurre en el ojo.

La suplementación de taurina en larvas de pejelagarto demostró tener un efecto favorecedor en el crecimiento, sobrevivencia y diferencias en las características morfológicas de la mucosa intestinal de las larvas. Así también, la expresión relativa de los genes implicados en la biosíntesis y transporte de taurina mostraron una regulación de estos genes cuando hay cambios en los niveles de las dietas.

En conjunto, se puede concluir que este trabajo provee información que nos permite aseverar, que si bien, *A. tropicus* tiene la capacidad de biosíntesis de taurina a través de las diferentes rutas metabólicas, su implementación en la dieta permitirá un mejor rendimiento durante el larvicultivo del organismo. Estos resultados permiten proveer el uso de un nuevo aditivo que pueda ser empleado para futuras investigaciones no solo en larvas de pejelagarto, sino también en juveniles y reproductores.

Recomendaciones

Conocer las regiones promotoras de los genes involucrados en la biosíntesis y transporte de taurina en el pejelagarto a través de la clonación completa de estos genes, permitirá ampliar el conocimiento en la forma en que estos genes son regulados.

Realizar la suplementación de taurina en la *Artemia*, podría ayudar a obtener mejores resultados en la sobrevivencia de larvas de pejelagarto.

Utilizar dietas de origen vegetal en combinación con la taurina para intentar reducir los costos de producción de larvas de pejelagarto.

Profundizar en el estudio morfo-fisiológico a nivel histológico para comprender mejor el efecto de la taurina cuando es suplementada.

El empleo de técnicas moleculares como el silenciamiento génico o CRISPR, junto con secuenciación masiva que permitan conocer mejor los procesos y genes que se ven afectados o comprometidos cuando estos genes son alterados o cuando la taurina es suplementada.

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Anexos

Del presente trabajo de doctorado se han originado los siguientes productos terminados:

Martínez-Burguete, T., Peña-Marín, E., Martínez-García, R., Llera-Herrera, R., & Alvarez-González, C. (2023). Physiological role of genes involved in taurine biosynthesis in fishes and in silico approach to determine transcription factors in their promoters' zone. Latin American Journal of Aquatic Research, 52(1). http://dx.doi.org/10.3856/vol52-issue1-fulltext-3026

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Identification of taurine biosynthesis pathways and its transporter in tropical gar. Martínez-Burguete, T., Peña-Marín S., Martínez-García R., Alvarez-Villagomez C.S., Llera-Herrera R.A., Alvarez-González C.A. XVI Simposium Internacional de Nutrición Acuícola. Presentación de póster oral. 29 de marzo al 3 de abril. 2022. On-line.

Martínez-Burguete, T., Peña-Marín, S., Llera-Herrera, R., y Alvarez-González, C. A. Identification and expression of transcripts involved in taurine biosynthesis during early ontogeny of tropical gar. XV Simposium Internacional de Nutrición Acuícola. Presentación de póster. 13 al 15 de noviembre. 2019. Mérida, Yucatán.