UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO
División Académica de Ciencias Biológicas

"BIOSÍSTESIS DE ÁCIDOS GRASOS POLIINSATURADOS EN EL PEJELAGARTO *(Atractosteus tropicus),* UN MODELO DE PEZ ANCESTRAL"

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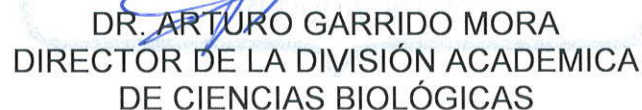

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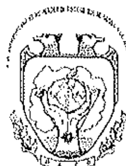

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A Dios todo poderoso por haberme dado la Vida y por iluminarme cada día.

Con inmenso cariño a mis padres:
María y Merardo
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RESUMEN

Los ácidos grasos poliinsaturados de cadena larga (LC-PUFA), como el ácido eicosapentaenoico (EPA, 20: 5n-3), el ácido araquidónico (ARA, 20: 4n-6) y el ácido docosahexaenoico (DHA, 22: 6n-3), son esenciales en múltiples procesos fisiológicos, especialmente importantes durante el desarrollo temprano de los vertebrados. La capacidad de biosíntesis de LC-PUFA es dependiente de dos enzimas importantes: acil desaturasas grasas (*Fads*) y elongación de proteínas de ácidos grasos de cadena muy larga (*Elovl*).

La presente tesis de investigación se enfoco en evaluar la capacidad de biosintesis de LC-PUFA en pejelagarto (*Atractosteus tropicus*). Con ese objetivo, en el primer capítulo se reportan las secuencias, la filogenia y los patrones de expresión de genes que codifican desaturasas (*fads*1 y *fads*2) y elongasas (*elovl*2 *y elovl*5). Para ello, se diseñaron oligonucleótidos específicos a partir del transcriptoma de *A. tropicus* para realizar qPCR durante el desarrollo embrionario y larvario, además de los órganos obtenidos de juveniles (intestino, músculo blanco, cerebro, hígado, corazón, tejido adiposo mesentérico, riñón, branquias, vejiga natatoria, estómago y bazo). Así mismo, se analizaron los perfiles de ácidos grasos durante el desarrollo temprano de *A. tropicus*, para definir los productos de biosíntesis asociados a las expresiones de los genes de interes. Posteriormente, se analizó el contenido de ácidos grasos durante la etapa embrionaria y larvaria, así como los alimentos utilizados para las larvas. Los resultados muestran la expresión de los genes *fads1*, *fads2*, *elovl2* y *elovl5* durante los estadios embrionarios, con picos de expresión a partir de los 15 dias post eclosión, relacionados con factores transcripcionales y dietéticos. Además, las *fads*1*, fads*2 y *elovl2* mostraron mayor expresión en el intestino, mientras que en el hígado hay una mayor expresión *de elovl*5. Estos resultados en conjunto con los perfiles de ácidos grasos sugieren que el pejelagarto activa su maquinaria biosintética de LC-PUFA permitiendo la producción de ARA, EPA y DHA para satisfacer las demandas fisiológicas en puntos cruciales del desarrollo durante el desarrollo temprano.

En un segundo capítulo se determinó el efecto del cambio en la proporción dietaria ácidos grasos poliinsaturados (PUFA) n-6 a n-3 como el ácido linoleico (LA, 18:2n-6) y el ácido α-linolenico (ALA, 18:3n-3) sobre el rendimiento productivo, índices somáticos, expresión diferencial de *fads1*, *fads2*, *elovl2* y *elovl5* y el perfil de ácidos grasos de carcasa y heces en juveniles del pejelagarto (*Atractosteus tropicus*). Para ello, se realizó un diseño experimental aleatorio con seis tratamientos por triplicado. Los tratamientos consistieron en cinco dietas experimentales con la sustitución de aceite de soya (SO) por aceite de linaza (LO) en las siguientes proporciones: DS100 (100% SO-0% LO), DS75 (75% SO-25% L0), DS50 (50% SO - 50% LO), D25 (25% SO-75% LO) y DS0 (0% SO-100% LO) y una sexta dieta utilizada como dieta de control (CD). Los resultados indican que las dietas DS100 y DS75 aumentaron significativamente la ganancia en peso, la tasa de crecimiento específica y la tasa de conversión proteica (WG, SGR y PER) en juveniles de *A. tropicus* y disminuyó significativamente su tasa de conversión alimenticia (FCR). Los índices hepatosomáticos (HSI) e indice viscerosomático (VSI) no mostraron diferencias significativas, mientras DS0 mostro incremento en la longitud relativa del intestino (RIL). Los resultados de expresión relativa de *elovl*2, *elovl*5, *fads*1 y *fads*2 en el hígado e intestino mostraron un incremento en el

tratamiento DS75 en comparación con los otros tratamientos Los resultados de perfiles de ácidos grasos en las carcasas mostró mayores niveles de EPA (20: 5n-3) y DHA (22: 6n-3) en la dieta con el 100% de aceite de linaza (DS0) de tal manera se confirmó la capacidad de biosíntesis de LC-PUFA en peces juveniles de *A. tropicus.*

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1.- INTRODUCCIÓN

Los ácidos grasos poliinsaturados de 18 carbonos (PUFA), como el ácido linoleico (LA, 18: 2n-6) y ácido α-linolénico (ALA, 18: 3n-3), no pueden sintetizarse de novo por los vertebrados, sin embargo, son sustratos esenciales para biosintetizar la ácidos grasos poliinsaturados de cadena larga (LC-PUFA) que incluyen el ácido docosahexaenoico (DHA, 22: 6n-3), ácido eicosapentaenoico (EPA, 20: 5n-3) y ácido araquidónico (ARA, 20: 4n-6) (Turchini et al., 2006; Fonseca-Madrigal et al., 2014; Garrido et al., 2019). En este sentido, los PUFA y LC-PUFA juegan un papel crucial en el crecimiento, la salud, la reproducción, las funciones corporales, la biología de las membranas, los procesos de señalización y las fuentes de energía (Ayisi et al., 2018).

La biosíntesis de LC-PUFA se realiza mediante dos enzimas clave, las acil desaturasas grasas (*fads*) y el alargamiento de proteínas de ácidos grasos de cadena muy larga (*elovl*). Las *fads* introducen un nuevo doble enlace (insaturación) entre el extremo carboxilo de una cadena de acilo graso y un doble enlace preexistente, mientras que las enzimas *elovl* catalizan la reacción inicial de condensación limitante de la velocidad de elongación de la cadena de acilo graso (Jakobsson et al., 2006; Guillou et al., 2010). En mamíferos, dos *fads* (*fads1* y *fads2*) y tres *elovl* (*elovl2*, *elovl4* y *elovl5*) representan las principales enzimas involucradas en la biosíntesis de LC-PUFA (Guillou et al., 2010; Castro et al., 2016), como se muestra en la **Figura 1.-** Rutas de biosíntesis de ácidos grasos poliinsaturados en vertebrados, incluyendo peces.

La biosíntesis de ARA a partir de LA, así como la biosíntesis de EPA a partir de ALA, se logra mediante dos vías distintas como sigue: una es Δ6 desaturación (*fads2*) - alargamiento (*elovl5*) - Δ5 desaturación (*fads1*) y otra es elongación (*elovl5*) - Δ8 desaturación (*fads2*) - Δ5 desaturación (*fads1*). Posteriormente, el DHA se puede biosintetizar a través de la denominada "vía de Sprecher", que consta de dos pasos de elongación consecutivos de EPA para producir ácido tetracosapentaenoico (24:5n-3), seguido de desaturación Δ6 (*fads2*) y un paso de acortamiento de cadena (β-oxidación parcial). Una ruta alternativa para la biosíntesis de DHA se puede operar en algunos teleósteos y consiste en un único alargamiento de EPA para producir ácido docosapentaenoico (22:5n-3), que se convierte directamente en DHA mediante la acción de una Δ4 desaturasa (Oboh et al. 2017).

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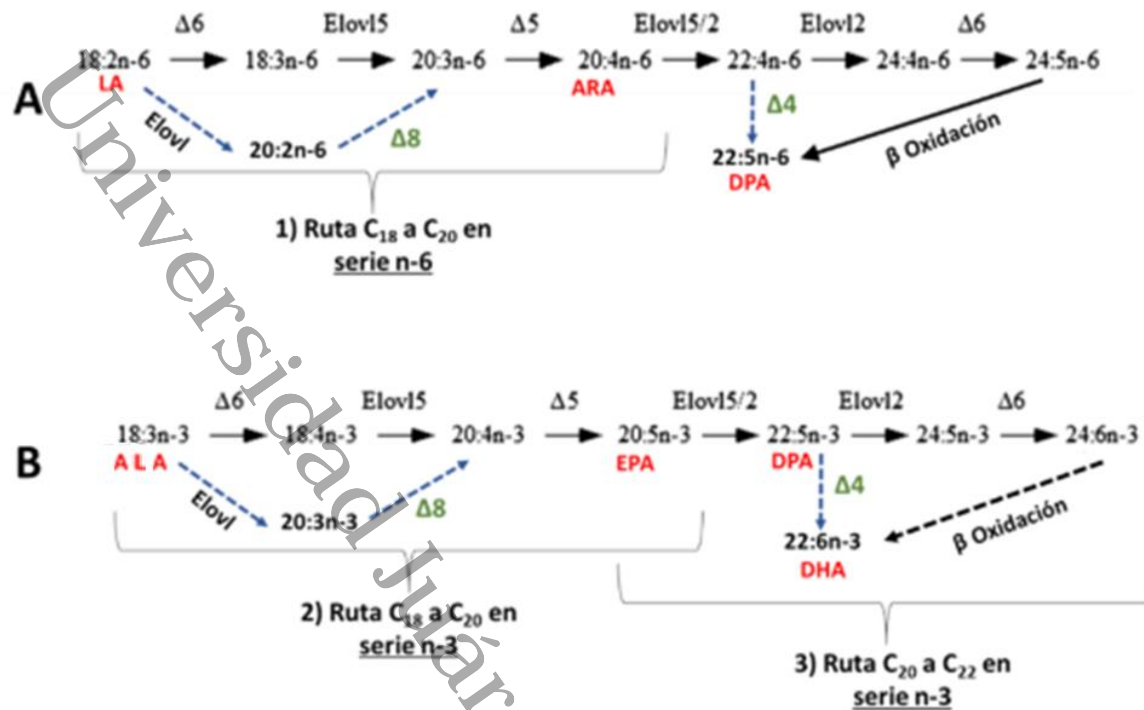

Figura 1.- Rutas de biosíntesis de ácidos grasos poliinsaturados en vertebrados, incluyendo peces A) de la serie n-6 y B) serie n-3 (Turchini et al., 2006; FonsecaMadrigal et al., 2014; Morais et al., 2015).

Los peces de agua dulce tienen una mayor capacidad para sintetizar LC-PUFA que los peces marinos, y se atribuye a la deficiencia de una o más enzimas clave en las vías biosintéticas (Tocher 2010; Ferraz et al., 2019). Además, los peces carnívoros marinos han perdido la capacidad de sintetizar LC-PUFA, como resultado de adaptarse a una dieta naturalmente rica en estos nutrientes (Turchini et al., 2006; Kabeya et al., 2017).

El aceite de pescado (FO) tiene una gran cantidad de LC-PUFA, y se incluye tradicionalmente en altos niveles de alimentos acuícolas de peces marinos, entregando altos contenidos de EPA y DHA. Sin embargo, esto ocasiona un fuerte impacto en las poblaciones naturales de peces en el ambiente marino (Tacon y Metian, 2015; FAO, 2020). Sin embargo, los aceites vegetales (VO) no contienen LC-PUFA, pero contienen PUFA como LA y ALA (Li et al., 2018), por lo que los aceites vegetales como los de soya y linaza son los ingredientes más utilizados en las dietas, los cuales aportan los sustratos necesarios para la biosíntesis de LC-PUFA, además disminuyen los costos de la elaboración de los alimentos (Turchini et al., 2009; Ayisi et al., 2019).

El pejelagarto (*Atractosteus tropicus*) es una especie carnívora de agua dulce nativa del sureste de México y América Central (Figura 2). *A. tropicus* pertenece a la familia

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Lepisosteidae, clasificada dentro del grupo Holostei, que incluye dos géneros, *Lepisosteus* con cuatro especies y *Atractosteus*, con tres especies. Los registros paleontológicos de estas especies sugieren que los gars actuales son el relicto de un antiguo clado de peces que, a diferencia de los teleósteos, no experimentó la duplicación completa del genoma (WGD) (Braasch et al., 2016).

Figura 2.- Pez juvenil de pejelagarto "*Atractosteus tropicus*".

Se sabe poco acerca de la capacidad de *A. tropicus* para utilizar de manera eficiente dietas basadas en aceites vegetales que sean más sostenibles. En consecuencia, es importante comprender las funciones fisiológicas de las acil desaturasas y elongasas grasas en *A. tropicus* y evaluar el efecto de los diferentes niveles de proporción de aceite de soya (PUFA n-3) con aceite de linaza (PUFA n-6) sobre el rendimiento del crecimiento, índice somático y perfil de ácidos grasos poliinsaturados en carcasses de juveniles del pejelagarto (*A. tropicus*). Ante ello la presente tesis de investigación caracteriza la capacidad de biosíntesis de ácidos grasos poliinsaturados en el pejelagarto (*Atractosteus tropicus*) y su eficiencia en el uso de diferentes fuentes lipídicas.

2.- ANTECEDENTES

2.1.- Diversidad de la maquinaria enzimática para la biosintesis LC-PUFA

Los peces muestran una gran diversidad de adaptaciones fisiológicas y metabólicas, debido a un proceso evolutivo, lo que genera una gran variabilidad en la capacidad de biosíntesis del LC-PUFA. Los peces teleósteos (modernos) han perdido el gen *fads1*, presentando exclusivamente *fads2*, la cual se ha diversificado para cumplir función bifuncional tipo Δ6Δ5 (Castro et al., 2012; 2016), como se muestra en Osteoglossomorpha (*Pantodon buchholzi*), Clupeocephala (*Danio rerio*) y tambaqui (*Colossoma macropomum*), pez conejo blanco (*Siganus canaliculatus*), pez plateado (*Chirostoma estor*), pez tenca (*Tinca tinca*), donde ésta enzima es la responsable de la desaturación de PUFA a LC-PUFA (Fonseca-Madrigal et al., 2014; Ferraz et al., 2018; Li et al., 2018, Lopes-Marques et al.,

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2018; Garrido et al., 2019; 2020). Por lo tanto, *fads2* se ha diversificado como resultado de la historia evolutiva específica de cada especie y factores ambientales que incluyen hábitat (marino versus agua dulce), nivel trófico y ecología, adquiriendo una capacidad de biosíntesis similar a las *fads1* (Li et al., 2018; Lopes-Marques et al., 2018). En la Tabla 1, se muestra un comparativo de la maquinaria enzimática involucrada en el metabolismo de biosíntesis ácidos grasos para peces marinos y peces de agua dulce. Las desaturasas Δ6Δ5 bifuncionales se caracterizan por biosintetizar LC-PUFA específicos, y la capacidad de biosíntesis de LC-PUFA es mayor en los teleósteos de agua dulce que en teleósteos marinos (Tocher et al., 2003; Xie et al., 2021), lo cual está relacionado con el escaso suministro de LC-PUFA en ecosistemas de agua dulce, así como también, el contenido de LC-PUFA de los organismos marinos depende de la zona geográfica (latitud, temperatura) y el nivel trófico (Zheng et al., 2005; Colombo et al., 2016). La influencia del nivel trófico en la capacidad de biosíntesis de LC-PUFA es un factor importante, ya que la mayoría de las especies marinas estudiadas consumen principalmente organismos (carnívoros/piscívoros), a diferencia de las especies de agua dulce que consumen principalmente planta/detritus (herbívoros) o plantas/detritus más animales (omnívoros) (Li et al., 2010), En ejemplo es el pez marino herbívora "conejo" (*Siganus canaliculatus*), el cual muestra la presencia de la enzima *fads2* con actividad tipo (Δ4 y Δ6/Δ5) y dos elongasas (*elovl4 y elovl5*) capaz de sintetizar LC-PUFA (Kabeya et al. 2017). Por lo tanto, los patrones de expresión genética para la biosíntesis de LC-PUFA están íntimamente relacionados con el hábitat y la biología especie (Lopes- Marques et al., 2018; Garrido, et al., 2019).

Tabla 1.- Comparativo de la maquinaria enzimática involucrada en el metabolismo de biosíntesis ácidos grasos para peces marinos y peces de agua dulce.

Peces Marinos			**Peces de Agua Dulce**		
Nombre científico	**Actividad de genes**	**Referencias**	**Nombre científico**	**Actividad de genes**	**Referencias**
Sparus aurata	**fads2*(Δ6), **fads2*(Δ8), ***elovl4*, ***elovl5*	Castro et al., 2016; Betancor et al., 2016	*Oreochromis niloticus*	**fads2(Δ6Δ5)*, **fads2(Δ4)*, ***elovl4*, ***elovl5*	Garrido et al., 2020
Larimichthys crocea	**fads2(Δ6)*, **fads (Δ4)*, ***elovl4*, ***elovl5*	Ji et al., 2020.	*Cyprinus carpio*	**fads2(Δ6)*, ***elovl5*	Eljasik et al., 2020

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Scophthalmus maximus	**fasd2(Δ6), **elvol5*	Castro et al., 2016; Kabeya et. al., 2017	*Danio rerio*	**fads2(Δ5Δ6), **elovl2, **elovl5, **elovl4*	Zhao et al., 2019
Rachycentron canadum	**fads2(Δ6), **elovl5, **elovl4*	Wang et al., 2016	*Channa striata*	**fads2(Δ6Δ5), *fads2(Δ4), **elovl5*	Kuah et al., 2016
Dicentrarchus labrax	**fads2(Δ6), **elovl5*	Fonseca-Madrigal et al., 2014	*Chirostoma estor*	**fads2(Δ6Δ5), *fads2(Δ4), **elovl5*	Fonseca-Madrigal et al., 2014
Siganus canaliculatus	**fads2(Δ5Δ6) *fads2(Δ4), **elovl5, **elovl4*	Kabeya et. al., 2017; Sun et al., 2019	*Tachysurus fulvidraco*	**fads2(Δ6), **elovl5*	Meng et al., 2017
Thunnus thynnus	**fads2(Δ6), **elovl5*	Betancor et al., 2016	*Lepisosteus oculatus*	**fads1(Δ5), *fads2(Δ6Δ8), **elovl2, **elovl5*	Lopes-Marques, et al., 2018
Solea senegalensis	*fads2(Δ6), fads2(Δ4), elovl5, elovl4*	Garrido et al.,2019; Torres et al., 2020			

Genes involucrados en la biosíntesis de lípidos, que poseen la capacidad de enlongar y desaturar ALA y LA a sus productos finales de LC-PUFA (ALA, EPA y DHA). *Desaturasas y **Elongasas.

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2.2.- Biosíntesis de LC-PUFA, a partir de PUFA en especies de agua dulce

La capacidad de biosíntesis de LC-PUFA, a partir de precursores PUFA se ha reportado en otras especies de agua dulce como *Cyprinus carpio* (Ren et al., 2012), *Chirostoma estor* (Fonseca-Madrigal et al., 2014), *Channa striata* (Kuah et al., 2016), *Oreochromis niloticus* (Chen et al., 2018), así mismo también se han realizado investigaciones dietarías con la sustitución de aceite de pescado por aceite vegetal en especies como *Tinca tinca* (Ljubojević et al., 2014), *Channa striata* (Kuah et al., 2016), *Colossoma macropomum* (Ferraz et al., 2019), *Barbonymus gonionotus* (Janaranjani et al., 2018), *Micropterus salmoides* (Chen et.al., 2019). El contenido de LC-PUFA n-3 en el tejido muscular está influenciado por la composición de ácidos grasos en la dieta (Ljubojević et al., 2014; Ayisi et al., 2019); en varias especies de agua dulce, se ha encontrado que las dietas ricas con aceites vegetales aumentan la biosíntesis de LC-PUFA mediante la expresión de genes *fads2/elovl5* en comparación con

una dieta rica con aceite de pescado (Ren, et al., 2012; Kuah, et al., 2016). Garrido et al. (2020), sugieren que *T. tinca* tiene alta tolerancia a las dietas con aceites vegetales que difieren notablemente en la composición de ácidos grasos, indicando que un equilibrio de 18:2n-6/18:3n-3 (disminuyendo 18:2n-6 y aumentando el contenido de 18:3n-3) podría incrementar el contenido de DHA, mejorando su valor nutricional y su potencial para la diversificación y sostenibilidad de la acuicultura.

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2.3.- Biosíntesis de LC-PUFA en diversos tejidos de peces

Diversos estudios han demostrado que el cerebro de teleósteos expresa genes que codifican enzimas involucradas en la biosíntesis de LC-PUFA como *fads2* (Δ6) y elongasas (*elovl5*) como se observó en *Gadus morhua L.* (Tocher et al., 2006) y *Esox lucius* (Carmona-Antoñanzas et al., 2013). Teniendo en cuenta que en todos los tejidos neurales se tiene una composición de ácidos grasos con una relación DHA: EPA muy alta, se ha sugerido que la retención *fads2* (Δ6) en peces marinos puede mantener los niveles de DHA en la membrana (por biosíntesis a través de metabolismo de EPA) de tejidos neurales en momentos de alta demanda fisiológica, incluso durante el desarrollo embrionario y larval (Abdul et al., 2016).

Existen estudios recientes de expresión de genes con respecto a la biosíntesis de LC-PUFA en donde las desaturasas (*fads1*(Δ5) y *fads2* (Δ6)) y elongasas (*elovl2* y *elovl5*) en *Chirostoma estor* (Fonseca-Madrigal et al., 2014), *Barbonymus gonionotus* (Janaranjani et al., 2018), *Sarpa salpa*, *Pegusa lascaris*, *Chelon labrosus* y *Atherina presbyter* (Garrido, et al., 2019), *Tinca tinca* (Garrido et al., 2020), presentan patrones de expresión altas de ARNm para desaturasas y elongasas en hígado e intestino en las especies de agua dulce, mientras que en las especies marinas los niveles de expresión más altos se muestran en el cerebro (Tocher et al., 2006).

Morais et al. (2009) reportan en salmón del Atlántico (*Salmo salar*) la sustitución del 100% de aceite de pescado con tres aceites vegetales (aceite de canola, aceite de linaza o aceite de soya) en la dieta, reportando actividad de expresión tisular en intestino, hígado, músculo blanco, riñón, bazo, corazón, cerebro, branquias y tejido adiposo, donde observaron una mayor expresión de *elovl5a*, *elovl5b* y *elovl2* en el intestino seguido de hígado y cerebro, indicando que la presencia de *elovl2* en el salmón permite la capacidad de esta especie para biosintetizar LC-PUFA. Garrido, et al. (2019), realizaron estudios de expresión génica tisular de *fads2* en varios peces, encontrando actividad génica en diversos tejidos como cerebro, branquias, corazón, intestino, hígado y músculo. Salema (*Sarpa salpa*) muestra un mayor

nivel de expresión de *fads2* en el hígado y el cerebro, similar a lo reportado en la suela de arena (*Pegusa lascaris*) que muestra la misma expresión de *fads2* en el hígado, el cerebro y el intestino, mientras que en el salmonete gris (*Chelon labrosus*) muestra una mayor expresión de *fads2* en el cerebro seguido por el hígado y el intestino; el pejerrey (*Atherina presbyter*) también muestra altas expresión de *fads*2 en el cerebro seguido de intestino e hígado, por lo tanto se ha considerado al hígado, intestino y cerebro como principales sitios metabólicos para la biosíntesis de LC-PUFA en estos peces.

Bhandari et al. (2016) reportan en los embriones de pez cebra (*Danio rerio*) una una alta expresión de los genes de *elovl1* (*elovl1a y elovl1b*) en la vejiga natatoria, y niveles aumentados de ácidos grasos de cadena larga de 14 a 20 carbonos, También observa que el consumo de lípidos de la yema se reduce considerablemente, mientras que las gotas de lípidos se acumulan dentro de la vejiga natatoria, lo que sugiere que ambos genes son clave para el desarrollo de la vejiga natatoria.

2.4.- Aceites vegetales en dietas para peces.

Los ingredientes más utilizados para la inducción a los PUFA son los aceites vegetales, y remplazan parcial o totalmente al aceite de pescado (FO) en la formulación de dietas. Los lípidos contenidos en las semillas de muchas plantas contienen una apreciable cantidad de PUFA, así como costos relativamente bajos y de producción estable con aumento de volumen. Los aceites de soya (*Glycine soja*), aceite de girasol (*Helianthus annus*), aceite de cártamo (*Carthamus tinctorius*) y aceite de semilla de uva (*Vitis vinífera*) contienen altas concentraciones de PUFA n-6 (>60%); mientras que en el aceite de germen de trigo (*Triticum vulgare*), aceite de maíz (*Zea mays*), aceite algodón (*Gossypium* sp.) y aceite de sésamo (*Sesamum indicum*) las concentraciones varían entre un 40-50%. Respecto a los PUFA n-3, el aceite de linaza (*Linum usitatissimum*) contiene hasta un 53% y el aceite de carmelina de un 35 a 45%. Otros ácidos grasos como el ácido oleico del aceite de oliva (*Olea europea*) con un contenido de 71%, también en el aceite de colza o canola (*Brassica napus*), aceite de cacahuete (*Arachis hypogaea*) (Turchini et al., 2009; USDA, 2010), el de coco (*Cocos nucifera*) que contiene 47% de ácido láurico y el aceite de palma (*Elaeis guineensis*) con 45% de ácido palmítico (USDA, 2010), son los más comunes en acuicultura como reemplazo del aceite de pescado.

Reportes en Lambari (*Astyanax altiparanae*), alimentados con diferentes proporciones de aceite de soya y aceite de linaza confirman la capacidad del Lambari para sintetizar EPA

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(20: 5n- 3) y DHA (22: 6n-3) (Pontes et al., 2019). Por otro lado, Sankian et al. (2019) indican que los peces mandarines (*Siniperca scherzeri*), pueden tolerar el reemplazo total de aceite de soya, ¿aceite de linaza o manteca de cerdo? sin ningún efecto adverso pronunciado sobre el rendimiento y la salud de los peces, esto probablemente se deriva de su capacidad para convertir PUFA (LA y ALA) a su correspondiente LC-PUFA. Así mismo, existen estudios realizados con aceite de soya como sustitución al aceite de pecado en la dieta de peces dulce acuícolas en donde representa una fuente de lípidos efectivas para las dietas formuladas obteniendo un mejor crecimiento en *Tinca tinca* (Ljubojević et al., 2014), *Oreochromis niloticus* (Godoy et al., 2019), *Astyanax altiparanae* (Pontes et al., 2019). La trucha Manchuria (*Brachymystax lenok*) tiene la capacidad de sintetizar LC-PUFA a partir de ALA; y la sustitución de FO por aceite de linaza (<75%) podría mejorar tanto el metabolismo de los lípidos como la resistencia a la oxidación (Yu et al., 2019).

La capacidad de biosíntesis de LC-PUFA a partir de PUFA en peces, depende no solo de la presencia de las enzimas elongasas (Elovl) y desaturasas (Fads), sino también de su actividad y especificidad en los diferentes sustratos directamente relacionados a la historia evolutiva, factores ambientales, nivel trófico y ecológico de la especie (Li et al., 2018; Lopes-Marques et al., 2018). Por lo que se han realizados grandes esfuerzos para determinar la capacidad de biosíntesis y degradación de los aceites vegetales en peces involucrados en la acuicultura.

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2.5.- Estudios de capacidad de biosíntesis de LC-PUFA en pejelagarto (*Atractosteus tropicus*)

Según los datos reportados por Lopes-Marques et al. (2018) a través del análisis filogenético en *Lepisosteus oculatus* se muestra la secuencia parcial de genes para *fads1* (Δ5) y *fads2* (Δ6), considerando que *A. tropicus*, tambien pertenece a la familia Lepisosteidae, se dedujo que estas especies tienen la capacidad de sintetizar LC-PUFA a partir de PUFA.

Existen pocos estudios en peces ancestrales que aborden el tema de las rutas de biosíntesis de LC-PUFA, análisis filogenético, rendimiento en el crecimiento, perfiles de ácidos grasos poliinsaturados y análisis en la expresión de biosíntesis de genes desaturasas (*fads1* (Δ5) y *fads2* (Δ6)) y elongasa (*elovl2* and *elovl5*), así como el entendimiento de la capacidad y rutas de biosíntesis de LC-PUFA a parir de (LA y ALA) en la dieta con diferentes proporciones de aceites vegetales (aceite de soya - aceite de linaza). siendo este el primer estudio realizado en ontogenia de larvas y en juveniles de *A. tropicus*.

3.- JUSTIFICACIÓN

El pejelagarto (*Atractosteus tropicus*), es una especie de gran importancia económica y ecologica, para el estado de Tabasco y el sur del país en general, con una gran importancia económica y gastronómica, por lo que se han realizado grandes esfuerzos para su inclusión en la acuacultura, sin embargo, necesita investigación base sobre las adaptaciones metabólicas y fisiológicas que permitan reforzar el desarrollo tecnológico de su cultivo, resaltando el desarrollo de un alimento específico que le proporcione los nutrientes necesarios que le permita un desarrollo adecuado y disminuya los costos de producción. En este sentido, este tipo de estudio permitirá generar conocimiento sobre las adaptaciones y capacidad de biosíntesis de LC-PUFA en un pez ancestral como lo es el pejelagarto y demostrar como dichas adaptaciones se relacionan con tetrápodos o peces modernos.

En la presente tesis de investigación y desde un punto de vista aplicado, el entendimiento de los procesos de regulación génica de las rutas de biosíntesis de LC-PUFA a partir de PUFA (LA y ALA) en la dieta con diferentes proporciones de aceites vegetales (aceite de soya - aceite de linaza), permitirá un uso eficiente de ingredientes alternativos a base de productos vegetales, conservando el contenido de LC-PUFA en los peces (Wang et al. 2014). Este estudio permitirá avanzar en la selección de ingredientes para elaborar dietas con altos grados de digestibilidad, que proporcione nutrientes biodisponibles, logrando una mayor sobrevivencia larvaria y una mejor tasa de crecimiento en juveniles, garantizando el éxito en el cultivo de esta especie.

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4.- OBJETIVOS

4.1.- Objetivo General

Caracterizar la capacidad de biosíntesis de ácidos grasos poliinsaturados en el pejelagarto (*Atractosteus tropicus*) y su eficiencia en el uso de diferentes fuentes lipídicas.

4.2.- Objetivos Específicos.

- Evaluar la expresión de los genes que participan en la biosíntesis de ácidos grasos poliinsaturados (*fads1* y *fads2*, *elovl2* y *elovl5*) y analizar el perfil de ácidos grasos poliinsaturados durante el desarrollo de la ontogenia temprana de pejelagarto (*A. tropicus*).

- Evaluar la expresión de los genes que participan en la biosíntesis de ácidos grasos poliinsaturados (*fads1* y *fads2*, *elovl2* y *elovl5*) en diferentes órganos (cerebro, branquias, corazón, hígado, musculo, tejido adiposo mesentérico, riñón, estómago, intestino, vejiga y bazo) en la etapa juvenil tardía de pejelagarto (*A. tropicus*).

- Evaluar el efecto de los diferentes niveles de proporción de aceite de soya (PUFA n-3) con aceite de linaza (PUFA n-6) sobre el rendimiento productivo, índice somático expresión relativa de *fads1* y *fads2*, *elovl2* y *elovl5* y perfil de ácidos grasos en carcasas y heces de juveniles del pejelagarto (*A. tropicus*).

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CAPITULO I

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Expression of long-chain polyunsaturated fatty acids biosynthesis genes during the early life-cycle stages of the tropical gar *Atractosteus tropicus*

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ABSTRACT

Long-chain (≥C_{20}) polyunsaturated fatty acids (LC-PUFA), including eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), are essential in multiple physiological processes, especially during early development of vertebrates. LC-PUFA biosynthesis is achieved by two key families of enzymes, fatty acyl desaturases (Fads) and elongation of very long-chain fatty acid (Elovl). The present study determined the expression patterns of genes encoding desaturases (*fads1* and *fads2*) and elongases (*elovl2* and *elovl5*) involved in the LC-PUFA biosynthesis during early life-stages of the tropical gar *Atractosteus tropicus*. We further analyzed the fatty acid profiles during early development of *A. tropicus* to evaluate the impact of Fads and Elovl enzymatic activities. Specific oligonucleotides were designed from *A. tropicus* transcriptome to perform qPCR (quantitative polymerase chain reaction) on embryonic and larval stages, along with several organs (intestine, white muscle, brain, liver, heart, mesenteric adipose, kidney, gill, swim bladder, stomach, and spleen) collected from juvenile specimens. Fatty acid content of feeds and embryonic and larval stages were analyzed. Results show that *fads1*, *fads2*, *elovl2* and *elovl5* expression was detected from embryonic stages with expression peaks from day 15 post hatching, which could be related to transcriptional and dietary factors. Moreover, *fads*1, *fads*2 and *elovl*2 showed a higher expression in intestine, while *elovl*5 showed a higher expression in liver, suggesting that the tropical gar activates its LC-PUFA biosynthetic machinery to produce ARA, EPA and DHA to satisfy physiological demands at crucial developmental milestones during early development.

1. Introduction

C_{18} polyunsaturated fatty acids (PUFA) like linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) cannot be synthesized de novo by vertebrates, including fishes, and are thus regarded as dietary essential fatty acids (Monroig et al., 2018). C_{18} PUFA are precursors of physiologically important long-chain (≥C_{20}) polyunsaturated fatty acids (LC-PUFA) including eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid

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(ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) (Castro et al., 2016; Monroig et al., 2018). In vertebrates, LC-PUFA play a crucial role in the membrane's structure fluidity and signaling processes, which are relevant in many physiological functions such as growth, neurological functions, inflammation, immune response, and reproduction (Colombo et al., 2016; Awisi et al., 2018).

Biosynthesis of LC-PUFA from C_{18} PUFA is mostly performed by two key enzyme families, Fatty acyl desaturases (Fads) and Elongation of very long-chain fatty acids (Elovl). Fads introduce a new double bond (unsaturation) between the carboxyl terminus of a fatty acyl chain and a pre-existing double bond, whereas Elovl enzymes catalyze the initial, rate-limiting condensation reaction of fatty acyl chain elongation (Jakobsson et al., 2006; Guillou et al., 2010). In mammals, two Fads (Fads1 and Fads2) and three Elovl (Elovl2, Elovl4, and Elovl5) are the main enzymes involved in LC-PUFA biosynthesis (Guillou et al., 2010; Castro et al., 2016). The biosynthesis of ARA from LA and EPA from ALA, is achieved by two distinct pathways as follows: one is Δ6 desaturation (Fads2) – elongation (Elovl5) – Δ5 desaturation (Fads1) and another is elongation (Elovl5) – Δ8 desaturation (Fads2) – Δ5 desaturation (Fads1). Subsequently, DHA can be biosynthesized via the so-called "Sprecher pathway", which comprises two consecutive elongation steps from EPA to produce tetracosapentaenoic acid (24:5n-3), followed by Δ6 desaturation (Fads2) and a chain shortening step (partial β-oxidation). An alternative pathway for DHA biosynthesis can be operated in some teleosts and consists of one single elongation from EPA to produce docosapentaenoic acid (22:5n-3), which is directly converted to DHA via the action of a Δ4 desaturase (Oboh et al., 2017).

In contrast to mammals, all *Fads* genes isolated from most Teleostei species, except for Elopomorpha species, are *Fads2* orthologs, suggesting that all teleosts have lost *Fads*1 genes during evolution (Li et al., 2018; Lopes-Marques et al., 2018; Garrido et al., 2019), suggesting that their substrate specificity has diversified because of the specific evolutionary history of each species, and environmental factors including their habitat (marine vs freshwater) (Ishikawa et al., 2019), trophic level and ecology (Kabeya et al., 2017b; Li et al., 2018). Therefore, in teleost, alternative pathways for LC-PUFA biosynthesis has been characterized where the enzymes encoded by *fads*2 can recognize and desaturase different substrates than their non-teleost vertebrate counterparts, thus the Δ6Δ8 desaturase specific activity typically contained in vertebrate Fads2 (Castro et al., 2016; Monroig et al., 2018), where teleost Fads2 also shows Δ4 and Δ6Δ5 desaturases activities (Monroig et al., 2011; Fonseca-Madrigal et al., 2014; Morais et al., 2015; Kabeya et al., 2017; Oboh et al., 2017; Li et al., 2018; Lopes-Marques et al., 2018). Overall, functional diversification of Fads2 is a particularly common trait among teleost species inhabiting freshwater where it has been hypothesized that a deficient supply of LC-PUFA through the diet could partly compensate for the loss of *fads1* (Kabeya et al., 2017a; Monroig et al., 2018). Through the functional diversification of the Fads2 family, many freshwater teleosts kept the fatty acid elongase *elovl2*, a gene absent in Neoteleostei genomes, a group including farmed marine finfish species. The *elovl2* gene encodes an elongase that is pivotal for the conversion of 22:5n-3 into 24:5n-3 and hence, plays a key role in DHA biosynthesis via the Sprecher pathway (Sprecher, 2000). The Fads2 family in marine teleost is less diverse compared to their freshwater counterparts, also, marine teleosts have lost elovl2, this could help to explain why they require LC-PUFA in their diet to guarantee a normal growth and development, preventing deficiency symptoms (Monroig et al., 2018). This is typically achieved in aquaculture by formulating diets that include high inclusion levels of marine ingredients, particularly fish oil, increasing the production of aquaculture feeds. On the contrary, C_{18} PUFA such as LA and ALA are provided by including vegetable oils in the diet, satisfying the essential fatty acid requirements for freshwater species since they have the enzymes to convert LA and ALA into LC-PUFA (e.g., Ferraz et al., 2019).

The tropical gar (*Atractosteus tropicus*) is a carnivorous freshwater species native to the southeast of Mexico and Central America that belongs to the Lepisosteidae family, classified within the Holostei group, which includes two genera, *Lepisosteus* with four species, and *Atractosteus*, with three species. From an evolutionary perspective, gars are paleontological records and a current relict of an ancient fish clade that diverged before the teleost-specific whole genome duplication (WGD or 3R) that occurred approximately 230–400 million year ago (mya) (Braasch et al., 2016; Venkatachalam et al., 2017), shows that gars and humans possess a *fads1* encoding a desaturase with Δ5 preference, and *fads2* encoding a desaturase with Δ6 preference as previously confirmed in L. *oculatus* (Lopes-Marques et al., 2018), where that species possesses Fads1 (Δ5) and Fads2 (Δ6Δ8) which are responsible for the LC-PUFA biosynthesis, demonstrating that the Lepisosteidae family conserves the desaturase machinery associated with tetrapods and that did not suffer the loss of Fads1 as found in more recently emerged fish lineages. While, as previous mentioned, teleosts only present Fads2 that shows mechanisms of bifunctionalization (Δ5Δ6). Currently wild populations of *A. tropicus* are being exploited although culture technology for *A. tropicus* developed in recent years has enabled a constant production that satisfies the demands of the regional market of southeastern Mexican states like Veracruz, Campeche, and Chiapas (Márquez-Couturier et al., 2006). Progresses have been made to understand important aspects of the *A. tropicus* nutrition and physiology, those include the characterization of digestive enzyme activities and the histological description of the digestive system through the species early development (Frías-Quintana et al., 2015), estimation of dietary lipid requirements (Huerta-Ortiz et al., 2018), the relative gene expression of fatty acid synthase (*fas*), acetyl-CoA carboxylase 1 (*acc*1) and carnitine palmitoyltransferase 1C (*cpt*1c) during larval ontogeny and across juvenile's organs (Jiménez-Martínez et al., 2019), as well as the effect of dietary lipid sources on growth, survival, cannibalism, and the relative gene expression *fas*, *acc*1 and *cpt*1c during early development (Jiménez-Martínez et al., 2020). However, little is known about the ability of *A. tropicus* to efficiently utilize vegetal oils-based diets that are more sustainable, which could help to reduce the feed production cost.

Therefore, each species possesses different capacity to biosynthesize LC-PUFA from PUFA, depending on factors such as the relative activity, availability, and affinity level of desaturases and elongases (Alhazzaa et al., 2018). However, in general terms, marine carnivorous fishes show more limited ability to synthesize LC-PUFA than fresh-water fishes and require the inclusion of C20 and C22 LC-PUFA in their diet (Yıldız et al., 2017; Alhazzaa et al., 2018), using fish oil to fulfill such fatty acid requirement. Nevertheless, fish oil is increasingly recognized as an environmentally unsustainable and economically unviable practice (Tocher, 2015; Chen et al., 2018). However, vegetables oils lack of n-3 LC-PUFA (C ≥ 20), therefore the replacement of FO by VO led the reduction in the n-3 LC-PUFA in diets (Yıldız et al., 2017). Consequently, the understanding of the physiological roles of fatty acyl desaturases and elongases in *A. tropicus* and assess their activity patterns during the entire life cycle is of strong interest for its ability to enable efficient and effective use of sustainable plant-based diet alternatives in aquaculture, as well to maintaining the nutritional quality of flesh (i.e., ARA, EPA, and DHA content of farmed fish) (Tocher, 2015; Yıldız et al., 2017). Here we investigated the expression levels of the fatty acyl desaturases *fads*1 (Δ5) and *fads*2 (Δ6), and fatty acyl elongases *elovl*2 and *elovl*5, involved in biosynthesis of LC-PUFA during development of the ancestral tropical gar *A. tropicus*. Moreover, we further determined the fatty acid profiles during the *A. tropicus* development to elucidate the contribution that activity of *fads* and *elovl* encoded enzymes have in the biosynthesis of the physiologically relevant LC-PUFA.

2. Materials and methods

2.1. Larviculture

A total of 700 *A. tropicus* embryos were obtained from a broodstock (one ~3.5 kg female and three ~1.5 kg males). The female was induced

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Table 1
Specific oligonucleotides used for quantitative real-time polymerase chain reaction (qPCR) in *Atractosteus tropicus*.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	(pb)	Amplification efficiency (%)
*fads*1 (Δ5)	TGACGTCACCAACTTCAGGC	TTGTCGATGTGGAAGGCAGT	105	95.17
*fads*2 (Δ6)	TCACTGGTTTGTCTGGGTGA	GCTCGATCTGGAAGTTGAGG	161	95.34
*elovl*2	CCACACTCTTGCTCACCCTA	TCCTCCTTGCCATGTTGCTA	173	96.12
*elovl*5	GGGATGGCTGTATTTCCAGA	TCCATTCGAGTGTCCATTCA	151	95.35
ef1	CCTGCAGGACGTCTACAAGATCG	GACCTCAGTGGTCACGTTGGA	120	99.82
18S rRNA	GGTAACGGGGAATCAGGGTT	TCCAATTACAGGGCCTCGAA	165	99.92

with a single dose of 35 μg per fish of luteinizing hormone-releasing hormone analogue (LHRHa) and was kept with the males in a 2000 L round plastic tank with polypropylene rope as artificial substrate emulating grass for egg adherence at the Tropical Aquaculture Laboratory (LAT) in the Academic Division of Biological Sciences (DACBIOL) from the Universidad Juárez Autónoma de Tabasco (UJAT). After spawning (16 h after hormonal induction), the broodstock were removed from the tank and the adhered eggs (embryonic stage) were kept within the tank until hatching (3 days after fertilization at 29 °C). The embryos with yolk sac were transferred into 70-L plastic tanks (420 per tank) connected to a 1500 L recirculation system equipped with a biological filter. Water quality was recorded daily with an oximeter (YSI 2030, Yellow Springs, Ohio, USA) and pH meter (HANNA HI 991.001, Nusfalau, Romania), and maintained during 30 days at a temperature of 29.1 ± 1.0 °C, dissolved oxygen of 6.5 ± 0.5 mg/L, and pH of 6.9 ± 0.2. After absorption of yolk sac (3 days post hatching, dph), feeding was offered five times a day (08:00, 11:00, 13:00, 15:00 and 18:00 h), starting with brine shrimp nauplii from mouth opening until 15 dph, and then co-fed between 16 and 18 dph with brine shrimp nauplii and trout diet (TD) containing 52% protein and 16% lipids (El Pedregal® Silver Cup, Toluca, México). Feeding densities of brine shrimp nauplii began with 50 nauplii per larva at mouth opening (3 dph), with a gradual increase to 800 nauplii per larvae at 15 dph. During co-feeding, densities were adjusted to remove 200 nauplii per larva every day until 18 dph. From 19 dph, only trout diet was offered. From that point onward, the feed particle size was adjusted according to the larval size (from 250-500, 500–750 and > 750 μm) and feed was provided at apparent satiation.

During larval development, 10 individuals (embryos or larvae) were collected from each experimental unit at 0 (embryo) and 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 (larvae) dph to perform the gene expression analysis. Larvae were collected and rinsed with distilled water prior to being preserved in RNA*later* (Life Technologies, Carlsbad, CA, USA). Then, samples were kept at room temperature for 24 h and stored at −80 °C until further analysis. For fatty acid (FA) analysis, 10 individuals (embryos or larvae) were collected from two different tanks and pooled at 5, 8, 9, 11, 13, 14, 18, 21, 24, 27 and 30 dph, frozen at −80 °C and subsequently lyophilized. All sampling procedures were performed prior to daily first feed supply at 8:00 h.

2.2. Tissue collection

Juveniles of *A. tropicus* were maintained in polyethylene tanks of 1.7 m^3 and were fed with trout diet. Three fish (average weight 22.33 g and 18.43 cm total length) were sacrificed using cold thermal shock (at 4 °C) after 24 h of fasting and subsequently dissected on ice to obtain individual organ samples from intestine, white muscle, brain, liver, heart, mesenteric adipose tissue, kidney, gill, swim bladder, stomach, and spleen. The tissue samples were immersed in RNA*later*, kept at room temperature during 24 h and stored at −80 °C until further analysis.

2.3. Sequence retrieval and phylogenetic analysis

The nucleotide sequences of *fads* and *elovl* genes and the reference genes *ef1* (elongation factor 1-alpha) and 18S rRNA used for this study were retrieved from a de novo transcriptome for *A. tropicus*, generated from Illumina short reads, reported in supplementary material by Martínez-Burguete et al. (2021) and supplementary_file_1. Transcripts were obtained by Basic Local Alignment Search Tool (BLAST), using as queries the genes reported for L. *oculatus* in the ENSEMBL databases (https://www.ensembl.org/) and NCBI (https://blast.ncbi.nlm.nih.gov/), using specific desaturases reported by Lopes-Marques et al. (2018) with accession numbers **MK005529** for *fads*1 and **MK005530** for *fads*2. The specific primer pairs were designed using Primer3web version 4.1.0 (Untergasser et al., 2012). The partial sequences obtained were translated into amino acid sequences using the ExPASy translation tool to detect the open reading frame (ORF), then the nucleotide sequences were compared to other using the NCBI-GenBank database (https://blast.ncbi.nlm.nih.gov/). The partial amino acid sequences of *A. tropicus* were obtained from the transcriptome of *A. tropicus* (NCBI BioProject accession number P**RJNA395289**, accessions **SRX3025453** to **SRX3025460**) for desaturases *fads*1 (Δ5), *fads*2 (Δ6) and elongases (*elovl*2 and *elovl*5). A set of amino acid sequences of *fads1*, *fads2*, *elovl2* and *elovl5* representatives from the main vertebrate clades were collected for phylogenetic analysis. Phylogenetic analysis was performed by constructing a tree using the Neighbor Joining (NJ) method with confidence in the resulting tree branch topology measured by bootstrapping through 1000 iterations using MEGA 7.0 software.

2.4. Primer design

Specific primers were designed for quantitative polymerase chain reaction (qPCR) from the partial sequences obtained from transcriptome, using Primer 3 Plus (http://primer3plus.com/web_3.0.0/primer3web_input.htm). Primers were designed with the following features: 20 to 25 nucleotide long, an annealing temperature (Ta) of ~60 °C, and a GC content of 40–60%. Finally, the secondary structure formation was assessed using Oligo Calculator version 3.27 (http://biotools.nubic.northwestern.edu/OligoCalc.html), to avoid intermolecular and intramolecular interactions that could adversely affect the annealing process. Table 1 includes sequences and other features from primers used in the present study.

2.5. Quantitative polymerase chain reaction (qPCR)

Total RNA from embryos/larvae pools and from juvenile selected tissues were extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommendations. RNA concentration and purity (through the 260/280 ratios) were measured with a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of RNA was confirmed after observing two discrete bands in a native agarose electrophoresis (1.5%).

One μg of total RNA was reverse transcribed into cDNA using Maxima First Strand cDNA Synthesis Kit (BioRad, Irvine, CA, USA) using random primers. The determination of transcript abundance of desaturases (*fads*1 and *fads*2) and elongases (*elovl*2 and *elovl*5) and reference genes was carried out in a thermocycler (CFX96 Real Time PCR, BioRad, CA). Each reaction contained 5 μL of qMix (SsoFast™ EvaGreen® Supermix, contains dNTPs, Sso7d fusion polymerase, $MgCl_2$, EvaGreen dye, stabilizers, Bio-Rad), 2 μL of cDNA, 2.2 μL of H_2O and 0.4 μL (10

Fig. 1. Alignment of the deduced amino acid sequence of desaturases (*fads*1 and *fads*2) from *A. tropicus* with desaturases from other organisms. Deduced amino acid sequences were aligned using ClustalX, with identity/similarity shading based on the BLOSUM62 matrix and a 75% cutoff. Identical residues are shaded black and similar residues are shaded grey. The asterisks on the base mark the heme-binding motif HPGG. The cytochrome *b*5-like domain is dot-underlined, two transmembrane regions are underlined dashes, and the three histidine-rich domains are solid-underlined in the figure (as shown in Monroig et al., 2010b and Lopes-Marques et al., 2018).

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Elovl2 [Equus caballus]
Elovl2 [Esox lucius]
Elovl2 [Gallus gallus]
Elovl2 [Homo sapiens]
Elovl2 [Mus musculus]
Elovl2 [Anguilla japonica]
Elovl2 [Crocodylus porosus]
Elovl2 [Danio rerio]
Elovl2 [Lepisosteus oculatus]
Elovl2 [Salmo salar]
Elovl2 [Xenopus tropicalis]
Elovl2 [Atractosteu tropicus]
Elovl [Anguilla japonica]
Elovl5 [Danio rerio]
Elovl5 [Homo sapiens]
Elovl5[Mus musculus]
Elovl5 [Salmo salar]
Elovl5 [Crocodylus porosus]
Elovl5 [Gallus gallus]
Elovl5 [Lepisosteus oculatus]
Elovl5 [Solea senegalensis]
Elovl [Sparus aurata]
Elovl5 [Oncorhynchus mykiss]
Elovl5 [Xenopus tropicalis]
Elovl5 [Atractosteus tropicus]

KXXEXXDT QXXFLHXXHH NXXXHXXMYXYY

TXXQXXQ

(caption on next page)

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Fig. 2. Alignment of the deduced amino acid sequence of elongases (*elovl*2 and *elovl*5) from *A. tropicus* with desaturases from other organisms. Deduced amino acid sequences were aligned using ClustalX, with identity/similarity shading based on the BLOSUM62 matrix and a 75% cutoff. Identical residues are shaded black and similar residues are shaded grey. Four different highly conserved motifs (KXXEXXDT, QXXFLHXXHH, NXXXHXXMYXYY and TXXQXXQ) are shown in this figure (as shown in Agaba et al., 2005 and Xue et al., 2014). A single histidine box, contained within the QXXFLHXXHH motif and five putative transmembrane domains (solid underlines).

μM) of each primer. Primers used in qPCR analyses are listed in Table 1. The qPCR program consisted of 3 min at 95 °C, followed by 38 cycles of 95 °C for 10 s, 60 °C for 30 s and an extension step at 70 °C for 5 s. All reactions were performed in duplicate. To normalize the gene expression of the evaluated genes, elongation factor 1-alpha (*ef1*) was used as a reference gene for embryo/larvae samples, whereas 18S rRNA was used as a reference gene for tissue juvenile samples because its transcription is not affected by experimental factors (Jiménez-Martínez et al., 2021). A standard curve for each pair of primers was created to estimate amplification efficiencies based on known amounts of cDNA (serial four-fold dilutions corresponding to cDNA transcribed from 100 to 0.1 ng of total RNA). The relative expression of genes was calculated by the ΔΔCt method (Pfaffl, 2001). Criteria of amplification, linear standard curve, and lack of primer dimers for qPCR for *A. tropicus* were as previously reported by Jiménez-Martínez et al., 2019; Jiménez-Martínez et al., 2021). Relative expression was calculated with efficiency-correction of the target and reference genes.

2.6. Fatty acid analyses during larval development

The FA profiles of embryos and larvae of *A. tropicus*, as well as those of brine shrimp nauplii and trout diet were determined by grounding and homogenizing the samples. Each sample comprised 100 mg of tissue and 0.2 mL of an internal standard (C19:0, Nonadecanoic acid, Fluka-72,332, 1 mg/mL) were added as a reference. FA were extracted with chloroform:methanol (2:1, *v*/v) according to Folch et al. (1957). The lipids extracted were saponified with 20% KOH:methanol (w:v) and free FA were recovered in hexane from the acidified saponifiable fraction (pH 1–2). Esterification with 10% BF_3 (boron trifluoride; Fluka Cat. No. 15716) in methanol was used to obtain fatty acid methyl esters (FAME) with an incubation of 60 min at 80 °C. FAME analysis was performed by capillary gas chromatography in a Perkin Elmer Clarus 500 GC equipped with a Perkin Elmer Elite-WAX (30 m × 0.25 mm × 0.25 μm film thickness, crossbond–PEG) capillary column and a flame ionization detector using hydrogen as carrier gas (flow rate of 40 mL/min). Temperatures of injector and detector were programmed at 280 and 250 °C, respectively. Column temperature was programmed to increase from 40 to 200 °C at 20 °C/min and from 200 to 250 °C at 2.5 °C/min. FAME identification was obtained by comparing the retention times of FAME peaks with those of reference standards (Supelco 37 Comp. FAME Mix). Results were reported as percentages (%) of total FAs.

2.7. Statistical analysis

The fatty acid profiles during early development, the relative expression levels of desaturases (*fads1* and *fads2*) and elongases (*elovl2* and *elovl5*) during early development and the relative expression levels of desaturases and elongases in the different tested organs of *A. tropicus* juveniles were compared through a Kruskal-Wallis test to determine if significant differences exist between treatments. Where statistical differences were detected, the post-hoc test of Nemenyi was performed to determine which treatments were different, using a significance value of $P \leq 0.05$. All statistical analyses were performed using STATISTICA™ v. 7.0 software (Statsoft, Tulsa, OK, USA).

3. Results

3.1. Sequence analysis

Fig. 1 shows a full-length ORF sequence obtained from the transcriptome of *A. tropicus* for *fads1* with 1350 bp which encode a putative protein of 449 amino acids, and a partial sequence of the *A. tropicus fads2* with 988 bp within the ORF. Alignments of the *A. tropicus* fatty acyl desaturases showed that both enzymes have conserved features, also a cytochrome *b*5-like domain containing a heme-binding motif (HPGG), three histidine boxes (HXXXH, HXXHH and QXXHH) and two transmembrane regions. Regarding elongases, Fig. 2 shows the full-length ORF obtained for *elovl2* and *elovl5* elongases. The ORF of *elovl2* comprised 927 bp that encoded a putative protein of 308 amino acid. Moreover, *elovl5* had 900 bp encoding 299 amino acid. The retrieved *elovl* sequences from *A. tropicus* contained well-conserved motifs of the Elovl family, including four highly conserved motifs (KXXEXXDT, QXXFLHXXHH, NXXXHXXMYXYY and TXXQXXQ), a single histidine box (HXXHH) contained within the QXXFLHXXHH motif and five putative transmembrane domains.

3.2. Phylogenetics

Fig. 3A shows a phylogenetic tree with all fish Fads proteins classified into two major groups with each *A. tropicus* Fads grouping, one for Fads1 and another one for Fads2 orthologs from other vertebrates. The *A. tropicus* Fads1 grouping formed a clade with a bootstrap of 100% between the tropical gar *A. tropicus* and the spotted gar L. *oculatus*, and with the Japanese eel (*Anguilla japonica*) and *Polypterus senegalus*, a bootstrap of 99% and 82%, respectively. Relatively more distant, Fads1 from American beaver (*Castor canadensis*), mouse (*Mus musculus*), horse (*Equus caballus*), grey mouse lemur (*Microcebus murinus*), human (*Homo sapiens*), wild pig (*Sus scrofa*), rooster (*Gallus gallus*) and frog (*Xenopus tropicalis*) formed a separate clade containing exclusively Fads1 sequences. Similarly, the phylogenetic analysis of Fads2 from both gar species (*A. tropicus* and L. *oculatus*) showed that these sequences, when clustered together, had a bootstrap value of 100%, as part of a branch grouping Fads2 from other vertebrates such as the Japanese eel (*A. japonica*), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), gilthead seabream (*Sparus aurata*), Senegalese sole (*Solea senegalensis*), human (*H. sapiens*) and mouse (*M. musculus*). The amino acid identity/similarity of desaturases varied between Fads1 and Fads2, where Fads1 shows an identity and similarity of 96.4 and 97.3% between *A. tropicus* and *L. oculatus*, while Fads2 shows an identity and similarity of 100% between *A. tropicus* and *L. oculatus*, although partial Fads2 sequences were compared (Table 2).

The phylogenetic tree constructed with Elovl sequences showed that all proteins were classified into two major groups with each *A. tropicus* Elovl clustering with the corresponding Elovl2 and Elovl5 orthologs from other vertebrate species (Fig. 3B). The *A. tropicus* Elovl2 was grouped more closely with Elovl2 from L. *oculatus* (bootstrap of 100%) and *A. japonica* (bootstrap of 89%). Finally, the *A. tropicus* Elovl5 was grouped with the L. *oculatus* Elovl5 with a bootstrap value of 100%, forming a clade with species such as: crocodile (*Cocrodylus porosus*), rooster (*G. gallus*), frog (*X. tropicalis*), human (*H. sapiens*) and homemade mouse (*M. musculus*) with a bootstrap value of 64% (Fig 3B). The amino acid identity/similarity of desaturases varied between Elovl2 and Elovl5, where Elovl2 showed identity/similarity of 98/99% between *A. tropicus* and *L oculatus*, while Elovl5 had identity/similarity scores of

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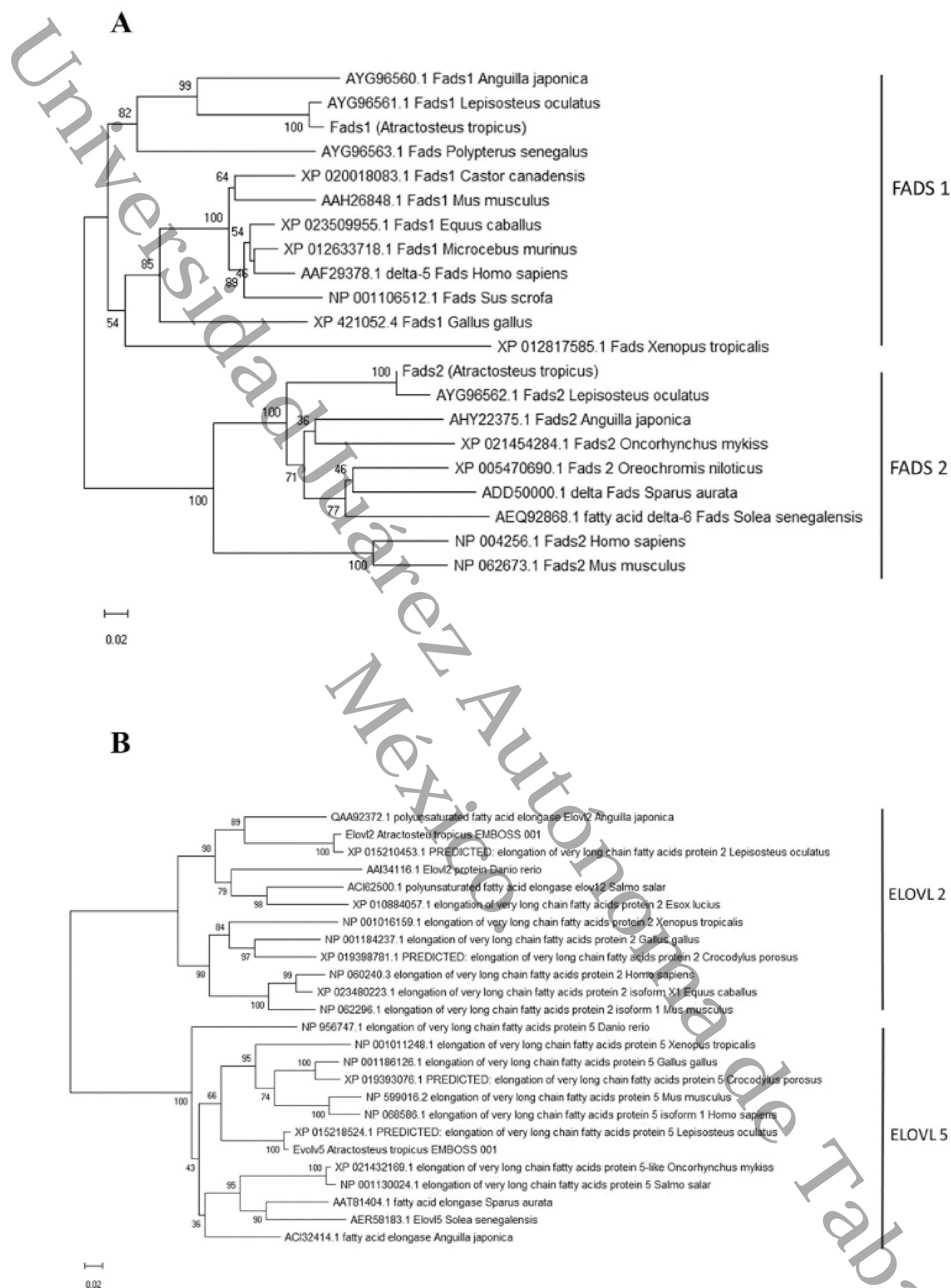

Fig. 3. Phylogenetic tree comparing putative amino acid sequences of desaturases Fads1 (Δ5) (a) and Fads2 (Δ6) (b) and elongases Elovl2 (c) and Elovl5 (d) from *A. tropicus* and other organisms. The tree was constructed using the Neighbor Joining method with MEGA 7. The horizontal branch length is proportional to amino acid substitution rate per site. The numbers represent the frequencies (%) with which the tree topology presented was replicated after 1000 iterations.

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Table 2
Comparison of Identity and Similarity percentages between the deduced amino acid sequence of desaturases (Fads1 and Fads2) and elongases (Elovl2 and Elov5) of *Atractosteus tropicus* and other species.

Species	Elovl Identity	Elovl Similarity	Species	Fads Identity	Fads Similarity
Elovl2 [*Equus caballus*]	77.7	85.2	Fads1 [*Anguilla japonica*]	77.8	84.3
Elovl2 [*Esox lucius*]	75.1	84.5	Fads1 [*Castor canadensis*]	70.3	81.9
Elovl2 [*Gallus gallus*]	73.4	83.5	Fads1 [*Equus caballus*]	69.5	82.2
Elovl2 [*Homo sapiens*]	71.0	83.8	Fads1 [*Gallus gallus*]	62.4	72.5
Elovl2 [*Mus musculus*]	70.4	85.5	Fads1 [*Mus musculus*]	68.6	80.6
Elovl2 [*Anguilla japonica*]	81.5	89.1	Fads1 [*Polypterus senegalus*]	71.7	82.3
Elovl2 [*Crocodylus porosus*]	72.7	85.2	Fads1 [*Sus scrofa*]	68.9	81.2
Elovl2 [*Danio rerio*]	71.6	81.3	Fads1 [*Xenopus tropicalis*]	15.6	19.8
Elovl2 [*Lepisosteus oculatus*]	**98.0**	**99.0**	**Fads1 [*Lepisosteus oculatus*]**	**96.4**	**97.3**
Elovl2 [*Atractosteu tropicus*]	**100**	**100**	**Fads1 [*Atractosteus tropicus*]**	**100**	**100**
Elovl2 [*Salmo salar*]	72.7	82.5	Fads1 [*Microcebus murinus*]	70.8	82.6
Elovl2 [*Xenopus tropicalis*]	73.7	83.5	**Fads2 [*Atractosteus tropicus*]**	**100**	**100**
Elovl5 [*Lepisosteus oculatus*]	**98.6**	**100.0**	**Fads2 [*Lepisosteus oculatus*]**	**100**	**100**
Elovl5 [*Atractosteus tropicus*]	**100**	**100**	Fads2 [*Anguilla japonica*]	78.3	87.5
Elovl5 [*Danio rerio*]	77.0	88.2	Fads2 [*Homo sapiens*]	68.1	80.1
Elovl5 [*Homo sapiens*]	76.3	86.0	Fads2 [*Mus musculus*]	67.6	79.8
Elovl5[*Mus musculus*]	75.7	86.0	Fads2 [*Oncorhynchus mykiss*]	76.7	86.8
Elovl5 [*Salmo salar*]	78.7	88.2	Fads 2 [*Oreochromis niloticus*]	75.0	86.4
Elovl5 [*Crocodylus porosus*]	78.7	88.9	Delta-5 Fads [*Homo sapiens*]	59.6	73.4
Elovl5 [*Gallus gallus*]	78.4	88.9	Delta-6 Fads [*Solea senegalens*]	71.7	84.4
Eovl5 [*Solea senegalensis*]	73.3	84.8	Delta-6 Fads [*Sparus aurata*]	75.8	84.9
Elovl [*Sparus aurata*]	78.7	86.8			
Elovl5 [*Oncorhynchus mykiss*]	79.1	88.2			
Elovl5 [*Xenopus tropicalis*]	80.4	87.2			

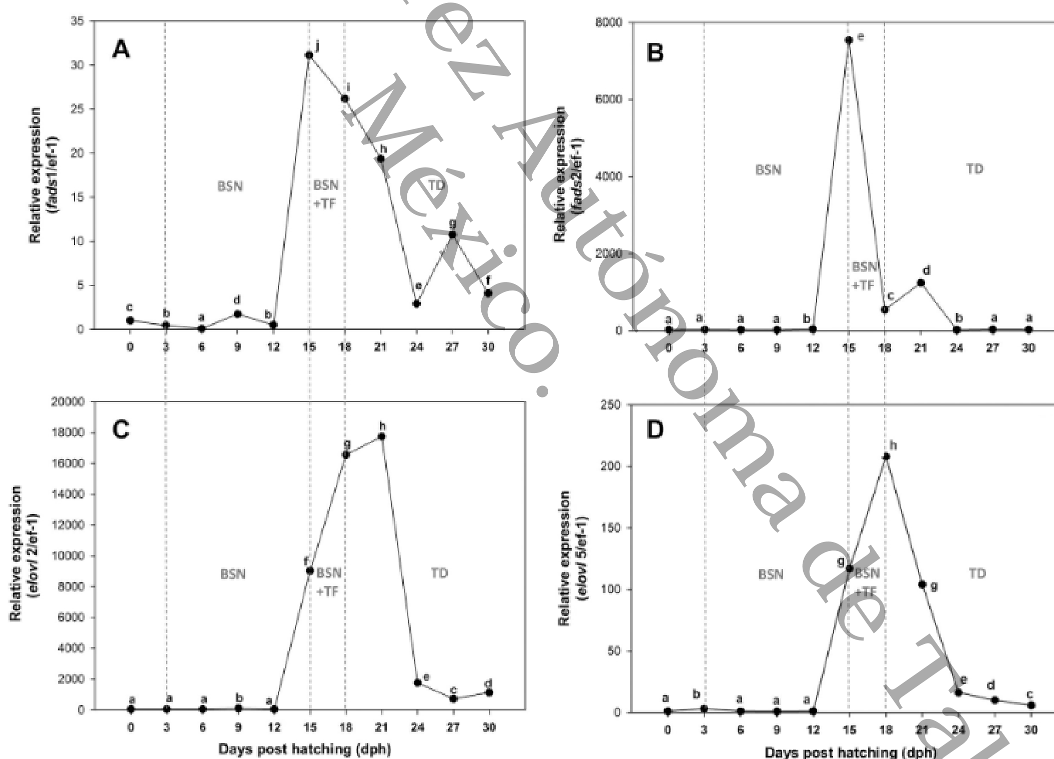

Fig. 4. Relative gene expression of desaturases *fads1* ($\Delta 5$) (A), *fads2* ($\Delta 6$) (B), elongases *elovl2* (C), and *elovl5* (D) during initial ontogeny of *A. tropicus*. Values in the graph represents means ± SD ($n = 3$). Means with different superscripts are significantly ($p \leq 0.05$) different. BSN, brine shrimp nauplii; TD, Trout diet.

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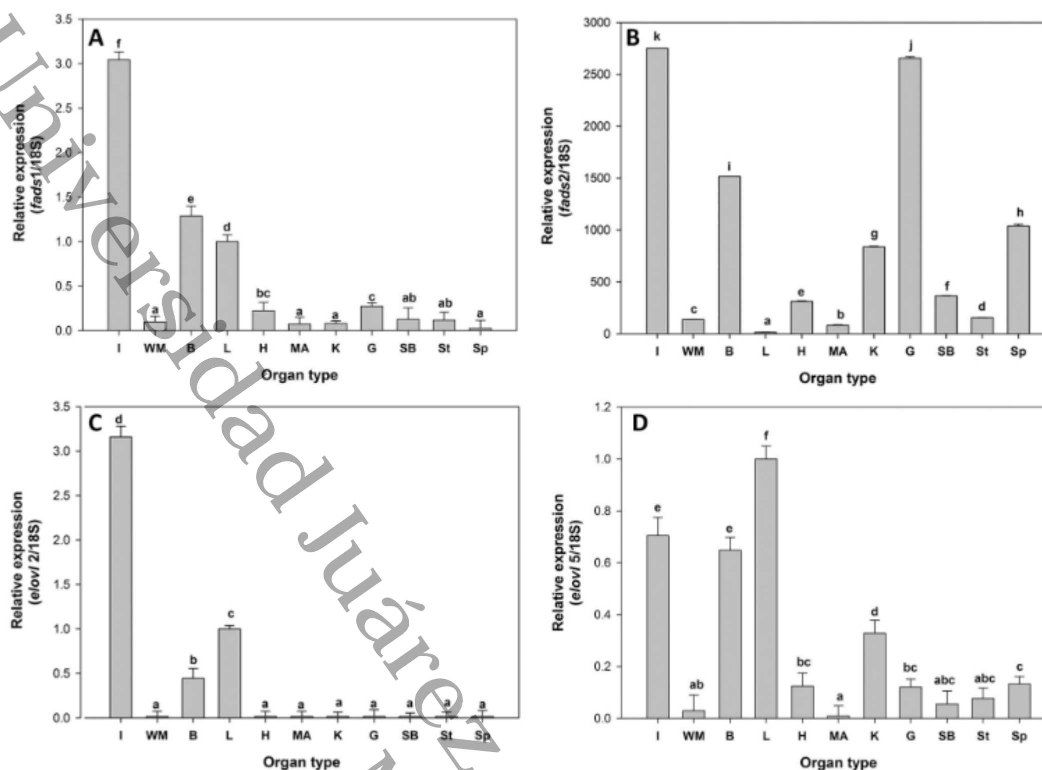

Fig. 5. Relative expression of desaturases *fads1* (Δ5) (a) and *fads2* (Δ6) (b) and elongases *elovl2* (c) and *elovl5* (d) in different juveniles' tissues: intestine (I), white muscle (WM), tissue brain (B), liver (L), heart (H), mesenteric adipose (MA), kidney (K), gill (G), swim bladder (SB), stomach (St) and spleen (Sp). Values in graph represents means ± SD (n = 3). Means with different superscripts are significantly ($p \leq 0.05$) different.

98.6/100% between *A. tropicus* and *L. oculatus* (Table 2).

3.3. Relative expression during larvae ontogeny

Transcripts of the *A. tropicus* desaturase *fads1* were detected from the embryo stage (0 dph) but the relative abundance remained low until 12 dph (Fig. 4A). A remarkable increase in relative abundance levels was detected at 15 dph, the point at which the highest expression of the *A. tropicus fads1* was observed ($p \leq 0.05$) (Fig. 4A). Subsequently decreasing levels of *fads1* transcripts were observed until the end of the larval period (30 dph) (Fig. 4A). Expression of the *A. tropicus fads2* was not detected at the embryo stage (0 dph). Low expression levels of the *A. tropicus fads2* continued until 12 dph and, as observed for *fads1*, a peak of *fads2* transcripts was detected at 15 dph ($p \leq 0.05$) (Fig. 4B), while a second peak of *fads*2 and *fads1* were detected at 21 and 27 dph, respectively. Low expression levels of the *A. tropicus elovl2* were detected from 0 to 12 dph. (Fig. 4C). However, there was a continuous increase from 15 dph to 21 dph, the time point at which the highest *elovl2* expression was observed (Fig. 4C). Subsequently, the expression of the *A. tropicus elovl2* dropped notably ($p \leq 0.05$) (Fig. 4C). As described above, the *A. tropicus* of *elovl*5 had very low expression levels until 12 dph, point beyond which an increase of expression was detected until reaching its maximum at 18 dph ($p \leq 0.05$) (Fig. 4D).

3.4. Tissue distribution of fads and elovl transcripts

The highest expression of the *A. tropicus fads1* gene was detected in intestine, followed by brain and liver, with lower expression levels detected in heart, gills, stomach, bladder, white mesenteric tissue, kidney, and spleen ($p \leq 0.05$) (Fig. 5A). For *fads2*, high expression levels in the intestine and gills were detected, followed by brain, spleen, and kidney, with low expression levels detected in other tissues including liver ($p \leq 0.05$) (Fig. 5B).

The highest expression of *elovl*2 was detected in intestine, followed by the liver and brain, with a very low expression detected in the other tissues analyzed ($p \leq 0.05$) (Fig. 5C). Regarding *elovl5*, liver, followed by intestine, brain and kidney, showed the highest expression levels in *A. tropicus* juveniles ($p \leq 0.05$) (Fig. 5D).

3.5. Fatty acid profiles during larval development

The FA profiles (% of total FA) of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) during *A. tropicus* early development, as well as diets (brine shrimp nauplii and trout feed), are shown in supplementary_file_2.

High levels of 20:5n-3 (EPA) and 22:6n-3 (DHA) were detected at 5 dph, decreasing on days 8 and 9 post hatching, while 18:3n-3 (ALA) showed the lowest levels at 5 dph, increasing at 11 dph, showing its highest percentage on day 21 ($p \leq 0.05$) (Fig. 6A). However, from 24 dph, 18:3n-3 decreased, while 20:5n-3 and 22:6n-3 increased until the 30 dph ($p \leq 0.05$). The highest levels of 18:2n-6 (LA) and 20:4n-6 (ARA) were shown at 5 dph, decreasing until 8 and 9 dph, however, these FA tended to increase until the 30 dph ($p \leq 0.05$) (Fig. 6B).

4. Discussion

Activity of the LC-PUFA biosynthetic pathways depends on multiple factors such as habitat (freshwater, brackish water, marine),

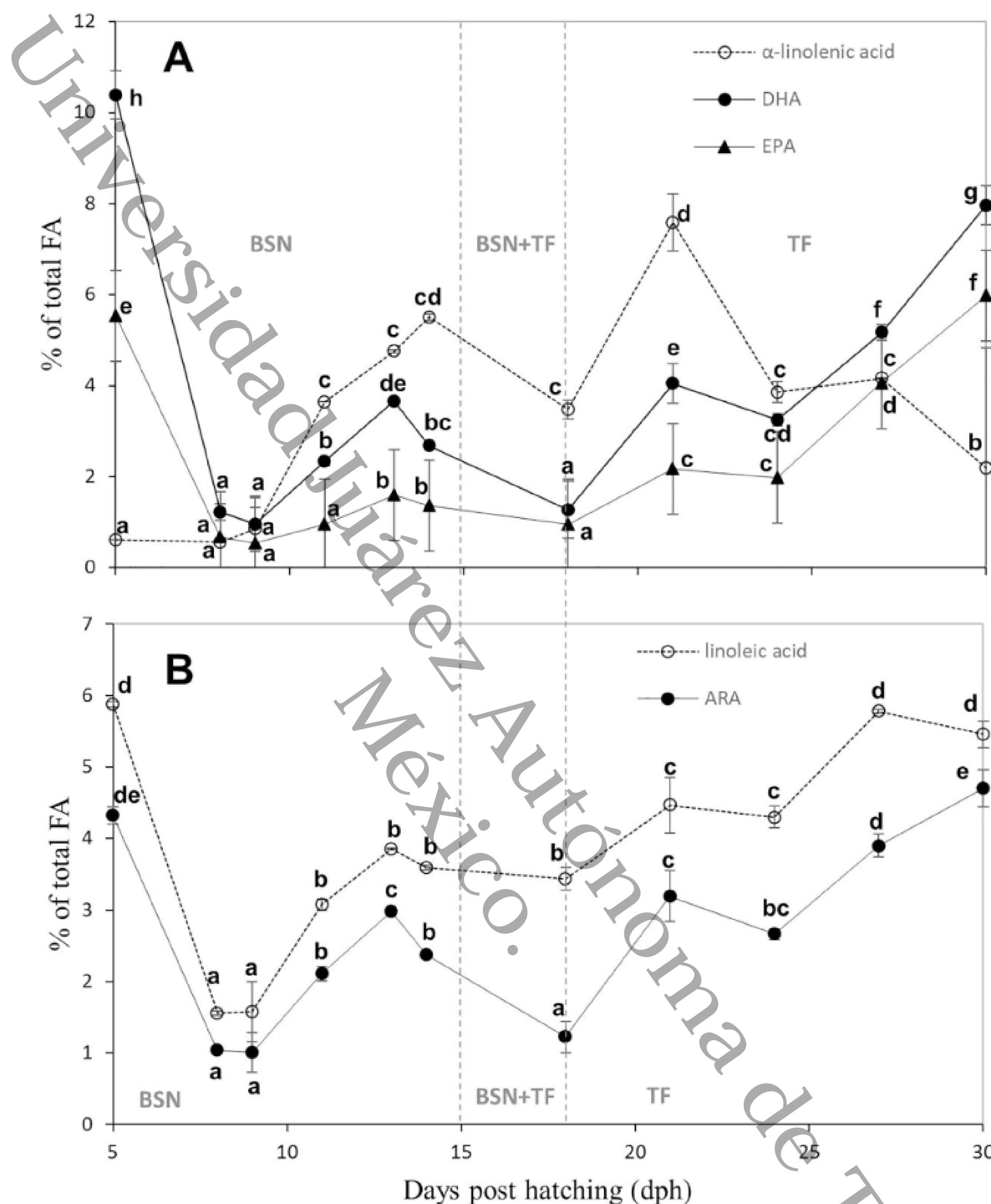

Fig. 6. Selected fatty acid profile (% total fatty acids) of *A. tropicus* during larval development, shown as n-3 LC-PUFA biosynthetic precursor (18:3n-3) and desaturation/elongation products (20:5n-3 and 22:6n-3) (Fig. 6a), and n-6 LC-PUFA biosynthetic precursor (18:2n-6) and desaturation/elongation product 20:4n-6 (Fig. 6b). Means ± SD ($n = 2$) with different superscripts are significantly ($p \leq 0.05$) different. BSN, brine shrimp nauplii; TD, Trout diet.

developmental stage, reproduction status, nutritional history, and stress status (Fonseca-Madrigal et al., 2014; Colombo et al., 2016; Kabeya et al., 2018; Lopes-Marques et al., 2018; Garrido et al., 2019). While remarkable progress has been made in elucidating the key components of LC-PUFA biosynthesis in teleost species with interest in aquaculture (e.g., Monroig et al., 2018), our current understanding of these pathways in ancestral fish such as gars is scarce. The present study allowed us to gain insight into the desaturase and elongase compensation by enabling the tropical gar *A. tropicus* to endogenously produce LC-PUFA and how such enzymatic machinery is modulated during *A. tropicus* development (Lopes-Marques et al., 2018; Garrido et al., 2019).

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In mammals, the desaturation reactions occurring along the LC-PUFA biosynthetic pathways are catalyzed by FADS1 and FADS2 (Guillou et al., 2010). Species of the orders Cyclostomata (*Lethenteron camtschaticum*), Chondrichthyes (*Callorhinchus milii, Scyliorhinus canicula*), Polypteriformes (*P. senegalus*), Lepisosteiformes (*L. oculatus*) and Elopomorpha (*A. japonica*) maintain the *fads1* ortholog that appears to be restricted to pre-3R and a certain early emerged post-3R lineages such as Elopomorpha (Castro et al., 2016; Lopes-Marques et al., 2018). In the present study, through sequence and phylogenetic analysis, it was revealed that *A. tropicus* possesses both *fads*1 and *fads*2 orthologs in its genome, which clustered very closely with orthologs characterized from the spotted gar L. *oculatus* (Lopes-Marques et al., 2018). It has previously been reported that the functional characterization of the L. *oculatus* Fads1 showed that this enzyme has Δ5 desaturase activity towards 20:3n-6 and 20:4n-3, which are converted into 20:4n-6 (ARA) and 20:5n-3 (EPA), respectively, whereas the L. *oculatus* Fads2 has Δ6 desaturase activity towards 18:2n-6 and 18:3n-3, which were desaturated to 18:3n-6 and 18:4n-3, respectively (Lopes-Marques et al., 2018). In addition, the *L. oculatus* Fads2 was able to convert 20:2n-6 and 20:3n-3 to 20:3n-6 and 20:4n-3, respectively, denoting a Δ8 desaturation capacity (Lopes-Marques et al., 2018). Therefore, given the high identity between Fads sequences from L. *oculatus* and *A. tropicus*, it is reasonable to speculate that the herein studied Fads1 and Fads2 from *A. tropicus* possess similar desaturation capabilities as described above for L. *oculatus*. Regarding elongases, our phylogenetic analysis revealed that *A. tropicus* possesses orthologs of both *elovl2* and *elovl5*. Unlike Fads, no functional characterization of Elovl enzymes from any species within the Lepisosteidae family including the spotted gar has been reported yet. However, the presence of active Elovl2 and Elovl5 enzymes in fish emerged before and after Lepisosteiformes (e.g., Wang et al., 2014; Monroig et al., 2016; Xu et al., 2020), therefore there is no reason to believe that *A. tropicus* Elovl2 and Elovl5 are not functional. Consequently, *A. tropicus* would possess, not only all desaturase but also elongase enzymatic capacities required for biosynthesizing the physiologically important ARA, EPA and DHA from the dietary essential C_{18} PUFA precursors LA and ALA (Castro et al., 2016; Monroig et al., 2018).

In the present study, the transcript abundance analyses of *fads1* gene during early development of *A. tropicus*, showed presence of transcript at 0 dph. Previous studies have shown that transcripts of key LC-PUFA biosynthesizing genes were also detected from the beginning of the zebrafish *D. rerio* embryogenesis (zygote) (Monroig et al., 2009). Since gene activation in zebrafish occurs after stages at which the presence of transcripts of *fads2*, *elovl*2 and *elovl*5 was detected, maternal transfer of mRNA was suggested as the mechanism accounting for such result (Monroig et al., 2009). Further evidence of maternal transfer of fads and elovl-like mRNA has been also reported in other fish species including *D. rerio* (Monroig et al., 2010a), cobia (*Rachycentron canadum*) (Monroig et al., 2011), *S. senegalensis* and *S. aurata*) (Morais et al., 2012; Torres et al., 2020). Similar to other fish species, it is likely that *fads1* transcripts were also transferred within the eggs of *A. tropicus* broodstock females. More clearly, the activation of the LC-PUFA biosynthesis in *A. tropicus* appears to be delayed until 15 dph, the point at which remarkably high expression peaks for all four genes investigated could be detected. The results from 15 dph coincided with a change in the feeding regime from *Artemia* nauplii to co-feeding with formulated trout diet. While such dietary change could have some stimulatory effects on LC-PUFA biosynthesis, it is also possible that the activation of the LC-PUFA biosynthesis pathways was due to the maturation of the digestive system, which was reported to be achieved around 15 dph (Frías-Quintana et al., 2015). Supporting this hypothesis, multiple studies have demonstrated that in fishes, the intestine, rather than a site for reacylation and remodeling of dietary lipids, plays other active roles, expressing the enzymes involved in the LC-PUFA biosynthesis, having a similar functional role as the liver for lipid metabolism (e.g., Bell et al., 2003; Fonseca-Madrigal et al., 2006; Morais et al., 2012; Galindo et al., 2021).

In the present study, *fads1*, *fads2* and *elovl2* showed a higher transcript abundance in intestine, while only *elovl5* showed a higher transcript abundance in liver. The expression of desaturases and elongases among fish tissues have shown differences in expression patterns between freshwater and marine fish (Monroig et al., 2018). In freshwater fish like zebrafish *D. rerio* (Monroig et al., 2009), pike silverside (*Chirostoma estor*) (Fonseca-Madrigal et al., 2014) and tench (*Tinca tinca*) (Garrido et al., 2020), *fads2*, *elovl2* and/or *elovl5* are highly expressed in liver and intestine. In marine fish like the Atlantic cod (*G. morhua*) (Tocher et al., 2006), cobia (*R. canadum*), (Zheng et al., 2009), nibe croaker (*Nibea mitsukurii*) (Yamamoto et al., 2010), Japanese flounder (*Paralichthys olivaceus*) (Kabeya et al., 2017a), chu's croaker (*Nibea coibor*) (Huang et al., 2017) and catadromous *A. japonica* (Xu et al., 2020), *fads*2, *elovl*2 and/or *elovl*5 are highly expressed in brain. However, there are exceptions to the rule, studies on *S. salar* and *O. mykiss* salmonids showed that *fads*2, *elovl*5 and/or *elovl*2 are highly expressed in liver, intestine and brain (Morais et al., 2009; Abdul Hamid et al., 2016). The expression patterns and activity of desaturases and elongases are influenced by factors such as phylogeny, and ecological and feeding habits (Garrido et al., 2019; Xie et al., 2020). Therefore, results of transcript abundance in different tissues of *A. tropicus* are in accordance with previous results in freshwater fish species. It is interesting to denote that the high expression of *elovl*5 registered in kidney it is not possible to explain in this work, however, these results coincide with reports in embryos of *D. rerio*, where *elovl5* is specifically expressed in the pronephric ducts (Monroig et al., 2009).

Regarding the FA profile changes during early ontogeny of *A. tropicus*, at 5 dph, high concentrations of EPA, DHA and ARA were detected with a tendency to decrease by 8 and 9 dph, a process in accordance with the total depletion of yolk sac. From 9 to 15 dph, larvae were feed only with brine shrimp nauplii, that shows high levels of ALA and LA (38% and 7% of total FAs) and low levels of EPA (1.43% of total FAs), where ALA and LA would potentially be substrates for Fads2. In this sense, the major expression peak of *fads*2 and *fads*1 were recorded at 15 dph, followed by peaks of *elovl*5 at 18 and *elovl*2 at 21 dph, following the LC-PUFA biosynthesis activation. Given the changes in PUFA profiles from *A. tropicus* larvae samples, from 23 dph onwards, an effect is observed in pathway n-3 with a decreased ALA and increased EPA and DHA. However, this effect is not reflected in pathway n-6 with LA and ARA. It should be noted that the trout diet showed a high LA (12.9%) and EPA (11.8%) content but an almost detectable ALA, ARA and DHA content, therefore the only way to increase this FA is from biosynthesis. In this sense, *elovl*2 expression peak at the 21 dph could be related to a second peak recorded on *fads*2 on the same day, being Elovl2 in charge to elongate 20:5n-3 to 24:5n-3, with the subsequent desaturation to 24:6n-3 by Fads2 (Δ6) in the so-called "Sprecher pathway" during DHA biosynthesis.

In conclusion, a high transcript abundance of *fads1*, *fads2*, *elovl2* and *elovl5* in several tissues of *A. tropicus*, with the intestine and the liver being the main organs involved in LC-PUFA biosynthesis. Therefore, the increased expression of *fads* and *elovl* genes during early development correspond to the end of the digestive system organogenesis as well to diet composition, where apparently this species possesses the enzymatic machinery to biosynthesize C_{18} to LC-PUFA C_{20} and C_{22}.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpb.2021.110628.

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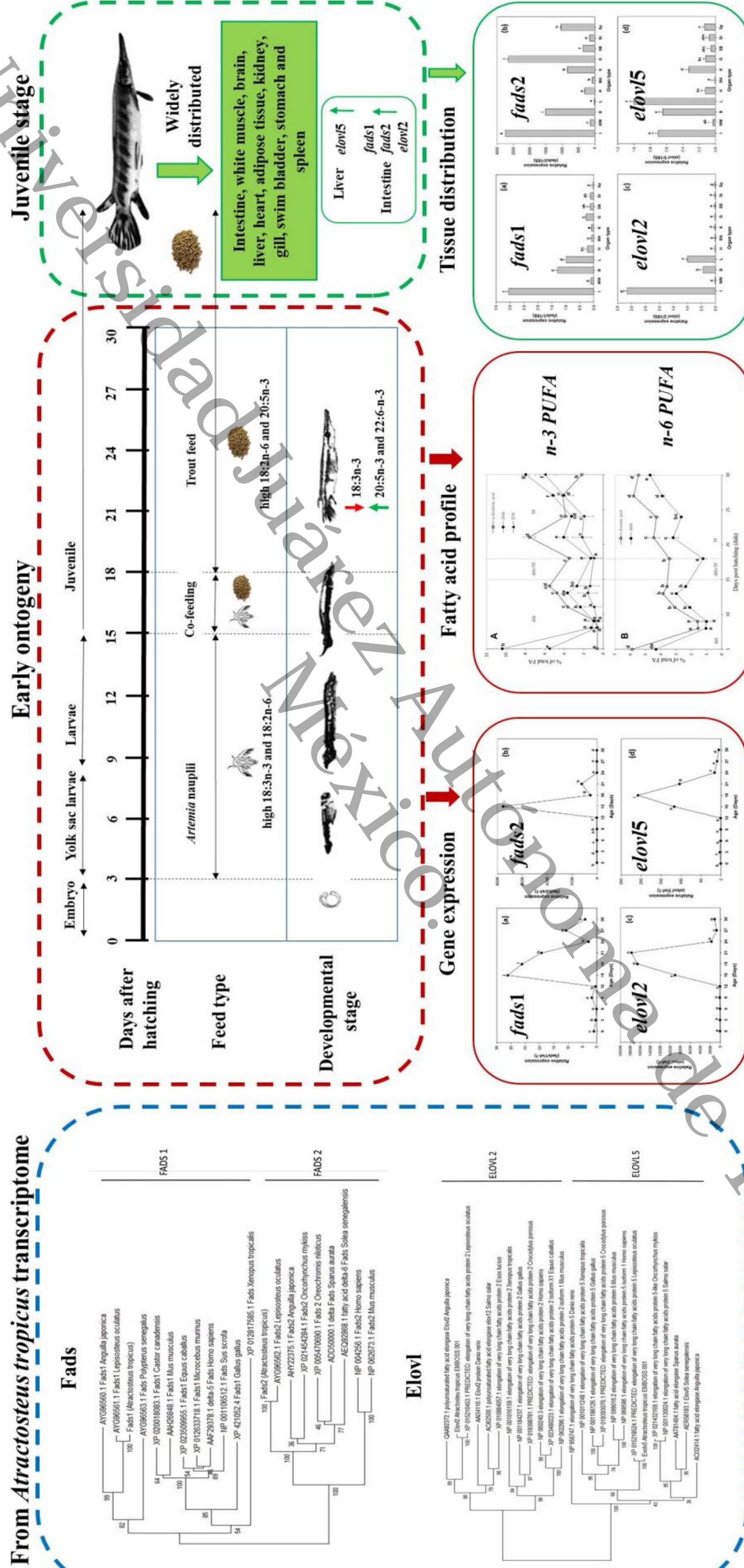

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Supplementary file 1

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ctgcctaagtaaagacagagaagagataccctggacagcgcaggtccagtctaaactacc
cgcccgcagctgccggctgtagtaccagcactcccaaattcacactgaataAATATGGAC
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sitio de restricción con BamHI
sitio de restricción con XbaI
codón de inicio ***ATG***
stop codón XX
Consenso XX

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México.

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México.

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sitio de restricción con BamHI
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codón de inicio ***ATG***
stop codón XX
Consenso XX

Universidad Juárez Autónoma de Tabasco.
México.

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México.

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sitio de restricción con BamHI
sitio de restricción con XbaI
Codon de inicio ***ATG***
stop codon XX
Consenso XX

Universidad Juárez Autónoma de Tabasco.
México.

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sitio de restricción con BamHI
sitio de restricción con XbaI
Codón de inicio ***ATG***
stop codon XX
Consenso XX

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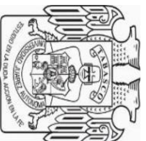

1 **Supplementary file 2.** Fatty acids content (% total fatty acids) (means ± SD; n=2) of tropical gar (*Atractosteus tropicus*) embryo and larvae at

2 different stages of development.

	Days after hatching (dah)											Feeds	
Fatty acids (%)	5	8	9	11	13	14	18	21	24	27	30	BSN	TD
C14:0	2.8±0.1^{b}	2.7±0.1^{b}	2.5±0.0^{b}	1.5±0.1^{c}	0.8±0.1^{d}	1.3±0.1^{c}	0.1±0.0^{e}	1.2±0.1^{c}	2.04±0.19^{c}	3.1±0.1^{ab}	3.4±0.0^{a}	0.0±0.0	7.2±0.1
C16:0	31.1±0.7^{c}	46.3±0.6^{a}	45.2±0.9^{a}	36.9±0.6^{b}	36.9±3.3^{b}	33.4±1.3^{bc}	37.3±1. 9^{b}	31.7±2.6^{c}	34.5±1.0^{b}	37.1±0.6^{b}	29.4±0.4^{c}	15.1±0.2	27.1±0.2
C18:0	11.1±0.4^{b}	16.7±0.1^{a}	18.1±0.4^{a}	17.9±0.1^{a}	18.4±1.6^{a}	16.4±0.7^{a}	18.3±1.3^{a}	17.3±1.2^{a}	17.6±0.1^{a}	17.2±0.4^{a}	13.4±0.3^{b}	6.7±0.1	7.2±0.1
∑ SFA	**47.3±1.3^{d}**	**68.7±0.9^{a}**	**69.8±1.5^{a}**	**59.7±1.1^{c}**	**59.9±7.3^{bc}**	**54.9±2.5^{cd}**	**60.3±5.6^{bc}**	**54.1±4.2^{cd}**	**57.9±1.6^{c}**	**61.3±2.1^{ab}**	**49.6±1.0^{d}**	**23.8±0.3**	**44.5±0.6**
C16:1	5.5±0.1^{a}	5.0±0.1^{a}	4.2±0.1^{a}	3.2±0.1^{a}	2.1±2.7^{a}	3.8±0.2^{a}	0.2±0.0^{b}	0.2±0.0^{b}	2.7±3.5^{a}	0.2±0.0^{b}	5.3±0.1^{a}	0.1±0.0	9.0±0.1
C18:1n9	16.6±0.2^{b}	18.4±0.2^{b}	18.8±1.1^{b}	21.7±9.2^{a}	17.8±0.9^{b}	22.8±0.7^{a}	26.9±0.1^{a}	21.0±0.4^{a}	20.6±0.7^{a}	12.2±0.1^{c}	16.1±0.2^{b}	25.0±0.1	19.6±0.1
C20:1n9	1.2±0.1^{b}	1.8±0.1^{a}	1.2±0.1^{b}	1.2±0.1^{b}	1.1±0.1^{b}	1.0±0.1^{c}	0.8±0.0^{c}	0.8±0.0^{c}	0.8±0.0^{c}	0.9±0.1^{c}	0.8±0.0^{c}	1.1±0.0	0.3±0.0
C24:1n9	0.1±0.0	0.1±0.0	0.2±0.0	0.4±0.0	0.3±0.1	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.1	0.3±0.0	0.1±0.1	0.0±0.0	0.1±0.0
∑ MSFA	**23.4±0.6^{ab}**	**25.6±0.4^{a}**	**24.6±1.1^{ab}**	**26.9±1.1^{a}**	**21.6±12.4^{ab}**	**28.3±1.4^{a}**	**28.6±0.4^{a}**	**22.7±0.3^{ab}**	**24.7±4.6^{ab}**	**13.9±0.5^{b}**	**22.4±0.7^{ab}**	**26.6±0.3**	**29.3±0.2**
C18:2n6	5.9±0.1^{a}	1.6±0.0^{d}	1.6±0.0^{d}	3.1±0.1^{c}	3.8±0.4^{c}	3.6±0.1^{c}	3.4±0.1^{c}	4.5±0.2^{b}	4.3±0.1^{b}	5.8±0.1^{a}	5.5±0.1^{a}	7.0±0.1	12.9±0.1
C18:3n3	0.6±0.00^{d}	0.6±0.0^{d}	0.8±0.1^{d}	3.6±0.2^{b}	4.8±0.6^{b}	5.5±0.2^{ab}	3.5±0.0^{b}	7.6±2.6^{a}	3.9±0.1^{b}	4.2±0.1^{b}	2.2±0.0^{c}	38.3±0.5	0.1±0.0
C20:3n6	1.1±0.1^{a}	0.2±0.0^{e}	0.2±0.0^{e}	0.4±0.0^{d}	0.5±0.0^{cd}	0.4±0.0^{d}	0.3±0.0^{e}	0.6±0.1^{c}	0.52±0.00^{cd}	0.7±0.0^{b}	0.7±0.0^{b}	0.1±0.0	0.2±0.0
C20:4n6	4.3±0.2^{ab}	1.0±0.0^{e}	1.0±0.0^{e}	2.1±0.2^{d}	3.0±0.4^{c}	2.4±0.1^{d}	1.2±0.2^{e}	3.2±0.3^{c}	2.7±0.0^{cd}	3.9±0.2^{b}	4.7±0.1^{a}	1.2±0.0	0.0±0.0
C20:5n3	5.5±0.1^{b}	0.7±0.0^{f}	0.5±0.0^{f}	0.9±0.1^{f}	1.6±0.2^{e}	1.4±0.0^{e}	0.9±0.1^{f}	2.2±0.0^{d}	1.9±0.0^{d}	4.1±0.0^{c}	5.9±0.1^{a}	1.4±0.0	11.8±0.1
C22:6n3	10.4±0.1^{a}	1.2±0.0^{g}	0.9±0.0^{g}	2.3±0.6^{f}	3.7±0.4^{e}	2.7±0.1^{ef}	1.3±0.2^{g}	4.0±0.4^{c}	3.2±0.1^{de}	5.2±0.2^{c}	7.9±0.1^{b}	0.0±0.0	0.2±0.3
∑ PUFA	**29.4±0.6^{ab}**	**5.7±0.2^{g}**	**5.6±0.2^{g}**	**13.4±1.4^{ef}**	**18.5±2.3^{cd}**	**16.9±0.6^{de}**	**11.1±0.6^{f}**	**23.1±3.7^{c}**	**17.4±0.4^{de}**	**24.8±0.8^{bc}**	**28.0±0.6^{b}**	**49.6±0.6**	**26.2±0.9**

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Σ n-3	16.7	2.5	2.4	7.1	10.3	9.8	5.8	14.2	9.3	13.7	16.3	40.2	12.1
Σ n-6	11.5	2.8	2.8	5.7	7.5	6.6	4.9	8.5	7.6	10.6	11.2	9.0	13.3
Σ n-9	17.8	20.5	20.4	23.6	19.5	24.1	28.1	22.3	21.7	13.6	16.9	26.2	20.1
Σ LC-PUFA	20.2	2.9	2.5	5.4	8.2	6.4	3.5	9.4	7.9	13.1	18.6	2.6	12.1
Σ n-3 LC-PUFA	15.9	1.9	1.5	3.3	5.3	4.0	2.2	6.2	5.2	9.2	13.9	1.4	12.1
∑(n-3/n-6)	1.4	0.9	0.8	1.2	1.4	1.5	1.2	1.7	1.2	1.3	1.5	4.5	0.9
DHA/EPA	1.8	1.8	1.8	2.5	2.3	1.9	1.3	1.8	1.6	1.3	1.3	0.0	0.0

3 Bold values are total % composition of a particular group of fatty acids. SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA,

4 polyunsaturated fatty acid. BSN, brine shrimp nauplii; TD, Trout diet.

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1 **CAPITULO II**

2

3 **Effects of dietary α-linolenic acid/linoleic acid ratio on growth performance, somatic index,**
4 ***fads* and *elovl* gene expression and fatty acid profile of the tropical gar (*Atractosteus***
5 ***tropicus*) juvenile.**

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19

20 **ABSTRACT**

21 Multiple physiological processes are dependent on long-chain (≥C_{20}) polyunsaturated fatty acids 22 (LC-PUFA), including eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) 23 and docosahexaenoic acid (DHA, 22:6n-3), where LC-PUFA biosynthesis is achieved by fatty 24 acyl desaturases (Fads) and elongation of very long-chain fatty acid (Elovl) (preset in tropical gar 25 *Atractosteus tropicus*), using as precursors C_{18} polyunsaturated fatty acids (PUFA) like linoleic 26 acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3). Therefore, present study determined the 27 effect of α-linolenic acid/linoleic acid ratio on growth performance, somatic index and fatty acid 28 profile from carcass and feces of tropical gar *Atractosteus tropicus*. A randomized experimental 29 design with six treatments in triplicate was used. The treatments consisted of five experimental 30 diets with the substitution of soybean oil (SO) for linseed oil (LO) in the following proportions: 31 DS100 (100% SO-0% LO), DS75 (75% SO-25% L0), DS50 (50% SO - 50% LO), D25 (25% 32 SO-75% LO) and DS0 (0% SO-100% LO) and a sixth diet used as a control diet (CD) with 33 100% fish oil. The results indicate that DS100 and DS75, significantly increased weight gain, 34 specific growth rate and protein conversion rate and significantly decreased feed conversion rate. 35 The lowest survival was recorded in CD, showing differences with DS100, DS75, DS50 and 36 DS25. Relative intestine length showed increase in DS0 in comparison with the other treatments. 37 Relative expression levels of *elovl*2, e*lovl*5, *fads*1 and *fads*2 in liver and intestine showed 38 increase in treatment DS75 compared to the other treatments. Likewise, the results of fatty acid 39 profiles in the carcasses showed higher levels of n-3 LC-PUFA, EPA (20: 5n-3) and DHA (22: 40 6n-3) in the diet with 100% linseed oil (DS0), thus confirming the capacity of LC-PUFA 41 biosynthesis in juvenile fish of *A. tropicus*. Therefore, *A. tropicus* show better growth 42 performance with sole dietary soybean oil compared to fish oil and different proportion of 43 soybean oil and linseed oil. The elongases and desaturases showed an upregulation in liver and 44 intestine of fish in treatment DS75. Fatty acid profile in carcass showed the higher levels of EPA 45 and DHA in DS0, highlighting the capacity of the specie to biosynthesize LC-PUFA from 46 PUFA.

47 **Key words**: LC-PUFA biosynthesis, *Atractosteus tropicus,* vegetable oils; essential fatty acids; 48 arachidonic acid; eicosapentaenoic acid; docosahexaenoic acid

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50 **Introduction**

51 The fish oil is rich in long-chain (≥C_{20}) polyunsaturated fatty acids (LC-PUFA), including
52 eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) and docosahexaenoic
53 acid (DHA, 22:6n-3), essential fatty acids for artificial diets used in aquaculture industry,
54 however the use of fish oil contribute in the natural fish populations impact (Tacón and Metian,
55 2015; FAO, 2020). Thus vegetable oils are needed for sustainable aqua feeds, which allow the
56 induction of polyunsaturated fatty acids (PUFA) in fish, reducing feed costs (Turchini et al .,
57 2009; Ayisi et al., 2019). Several vegetables oils are use in aqua feed industry, including soybean
58 oil (*Glycine soja*), sunflower oil (*Helianthus annus*), safflower oil (*Carthamus tinctorius*) and
59 grape seed oil (*Vitis vinifera*), oils rich (>60%) in n-6 PUFA linoleic acid (LA, 18:2n-6) fatty
60 acids (FA); while in wheat germ oil (*Triticum vulgare*), corn oil (*Zea mays*), cottonseed oil
61 (*Gossypium* sp.) and sesame oil (*Sesamum indium*) concentrations vary between 40-50% of LA
62 (USDA, 2010; Ayisi et al., 2019). Regarding n-3 PUFA, α-linolenic acid (ALA, 18:3n-3),
63 linseed oil (*Linum usitatissimum*) contains up to 53% and camelina oil contains between 35-45%
64 (Turchini et al., 2009; USDA, 2010). However, replacing fish oil with vegetable oils in aqua
65 feeds, the FA profile in the diet should be the central aspect because there is a strong correlation
66 between the fatty acid profile of the fish and the diet provided (Tocher, 2010) as well to specie
67 specific capacity to biosynthesize LC-PUFA from PUFA (Pontes et al., 2019). The ability of LC-
68 PUFA biosynthesis from PUFA in fish depends not only on the presence of elongase (*elovl*) and
69 desaturase (*fads*) enzymes, but also on their activity and specificity on different substrates
70 directly related to the evolutionary history, environmental factors, trophic and ecological level of
71 the species (Li et al., 2018; Lopes-Marques et al., 2018). Thus, great efforts have been made to
72 determine the biosynthesis and degradation capacity of vegetable oils in fish involved in
73 aquaculture.

74 Reports in lambari (*Astyanax altiparanae*), shows the ability of the species to synthesize EPA
75 and DHA when different proportions of soybean oil and linseed oil are offered (Pontes et al.,
76 2019). Sakian et al. (2019) indicate that mandarin fish (*Siniperca scherzeri*), tolerate total
77 replacement of fish oil by soybean oil and linssed oil without any adverse effect on fish
78 performance and health, attributed to the ability to convert PUFA to LC-PUFA. Studies in
79 Manchurian trout (*Brachymystax lenok*), show that possess the ability to synthesize LC-PUFA

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80 from ALA, where the substitution of fish oil with linseed oil (<75%) could improve lipid
81 metabolism and oxidation resistance (Yu et al., 2019).
82 The tropical gar (*Atractosteus tropicus*), is a carnivorous freshwater species, belongs to the
83 family Lepisosteidae, belonging to ancestral fish, used as study models (Braasch et al., 2008).
84 Tropical gar is a species of commercial importance in the state of Tabasco, México, therefore,
85 nutritional studies that allow us to achieve in growth performance, profitability and aqua feeds
86 sustainability is needed. Currently, in aquaculture nutrition, the use of a mixture of soybean and
87 linseed oil |has been considered as an alternative to replace fish oil if the fish possess the
88 necessary enzymes (elongases and desaturases) to synthesize highly polyunsaturated fatty acids
89 ARA (20: 4n-6), EPA (20: 5n-3) and DHA (22: 6n-3) (Pontes, et al., 2019). Previous studies in *A*
90 *tropicus* (De la Cruz-Alvarado et al., 2021) showed that is able to activate the metabolic
91 machinery to biosynthesize LC-PUFA from PUFA during larval development due to the
92 presence of elongases (*elovl*2 and *elovl*5) and desaturases (*fads*1 (Δ5) and *fads*2 (Δ6)). Therefore,
93 the aim of the present study was to determine the effect of dietary α-linolenic acid/linoleic acid
94 ratio from linseed oil and soybean oil on growth performance, somatic index, expression of *fads*
95 and *elovl* genes and fatty acid profile of carcasses and feces of tropical gar *A. tropicus*.
96

97 **Materials and methods**

98 **2.1. Biological material**

99 The juveniles of *A. tropicus* were obtained from a batch of breeders (a female of ~ 2.5 kg and
100 three males of ~ 1.5 kg), where the female was induced using an analog of the luteinizing
101 hormone releasing hormone (LHRHa) (single dose of 35 μg per fish) and were kept in a 2000 L
102 round plastic tank at the Tropical Aquaculture Laboratory (LAT-DACBIOL-UJAT) until
103 spawning. After spawning, the larviculture procedure was continued according to protocol
104 described by De la Cruz-Alvarado et al. (2021). A total of 234 juveniles (0.84 ± 0.01 g mean
105 weight) with 45 days after hatching (DAH) were used for the experimental design.

106 2.2. Diets and experimental design

107 For the experimental diets, the animal protein meals (poultry by-products meal, pork meal and
108 fish meal) were washed three times with alcohol with the aim of remove the greatest amount of
109 lipids as described by Kandil et al. (2011). Briefly, two separate washes where performed to each
110 animal meal using 70% alcohol during 60 min, after every wash, the meals were filtered with a

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111 150 µm mesh grind. Then, a third wash was performed with 96% alcohol during 60 minutes, and
112 filtered again. Drying were carried out prior to their use for diet manufacture using a forced-air
113 furnace during 12 hours at 60°C. Six isoproteic (45% crude protein), isolipidic (15% lipid) and
114 isoenergetic (17.67 kJ/kg) experimental diets were formulated using the MIXITWIN v.5.0
115 software (Microsoft Windows, Redmond, WA) as shown in Table 1. Five experimental diets
116 consisted on substitution of soybean oil (SO) by linseed oil (LO) in the following proportions:
117 DS100 (100% SO-0% LO), DS75 (75% SO-25% L0), DS50 (50% SO- 50% LO), D25 (25% SO-
118 75% LO) and DS0 (0% SO-100% LO) and a sixth diet used as control diet (CD) with 100% fish
119 oil.

120 Diets preparation was according to Álvarez-González et al. (2001). Briefly, the macronutrients
121 were stirred during 15 minutes, then micronutrients were added and mixed during 15 minutes.
122 Liquid ingredients were incorporated and blended during 15 minutes and finally, water was
123 added (~400 ml/kg per diet) and stirred by other 15 minutes. Pellets were prepared in a meat mill
124 (Torrey, M-22RI, Monterrey, N.L, México) with a 5 mm screen, and then all pellets were dried
125 for 60°C during 12 hr in a forced-air furnace. The pellets were crushed manually and sieved to
126 obtain an adequate size for juveniles, and finally, the experimental diets were kept at −20°C for
127 later use. The experimental diets were analyzed for proximal composition: moisture, ash, lipid
128 and protein content according to AOAC (2000). The experiment was carried out in 70 L plastic
129 circular tanks in triplicate (18 experimental units) during 30 days, using 13 fish per experimental
130 unit. The system was connected to a recirculation system activated by a 0.5 HP water pump
131 (Jacuzzi , JWPA5D-230A, Delavan, WI) recording temperature (28.1 ± 1.2 °C, mean ± standard
132 deviation, SD), dissolved oxygen (6.5 ± 0.2 mg / L) measured with an oxygen meter (YSI 85,
133 Ohio, USA) and pH (7.1 ± 0.2) with a potentiometer (HANNAHI 991001, Romania, Europe).
134 Juveniles were feed three times a day (9:00, 13:00 and 17:00) at satiety. Cleaning of the units
135 were performed by siphoning three times a day to remove excreta 1 h post feeding.

136

137 **2.3. Growth performance, somatic index and survival**

138 Biometrics were performed every 15 days to determine the individual wet weight with analytical
139 balance (Ohaus mod. CS2000, China) and total length by photographing each fish in a
140 transparent flat container and using the ImageJ 1.51j8 software (U.S. National Institutes of

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141 Health, Bethesda, MD) for specific length calculations. Previous to each biometry, fish were
142 staved during 24 h to ensure gut empting.
143 At the end of the experiment, the weight gain (WG; %) = [(Wf - Wi)/ Wi] x100; the specific
144 growth rate (SGR; %) = 100 x [(ln Wf - ln Wi) / T]; where: Wi and Wf are the initial and final
145 weight of the fish respectively, and T is the number of days in the feeding period; the feed
146 conversion rate (FCR) = dry feed consumed (g) / gain in wet weight (g); condition factor (K):
147 [(wet weight (g) × total length ^{3} (cm)] × 100; the protein efficiency rate (PER): fish weight gain
148 (g) / dry protein fed (g) and survival (S) = (final organisms / initial organisms) X 100 were
149 determined.
150 For somatic index calculation, three fishes per tank (9 per treatment) were sacrificed using cold
151 thermal shock (-4 °C) and dissected to measure weight of visceral package and liver, as well to
152 intestine longitude, used to calculate the hepatosomatic index (HSI) = (liver weight (g) × 100)
153 /total body weight (g), the viscerosomatic index (VSI) = (viscera weight (g) × 100) /total body
154 weight (g) and the relative intestine length (RIL) = [intestine length (cm)/total organism length
155 (cm)] × 100.
156 For fatty acid profile analyses, three individuals were collected from each experimental unit (9
157 per treatment) and were sacrificed using cold thermal shock (-4 °C) to be frozen at -80 ° C and
158 lyophilized. In addition, the feces from previous dissected fishes were collected and subsequently
159 frozen at -80 ° C and lyophilized.
160 For gene qPCR analysis, two juveniles of *A. tropicus* were collected from each tank (6 per
161 treatment) and sacrificed using cold thermal shock (at −4° C) and subsequently dissected on ice
162 to obtain liver and intestine, then each type of tissue was mixed by tank in same Eppendorf tubes
163 with 1.5 ml of RNA Later (Life Technologies) and stored at -20° C until further analysis.
164

165 **2.4. Real-time gene expression**

166 Total RNA extraction was carried out according to the trizol reagent method (Invitrogen).
167 Subsequently, the concentration and purity of the RNA were estimated by the 260/280 ratio
168 using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). To determine the
169 integrity of the nucleic acids, electrophoresis was performed on a 1% agarose gel using a 1 µl
170 sample aliquot mixed with 1 µl of run buffer from each of the samples at 100 volts in an
171 electrophoresis Mini Sub Cell GT chamber (Bio-Rad) for 30 min. Bands were visualized in a gel

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172 documentation system, Molecular Imagen Gel Doc XR + Imaging System (Bio-Rad). Once the
173 integrity of the genetic material of each of the samples was evaluated, the corresponding aliquots
174 were sampled based on their calculated concentration. Subsequently, 1 µg of RNA was used for
175 reverse transcription with the iScriptTM Select cDNA Synthesis Kit 170-8896 (Bio-Rad); 2 µl of
176 cDNA for qPCR was used consecutively.
177 Expression analysis of the genes *fads*1, *fads*2, *elovl*2 and *elovl*5 was performed in a 96-well
178 thermocycler (CFX96 Real-Time System Thermal Cycle; C1000, CA, USA) using specific
179 primers for RT-qPCR reported in table 2 and previous reported by De la Cruz-Alvarado et al.,
180 2021 with accession numbers (#######). The 10 µl reaction mixture included 5 µl of Eva Green,
181 2 µl of cDNA, 2.85 µl of H2O milli-Q and 0.15 µl of each primer (15 µmol/L). The qPCR was
182 performed under the following conditions: 2 min at 95°C, followed by 38 cycles at 95°C for 10 s,
183 60°C for 30 s and extension at 70°C for 5 s. In each run of qPCR, a negative control was added
184 with the same components of the reaction using distilled water instead of cDNA. For
185 normalization of cDNA, 18S rRNA was tested as constitutive gene because it was more stable
186 tan β-actin and its analysis was carried out in parallel with all the samples. The relative
187 expression of the genes was calculated by the delta-delta ct method (Livak & Schmittgen, 2001).
188

189 **2.5. Fatty Acid Analysis**

190 Experimental diets, final carcass and feces were analyzed to determine the content of saturated
191 fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA),
192 expressed as a percentage of total fatty acids at the Laboratory of Lipid Metabolism, Centro de
193 Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Baja California Sur, México
194 (Supplementary file 1). For the analysis, one gram of dry weight of each sample was placed in a
195 glass bottle with 6 ml chloroform: methanol (2:1 v/ v), 10 µl of antioxidant (BHT) and 10 µl of
196 23:0 fatty acid (FA) as internal standard. The samples were macerated directly into the solvent
197 using a glass mortar and pestle. All lipids were trans esterified using boron trifluoride methanol
198 (14% BF3 methanol, Supelco) and analysed in a Hewlett-Packard CG 6890-N gas
199 chromatograph equipped with a DB-23 (30 mm in length × 0.25 mm in internal diameter, 0.25
200 µm film thickness), a fused silica capillary column (J&W Scientific), ionization flame detector
201 (FID) at 280°C, with helium as carrier gas, and a 110–220°C temperature gradient (3°C/min).
202 Fatty acids were identified by comparing their retention times with those of standards

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203 (Component FAME Mix 47885-U de supelco; Tricosanoic acid T6543 de sigma 99% de purity,
204 St Louis, MO, USA) with the concentration of each fatty acid corrected by correlation with the
205 response of the corresponding standard. Data were analyzed using GC ChemStation Rev.
206 A.10.02 (1757, Agilent Technologies, 2003) (Palacios, Ibarra, & Racotta, 2000).
207

208 **2.5. Statistical analysis**

209 The growth performance parameters, somatic index, relative expression of *elovl* and *fads* genes
210 and the percentage of total fatty acid (each fatty acid) in carcass and feces were evaluated by
211 means of a Kruskal-Wallis test to determine significant differences between treatments. When
212 statistical differences were detected, the Nemenyi posterior test was performed to determine
213 which treatments were different, using a significance value of $p < 0.05$. All statistical analyzes
214 were performed using the STATISTICATM v. 7.0 software (Statsoft, Tulsa, OK, USA).
215

216 **Results**

217 **3.1. Growth performance, somatic index and survival**

218 The evaluation of growth performance, somatic index and survival of juvenile *A. tropicus*
219 showed significant differences between dietary treatments ($p < 0.05$). Fish fed DS100 and DS75
220 had higher growth with mean final weight (2.77 ± 0.24 g and 2.60 ± 0.08 g) respectively (Figure
221 1A) and DS100 presenting higher mean total length (10.50 ± 1.7) with significant differences
222 than the rest of the treatments ($p < 0.05$) (Figure 1B). DS100 and DS75 showed the highest value
223 of WG (%) and SGR (%) with respect to the other treatments ($p < 0.05$). Likewise, the lowest
224 value of FCR (1.54 ± 0.35) was shown in DS100, presenting statistically significant differences
225 with respect to the other diets ($p < 0.05$). DS100 reached the highest PER value (1.46 ± 0.31)
226 with respect to the other treatments ($p < 0.05$). With respect to the K value in all diets, there were
227 no significant differences ($p > 0.05$). The DS100, DS75 and DS50 treatments showed 100%
228 survival ($p < 0.05$). The HIS and VSI showed a tendency to increase wit linseed oil increasing,
229 without statistical differences ($p > 0.05$), however RIL showed higher values on DS0 treatment (p
230 <0.05) than the other treatments (Table 3).
231
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234 ***fads* and *elovl* gene expression**

235 The elongases (*elovl*2 and e*lovl*5) and desaturases (*fads*1 and *fads*2) relative gene expression in
236 response to dietary treatments was determined in liver and intestine of *A. tropicus* (figure 2 and
237 3). Results showed significantly higher (P <0.05) levels of *elovl*2, e*lovl*5,*fads*1 and *fads*2 relative
238 gene expression in liver and intestine of fish fed treatment DS75 compared to the other
239 treatments.

240

241 **Fatty acid profiles**

242 The FA profiles (% of total FA) of saturated fatty acids (SFAs), monounsaturated fatty acids
243 (MUFA) and polyunsaturated fatty acids (PUFA) in the experimental diets are in supplementary
244 file 1. Saturated fatty acids (∑SFA) were higher in fish fed the DC diet (46.3±0.4), whereas
245 polyunsaturated fatty acids (∑ PUFA) were higher in fish fed DS100 diets (38.5±0.1) than in fish
246 fed the DC diet. The highest level of linoleic acid (LA, 18: 2n-6) was in the DS100 diet
247 (29.0±0.1) with statistically significant differences from the other diets (p<0.05). For α-linolenic
248 acid (ALA, 18: 3n-3) higher levels were obtained in the DS50 (11.1 ± 0.1) and DS25 (11.1 ± 0.6)
249 diets, (p <0.05). The highest level of arachidonic acid (ARA, 20: 4n-6) was presented by DC (0.7
250 ± 0.0) with significant differences with the other diets (p <0.05). Likewise, the highest levels of
251 eicosapentaenoic acid (EPA, 20: 5n-3) were presented by the CD (2.0 ± 0.1), DS100 (1.2 ± 0.0)
252 and DS75 (1.8 ± 0.1) showing differences with respect to the other diets, (p <0.05). In addition,
253 the highest docosahexaenoic acid (DHA, 22: 6n-3) values were obtained in CD (2.7 ± 0.1),
254 followed by DS100 (2.3 ± 0.0) and DS75 (2.1 ± 0.1) (p <0.05). The n-6 polyunsaturated fatty
255 acids (n-6 LC-PUFA) of DS100 (30.5±0.1) were significantly higher than those of other
256 treatments. The level of n-3 polyunsaturated fatty acids (n-3 LC-PUFA) was significantly higher
257 in DS50 (14.4±0.8) and DS25 (13.6±0.1) diets, (p<0.05). Finally, the highest value of the LA /
258 ALA ratio was in the DS100 diet (7.8 ± 0.1) showing statistically significant differences with
259 respect to the other diets (p <0.05).

260 The percentage of total carcass fatty acids in juvenile tropical gar (*A. tropicus*) is shown in
261 supplementary file 1. Saturated fatty acids (∑SFA) were higher in fish fed the DC diet (35±0.4),
262 while polyunsaturated fatty acids (∑ PUFA) were higher in fish fed DS0 diets (41.1±0.6) than in
263 fish fed the DC diet, there being significant differences between treatments, (p<0.05). Selected
264 PUFA and LC-PUFA n-3 and n-6 (% total fatty acids) from carcass of *A. tropicus* juveniles fed

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265 with different inclusion percentages of soybean oil and linseed oil are shown in figure 4. The n-6
266 LC-PUFA of DS100 (27.6±0.3) were significantly higher than those of other treatments. In
267 addition, the n-3 LC-PUFA was significantly higher in the DS0 diet (24.1±0.5), ($p<0.05$).
268 Finally, higher values of the LA / ALA ratio were presented in the fish carcasses with DS100
269 (14.9±0.1) presenting statistically significant differences between treatments ($p <0.05$).
270 DS100 (15.6 ± 0.2) presented the highest value of linoleic acid (18: 2n-6) which presented
271 differences with respect to the other treatments ($p <0.05$). The highest level of ALA was detected
272 in the DS50 diet (2.9 ± 0.0) with differences between diets ($p <0.05$). While arachidonic acid
273 (ARA, 20: 4n-6), eicosapentaenoic acid (EPA, 20: 5n-3) and docosahexaenoic acid (DHA, 22:
274 6n-3) levels showed the highest levels in DS0 (7.5 ± 0.2, 5.5 ± 0.2 and 13.6 ± 0.4 respectively),
275 showing differences with respect to the other treatments, ($p <0.05$).
276 The percentage of total feces fatty acids in juvenile tropical gar (*A. tropicus*) is shown in
277 supplementary file 1. Saturated fatty acids (∑SFA) had the highest level in fish fed the DC diet
278 (62.8±0.8), while polyunsaturated fatty acids (∑ PUFA) had the highest levels in fish fed DS75
279 diets (19.5±0.3) existing significant differences between treatments ($p<0.05$). Selected PUFA
280 and LC-PUFA n-3 and n-6 (% total fatty acids) from feces of *A. tropicus* juveniles fed with
281 different inclusion percentages of soybean oil and linseed oil are shown in figure 5. The n-6 LC-
282 PUFA of DS75 (13.5±0.1) were significantly higher than those of other treatments. Regarding
283 the level of n-3 LC-PUFA was significantly higher in DS75 (6.1±0.1), DS25 (6.3±0.1a) and DS0
284 (6.2±0.1) diets, ($p<0.05$). Finally, the highest value of the LA / ALA ratio was in the DS100 diet
285 (8.4±0.1) showing statistically significant differences with respect to the other diets ($p <0.05$).
286 Selected PUFA and LC-PUFA n-3 and n-6 (% total fatty acids) from feces of *A. tropicus*
287 juveniles fed with different inclusion percentages of soybean oil and linseed oil are shown in
288 figure 5. Linoleic acid (LA, 18: 2n-6) the highest level was in the DS75 (10.2±0.1) showing
289 differences with respect to the other diets ($p <0.05$). For ALA, higher levels were obtained in the
290 DS25 (4.2±0.0) and DS0 (4.1±0.0) ($p <0.05$). In the case of ARA, the DS100 treatment (1.9±0.1)
291 presented the highest level, showing differences with the other diets ($p <0.05$). In addition, the
292 highest levels of eicosapentaenoic acid (EPA, 20: 5n-3) were presented by DS75 (1±0.0),
293 showing differences with respect to the other diets, ($p <0.05$). Likewise, DHA showed the
294 highest values were obtained in CD (1.6±0.0) ($p <0.05$).
295

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296 **Discussion**

297 Previous studies in several fish species use different lipid plant sources that did not affect the
298 growth performance. as reported in *Oreochromis niloticus* (Nile tilapia), *Pangasius*
299 *hypophthalmus* (panga), *Maccullochella peelii peelii* (Murray cod) and *Trachinotus carolinus*
300 (Florida pompano) (Ng et al., 2013; Asdari et al., 2011; Turchini et al., 2011; Rombenso et al.,
301 2016). Therefore, this study was conducted to evaluate the effect of dietary omega 3 to omega 6
302 PUFA on growth performance, somatic index and fatty acid profile on carcass and feces of
303 juveniles, using soybean oil and linseed oil as PUFA source and using fish oil (rich in LC PUFA)
304 as control.

305 Our research show that replace of total fish oil by soybean oil (DS100) increased WG (%), SGR
306 and PER in juvenile *A. tropicus* and decreased its FCR. Replacement of dietary fish oil with
307 soybean oil increased growth performance in largemouth bass (*Micropterus salmoides*) and
308 rabbitfish (*Siganus canaliculatus*) (Chen et al., 2019; Xu et al., 2012). The most common
309 vegetable oils used is soybean oil, which offers competitive prices, high availability in the world
310 market and is rich in linoleic acid (LA,18:2n-6) (Ayisi et al., 2019). It is worth noting that
311 likewise, the DS75 diet, which contains 75% soybean oil with 25% linseed oil, presents similar
312 results to the DS100 diet with respect to growth performance and somatic index, therefore, this
313 mixture of vegetable oils would also favor its use for feeding juvenile *A. tropicus*. According to
314 what reported by Pontes et al. (2019) in *Astyanax altiparanae* showingthat soybean oil and
315 linseed oil mixture in the diet could replace fish oil, if the fish possesses elongase and desaturase
316 enzymes.

317 Soybean oil is deficient in omega 3 PUFA and in contrast linseed oil contains more than 50% of
318 α-linolenic acid (18:3n-3), however, it is produced on a smaller scale and therefore more
319 expensive compared to soybean oil and other vegetable lipid sources (Ayisi et al., 2019). There
320 are studies conducted with soybean oil as a replacement for fish oil in the diet of aquaculture
321 freshwater fish, where there was no differential effect on fish growth, such as *Tinca tinca*
322 (Ljubojević et al., 2014), *Micropterus salmoides* (Chen et al., 2019), *Oreochromis niloticus*
323 (Godoy et al., 2019), *Astyanax altiparanae* (Pontes et al., 2019), *Rhamdia quelen* (Hilbig et al.,
324 2019).

325 The results of the percentages of total fatty acids in experimental diets used for juvenile *A.*
326 *tropicus* show that the soybean oil diet (DS100) presents a decrease in saturated fatty acids

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327 (∑SFA) and polyunsaturated fatty acids of the n-3 series (n-3 LC-PUFA), while the n-6 series
328 increased polyunsaturated fatty acids (n-6 LC-PUFA). It is known that the fatty acid profile
329 content of the diet has affected the fatty acid profile content of the fish body, in agreement with
330 what has been reported in other fish species (Xu et al., 2012, 2015; Emre et al., 2016; González-
331 Félix et al., 2016; Chen et al., 2019, Pontes et al., 2019).
332 The percentage content of linoleic acid (LA, 18: 2n-6) and α-linolenic acid (ALA, 18: 3n-3) in
333 the carcasses of juvenile *A. tropicus* fish increased with soybean oil in the DS100 diet, in
334 agreement with that reported by Emre et al. (2016) and Chen et al. (2019), who reported that
335 replacing fish oil in the diet with soybean oil increased the levels of linoleic acid and α-linolenic
336 acid in muscles. Likewise, in the results the content of long chain polyunsaturated fatty acids
337 (LC-PUFA) such as ARA (20: 4n-6), EPA (20: 5n-3) and DHA (22: 6n-3) in the carcasses of
338 juvenile *A. tropicus* fish decreased with the increase of soybean oil in the diet, therefore, the
339 results show that the replacement of fish oil in the diet with soybean oil results in a decrease of
340 LC-PUFA content in fish tissues. Similar data are reported by Emre et al. (2016) and Chen et al.
341 (2019) in replacing dietary fish oil with soybean oil in *Argyrosomus regius* (croaker)
342 *Micropterus salmoides* (largemouth bass) respectively, where the quality changed and fatty acid
343 levels of ARA, EPA and DHA were reduced in various tissues such as muscle and liver.
344 Although vegetable oils do not have long chain polyunsaturated fatty acids (CL-PUFA), some
345 tropical freshwater fish species can synthesize arachidonic acid (ARA; 20: 4n-6) from 18:2n-6,
346 eicosapentaenoic acid (EPA; 20: 5n- 3) and docosahexaenoic acid (DHA; 22: 6n-3) from 18:3n-3
347 (Paulino et al., 2018). The synthesis of ARA, EPA and DHA is carried out by desaturation and
348 elongation reactions, catalyzed by enzymes, desaturases (Δ5 and Δ6) and elongases (Garrido et
349 al., 2020). Given this, adequate amounts of the precursor fatty acids must be supplied in the fish
350 diet to achieve an adequate 18:2n-6/18:3n-3 ratio. As reported by De la Cruz et al. (2021), the
351 tropical gar (*A. tropicus*), is an ancestral fish species, belonging to the Lepisosteidae family,
352 carnivorous and freshwater, where the presence of functional elongases (elovl2 and elovl5) and
353 desaturases (fads1 (Δ5) and fads2 (Δ6)) of the transcriptome, enzymes necessary to perform the
354 LC-PUFA biosynthesis cascade, has been detected, therefore, this species has the ability to
355 biosynthesize CL-PUFA. Therefore, in this research study, the biosynthesis capacity of *A.*
356 *tropicus* was confirmed by the results of the bioassay, which is shown in Table 4, corresponding
357 to the total percentage of fatty acids in the carcasses of juvenile fish, where the level of n-3

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358 polyunsaturated fatty acids (n-3 LC-PUFA) was significantly higher in the DS0 diet (100%
359 linseed oil), likewise eicosapentaenoic acid (EPA, 20: 5n-3) and docosahexaenoic acid (DHA,
360 22: 6n-3) showed the highest levels in DS0, with respect to the other treatments. These results
361 are consistent with studies indicating significant increase in n-3 fatty acids (n-3 LC-PUFA) and
362 DHA content as linseed oil in the diet increases (Li et al., 2015). Similar results are reported by
363 Omolo et al. (2017) who observed that *Oreochromis niloticus* (Nile tilapia) fed linseed oil had a
364 significant increase in muscle level n-3 fatty acids and DHA content. Therefore, it was suggested
365 that tilapias are able to convert dietary α-linolenic acid (ALA, 18: 3n-3) to LC-PUFA (Sargent et
366 al., 2002) and thus can elongate and desaturate α-linolenic acid (ALA, 18: 3n-3) to tissue DHA.
367 However, the level of conversion of C18 PUFA to n-3 long chain polyunsaturated fatty acids (n-
368 3 LC-PUFA) varies among species (Sargent et al., 2002).
369 In conclusion, 100% soybean oil diet (DS100) and mixture of 75% soybean oil with 25% linseed
370 oil diet (DS75) can successfully replace fish oil in diets of *A. tropicus* juveniles, showing higher
371 growth performance and feed conversion than treatment fish oil diet (DC). The elongases and
372 desaturases showed an upregulation in liver and intestine of fish in treatment DS75. Even 100%
373 linseed oil diet (DS0) showed the lower growth performance, the higher levels of EPA and DHA
374 found in the carcasses confirm the capacity of LC-PUFA biosynthesis in juvenile fish of *A.*
375 *tropicus*.
376

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486 **Table legends**

487 **Table 1.** Experimental diets for juveniles of *A. tropicus* fed with different inclusion percentages
488 of soybean oil and linseed oil. Selected fatty acid profile (% total fatty acids) in experimental
489 diets (mean ± SD). Linoleic acid (18: 2n-6), α-linolenic acid (18:3n-3), arachidonic acid, ARA
490 (20:4n-6), eicosapentaenoic acid, EPA (20:5n-3), and docosahexaenoic acid, DHA (22:6n-3).

Ingredients (g 100 g^{-1} dieta)	**DC**	**DS100**	**DS75**	**DS50**	**DS25**	**DS0**
Fish meal[a]	274.9	274.9	274.9	274.9	274.9	274.9
poultry by-products meal	135.0	135.0	135.0	135.0	135.0	135.0
Pork meal[a]	135.0	135.0	135.0	135.0	135.0	135.0
Soybean meal[a]	135.0	135.0	135.0	135.0	135.0	135.0
Corn starch[b]	76.6	76.6	76.6	76.6	76.6	76.6
Fish oil[a]	104.9	0.0	0.0	0.0	0.0	0.0
Soybean oil[c]	0.0	104.9	78.6	52.4	26.2	0.0
Linseed oil[c]	0.0	0.0	26.2	52.4	78.6	104.9
Grenetin[d]	18.0	18.0	18.0	18.0	18.0	18.0
Vitamin C[e]	4.5	4.5	4.5	4.5	4.5	4.5
Vitamin E[e]	0.9	0.9	0.9	0.9	0.9	0.9
Taurine[f]	1.8	1.8	1.8	1.8	1.8	1.8
Premix of vit-min[g]	13.5	13.5	13.5	13.5	13.5	13.5
Chemical composition (g/100g dry matter)						
Crude protein	45.5±0.01	45.8±0.02	45.6±0.01	45.5±0.01	45.4±0.03	45.2±0.02
Ether extract	15.3±0.02	15.4±0.01	15.4±0.03	15.1±0.01	15.0±0.02	15.1±0.01
Ash	13.2±0.22	13.4±0.20	13.4±0.21	13.1±0.20	13.2±0.22	13.2±0.20
Energy (Kj/g)	17.7 ±10	17.7 ±08	17.6 ±07	17.5 ±08	17.6 ±10	17.6 ±09
Fatty acid composition (% of total fatty acids)						
18:2n-6 LA	7.4±0.1[f]	29±0.1[a]	24±0.2[b]	18.9±0.9[c]	14.2±0.1[d]	10±0.0[e]
18:3n-3 ALA	1.4±0.0[e]	3.7±0.0[d]	8±0.1[c]	11.1±0.6[a]	11.1±0.1[a]	9.3±0.1[b]
20:4n-6 ARA	0.7±0.0[a]	0.5±0.0[b]	0.5±0.0[bc]	0.4±0.0[c]	0.3±0.0[d]	0.3±0.0[d]

20:5n-3 EPA	2.0±0.1[a]	2.0±0.0[a]	1.8±0.1[a]	1.5±0.1[b]	1.1±0.0[c]	0.8±0.1[d]
22:6n-3 DHA	2.7±0.1[a]	2.3±0.0[b]	2.1±0.1[b]	1.7±0.1[c]	1.2±0.0[d]	0.9±0.0[e]
∑ PUFA	**15.5±0.3[e]**	**38.5±0.1[a]**	**37.3±0.5[ab]**	**34.6±1.6[b]**	**28.8±0.2[c]**	**22±0.2[d]**
Ratio LA/ALA	5.4±0.1[b]	7.8±0.1[a]	3.0±0.0[c]	1.7±0.0[d]	1.3±0.0[e]	1.1±0.0[e]
Ratio DHA/EPA	1.3±0.0[a]	1.1±0.0[b]	1.2±0.0[b]	1.1±0.0[b]	1.1±0.0[b]	1.2±0.1[b]
Ratio ARA/EPA	0.3±0.0[b]	0.3±0.0[d]	0.3±0.0[cd]	0.3±0.0[cd]	0.3±0.1[bc]	0.4±0.0[a]

491 [a]PROTMAGRO S.A. de C.V., Guadalajara, Jalisco;

492 [b]IMSA, Industrializadora de Maíz S.A de C.V. Guadalajara, Jalisco, México;

493 [c] SESAJAL S.A. de C.V., Guadalajara, Jalisco, México

494 [d]D´gari, Productos alimenticios y dietéticos relámpago, S.A. de C.V., Tlalpan, México D.F.

495 [e]Pedregal (Trout diet Silver Cup), Toluca, Edo. Mex. México.

496 [f]Sigma - Aldrich # catalago T0625

497 [g]ROVIMIX R C-EC (Roche) 35 %.vitamin premix composition g, mg or International Units per
498 kg of diet: vitamin A, 10,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 100,000 IU; vitamin
499 K3, 4.0 g; thiamine B1, 8.0 g; riboflavin B2, 8.7 g; pyridoxine B6, 7.3 g; vitamin B12, 20.0 mg;
500 niacin, 50.0 g; pantothenic acid, 22.2 g; inositol, 0.15 mg; nicotinic acid, 0.16 mg; folic acid, 4.0
501 g; biotin, 500 mg; vitamin C, 10.0 g; choline 0.3 mg, excipient q.s. 2 g; manganese, 10 g;
502 magnesium, 4.5 g; zinc, 1.6 g; iron, 0.2 g; copper, 0.2 g; iodine, 0.5 g; selenium, 40 mg; cobalt
503 60 mg. Excipient q.s. 1.5 g

504

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505 **Table 2** Specific oligonucleotides used for real-time polymerase Chain Reaction (qPCR) in *A.*
506 *tropicus*.

Primer names	Forward primer (5'-3')	Reverse primer (5'-3')	Size (pb)	Step
***fads*1 (Δ5)**	TGACGTCACCAACTTCAGGC	TTGTCGATGTGGAAGGCAGT	105	RT - PCR
***fads*2 (Δ6)**	TCACTGGTTTGTCTGGGTGA	GCTCGATCTGGAAGTTGAGG	161	RT - PCR
***elovl*2**	CCACACTCTTGCTCACCCTA	TCCTCCTTGCCATGTTGCTA	173	RT - PCR
***elovl*5**	GGGATGGCTGTATTTCCAGA	TCCATTCGAGTGTCCATTCA	151	RT - PCR
18S rRNA	GGTAACGGGGAATCAGGGTT	TCCAATTACAGGGCCTCGAA	156	qPCR

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509

510 **Table 3.** Growth performance, somatic index and survival of *A. tropicus* juveniles fed with
511 different inclusion percentages of soybean oil and linseed oil.

Indexes	DC	DS100	DS75	DS50	DS25	DS0
WG (%)	131.02 ± 15.87^{b}	228.56 ± 29.93^{a}	207.86 ± 9.23^{a}	150.30 ± 26.32^{b}	110.93 ± 39.25^{b}	41.21 ± 31.21^{c}
SGR (%)	2.79 ± 0.23^{a,b}	3.96 ± 0.30^{a}	3.75 ± 0.10^{a}	3.05 ± 0.35^{ab}	2.45 ± 0.60^{b}	1.09 ± 0.76^{c}
FCR	2.19 ± 0.11^{c}	1.54 ± 0.35^{d}	2.09 ± 0.19^{c}	2.11 ± 0.57^{c}	2.72 ± 0.39^{b}	3.04 ± 0.13^{a}
PER	1.00 ± 0.05^{ab}	1.46 ± 0.31^{a}	1.05 ± 0.09^{a,b}	1.08 ± 0.30^{ab}	0.71 ± 0.11^{b}	0.88 ± 0.51^{b}
K	0.283 ± 0.01	0.267 ± 0.12	0.301 ± 0.05	0.288 ± 0.04	0.272 ± 0.01	0.253 ± 0.03
HSI	1.79 ± 0.41	1.82 ± 0.21	1.73 ± 0.25	2.11 ± 0.26	2.18 ± 0.33	2.16 ± 0.47
VSI	5.93 ± 0.58	5.67 ± 0.87	6.19 ± 0.58	6.31 ± 0.59	6.70 ± 0.91	6.50 ± 1.23
RIL	22.72 ± 2.29^{b}	23.33 ± 1.87^{b}	22.29 ± 2.58^{b}	23.66 ± 2.97^{b}	22.90 ± 1.59^{b}	28.31 ± 2.58^{a}
S (%)	84.62 ± 0.0^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	94.87 ± 4,45^{a}	92.31 ± 7.69^{ab}

512 WG: weight gain; SGR: specific growth rate; FCR: feed conversion rate; PER: protein efficiency
513 rate; K: condition factor and S: Survival. HSI: hepatosomatic index; VSI: viscerosomatic index;
514 RIL: relation of intestine length and total organism length. K: condition factor. Means ± SD (n =
515 9) with different superscripts are significantly ($p \leq 0.05$) different.

516

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517 **Figures legends**

518

519 **Figure 1** Growth in weight (g) (A) and total length (cm) (B) of *A. tropicus* juveniles fed with
520 different inclusion percentages of soybean oil and linseed oil. Values are mean ± SD. Significant
521 differences within the diets are indicated by different letters ($p < 0.05$).

522

523 **Figure 2.** Relative gene expression of elongases *elovl*2 (A) and *elovl*5 (B) and desaturases *fads*1
524 (Δ5) (C) and *fads*2 (Δ6) (D) in liver of *A. tropicus* juveniles feed with different inclusion
525 percentages of soybean oil and linseed oil. Values in the graph represents means ± SD (n = 3).
526 Means with different superscripts are significantly ($p \leq 0.05$) different.

527

528 **Figure 3.** Relative gene expression of elongases *elovl*2 (A) and *elovl*5 (B) and desaturases *fads*1
529 (Δ5) (C) and *fads*2 (Δ6) (D)in intestine of *A. tropicus* juveniles feed with different inclusion
530 percentages of soybean oil and linseed oil. Values in the graph represents means ± SD (n = 3).
531 Means with different superscripts are significantly ($p \leq 0.05$) different.

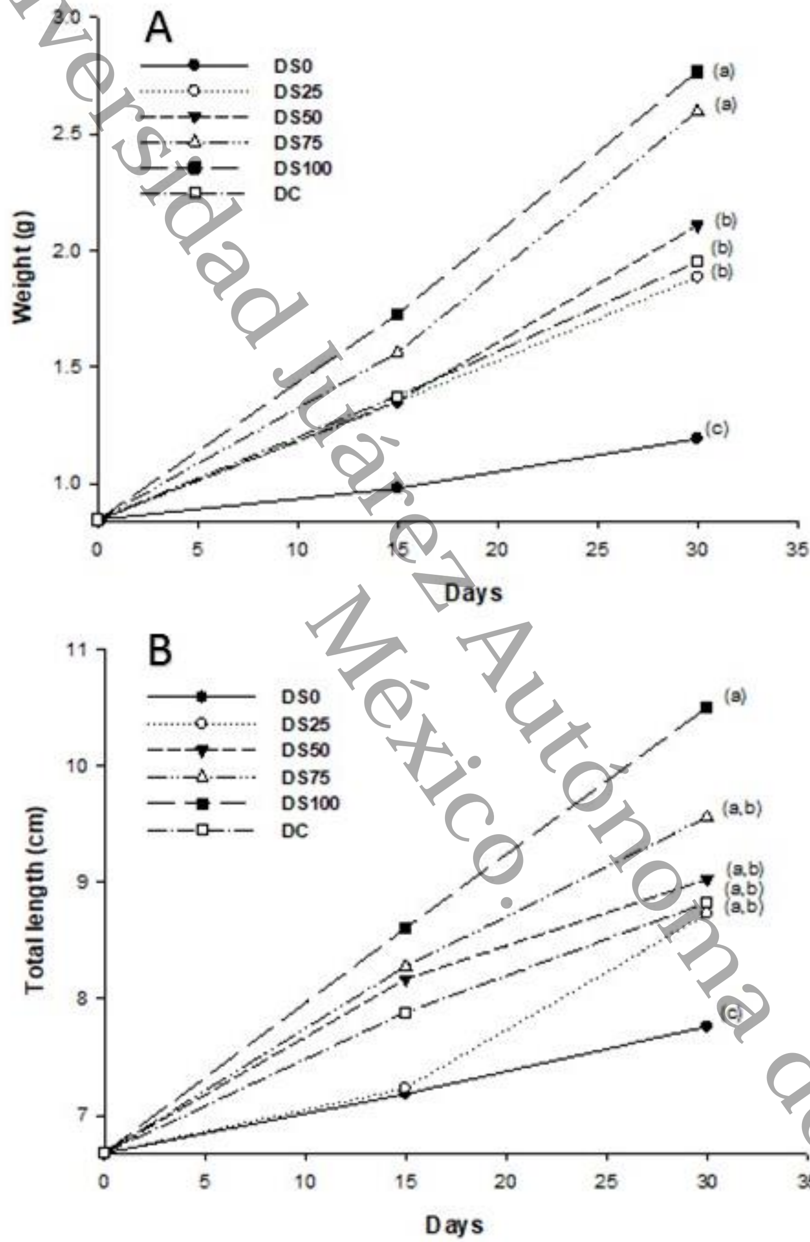
532

533 **Figure 4.** Selected fatty acid profile (% total fatty acids) from carcass of *A. tropicus* juveniles fed
534 with different inclusion percentages of soybean oil and linseed oil. A) α-linolenic acid (ALA,
535 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid, (DHA, 22:6n-3), B)
536 Linoleic acid (LA, 18: 2n-6), arachidonic acid (ARA, 20:4n-6). Means ± SD (n = 2) with
537 different superscripts are significantly ($p \leq 0.05$) different.

538

539 **Figure 5.** Selected fatty acid profile (% total fatty acids) from feces of *A. tropicus* juveniles fed
540 with different inclusion percentages of soybean oil and linseed oil. A) α-linolenic acid (ALA,
541 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid, (DHA, 22:6n-3), B)
542 Linoleic acid (LA, 18: 2n-6), arachidonic acid (ARA, 20:4n-6). Means ± SD (n = 2) with
543 different superscripts are significantly ($p \leq 0.05$) different.

544 **Figure 1**



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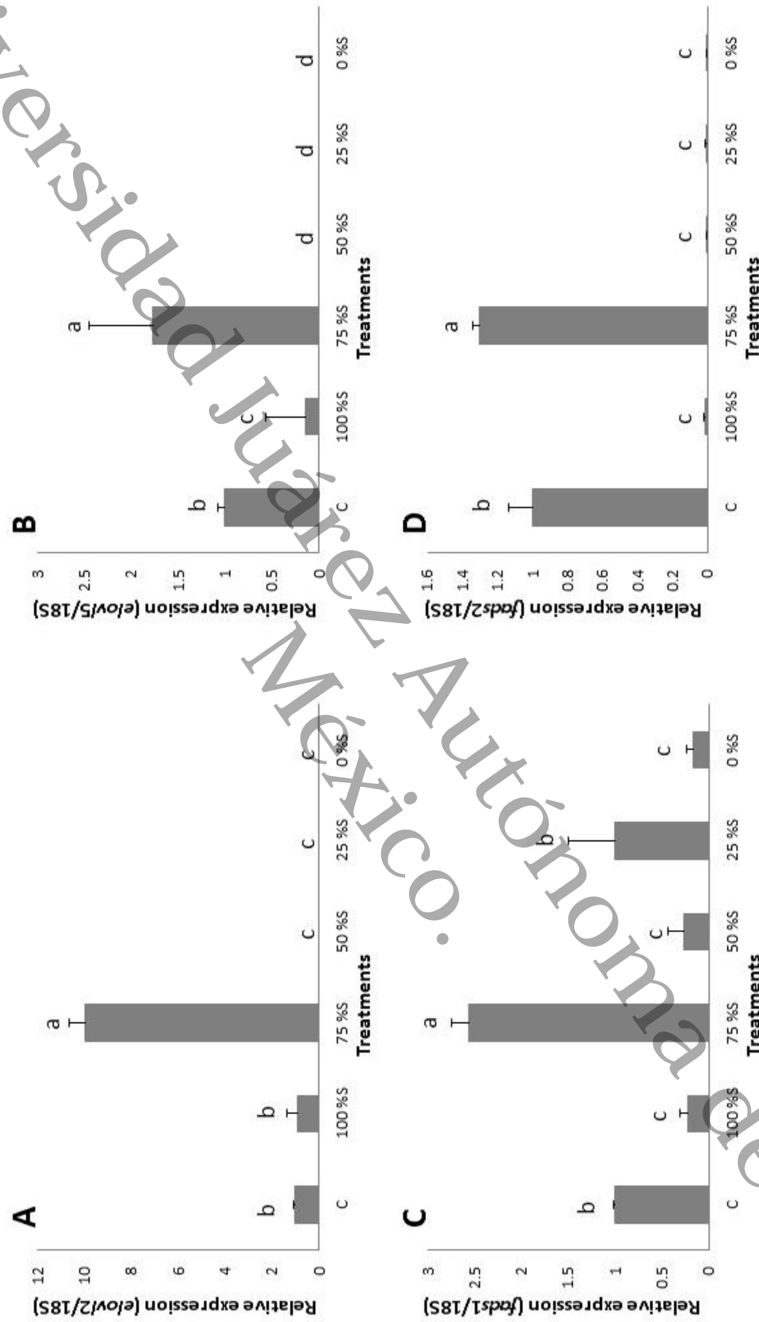
546 **Figure 2**



547

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548 **Figure 3**

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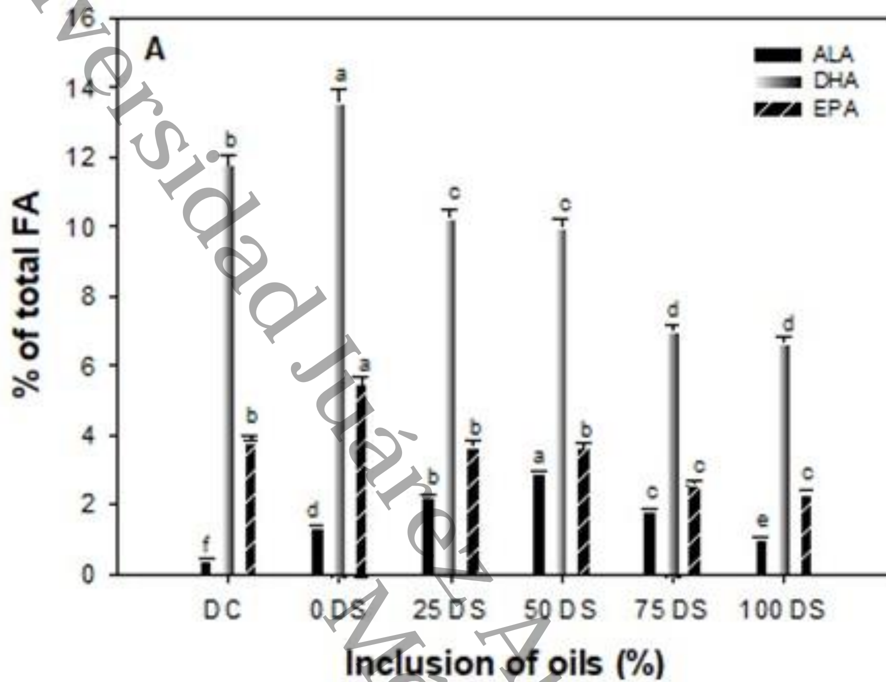
549 **Figure 4**



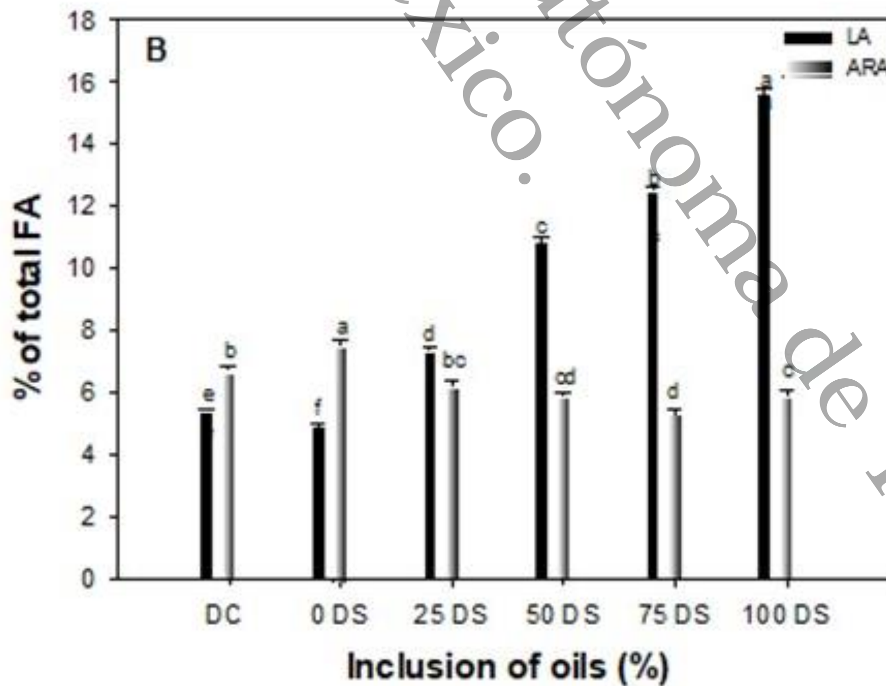


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Figure 5

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Supplementary file 1.

Percentage of total fatty acids (mean ± standard deviation, SD) in experimental diets used for *A. tropicus* juveniles. Diet designations as in Table 1. Linoleic acid (18: 2n-6), α-linolenic acid (18:3n-3), arachidonic acid, ARA (20:4n-6), eicosapentaenoic acid, EPA (20:5n-3), and docosahexaenoic acid, DHA (22:6n-3).

Fatty acids (%)	DC	DS100	DS75	DS50	DS25	DS0
14:0	4.1±0.0[a]	1.2±0.0[e]	1.3±0.0[de]	1.3±0.0[cd]	1.4±0.0[c]	1.6±0.0[b]
15:0	0.6±0.0[a]	0.1±0.0[d]	0.1±0.0[cd]	0.2±0.0[cd]	0.2±0.0[c]	0.2±0.0[b]
16:0	25.9±0.0[a]	16.4±0.0[d]	16.4±0.2[d]	16.8±0.4[d]	18.1±0.1[c]	19.9±0.1[b]
17:0	0.8±0.0[a]	0.3±0.0[c]	0.3±0.0[c]	0.4±0.1[bc]	0.4±0.0[bc]	0.4±0.0[b]
18:0	13.4±0.3[a]	10±0.2[c]	10.6±0.1[bc]	11.8±1.3[abc]	12.7±0.2[ab]	13.9±0.1[a]
20:0	0.7±0.0[a]	0.3±0.0[c]	0.4±0.0[c]	0.4±0.0[c]	0.4±0.0[b]	0.4±0.1[b]
21:0	0.1±0.0[a]	0±0.0[b]	0±0.0[b]	0±0.0[b]	0±0.0[b]	0±0.0[b]
22:0	0.4±0.0[a]	0.3±0.0[b]	0.3±0.0[b]	0.3±0.0[ab]	0.3±0.0[ab]	0.3±0.0[c]
24:0	0.3±0.0[ab]	0.1±0.0[b]	0.2±0.0[ab]	0.2±0.0[ab]	0.2±0.0[ab]	0.2±0.0[a]
∑ SFA	46.3±0.4[a]	29±0.0[d]	29.7±0.4[d]	31.5±1.2[cd]	33.9±0.2[c]	37.1±0.0[b]
16:1n-9	0.4±0.0[a]	0.2±0.0[c]	0.2±0.0[c]	0.2±0.0[bc]	0.2±0.0[bc]	0.3±0.0[b]
16:1n-7	6.1±0.1[a]	2.7±0.0[d]	2.7±0.0[d]	2.7±0.0[d]	3±0.0[c]	3.4±0.0[b]
17:1n-8	0.3±0.0[a]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]
18:1n-9	24.6±0.1[e]	25.5±0.0[d]	26±0.1[d]	26.8±0.1[c]	29.5±0.1[b]	32.1±0.1[a]
18:1n-7	3.5±0.0	2.3±0.0	2.2±0.0	2.2±0.1	2.3±0.0	2.5±0.0
18:1n-5	0.1±0.0[ab]	0±0.0[b]	0±0.0[ab]	0.1±0.0[a]	0.1±0.0[ab]	0.1±0.0[ab]
20:1n-11	0.5±0.0[bc]	0.2±0.0[d]	0.4±0.0[cd]	0.4±0.0[c]	0.6±0.0[b]	0.8±0.0[a]
20:1n-9	1±0.0[a]	0.5±0.0[d]	0.5±0.0[cd]	0.5±0.0[d]	0.6±0.0[c]	0.6±0.0[b]
22:1n-11	0.3±0.0[a]	0.1±0.0[b]	0.1±0.0[b]	0.2±0.0[b]	0.2±0.0[ab]	0.2±0.1[ab]
22:1n-9	0.3±0.0[a]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.1[b]
24:1n-9	0.7±0.0[a]	0.4±0.0[bc]	0.4±0.0[bc]	0.3±0.1[c]	0.5±0.0[bc]	0.5±0.0[b]
∑ MSFA	38.2±0.1[b]	32.5±0.1[d]	33±0.2[d]	34±0.2[c]	37.4±0.1[b]	41±0.2[a]
18:2n-6 LA	7.4±0.1[f]	29±0.1[a]	24±0.2[b]	18.9±0.9[c]	14.2±0.1[d]	10±0.0[e]
18:3n-6	0.1±0.0[a]	0.1±0.0[ab]	0.1±0.0[ab]	0.1±0.1[a]	0±0.0[b]	0±0.0[b]
18:3n-3 ALA	1.4±0.0[e]	3.7±0.0[d]	8±0.1[c]	11.1±0.6[a]	11.1±0.1[a]	9.3±0.1[b]
18:4n-3	0.1±0.0[bc]	0.1±0.0[bc]	0.1±0.0[c]	0.1±0.0[bc]	0.1±0.0[ab]	0.1±0.0[a]
20:2n-6	0.3±0.0[a]	0.2±0.0[b]	0.2±0.0[b]	0.2±0.0[b]	0.2±0.0[b]	0.2±0.0[b]
20:3n-3	0.1±0.0[a]	0±0.0[b]	0±0.0[b]	0±0.0[b]	0±0.0[b]	0±0.0[b]
20:4n-6 ARA	0.7±0.0[a]	0.5±0.0[b]	0.5±0.0[bc]	0.4±0.0[c]	0.3±0.0[d]	0.3±0.0[d]
20:5n-3 EPA	2±0.1[a]	2±0.0[a]	1.8±0.1[a]	1.5±0.1[b]	1.1±0.0[c]	0.8±0.1[d]
21:4n-6	0.1±0.0[a]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]
22:5n-6	0.4±0.0[a]	0.4±0.0[a]	0.4±0.0[a]	0.3±0.0[b]	0.2±0.0[c]	0.2±0.0[d]
22:6n-3 DHA	2.7±0.1[a]	2.3±0.0[b]	2.1±0.1[b]	1.7±0.1[c]	1.2±0.0[d]	0.9±0.0[e]
∑ PUFA	15.5±0.3[e]	38.5±0.1[a]	37.3±0.5[ab]	34.6±1.6[b]	28.8±0.2[c]	22±0.2[d]

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∑ n-9 LC-MUFA	27±0.1[d]	26.7±0.1[d]	27.1±0.2[d]	28±0.2[c]	30.8±0.1[b]	33.6±0.2[a]
∑ n-6 LC-PUFA	9.4±0.1[f]	30.5±0.1[a]	25.4±0.2[b]	20.3±0.8[c]	15.3±0.1[d]	11±0.1[e]
∑ n-3 LC-PUFA	6.2±0.2[d]	8.1±0.1[c]	12±0.3[b]	14.4±0.8[a]	13.6±0.1[a]	11.2±0.2[b]
(n-3)/(n-6)	0.7±0.0[d]	0.3±0.0[f]	0.5±0.0[e]	0.7±0.0[c]	0.9±0.0[b]	1±0.0[a]
Ratio LA/ALA	5.4±0.1[b]	7.8±0.1[a]	3±0.0[c]	1.7±0.0[d]	1.3±0.0[e]	1.1±0.0[e]
Ratio DHA/EPA	1.3±0.0[a]	1.1±0.0[b]	1.2±0.0[b]	1.1±0.0[b]	1.1±0.0[b]	1.2±0.1[b]
Ratio ARA/EPA	0.3±0.0[b]	0.3±0.0[d]	0.3±0.0[cd]	0.3±0.0[cd]	0.3±0.1[bc]	0.4±0.0[a]

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Percentage of total fatty acids (mean ± standard deviation, SD) in final carcass of tropical gar (*A. tropicus*) juveniles feed with experimental diets. Linoleic acid (18: 2n-6), α-linolenic acid (18:3n-3), arachidonic acid, ARA (20:4n-6), eicosapentaenoic acid, EPA (20:5n-3), and docosahexaenoic acid, DHA (22:6n-3).

Fatty acids (%)	DC	DS100	DS75	DS50	DS25	DS0
14:0	1.5±0.0[a]	0.8±0.0[c]	1±0.0[b]	0.8±0.0[c]	1±0.0[b]	1±0.0[d]
15:0	0.4±0.0[a]	0.2±0.0[e]	0.2±0.0[bc]	0.2±0.0[de]	0.3±0.0[b]	0.2±0.0[cd]
16:0	20.6±0.2[a]	19.1±0.1[bc]	20.2±0.1[a]	18.6±0.1[cd]	19.1±0.1[b]	18.1±0.1[d]
17:0	0.6±0.0[a]	0.4±0.0[bc]	0.4±0.0[b]	0.4±0.0[c]	0.4±0.1[bc]	0.4±0.0[bc]
18:0	11±0.0[ab]	10.2±0.3[b]	10.1±0.2[b]	10.2±0.3[b]	10.1±0.2[b]	11.3±0.2[a]
20:0	0.2±0.0[b]	0.1±0.0[d]	0.2±0.0[c]	0.2±0.0[d]	0.2±0.0[c]	0.2±0.0[a]
21:0	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[c]	0.1±0.0[b]	0.1±0.0[c]	0.2±0.0[a]
22:0	0.1±0.0[b]	0.1±0.0[bc]	0.1±0.0[d]	0.1±0.0[c]	0.1±0.0[b]	0.2±0.0[a]
24:0	0.2±0.0[ab]	0.1±0.0[bc]	0.1±0.0[c]	0.1±0.0[c]	0.1±0.0[c]	0.2±0.0[a]
∑ SFA	35±0.4[a]	31.3±0.4[bc]	32.4±0.4[b]	30.7±0.4[c]	31.4±0.4[bc]	31.4±0.4[bc]
16:1n-9	1.5±0.0[a]	1±0.0[d]	1±0.0[d]	1.2±0.0[c]	1.2±0.0[c]	1.4±0.0[b]
16:1n-7	3.6±0.0[a]	2.3±0.0[e]	2.6±0.0[c]	2.3±0.0[d]	2.8±0.0[b]	2.1±0.0[f]
17:1n-8	0.2±0.0[a]	0.1±0.0[b]	0.1±0.0[b]	0.1±0.0[b]	0.2±0.0[b]	0.1±0.0[b]
18:1n-9	18.5±0.0[d]	20.1±0.0[c]	21.4±0.0[a]	20.4±0.0[bc]	20.4±0.1[b]	17.1±0.0[e]
18:1n-7	5.9±0.0[a]	4.4±0.0[e]	4.8±0.0[c]	4.6±0.0[d]	5±0.0[b]	5±0.0[b]
18:1n-5	0.1±0.0[ab]	0±0.0[cd]	0.1±0.0[abc]	0±0.0[d]	0.1±0.0[bcd]	0.1±0.0[a]
20:1n-11	0.1±0.0[d]	0.1±0.0[b]	0.2±0.0[a]	0.1±0.0[bc]	0.1±0.0[bc]	0.1±0.0[c]
20:1n-9	1±0.0[a]	0.6±0.0[d]	0.7±0.0[c]	0.7±0.0[c]	0.8±0.0[b]	0.8±0.0[b]
22:1n-11	0.1±0.0[ab]	0.1±0.0[bc]	0±0.0[c]	0.1±0.0[bc]	0.1±0.0[a]	0.1±0.0[bc]
22:1n-9	0.1±0.0[ab]	0±0.0[b]	0±0.0[ab]	0.1±0.0[ab]	0.1±0.0[a]	0.1±0.0[ab]
24:1n-9	0.5±0.0[b]	0.5±0.0[b]	0.5±0.0[b]	0.5±0.0[b]	0.6±0.0[b]	0.7±0.0[a]
∑ MSFA	32±0.1[a]	29.9±0.1[c]	32±0.1[a]	30.7±0.1[b]	32±0.1[a]	28.2±0.1[d]
18:2n-6 LA	5.4±0.1[e]	15.6±0.2[a]	12.5±0.1[b]	10.9±0.1[c]	7.3±0.1[d]	4.9±0.1[f]
18:3n-6	1±0.1[cd]	3.2±0.4[a]	2.6±0.3[ab]	0.3±0.0[d]	1.8±0.2[bc]	1.2±0.0[cd]
18:3n-3 ALA	0.4±0.0[f]	1.1±0.0[e]	1.9±0.0[c]	2.9±0.0[a]	2.3±0.0[b]	1.4±0.0[d]
18:4n-3	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]	0±0.0[a]	0.1±0.0[a]	0.1±0.0[a]
20:2n-6	0.4±0.0[b]	0.6±0.0[a]	0.5±0.0[ab]	0.5±0.0[ab]	0.5±0.0[b]	0.5±0.0[b]
20:3n-3	0.2±0.0[ab]	0.2±0.0[ab]	0.1±0.0[b]	0.2±0.0[a]	0.2±0.0[a]	0.2±0.0[a]
20:4n-6 ARA	6.7±0.2[b]	6±0.1[c]	5.3±0.1[d]	5.9±0.1[cd]	6.2±0.2[bc]	7.5±0.18[a]
20:5n-3 EPA	3.9±0.1[b]	2.4±0.1[c]	2.6±0.1[c]	3.7±0.1[b]	3.7±0.1[b]	5.5±0.2[a]
21:4n-6	0.2±0.0[c]	0.2±0.0[c]	0.2±0.0[c]	0.4±0.1[b]	0.5±0.0[ab]	0.6±0.0[a]

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22:5n-6	0.2±0.0[b]	0.2±0.0[d]	0.1±0.0[e]	0.2±0.1[c]	0.2±0.0[d]	0.3±0.0[a]
22:6n-3 DHA	11.8±0.3[b]	6.7±0.2[d]	7±0.2[d]	10±0.3[c]	10.3±0.3[c]	13.6±0.4[a]
∑ **PUFA**	34.3±0.5[d]	39.6±0.1[b]	36.3±0.5[c]	39.4±0.5[b]	37.2±0.5[c]	41.1±0.6[a]
∑ n-9 LC-MUFA	21.4±0.1[e]	22.3±0.1[d]	23.6±0.1[a]	22.7±0.1[c]	23.1±0.1[b]	20±0.1[f]
∑ n-6 LC-PUFA	15.9±0.2[f]	27.6±0.3[a]	23±0.2[b]	20.2±0.2[c]	18.3±0.2[d]	17.1±0.2[e]
∑ n-3 LC-PUFA	18.5±0.4[b]	11.9±0.2[d]	13.4±0.3[c]	19.2±0.4[b]	19±0.4[b]	24.1±0.5[a]
(n-3)/(n-6)	1.2±0.0[b]	0.4±0.0[f]	0.6±0.0[e]	1±0.0[d]	1±0.0[c]	1.4±0.0[a]
Ratio LA/ALA	13.1±0.1[b]	14.9±0.1[a]	6.3±0.0[c]	3.7±0.0[d]	3.2±0.0[e]	3.5±0.0[de]
Ratio DHA/EPA	3.1±0.0[a]	2.9±0.0[b]	2.7±0.0[b]	2.8±0.0[b]	2.8±0.0[b]	2.5±0.0[c]
Ratio ARA/EPA	1.8±0.0[c]	2.6±0.1[a]	2.1±0.0[b]	1.6±0.0[c]	1.7±0.0[c]	1.4±0.0[d]

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Percentage of total fatty acids (mean ± standard deviation, SD) in feces of tropical gar (*A. tropicus*) juveniles fed with experimental diets. Linoleic acid (18: 2n-6), α-linolenic acid (18:3n-3), arachidonic acid, ARA (20:4n-6), eicosapentaenoic acid, EPA (20:5n-3), and docosahexaenoic acid, DHA (22:6n-3).

Fatty acids (%)	**DC**	**DS100**	**DS75**	**DS50**	**DS25**	**DS0**
14:0	3.8±0.0^{a}	1.7±0.0^{c}	1.5±0.0^{d}	1.5±0.0^{d}	1.6±0.0^{cd}	1.8±0.0^{b}
15:0	0.7±0.0^{a}	0.3±0.0^{b}	0.3±0.0^{b}	0.2±0.0^{b}	0.3±0.0^{b}	0.2±0.0^{b}
16:0	30.4±0.2^{a}	26.7±0.1^{b}	25.7±0.1^{c}	26.5±0.2^{b}	25.1±0.2^{c}	23.2±0.1^{d}
17:0	1.2±0.0^{a}	0.7±0.0^{b}	0.6±0.0^{c}	0.6±0.0^{c}	0.6±0.0^{c,d}	0.5±0.0^{d}
18:0	24.2±0.6^{b}	30.3±0.7^{a}	25±0.6^{b}	29.1±0.7^{a}	23.5±0.6^{b}	18.9±0.5^{c}
20:0	1±0.0^{a}	0.9±0.0^{b}	0.8±0.0^{d}	0.8±0.0^{c}	0.7±0.0^{e}	0.5±0.0^{f}
21:0	0.2±0.0^{a}	0.1±0.0^{b}	0.1±0.0^{b,c}	0.1±0.0^{b,c,d}	0.1±0.0^{c,d}	0.1±0.0^{d}
22:0	0.8±0.0^{a}	0.8±0.0^{a}	0.6±0.0^{c}	0.6±0.0^{b}	0.5±0.0^{d}	0.4±0.0^{e}
24:0	0.4±0.0^{a,b}	0.4±0.0^{a}	0.3±0.0^{a,b}	0.4±0.0^{a,b}	0.4±0.0^{a,b}	0.3±0.0^{b}
∑ SFA	62.8±0.8^{a}	61.9±0.8^{a,b}	54.9±0.7^{c}	59.8±0.7^{b}	52.7±0.7^{c}	46±0.6^{d}
16:1n-9	0.5±0.0^{a}	0.4±0.0^{b}	0.3±0.0^{c}	0.3±0.0^{d}	0.3±0.0^{c,d}	0.4±0.0^{a,b}
16:1n-7	3.5±0.0^{b}	1.6±0.0^{f}	2.1±0.0^{d}	1.9±0.0^{e}	2.3±0.0^{c}	3.6±0.0^{a}
17:1n-8	0.2±0.0^{a}	0.1±0.0^{c}	0.1±0.0^{d}	0.1±0.0^{d}	0.1±0.0^{c}	0.2±0.0^{b}
18:1n-9	16.2±0.0^{e}	17.2±0.1^{d}	19.7±0.1^{c}	19.6±0.1^{c}	23±0.1^{b}	28.3±0.1^{a}
18:1n-7	3.1±0.0^{a}	2.1±0.0^{f}	2.4±0.0^{d}	2.2±0.0^{e}	2.5±0.0^{c}	3±0.0^{b}
18:1n-5	0.2±0.0^{a}	0.2±0.0^{a}	0±0.0^{c}	0±0.0^{c}	0±0.0^{c}	0.1±0.0^{b}
20:1n-11	0.7±0.0^{b}	0.6±0.0^{c}	0.4±0.0^{d}	0.5±0.0^{d}	0.6±0.0^{b,c}	1.2±0.0^{a}
20:1n-9	0.8±0.0^{a}	0.5±0.0^{e}	0.6±0.0^{c}	0.6±0.0^{d}	0.7±0.0^{b}	0.7±0.0^{b}
22:1n-11	0.3±0.0^{a}	0.1±0.0^{b}	0.2±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.1±0.0^{b}
22:1n-9	0.3±0.0^{a}	0.1±0.0^{b}	0.1±0.0^{b}	0.1±0.0^{b}	0.1±0.0^{b}	0.1±0.0^{b}
24:1n-9	2.4±0.1^{a}	0±0.0^{c}	0±0.0^{c}	0±0.0^{c}	1.1±0.0^{b}	1±0.1^{b}
∑ MSFA	28.4±0.1^{c}	23.3±0.1^{f}	26.3±0.1^{d}	25.8±0.1^{e}	31.4±0.1^{b}	38.7±0.1^{a}
18:2n-6 LA	3.1±0.0^{e}	8.4±0.1^{b}	10.2±0.1^{a}	8.2±0.1^{b}	7.3±0.1^{c}	7±0.1^{d}
18:3n-6	0.1±0.0^{d}	0.6±0.1^{a}	0.4±0.1^{b}	0.2±0.0^{c,d}	0.3±0.0^{c}	0.3±0.0^{c}
18:3n-3 ALA	0.7±0.0^{e}	1±0.0^{d}	3.7±0.1^{b}	2.8±0.0^{c}	4.2±0.0^{a}	4.1±0.0^{a}
18:4n-3	0.2±0.0^{b,c}	0.4±0.0^{a}	0.1±0.0^{e}	0.1±0.0^{d,e}	0.1±0.0^{c,d}	0.2±0.0^{b}
20:2n-6	0.2±0.0^{c}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{b}	0.2±0.0^{b,c}	0.2±0.0^{d}
20:4n-6 ARA	1.7±0.0^{b}	1.9±0.1^{a}	1.4±0.0^{cd}	1±0.0^{e}	1.4±0.0^{d}	1.5±0.0^{bc}
20:5n-3 EPA	0.9±0.0^{ab}	0.7±0.0^{c}	1±0.0^{a}	0.7±0.0^{c}	0.9±0.0^{b}	0.8±0.0^{b}
21:4n-6	0.1±0.0^{e}	0±0.0^{f}	0.1±0.0^{c}	0.1±0.0^{d}	0.2±0.0^{a}	0.1±0.0^{b}
22:5n-6	0.4±0.0^{a}	0.1±0.0^{d}	0.3±0.0^{b}	0.3±0.0^{c}	0.3±0.0^{b}	0.3±0.0^{c}

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22:6n-3 DHA	1.6±0.0[a]	1.4±0.0[b]	1.3±0.0[c]	1±0.0[d]	1±0.0[d]	1±0.0[d]
∑ PUFA	9.5±0.1[d]	15.6±0.2[c]	19.5±0.3[a]	15.2±0.2[c]	16.6±0.2[b]	16±0.2[b,c]
∑ n-9 LC-MUFA	20.1±0.1[d]	18.3±0.1[e]	20.8±0.1[c]	20.6±0.1[c]	25.2±0.1[b]	30.5±0.1[a]
∑ n-6 LC-PUFA	6.3±0.1[e]	12.5±0.1[b]	13.5±0.1[a]	10.7±0.1[c]	10.4±0.1[c,d]	10±0.1[d]
∑ n-3 LC-PUFA	3.3±0.1[c]	3.5±0.1[c]	6.1±0.1[a]	4.6±0.1[b]	6.3±0.1[a]	6.2±0.1[a]
(n-3)/(n-6)	0.5±0.0[c]	0.3±0.0[f]	0.4±0.0[d]	0.4±0.0[e]	0.6±0.1[b]	0.6±0.0[a]
Ratio LA/ALA	4.4±0.0[b]	8.4±0.1[a]	2.8±0.0[c]	2.8±0.0[c]	1.8±0.0[d]	1.7±0.0[d]
Ratio DHA/EPA	1.8±0.0[b]	2.2±0.0[a]	1.3±0.0[d]	1.4±0.0[c]	1.2±0.0[e]	1.3±0.0[d]
Ratio ARA/EPA	1.9±0.0[b]	3±0.0[a]	1.5±0.0[c]	1.6±0.0[c]	1.6±0.0[c]	1.9±0.0[b]

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7.- DISCUCIÓN GENERAL

La capacidad de biosintésis de ácidos grasos de cadena larga (LC-PUFA) depende de múltiples factores como el hábitat (agua dulce, agua salobre, marina), etapa de desarrollo, estado de reproducción, historial nutricional y estado de estrés (Fonseca-Madrigal et al., 2014; Colombo et al., 2016; Kabeya et al., 2017; Lopes-Marques et al., 2018; Garrido, et al., 2019). La comprensión de las rutas de la biosíntesis de LC-PUFA en peces ancestrales como el gars es escasa. Este estudio nos permitió conocer algunos métodos de regulación de los genes que regulan las enzimas desaturasa y elongasa que permite al pejelagarto "*Atractosteus tropicus"* producir de forma endógena LC-PUFA (Lopes-Marques et al., 2018; Garrido, et al., 2019).

En la presente tesis, durante el estudio del primer capítulo, correspondiente al análisis secuencial y filogenético, se reveló que *A. tropicus* posee ortólogos *fads*1 y *fads*2 en su genoma, que se agruparon muy de cerca con ortólogos caracterizados a partir de *Lepisosteus oculatus* (Lopes-Marques et al., 2018). De tal manera, que la caracterización funcional *fads1* de *L. oculatus* mostró que esta enzima tiene actividad Δ5 desaturasa hacia 20: 3n-6 y 20: 4n-3, que se convierten en 20: 4n-6 (ARA) y 20: 5n-3 (EPA), respectivamente, mientras que *fads2* en *L. oculatus* tiene actividad Δ6 desaturasa hacia 18: 2n-6 y 18: 3n-3, que son desaturadas a 18: 3n-6 y 18: 4n-3, respectivamente (Lopes-Marques et al., 2018). Además, en *L. oculatus* el *fads2* fue capaz de convertir 20: 2n-6 y 20: 3n-3 en 20: 3n-6 y 20: 4n-3, respectivamente, lo que denota, una capacidad de desaturación tipo Δ8 (Lopes-Marques et al., 2018). Por lo tanto, dada la similaridad entre las secuencias de *fads* de *L. oculatus* y *A. tropicus*, es razonable predecir que las *fads1* y *fads2* de *A. tropicus* poseen capacidades similares de desaturación a las descritas anteriormente para *L. oculatus*. Con respecto a las elongasas, el análisis filogenético muestra que *A. tropicus* posee verdaderos ortólogos tanto de *elovl2* como de *elovl5*. A diferencia de las *fads*, todavía no se ha informado de una caracterización funcional de las enzimas *elovl* de ninguna especie dentro de la familia Lepisosteidae. Por consiguiente, *A. tropicus* poseería, no solo toda la desaturasa, sino también las capacidades enzimáticas de elongasa necesarias para biosintetizar ARA, EPA y DHA a partir de los precursores de PUFA (LA y ALA) esenciales en la dieta (Castro et al., 2016; Monroig et al., 2018).

Los análisis de expresión génica del gen *fads1* durante el desarrollo temprano de *A. tropicus*, mostraron la presencia de transcripción a los 0 días pos eclosion (dph). Así mimo, estudios anteriores han demostrado que las transcripciones de genes clave de biosíntesis de LC-PUFA también se detectaron desde el comienzo de la embriogénesis del pez cebra "*D. rerio"* (cigoto) (Monroig et al., 2009). Debido a que la activación de genes en el pez cebra

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ocurre después de etapas en las que se detectó la presencia de transcripciones de *fads2*, *elovl2* y *elovl5*, se reporta la transferencia materna de ARNm como el mecanismo que explica tal resultado (Monroig et al., 2009). También se ha informado de más pruebas de la transferencia materna de ARNm de tipo *fads* y *elovl* en especies de peces, *D. rerio* (Monroig et al., 2010a), cobia "*Rachycentron canadum*" (Monroig et al., 2011), *S. senegalensis* y *S. aurata* (Morais et al., 2012; Torres et al., 2020). La activación de la biosíntesis de LC-PUFA en *A. tropicus* en el 15 dph presenta picos de expresión notablemente alto para los cuatro genes investigados. Esto coincide con un cambio en el régimen de alimentación de nauplios de Artemia a la alimentación conjunta con la dieta formulada para truchas. Si bien tal cambio en la dieta podría tener algunos efectos estimulantes sobre la biosíntesis de LC-PUFA, también es posible que la activación de las vías de biosíntesis de LC-PUFA se deba a la maduración del sistema digestivo en *A. tropicus*, que se logró alrededor de 15 dph, de acuerdo a lo reportado por Frias-Quintana et al. (2015). Así mismo, existen estudios que han demostrado que el intestino juega un papel activo en la biosíntesis de LC-PUFA en peces (Bell et al. 2003; Fonseca-Madrigal et al. 2006; Morais et al., 2012; Galindo et al., 2021). Efectivamente, en los análisis de distribución de tejidos de los genes *fads* y *elovl* confirmaron que el intestino también es un sitio activo para la biosíntesis de LC-PUFA en juveniles de *A. tropicus.*

En la presente tesis, los genes f*ads1*, *fads2* y *elovl2* mostrron la mayor expresión en el intestino, mientras que solo *elovl5* mostró la máxima expresión relativa en el hígado. La expresión relativa de desaturasas y elongasas entre tejidos de peces ha mostrado diferencias en el patrón de expresión entre peces de agua dulce / salmónidos y peces marinos (Monroig et al., 2018). Al respecto, estudios en especies de peces de agua dulce como pez cebra (*D. rerio)* (Monroig et al., 2009), lucio pejerrey (*Chirostoma estor*) (Fonseca-Madrigal et al., 2014) y tenca (*Tinca tinca*) (Garrido et al., 2020), la expresion de los genes fads2, elovl2 y / o elovl5 se expresan en gran medida en el hígado y el intestino. Por ello, los patrones de expresión y la actividad de los genes de las desaturasas y elongasas están influenciados por factores como la filogenia, los hábitos ecológicos y los hábitos alimentarios (Garrido et al., 2019; Xie et al., 2020). De tal forma, los resultados en *A. tropicus* están de acuerdo con los resultados de especies de peces de agua dulce anteriormente mencionados. Los resultados de la alta expresión de *elovl5* registrada en riñón, coinciden con reportes en embriones de *D. rerio*, los cuales muestran expresión específicamente en los conductos pronefricos (Monroig et al., 2009).

En la actualidad, no existen estudios que han evaluado el efecto de la proporción de PUFA n-3 y n-6 sobre el rendimiento del crecimiento, índice somático y el perfil de ácidos

grasos de carcasses en peces juveniles del pejelagarto "*Atractosteus tropicus",* sin embargo existen estudios previos en varias especies de peces, con respecto al uso de diferentes fuentes vegetales de lípidos que no afectaron el rendimiento del crecimiento del pez, como lo reportado por Ng et al. (2013); Asdari et al. (2011); Turchini et al. (2011); Rombenso et al. (2016) en especies de tilapia del Nilo *(Oreochromis niloticus)*, panga *(Pangasius hypophthalmus)*, bacalao Murray *(Maccullochella peelii peelii)* y Florida pompano *(Trachinotus carolinus*) respectivamente. Ante ello, en el segundo capítulo de la presente tesis se realizaron estudios para probar la viabilidad del aceite de soya y/o aceite de linaza con respecto al aceite de pescado en las diferentes dietas elaboradas, determinando los efectos del cambio en la proporción de PUFA n-3 y n-6 sobre el rendimiento del crecimiento, índices somáticos y perfil de ácidos grasos del metabolismo lipídico de juveniles en *A. tropicus*.

En el segundo capítulo, se mostró que el reemplazo del aceite de pescado en la dieta por el 100% de aceite de soya (DS100), aumentó significativamente la ganancia en peso, la tasa de crecimiento específica y la tasa de conversión proteica (WG, SGR y PER) y disminuyó significativamente su FCR en juveniles de *A. tropicus*, lo que nos indicó que el reemplazo de aceite de pescado dietético por aceite de soya puede aumentar el rendimiento del crecimiento en peso y longitud, así como un mayor aprovechamiento de alimento y proteína, la cual favorecería en la utilización para la alimentación de *A.tropicus* con aceites vegetales, permitiendo la disminución de costos en la producción. Así mismo, estudios similares por Chen et al. (2019) y Xu et al. (2012) en el reemplazo de aceite de pescado en la dieta con aceite de soya reportan el aumento en el rendimiento del crecimiento en lobina negra *(Micropterus salmoides*) y en pez conejo (*Siganus canaliculatus*) respectivamente. De tal forma, cabe resaltar que los aceites vegetales más comunes utilizados es el aceite de soya, el cual ofrece precios competitivos, alta disponibilidad en el mercado mundial y es rico en ácido linoleico (LA,18:2n-6) (Ayisi et al., 2019). Así mismo, la dieta DS75, la cual contiene 75% de aceite de soya con 25% de aceite de linaza, presenta resultados semejantes a la dieta DS100 con respecto al rendimiento del crecimiento e índice somático, por lo tanto, esta mezcla de aceites vegetales también favorecería su utilización para la alimentación de juveniles de *A. tropicus.* De acuerdo con lo reportado por Pontes et al. (2019) muestran que el uso de la mezcla de aceites de soya y linaza en la dieta sería una alternativa al aceite de pescado, si el pez posee enzimas elongasas y desaturasas, como es en el caso de Lambari *(Astyanax altiparanae*).

Es importante tener en cuenta que, al reemplazar el aceite de pescado por aceites vegetales en alimentos para acuicultura, el perfil de ácidos grasos en la dieta debe ser el

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aspecto central para considerar porque existe una fuerte correlación entre el perfil de ácidos grasos del pescado y la dieta proporcionada (Pontes et al., 2019). Ante ello, hay que considerar que, a diferencia del aceite de pescado, el aceite de soya es deficiente en ácidos grasos de la serie n-3 y en contraste con la mayoría de los aceites vegetales, el aceite de linaza contiene más del 50% de ácido α-linolénico (18:3n-3), sin embargo, se produce a menor escala y, por lo tanto, más caro en comparación con el aceite de soya y otras fuentes de lípidos vegetales (Ayisi et al., 2019). Del mismo modo, existen estudios realizados con aceite de soya como sustitución al aceite de pescado en la dieta de peces dulce acuícolas, en donde no existió un efecto diferencial en el crecimiento de los peces, como *Tinca tinca* (Ljubojević et al., 2014), *Micropterus salmoides* (Chen et.al., 2019), *Oreochromis niloticus* (Godoy et.al., 2019), *Astyanax altiparanae* (Pontes et.al., 2019), *Rhamdia quelen* (Hilbig et al., 2019).

En el segundo capitulo también se midio la expresión diferencial de elongasas y desaturasas en hígado e intestino de juveniles alimentados con las dietas experimentales del mismo capitlo, donde se corroboro que el tipo de aceite dietario es un modulador de la regulación y expresión de los genes involucrados en la biosinthesis de LC-PUFA, además que se corrobora la capacidad de la especie para biosintetizar EPA y DHA a partir de ALA. Es importante el denotar que para los cuatro genes medidos (*fads*1, *fads*2, *elovl*2 y *elovl*5), mostraron una sobreexpresión con el tratamiento DS75 tanto en higado como intestino.

En el segundo capítulo, el contenido del porcentaje de ácido linoleico (LA, 18: 2n-6) y ácido α-linolénico (ALA, 18: 3n-3) en las carcasses de los peces juveniles de *A. tropicus*, aumentó con el aceite de soya en la dieta DS100, de acuerdo con lo reportado por Emre et al. (2016) y Chen et al. (2019), quienes informaron que el reemplazo del aceite de pescado en la dieta por aceite de soya aumentó los niveles de ácido linoleico y ácido linolénico en los músculos. Así mismo, en los resultados del contenido de ácidos grasos poliinsaturados de cadena larga (LC-PUFA) como ARA (20: 4n-6), EPA (20: 5n-3) y DHA (22: 6n-3) en las carcasses de los peces juveniles de *A. tropicus* disminuyó con el aumento del aceite de soya en la dieta, por lo tanto, los resultados muestran que el reemplazo del aceite de pescado en la dieta por aceite de soya resulta en una disminución del contenido de LC-PUFA en los tejidos de pescado. Resultados similares reportan Emre et al. (2016) y Chen et al. (2019) en el reemplazo de aceite de pescado en la dieta con aceite de soya en corvina *(Argyrosomus regius*) y lobina negra *(Micropterus salmoides*) respectivamente, en donde cambio la calidad y se redujo los niveles de ácidos grasos de ARA, EPA y DHA en diversos tejidos como el músculo e hígado.

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Aunque los aceites vegetales no presentan LC-PUFA, algunas especies de peces tropicales de agua dulce pueden sintetizar ácido araquidónico (ARA; 20: 4n-6) de 18:2n-6, ácido eicosapentaenoico (EPA; 20: 5n- 3) y ácido docosahexaenoico (DHA; 22: 6n-3) de 18:3n-3 (Paulino et al., 2018). De acuerdo con lo reportado por De la Cruz-Alvarado et al. (2021) correspondiente al Capítulo I de la presente tesis, en el pejelagarto (*A. tropicus*) se ha detectado la presencia de elongasas funcionales (elovl2 y elovl5) y desaturasas (fads1 (Δ5) y fads2 (Δ6)) del transcriptoma, por lo tanto, esta especie tiene la capacidad de biosíntesis de LC-PUFAL. Ante ello, en el segundo capítulo de investigación se confirma mediante los resultados del bioensayo la capacidad de biosíntesis de *A. tropicus,* correspondiente a la expresión de los genes involucrados y al porcentaje total de ácidos grasos de las carcasses de los peces juveniles, en donde el nivel de LC-PUFA n-3 fue significativamente más alto en la dieta DS0 (100% de aceite de linaza), así mismo el ácido eicosapentaenoico (EPA, 20: 5n-3) y ácido docosahexaenoico (DHA, 22: 6n-3) con respecto a los otros tratamientos. Estos resultados son consistentes con estudios que indican el aumento significativo en los ácidos grasos LC-PUFA n-3 y el contenido de DHA a medida que aumenta el aceite de linaza en la dieta (Li et al., 2015). Resultados similares son reportados por Omolo et al. (2017) quienes observaron que tilapia del nilo (*Oreochromis niloticus*) alimentada con aceite de linaza tuvo un aumento significativo de DHA a nivel muscular. Por lo tanto, se sugirió que la tilapia es capaz de convertir la dieta con ácido α-linolénico (ALA, 18: 3n-3) a LC-PUFA (Sargent et al., 2002) y por lo tanto pueden alargar y desaturar el ácido α-linolénico (ALA, 18: 3n-3) a DHA tisular.

Es asi que, esta tesis asienta las bases de conocimiento sobre la capacidad de biosíntesis de acidos grasos poliinsaturados en pejelagarto (*Atractosteus tropicus*) y que da apertura a muchas otras investigaciones en la especie, siempre con una visión multidisiplinaria, en proo de la ciencia básica que contribuya al desarrollo de la zootecnia de la especie, asi como su conservación.

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8.- CONCLUSIÓN

Los genes fads1, fads2, elovl2 y elovl5 muestran expresión en varios tejidos de *A. tropicus*, siendo el intestino y el hígado los principales órganos implicados en la biosíntesis de LC-PUFA. Por lo tanto, el aumento de expresión de los genes de fads y elovl durante el desarrollo temprano corresponde al final de la organogénesis del sistema digestivo, así como a la composición de la dieta, donde aparentemente las especies poseen la maquinaria enzimática para biosintetizar C18 a LC-PUFA C20 y C22.

A partir de la mezcla de 75% de aceite de soya con 25% de aceite de linaza (DS75) y el 100% de aceite de soya (DS100) se puede reemplazar con éxito al aceite de pescado en las dietas para los ácidos grasos esenciales adecuados en peces juveniles de *A. tropicus*, sin efectos adversos sobre el rendimiento del crecimiento, obteniendo mayor aprovechamiento de alimento y permitiendo la disminución de costos en la producción.

Los resultados con mayores niveles de EPA y DHA en la dieta con el 100% de aceite de linaza en las carcasses confirman la capacidad de biosíntesis de PUFA-CL en peces juveniles de *A. tropicus.*

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