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FACULTAD DE CIENCIAS MARINAS

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Metabolismo de aminoácidos azufrados y su relevancia como nutrientes limitantes en el uso de proteínas alternativas en la alimentación de peces marinos: Efectos sobre el desempeño productivo, estatus tisular de taurina y metabolismo lipídico.

T E S I S

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS PARA OBTENER EL GRADO DE

DOCTOR EN OCEANOGRAFÍA COSTERA

PRESENTA

OMAR EZEQUIEL AGUILLÓN HERNÁNDEZ

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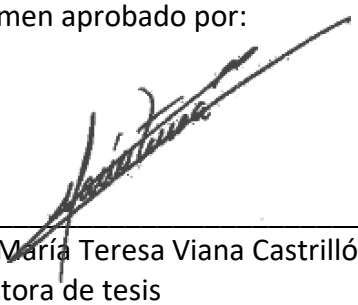

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El presente trabajo tuvo la intención de establecer la importancia de ciertos aminoácidos limitantes en peces, de acuerdo con sus distintos papeles metabólicos en contraste con su papel en la síntesis proteica para explicar el crecimiento. Información importante para balancear las dietas de acuerdo con el perfil de aminoácidos de las distintas fuentes proteicas. El uso de proteínas alternativas a la harina de pescado, en la elaboración de dietas destinadas al cultivo de peces carnívoros es una práctica creciente en la actualidad. Sin embargo, algunos recursos proteicos pueden ser pobremente utilizados por los organismos debido, entre otros factores, a un desbalance en su composición aminoacídica. La disminución de un solo aminoácido por debajo del requerimiento puede disminuir la retención de la proteína e incluso puede reducir el consumo y con ello el crecimiento. Cuando esta situación ocurre se califica a dicho aminoácido como limitante y se busca incrementar su concentración mediante la mezcla de diferentes fuentes proteicas, o bien con la suplementación con aminoácidos sintéticos cristalinos. Es bien sabido que los principales aminoácidos limitantes son la lisina y la metionina, aminoácidos que por lo general se encuentran en baja cantidad, comparado a la harina de pescado, en la gran mayoría de fuentes proteicas alternativas. La lisina, es un aminoácido cuya función principal radica en ser sustrato de la síntesis proteica y estimular o inhibir mediante ciertos mecanismos celulares dicha síntesis. Sin embargo, se ha sabe que los productos del catabolismo de la lisina

pueden estar involucrados en diversas aplicaciones, como en la función cerebral además de la generación de carnitina. Por otro lado, la metionina genera diversas moléculas funcionales (S-adenosil metionina, cisteína y taurina) cuyo papel metabólico podría limitar su participación como sustrato de la síntesis proteica. De las moléculas funcionales que pueden generarse a partir de metionina, en los organismos animales, es la taurina. Constituye un derivado aminoacídico que presenta diversas funciones metabólicas, que, en su ausencia, puede incluso afectar el desempeño productivo en organismos acuáticos. Por tal motivo, se considera como un nutriente esencial para diversas especies cultivadas, aún si éste no conforma la parte estructural de la proteína. Ya que su presencia podría afectar las funciones mismas de la metionina, como lo es el metabolismo lipídico y crecimiento, el uso de fuentes proteicas con baja cantidad de aminoácidos azufrados y sin taurina, como los de origen vegetal, realza la importancia de entender la capacidad de síntesis del derivado respecto del precursor para formular adecuadamente una dieta. Por tal motivo en el **Capítulo 1** el objetivo fue demostrar el nivel de esencialidad entre la taurina y la metionina en un pez carnívoro como la *Totoaba macdonaldi*. Para obtener más información sobre el papel metabólico de ambos nutrientes, como variables de respuesta se eligieron además del crecimiento somático, la concentración corporal de taurina, el nivel de grasa y de colesterol, así como la expresión de genes relacionados con su síntesis y captación (*csad* y *taut)* en los órganos encargados de controlar su homeostasis corporal. Es así como mediante un modelo factorial 2X2 (concentración de metionina y taurina 1%) se observó que el crecimiento parece estar relacionado con el mantenimiento de la concentración tisular de taurina. Además, se observó que su efecto sobre el crecimiento es independiente del nivel de metionina y que junto con ésta puede generar un efecto significativo sobre la expresión genética de la IGF-1 asociada al crecimiento somático, existiendo interacción entre nutrientes. Por otro lado, ambos nutrientes mostraron un papel metabólico distinto sobre el colesterol y sobre ácidos grasos mono y poliinsaturados ya que, bajo la presencia de taurina, disminuyó significativamente el colesterol hepático y corporal en la *T. macdonaldi,* mientras que hubo un incremento en la ganancia de grasa cruda total. Por otro lado, bajo la presencia de metionina se incrementó la concentración de colesterol hepático e incrementó la proporción de ácidos grasos monoinsaturados n9 y n7, sin afectar la concentración lipídica total. Los cambios generados sobre el contenido de colesterol y depositación de ácidos grasos generó diferentes preguntas sobre la importancia metabólica de la metionina y si esta influía en su retención en el organismo. Por lo que en el **Capítulo 2** se evaluó la prioridad metabólica de la metionina a diferentes niveles (0.7, 0.9, 1.2, y 1.7%) en una dieta restringida tanto en colesterol como de lípidos en juveniles de jurel (*Seriola dorsalis*). Como variables de respuesta, se analizó, aparte del crecimiento, la expresión de genes relacionados con el metabolismo de la metionina (*mat* y *bhmt*), lipogénesis (*srbp-1* y *hmg-coa*), síntesis proteica (*mtor*) y crecimiento (*igf-1*). Los resultados mostraron que el crecimiento somático, el contenido proteico y de colesterol no se relacionaron con la enzima que re sintetiza la metionina BHMT (betaina homocisteina metil transferasa), pero sí con la enzima

encargada del catabolismo de la metionina adenosiltransferasa (MAT). Por tanto, el incremento en estos parámetros no requeriría de una mayor retención tisular de metionina. Sin embargo, los cambios en la depositación de ácidos grasos mostraron una relación más compleja la cual requerirá seguir siendo investigada. Por su cuenta, la metionina como aminoácido limitante tiene una gran relevancia a nivel metabólico ya que regula diversos procesos necesarios para el crecimiento somático, así como la composición química del organismo. Mientras que la taurina como derivado no es un factor que genere, una demanda extra de metionina, aparentemente, otros derivados metabólicos como la fosfatidil colina y la metilación del DNA, podrían constituir un factor en la demanda de metionina debido a la probable disminución de su retención.

Palabras clave: Metabolismo lipídico, peces carnívoros, metionina, *Seriola*, *Totoaba macdonaldi*.

ABSTRACT of the thesis presented by **Omar Ezequiel Aguillón Hernández** as a partial requirement to obtain the DOCTOR OF SCIENCE IN COASTAL OCEANOGRAPHY. Ensenada, Baja California, México. January, 2021.

Sulfur amino acid metabolism and its relevance as limiting nutrients in the use of alternative proteins in marine fish feeding: Effects on productive performance, taurine tissue status and lipid metabolism.

The present work intends to establish the importance of certain limiting amino acids in fish, according to their different metabolic roles in contrast to their protein synthesis role to explain growth. Information that is crucial to formulate diets according to the amino acid profile of the different protein sources. Using alternative proteins to fishmeal for feeds to culture carnivorous fish is a growing practice today. However, some protein resources can be poorly used due to an imbalance in their amino acid composition, among other factors. Decreasing a single amino acid below the requirement can decrease protein retention and reduce consumption and, thus, growth. When this situation occurs, that particular amino acid is classified as limiting, and it is inquired to increase its concentration by mixing different protein sources or supplementing with crystalline synthetic amino acids. It is well known that the primary limiting amino acids are lysine and methionine, amino acids that are generally found in low amounts, compared to fishmeal, in the vast majority of alternative protein sources. Lysine is an amino acid whose primary function is to be a substrate for protein synthesis and stimulate or inhibit protein synthesis through specific cellular mechanisms. It is known that lysine and its catabolism derivatives might be involved in various applications, such as brain function, in addition to carnitine synthesis. On the other hand, methionine generates various functional molecules (S-adenosyl methionine, cysteine, and taurine) whose metabolic role could limit its function as a protein synthesis substrate. Taurine is one of the functional molecules generated from methionine in animal organisms. It is an amino acid derivative that has various metabolic functions, which, in its absence, can even affect the productive performance of aquatic organisms. Thus, it is considered an essential nutrient for various aquatic species, even if it does not form the structural part of the protein. Since its presence could affect the very functions of methionine, such as lipid metabolism and growth, the use of protein sources with low amounts of sulfur amino acids and without taurine, such as those of plant origin, highlights the importance of understanding the capacity synthesis of the derivative concerning the precursor to formulating a diet properly. For this reason, in **Chapter 1**, the objective was to demonstrate the level of essentiality between

taurine and methionine in a carnivorous fish such as *Totoaba macdonaldi*. To obtain more information on the metabolic role of both nutrients, as response variables, in addition to somatic growth, the body concentration of taurine, the level of fat and cholesterol, as well as the expression of genes related to their synthesis and uptake were chosen (*csad* and *taut*) in the organs responsible for controlling their body homeostasis. Thus, using a 2X2 factorial model (1% methionine and taurine concentration), it was observed that growth seems to be related to the maintenance of the tissue concentration of taurine. Also, it was observed that its effect on growth is independent of the level of methionine, and both resulted in a significantly higher effect on the genetic expression of IGF-1, associated with somatic growth, with a significant interaction between nutrients. On the other hand, both nutrients showed a different metabolic role in cholesterol and mono and polyunsaturated fatty acids. Under the presence of taurine, liver and body cholesterol significantly decreased in *T. macdonaldi*, while there was an increase in the total crude fat gain. On the other hand, under e presence of methionine, the hepatic cholesterol concentration increased, and the proportion of monounsaturated fatty acids n9 and n7 increased without affecting the total lipid concentration. The changes generated on the cholesterol content and fatty acid deposition raised different questions about methionine's metabolic importance and if it influenced its retention in the body. Therefore, in **Chapter 2**, methionine's metabolic priority was evaluated at different levels (0.7, 0.9, 1.2, and 1.7%) in a diet restricted both in cholesterol and lipids in juvenile horse mackerel (*Seriola dorsalis*). As response variables, apart from growth, the expression of genes related to methionine metabolism (*mat* and *bhmt*), lipogenesis (*srbp-1* and *hmg-coa*), protein synthesis (*mtor*), and growth (*igf-1*). The results showed that somatic growth, protein, and cholesterol content were not related to the enzyme that re-synthesizes methionine BHMT (betaine homocysteine methyl transferase), but they were related to the enzyme responsible for the catabolism of methionine adenosyl transferase (MAT). Therefore, the increase in these parameters would not require greater tissue retention of methionine. However, the fatty acid deposition changes showed a more complex relationship, which will require further investigation. On its own, methionine as a limiting amino acid has great relevance at the metabolic level since it regulates various processes necessary for somatic growth and the chemical composition of the body. While taurine as a derivative is not a factor that generates an extra demand for methionine, other metabolic derivatives such as phosphatidylcholine and DNA methylation could constitute a factor in the demand for methionine, causing its decreasing in retention.

Keywords: Lipid metabolism, carnivore fish, methionine, *Seriola*, *Totoaba macdonaldi*.

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A mis seres queridos, familiares, amigos y personas que me han apoyado para mantenerme en una travesía donde la perseverancia es una virtud imprescindible.

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ÍNDICE

ÍNDICE DE CUADROS Y FIGURAS

I. INTRODUCCIÓN GENERAL

La demanda de productos acuícolas para la alimentación humana continúa incrementándose, y con ello, la demanda por alimentos destinados a esta actividad y sus principales ingredientes. Dentro de los insumos utilizados en la generación de dichos alimentos, los proteicos son los más costosos y representan a menudo más del 50% de la dieta (Miles and Chapman, 2014). Por tal motivo, el objetivo en la alimentación de peces no sólo es el reducir el consumo de harina de pescado, sino también buscar fuentes proteicas alternativas. Que además de reducir el contenido proteico total, se asegure un máximo crecimiento y la retención de nitrógeno se vuelve algo esencial. El concepto desarrollado por Fuller et al. (1989) sobre la proteína ideal, se presenta como un punto de partida para suministrar el contenido justo de proteína y evitar su desperdicio. La proteína ideal considera la presencia de aminoácidos indispensables y dispensables en valores igualmente limitantes para los requerimientos de una especie dada (Gómez-Requeni et al., 2004; Yamamoto et al., 2005; Gaylord and Barrows 2009; Milgen and Dourmad, 2015). Definiendo como aminoácido limitante a aquel que, si se encuentra debajo del requerimiento mínimo de la especie, limitará el crecimiento. En este sentido las proteínas pueden presentar más de un aminoácido limitante y su importancia comúnmente se relaciona con el impacto que generan sobre el crecimiento, presentándose como primer y segundo limitantes o como co-limitantes (Fig 1). Aunque es universalmente aceptado el concepto de aminoácido limitante, la variable de respuesta asociada al crecimiento y su interacción con los aminoácidos ha recibido un nuevo enfoque, al reconocer que dichos compuestos pueden afectar la tasa de síntesis proteica y división celular mediante la regulación de mecanismos endócrinos y celulares que magnifican el impacto de estos nutrientes sobre el crecimiento más allá de su función simple como substrato

para la elaboración de proteínas (Wu et al., 2014). Debido a esto, el requerimiento de aminoácidos en diversas especies se ha replanteado tomando en cuenta otras variables de respuesta como su impacto sobre el eje somatotrópico, la tasa de síntesis y degradación proteica, el metabolismo energético y lipídico. De tal forma que, en su conjunto, el requerimiento de aminoácidos no esté basado en la composición aminoacídica corporal (Cuadro 1) (Lebret et al., 2018, Berri et al., 2020) sino a una estimación con base a la retención de aminoácidos más aquellos que son utilizados en el metabolismo intermediario, envueltos en diversas funciones esenciales. La idea anterior nos lleva a nuestro primer tópico, que es la determinación del requerimiento de aminoácidos en animales.

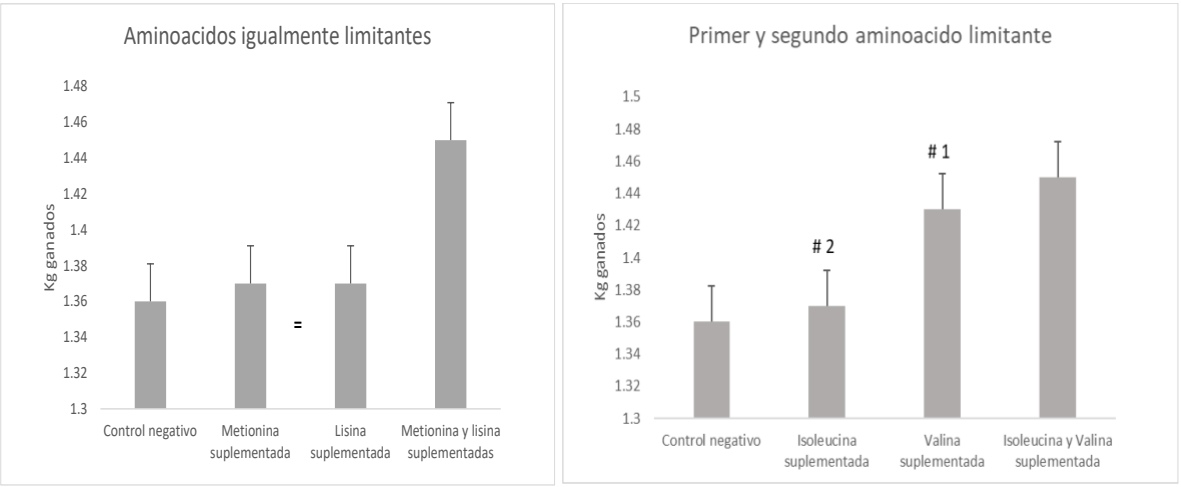
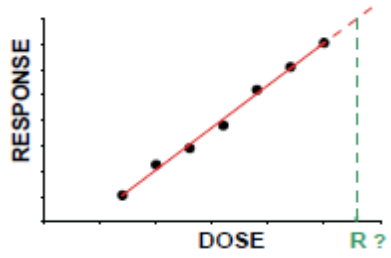
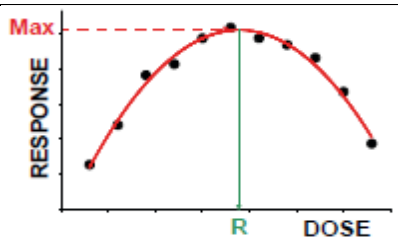
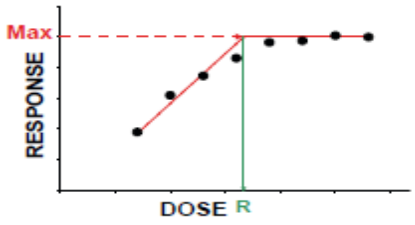
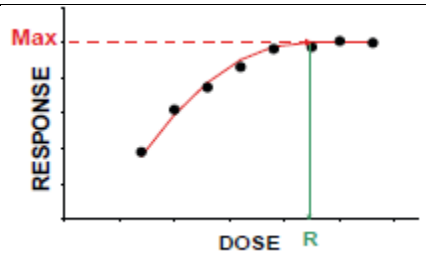


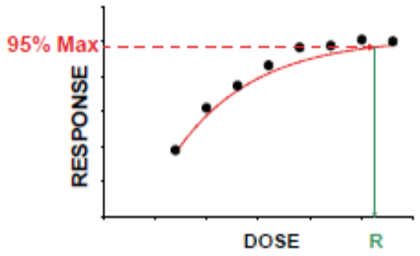
Figura 1. Tipos de interacción entre aminoácidos limitantes y crecimiento (Shimada, 2005)

1. **Determinación del requerimiento de aminoácidos en animales**

Por lo general, el requerimiento total de aminoácidos determina la cantidad de proteína para una especie. Y para tal fin, durante décadas los ensayos dosis respuesta han servido para su estimación. Ensayos que consisten en pruebas de alimentación basándose en el crecimiento obtenido utilizando diferentes niveles del o de cada uno de los aminoácidos (Ajinomoto, 2012). Estos métodos, utilizan a menudo modelos no lineales que mediante un análisis de regresión permiten estimar la concentración mínima para obtener el máximo crecimiento (Cuadro 1).

Cuadro 1. Respuestas “tipo” observadas en los ensayos del requerimiento de aminoácidos y métodos estadísticos de análisis.

Forma de la respuesta	**Modelo matemático**	**Representación gráfica**
Lineales		
Incremento lineal	función linear Y=aX+b Requerimiento= Indeterminado	
Incrementa y después disminuye al alcanzar su máxima respuesta	Función cuadrática $Y=aX^2+bX+c$ Requerimiento=-b/2a	
No lineales		
Incrementa y se estabiliza después de la Máxima respuesta	Broken-line Y=Y máxima = +U.(R-X) Requerimiento= Ymax	
	Plateu curvilíneo Y=Y máxima = +U.(R-X) Requerimiento= Ymax	

	Asintótica Y=Y máxima= -a.exp(-bX)	
Y= Respuesta (normalmente crecimiento), X= Dosis abc y u son parámetros de las fórmulas		

Aparte del crecimiento, estos métodos pueden también estimar las concentraciones plasmáticas del aminoácido a ensayar, la producción de amonio y el contenido proteico, y así asociar el crecimiento a su aprovechamiento (Madrid et al., 2019; Tulli et al., 2010; Tibaldi and Tulli, 1999). Lo anterior nos introduce al balance de nitrógeno, lo que nos da la diferencia entre el nitrógeno consumido y el excretado (McDonald et al., 2010) (Fig. 2), que sirve como referente de un exceso o deficiencia de algún aminoácido. Por otro lado, cuando existe restricción de algún aminoácido, aparte de menor crecimiento, la deficiencia dará como resultado el uso excesivo de los nutrientes como fuente energética aumentando la producción de amonio (Cowey, 1972). Así que, mediante el balance de nitrógeno se puede tener una estimación con mayor precisión, que nos permita diferenciar entre la suficiencia y excesos de los aminoácidos dietarios, para establecer sus requerimientos con mayor exactitud (Bae et al., 2011). La meta en el requerimiento de aminoácidos es obtener la máxima retención de nitrógeno y proteína (Fig. 2), el cual se relaciona positivamente con la similitud entre el perfil de aminoácidos y los requerimientos de la especie (Moore and Soeters, 2015). Por otro lado, se sabe que existe una asociación entre los requerimientos de aminoácidos y el perfil de aminoácidos del organismo, observando que, aunque los requerimientos cambian cuantitativamente entre diferentes etapas de desarrollo en

las especies animales, las proporciones entre ellos se guarda (Richard and Frank, 2011). Con base en lo anterior expuesto, y a través de un ensayo de dosis respuesta, se utiliza a la lisina como el 100%, calculando el requerimiento del resto, guardando su proporcionalidad con respecto al aminoácido (Fuller et al., 1989) (Cuadro 2).

Cuadro 2. Comparativo entre la proporción corporal de aminoácidos indispensables en el bagre de canal (*Ichtialurus punctatus*) y su requerimiento según la NRC.

Aminoácidos	Perfil de aminoácidos esenciales (Expresados como porcentaje de la lisina)	% Aminoácido en alimento (3000 kcal ED/kg de alimento) *
Lisina	100	1.43
Fenilalanina + Tirosina	98	1.4
Arginina	84	1.2
Leucina	68	0.98
Valina	59	0.84
Isoleucina	51	0.73
Metionina + Cisteína	45	0.64
Treonina	39	0.56
Histidina	29	0.42
Triptófano	10	0.14

*Requerimientos para el bagre de canal *Ichtialurus punctatus* según la NRC (2011)
Para realizar el comparativo, se consideró el requerimiento de la lisina (1.43) como punto de partida para calcular el requerimiento del resto.

La retención de la mayor parte de los aminoácidos dietarios para constituir proteínas depende de la demanda energética en las especies, aspecto a considerar para lograr tal fin (Cowey, 1972). En la demanda total de energía de una especie se debe tomar en cuenta al menos tres diferentes procesos biológicos a los que se destina la dieta (Figura 2). El primero es el mantenimiento metabólico (metabolismo basal), el cual se puede calcular mediante el gasto de oxígeno y producción de CO_2 del organismo en reposo. En segundo lugar, la destinada a crecimiento; la cual se puede determinar por el contenido energético total de producto que se plantea obtener (en el caso de piscicultura, el pez entero en g o kg). Por último, el incremento calórico alimenticio el

cual es la energía invertida por el organismo para liberar los nutrientes presentes en la dieta. Esta última relacionada con la digestibilidad de los componentes dietarios. Por lo anterior cabe destacar que al considerar la energía en la determinación del requerimiento de aminoácidos se debe discernir entre la que es digestible y no, con su contenido en bruto. Dicho esto, la digestibilidad de los aminoácidos es determinante para aumentar la precisión en el requerimiento de aminoácidos. Debido a lo anterior, es que se han generado cambios en la determinación de requerimientos de aminoácidos en el campo de la nutrición animal, surgiendo conceptos como son la digestibilidad ileal de la proteína. Llamada así por considerar la digestibilidad de los aminoácidos en el íleo (segmento intestinal de los mamíferos en la zona post absorción), por donde en mamíferos es el ingreso de los aminoácidos dietarios, obteniendo los requerimientos como gramos de aminoácidos digestibles (Stein et al., 2007). En un inicio se consideró el requerimiento de aminoácidos no sintetizables en las especies animales (Wu et al., 2014), asumiendo la síntesis del resto de aminoácidos de forma eficiente. Sin embargo, la falta de evidencia sobre la síntesis de algunos aminoácidos ha generado que se considere también la fracción de aminoácidos sintetizables en los requerimientos de la especie. Ya sea como fuente de nitrógeno para la síntesis del resto, o como requerimientos específicos asociados a sus funciones (Wu, 2009). Este aspecto ha sido de importancia para replantear la idea de proteína ideal, agregando en el concepto la relación de aminoácidos indispensables y dispensables (esenciales/no esenciales).

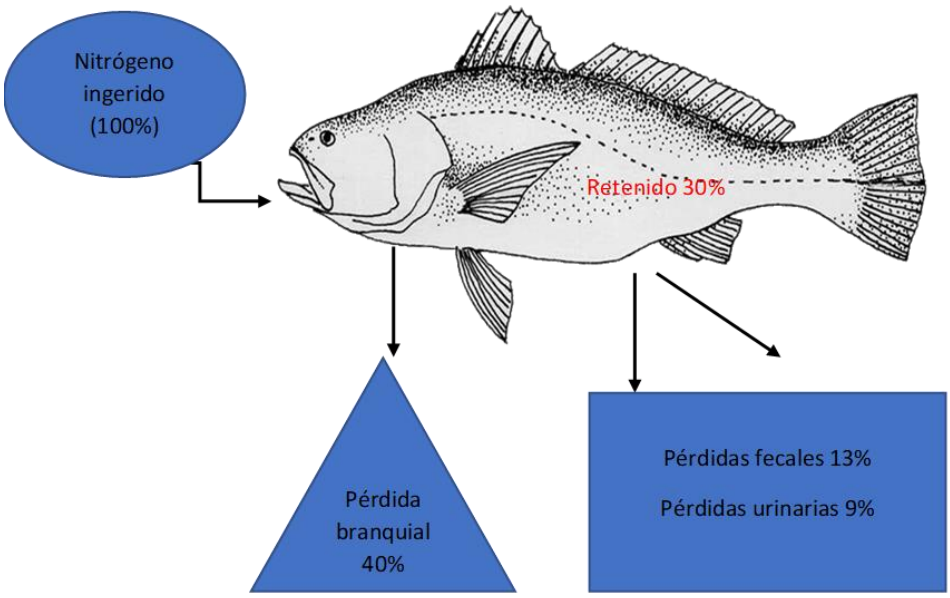


Figura 2. Balance de nitrógeno en peces (Luquet, 1982).

Si bien los conceptos aquí resumidos son válidos para la determinación del requerimiento de aminoácidos en la mayoría de las especies animales. En los organismos acuáticos, y en especial los peces carnívoros, presentan diferencias sustanciales en su metabolismo lo que dificulta el establecimiento del requerimiento de aminoácidos. Lo que se tratará en el siguiente punto.

2. Propiedades específicas del metabolismo proteico y energético en peces carnívoros

Ya que los peces son organismos poiquilotermos, su costo metabólico de mantenimiento es bajo. Al igual que el resto de los organismos, el crecimiento responde al perfil de los aminoácidos y al contenido de proteína en la dieta (Cowey, 1972), pero no de la manera tradicional en que se

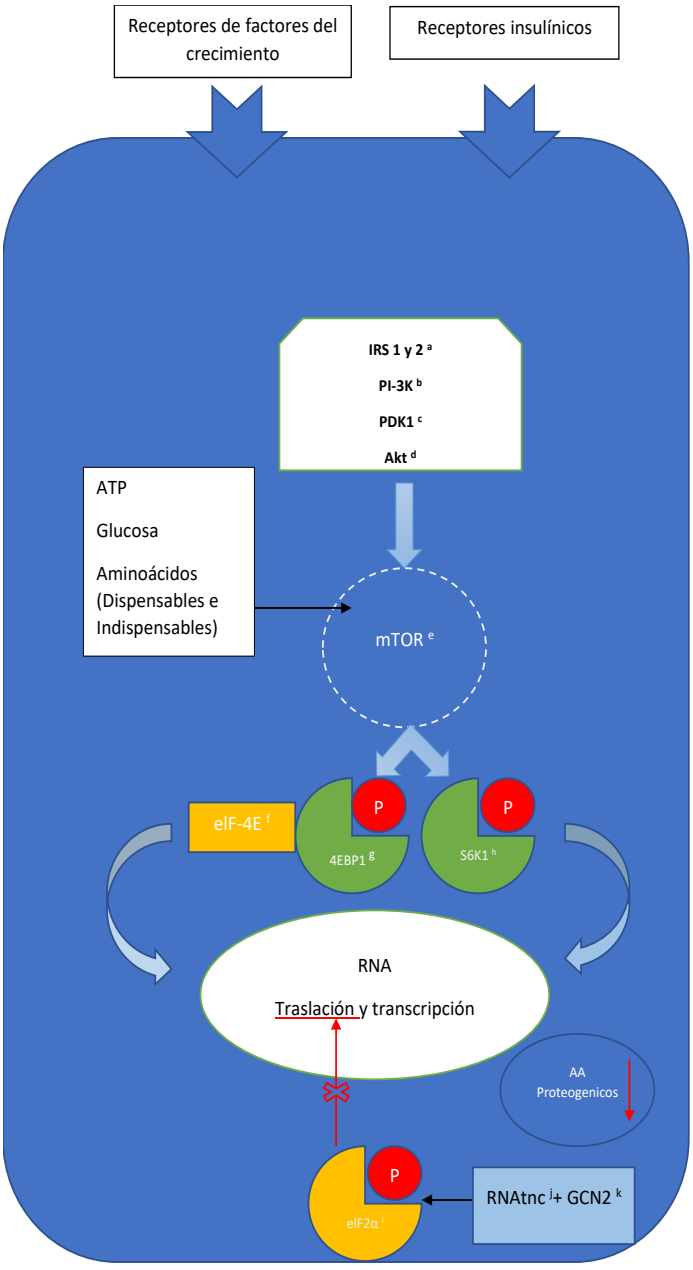
observa en otras especies animales. Mientras que en los cerdos o aves gran parte de la proteína en exceso se degrada a compuestos orgánicos más inocuos (como la urea o ácido úrico) (McDonald et al., 2010), en los peces carnívoros los aminoácidos son utilizados como fuente energética produciendo amonio como resultado. El crecimiento obtenido tarda más en alcanzar la meseta en la curva de crecimiento (Sankian et al., 2017; Amoah, 2011). Esto indica que el pez no tiene limitantes para utilizar los aminoácidos como fuente energética, lo cual se explica por su capacidad de eliminar amonio directamente al medio, disminuyendo considerablemente el costo en la formación de urea. Sin embargo, no explica el porqué la eficiencia proteica se mantiene aún con un elevado contenido proteico (Cowey, 1972; Kim et al., 2016; Rueda-López et al., 2011). Para que exista depositación proteica, deben de existir sustratos para la generación de proteínas y suficiente energía para sostener este proceso, siendo necesario contar con suficiente ATPs y aminoácidos (Buttery and Lindsay, 1980). Además de contar con un control endócrino altamente regulado por diversos factores, entre los cuales se encuentran los nutrientes (Fig 3). Aquí vale la pena hacer un paréntesis, ya que existe una enorme diferencia en la regulación del metabolismo energético entre peces carnívoros y animales de producción terrestres. Diferencia dada, no por su condición poiquiloterma, sino por su carácter carnívoro estricto. De acuerdo con Figueiredo-silva et al. (2012), pareciese que los peces son intolerantes a la glucosa, ya que a nivel celular a pesar de estar regidos por el binomio insulina-glucagón, presentan una respuesta muy diferente a los nutrientes a la observada en mamíferos.

En el gato, un modelo carnívoro por excelencia, la glucocinasa (enzima que fosforila a la glucosa para realizar el proceso de glucólisis y sensor a nivel pancreático, y estimula la liberación de insulina), se expresa pobremente además de tener una actividad baja. También, la actividad

gluconeogénica y de degradación de aminoácidos puede permanecer en el hígado, aun después de alimentarse (Verbrugghe and Hesta, 2017). En los peces carnívoros, se observan condiciones similares en donde se mantiene la gluconeogénesis y la degradación de proteína aun después de alimentados, pero de forma independiente del contenido de carbohidratos en la dieta (Panserat et al., 2013) a diferencia de los homeotermos. Diferencia crucial, ya que da lugar a la fabricación de glucosa endógena, como si la glucosa de la dieta no fuera detectada. Sin embargo, dicha gluconeogénesis puede responder al estímulo insulínico como sucede en mamíferos (Dai et al., 2015). De acuerdo con Andoh (2007), algunos aminoácidos y no la glucosa pueden ser secretagogos de mayor importancia que la insulina, generando un requerimiento de dichos aminoácidos asociados a esa función. Dicha funcionalidad de ciertos aminoácidos se ha evidenciado en experimentos donde modificaciones en el perfil de aminoácidos resultan en cambios significativos en el metabolismo intermediario (Figuereido-Silva et al., 2010; Caballero-Solares et al., 2015; Dias et al., 2005; Gomez-Requeni et al., 2003). Este hecho, corrobora la necesidad de investigar el requerimiento de aminoácidos como reguladores del estatus energético. Lo anterior, nos permitirá identificar cuáles aminoácidos y en qué cantidad participan en la regulación energética para lograr un máximo aprovechamiento proteico.

Además del efecto insulínico ya mencionado, existen algunos aminoácidos funcionales que influyen sobre órganos en la regulación hormonal del crecimiento, como es el caso del hígado, el cual genera la IGF-1 (Factor de crecimiento similar a la insulina 1). En este sentido tanto la presencia de aminoácidos como el estado energético puede condicionar la producción de dicha hormona a través de los receptores de la GH (Dauncey and Bates, 1994) (Fig 3). Este posible traslape de la regulación del estatus energético y el estímulo de la síntesis proteica es lo que

permitiría explicar el paradigma del crecimiento en peces carnívoros, en el cual como se mencionó anteriormente, a mayor proteína dietaría mayor es el crecimiento.



[a]IRS= Sustrato del receptor insulínico 1 y 2
[b]PI-3K= Fosfoiocitidina 3 cinasa
[c]PDK1=Cinasa dependeinte de fosfoiocitidina
[d]Akt=Alfa serina/treonina proteína cinasa
[e]mTOR=Receptor mamífero de la rampamicina
[f]elF-4E=Factor de iniciación de la traslación eucariótica número 4
[g]4EBP1=Proteína de unión al factor de iniciación de la translación eucariótico 4
[h]S6K1= S6 ribosomal cinasa protéica betta-1
[i]elF-2E=Factor de la traslación eucariótica número 2
[j]RNAtnc=RNA de transferencia no cargados
[k]GCN2=Control general no suprimible número 2
P=Fosforilación

Figura 3. Esquema de los mecanismos celulares de control de crecimiento y síntesis proteica mediado por nutrientes

3. Regulación del eje somatotrópico por aminoácidos

El eje somatotrópico es un regulador del crecimiento importante. Estimula el desarrollo muscular, síntesis de proteínas e inhibe la degradación de proteínas (Fuentes et al., 2013). Los componentes de dicho eje incluyen la hormona de crecimiento (GH), el receptor de la hormona del crecimiento (GHR), los factores de crecimiento similares a la insulina (IGF-I e IGF-II), receptores de IGF y proteínas de unión a la IGF. En el hígado de la trucha arco iris la GHR está regulada por la temperatura y el estado nutricional (Gabillard et al., 2006). Estos receptores promueven a nivel celular la síntesis de IGF y el sitio principal de su síntesis es el hígado (Reinecke, 2006). Las hormonas polipeptídicas IGF (I y II), son estructuralmente similares a la insulina y, en cierta medida, ejercen efectos similares a la insulina. Sin embargo, sus funciones fisiológicas no son del todo similares, así como sus patrones de expresión en función del tejido. Las IGFs tienen efectos autocrinos, paracrinos y endocrinos, siendo estos últimos modulados por la proteína de unión, IGFBPs. De hecho, los IGF circulantes están principalmente vinculados a las IGFBP, y menos del 1% del plasma IGF-I está presente en forma libre (Reinecke, 2006).

La regulación del eje somatotrópico se basa, en parte, en interacciones complejas entre sus componentes que pueden ejercer una retroalimentación positiva o negativa entre sí. Por ejemplo, dependiendo de la dosis de GH en circulación, la expresión hepática y el nivel plasmático de IGF-I mejoran (Funkenstein et al., 1989; Niu et al., 1993; Duan et al., 1993), lo que muestra una correlación con las tasas de crecimiento en varias especies de peces (Reinecke and Collet, 1998). La GHR podría participar en la integración de los cambios de los niveles plasmáticos de GH, así como en la regulación de la IGF-I (Saera-Vila et al., 2007). A la inversa, cuando la IGF-I está en circulación, se asegura un control de retroalimentación negativo sobre la secreción de GH

(Pérez-Sánchez et al., 1992). El rendimiento productivo como ganancia en peso de los peces (acreción muscular), a menudo se correlaciona con las concentraciones plasmáticas de IGF-I (Reinecke and Collet, 1998; Cleveland and Burr, 2011), probablemente derivadas del hígado (Snyder et al., 2012).

Tanto en peces como en mamíferos, el eje GH-IGF, aparte del rendimiento productivo, se regula también por el estado nutricional (Renaville et al., 2002; Chauvigné et al., 2003), siendo afectado por la ración alimenticia (Pérez-Sánchez et al., 1995; Metón et al., 2000), composición de la dieta (Metón et al., 2000) y materias primas (Gómez-Requeni et al., 2005).

Con relación a los aminoácidos, existe evidencia de su efecto sobre la expresión hepática de *igf* en peces (Rolland et al., 2015; Espe et al., 2015; Zhou et al., 2019). Es así que mientras la arginina puede incrementar la secreción de GH (Pohlenz et al., 2013), la metionina regula la expresión de la GHR (Rolland et al., 2015). Mientras que de acuerdo con Zhao et al. (2020) la leucina presenta un mecanismo independiente de la GH en la regulación de la producción de IGF-1 en teleósteos.

4. Regulación de la síntesis proteica por aminoácidos

El proceso continuo de síntesis y degradación de proteínas en las células se denomina recambio proteico (Wu, 2009). A diferencia de otros nutrientes (lípidos y glucosa), no existe un almacenamiento interno de aminoácidos *per se*, ya que la función principal de la proteína es la síntesis de tejido nuevo (Brosnan, 2003). Metabólicamente hablando (energía), las proteínas son las moléculas más costosas (energéticamente) para sintetizar (Smith et al., 1999). De tal manera que para un pez en crecimiento se estima que el costo oscila entre el 11 y el 24% del gasto de

energía en el metabolismo basal, y hasta en un 42% del incremento calórico alimenticio (Wood, 2001). Además, como las proteínas son componentes esenciales en el cuerpo, requieren un proceso de mantenimiento continuo y costoso (de constante síntesis y degradación, es decir, recambio proteico) (Rolland et al., 2015).

La capacidad de los aminoácidos para regular la síntesis de proteínas es mediante vías moleculares (Kimball and Jefferson, 2006). De tal forma que si falta un aminoácido la síntesis de proteínas no se lleva a cabo, tanto por no completar la secuencia, como también por presentar una regulación negativa del proceso (Fig 3). La existencia de controles moleculares que impiden el inicio de la síntesis de proteínas parece lógica, especialmente cuando se considera el costo energético del proceso. Los aminoácidos, además de modular la síntesis de proteínas a través de la ruta del receptor de la rampamicina TOR (Figura 3), pueden afectar las vías de degradación de proteínas a nivel transcripcional (Rolland et al., 2015). También se sabe que la degradación de proteínas es la vía que influye predominante a la depositación de las mismas, tanto en los humanos (Fereday et al., 1998), como en los peces (Cleveland and Burr, 2011).

El músculo blanco es el reservorio más grande de proteínas corporales, el cual en las truchas representa el 50% (Ballantyne, 2001). La tasa de síntesis de proteínas en el músculo es mucho más baja que en el hígado, lo que refleja las diferentes funciones de estos órganos, siendo este último, el órgano metabólico más importante (Carter and Houlihan, 2001). El efecto de la acumulación de proteínas en el músculo está dado por una mayor síntesis que degradación, regulado por el estado energético del animal. Es así como en una trucha arcoíris en ayunas, la tasas de síntesis de proteínas disminuye y la proteólisis se ve aumentada por la actividad de las proteasas (Salem et al., 2007). Por el contrario, durante la alimentación, la síntesis proteica en el

hígado se incrementa aún después de dos horas de haber ingerido el alimento (McMillan and Houlihan, 1989). Los factores nutricionales, como la ración y componentes alimenticios (específicamente los aminoácidos), afectan también la síntesis de proteínas a través de la regulación diferencial de las vías de degradación en peces (Cleveland and Burr, 2011; Snyder et al., 2012). Lo anterior se suma a la evidencia creciente de que los aminoácidos son capaces de modular el crecimiento, no solo por ser precursores de la síntesis de proteínas, sino también porque ser capaces de regular las funciones principales a través de vías sensibles a los nutrientes. De tal manera que los mecanismos que controlan el crecimiento y el metabolismo energético en peces son temas que introducen a un nuevo concepto en cuanto al su requerimiento, ya que éste se basa en sus funciones como moléculas reguladoras de la homeostasis.

5. Aminoácidos funcionales

Si bien la ingesta de proteína da como resultado el ensamblaje de proteínas y péptidos, el estudio de su función como reguladores del metabolismo es un punto importante. En este sentido tanto aminoácidos esenciales como no esenciales están involucrados en procesos metabólicos como:

1) Recambio proteico intracelular

2) Síntesis y degradación de otros aminoácidos en tejidos y células

3) Generación de pequeños péptidos, compuestos nitrogenados y azufrados

4) Metabolismo lipídico y glicémico

5) Metilación y síntesis de DNA

6) Señalización redox celular

Todos estos procesos mencionados por Wu (2009), afectan el desempeño productivo del organismo sin involucrar directamente la formación de proteínas tisulares, lo cual modifica el requerimiento de los aminoácidos más allá del requerido para la síntesis de tejido nuevo.

II. ANTECEDENTES

Con base en los conceptos recientemente mencionados, la metionina al constituir un aminoácido que genera diversos compuestos de importancia metabólica es uno de los aminoácidos más susceptibles a cambiar su requerimiento dependiendo de la presencia de otros; de las variables de respuesta estudiadas; así como de las fuentes proteicas utilizadas (digestibilidad de aminoácidos). Sin embargo, en la tilapia (*Oreocromis sp*.), el requerimiento de metionina se acerca mucho al calculado mediante el perfil de aminoácidos corporales (Jauncey et al., 1983; Santiago, 1985). Mientras que, en el bagre su requerimiento discrepa del calculado (Cai and Burtle, 1996), sobre todo cuando se utilizan proteínas vegetales. Diferencia que en opinión de Cai y Burtle, (1996) pudo deberse a la baja digestibilidad de las proteínas vegetales. En especies carnívoras como el salmón del Atlántico (*Salmo salar,*) el requerimiento de aminoácidos azufrados mediante el cálculo del perfil de aminoácidos corporales también se subestima (de Almeida et al., 2014), diferencia que es aún más importante cuando se incluye a la taurina como esencial a pesar de ser un derivado del metabolismo de los aminoácidos azufrados que no forma parte de la estructura proteica (Espe et al., 2008). Se estima que el requerimiento elevado de la metionina en peces carnívoros se asocia a una alta actividad de enzimas relacionadas a su catabolismo (de Almeida et al., 2014). Es importante señalar las peculiaridades del metabolismo de la metionina, así como las interacciones que tiene con algunos nutrientes específicos. Su degradación en la primera etapa es dependiente de moléculas donadoras de grupo metilo como son la betaina, colina y tetrahidrofolato, lo cual ejerce efectos sobre los procesos relacionados al metabolismo de los grupos metilo (Oda, 2006). En su segunda etapa, que es la transulfuración, las enzimas involucradas están reguladas por las concentraciones de sus propios productos

metabólicos y vitaminas del complejo B como la riboflavina (B6) (Espe et al., 2020). Además, para la síntesis de cisteína se requiere una molécula de serina por cada molécula a sintetizar, para convertirlo en un aminoácido indispensable. Sin embargo, en dicho proceso es consumido, por lo que se observa un incremento en las funciones asociadas a la cisteína cuando este aminoácido es suplementado (Zhou et al., 2011). Por tanto, la metionina es uno de los aminoácidos que más interacciones tiene con diversos nutrientes (Fig. 4), por lo que su requerimiento debe ajustarse a un mayor número de variables de respuesta, además del crecimiento (retención). Por otra parte, la taurina merece una mención especial ya que su importancia en peces carnívoros ha quedado demostrada en diversos trabajos (Gaylord et al., 2007; López et al., 2015; Kim et al., 2017). Al igual que la metionina y sus productos metabólicos, la taurina es una molécula con múltiples funciones que a su vez interactúa con nutrientes como el colesterol, donde la taurina parece ser la principal molécula con la cual se conjuga con el colesterol para eliminarse vía biliar en peces marinos carnívoros (Gu et al., 2017). Por otro lado, el colesterol y la taurina puede influir en la actividad de enzimas lipolíticas y lipogénicas (Hayes and Sturman, 1981) impactando la composición química de los organismos. Además de su papel antioxidante que ha sido reportado en diversas especies de peces (Aguillón-Hernández et al., 2017; Bañuelos-Vargas et al., 2013). Sin embargo, la relevancia de dicho compuesto radica en su capacidad de síntesis, la cual tiene una gran variabilidad entre las diversas especies de peces, por lo que su inclusión solo será necesaria cuando la especie tenga una capacidad reducida o nula de sintetizarla (Knopf et al., 1978) y no consuma harina de pescado en su dieta. Existen diversos reportes que han demostrado el beneficio de la suplementación de taurina, al resultar en ahorro proteico al no ser necesaria su

síntesis a través de los aminoácidos azufrados (metionina) indispensables para el crecimiento (Michelato et al., 2018).

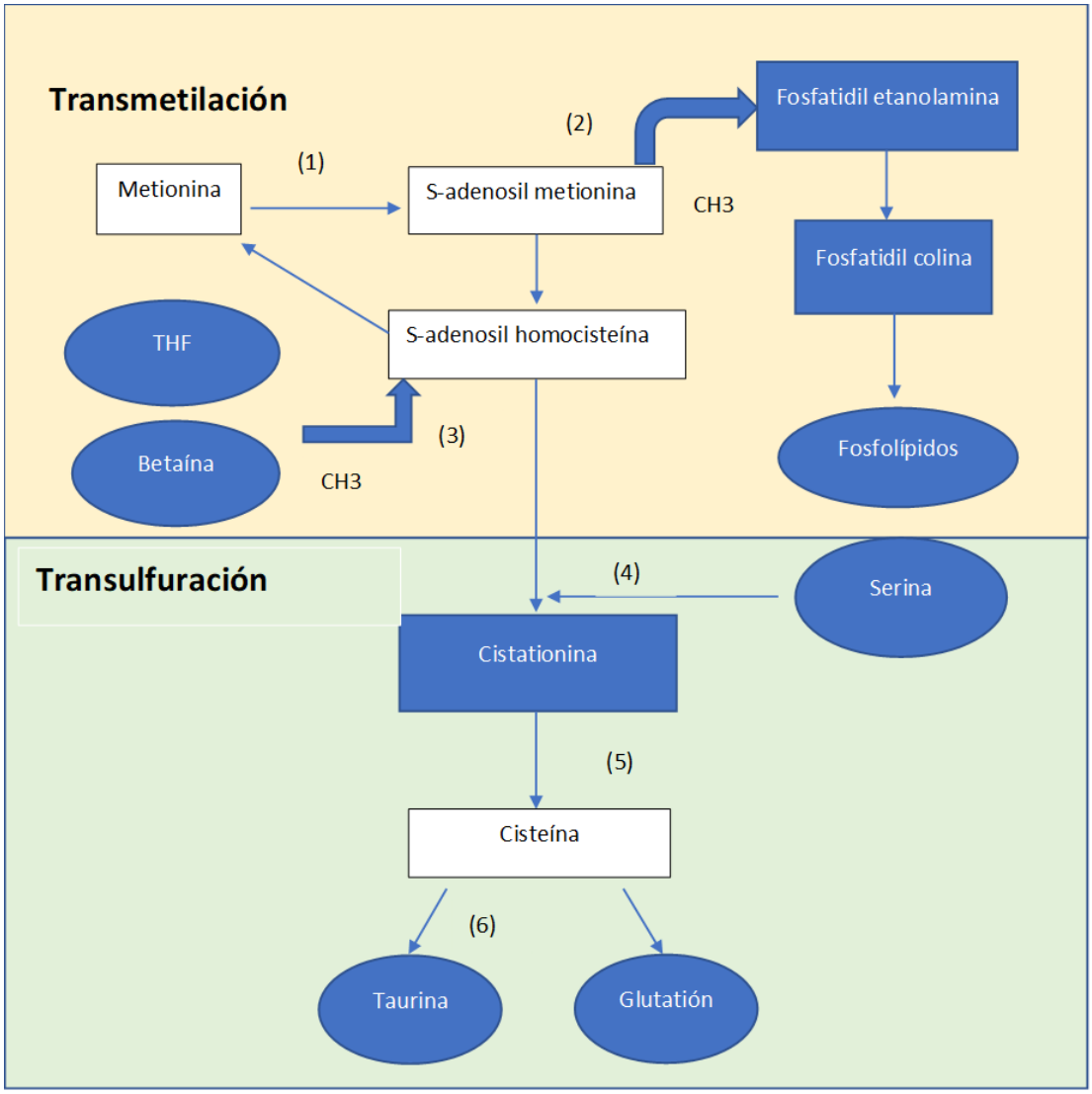


Figura 4. Esquema del metabolismo de la metionina, sus principales e interacciones con otros nutrientes.

Números indican la actividad de una enzima específica: 1. Metionina adenosil transferasa (MAT), 2. Fosfatidil etanolamina metil transferasa (PEMT), 3. Metionina sintasa 1 y 2 (MS, BHMT), 4. Cistationina beta sintasa (CBS), 5. Cistationina gama liasa (CTH) y 6. Cisteinosulfinato descarboxilasa (CSAD).

La expresión de enzimas relacionadas al metabolismo específico de los aminoácidos azufrados ha permitido determinar los efectos de diferentes nutrientes azufrados sobre el metabolismo proteico, energético y la capacidad antioxidante. Sin embargo, las interacciones metabólicas varían entre las diferentes especies de peces (Watson, 2013). Por otro lado, no todas las vías metabólicas descritas en organismos terrestres parecen estar presentes en las distintas especies de peces, aspecto que ha sido motivo de estudio. Para explicar el crecimiento somático observado a través del estudio de la expresión de mediadores del crecimiento, ha dado lugar al papel de los aminoácidos dietarios como mediadores de los componentes del eje somatotrópico. Efecto no despreciable ya que justifica la importancia de un aminoácido sobre el resto como promotor del crecimiento (Bower and Johnston, 2010).

III. JUSTIFICACIÓN

Hoy en día no se conoce lo suficiente sobre los nutrientes asociados al metabolismo, mismos que podrán cambiar el requerimiento de cada uno de los aminoácidos. El conocer dichas interacciones, así como estar al tanto de manera precisa del requerimiento de los aminoácidos azufrados, con relación a sus prioridades metabólicas, nos permitirá conocer con mayor exactitud los requerimientos de aminoácidos.

El actual trabajo tiene como objetivo sentar las bases del requerimiento de aminoácidos azufrados mediante los cambios en el contenido de taurina corporal, colesterol, ácidos grasos, aminoácidos y la expresión de enzimas de la transmetilación, transulfuración. Además del proceso de homeostasis de la taurina, para obtener una visión tangible de la importancia de los aminoácidos azufrados como nutrientes en la nutrición de peces marinos carnívoros.

IV. HIPÓTESIS GENERAL

El efecto limitante de la metionina en dietas formuladas con proteínas alternativas se debe a la demanda existente de síntesis de productos azufrados funcionales y a su importancia como regulador de procesos metabólicos de importancia para el crecimiento.

V. OBJETIVO GENERAL

Demostrar la importancia de la metionina como aminoácido limitante en el crecimiento y a nivel metabólico.

1. Objetivo específico: Capítulo 1: Determinar la importancia de la taurina sobre la demanda nutricional de metionina en *Totoaba macdonaldi* y su importancia sobre el desempeño productivo y composición corporal.

2. Objetivo específico: Capítulo 2: Determinar la prioridad metabólica formulando con distintos niveles de metionina, de la deficiencia al exceso, sobre tres de las principales funciones de la metionina: Crecimiento, síntesis proteica y síntesis lipídica; mediante la evaluación de la expresión de genes reguladores de estas vías en el jurel, *Seriola dorsalis*.

VI. RESULTADOS

1. Capítulo 1:

La suplementación de metionina no incrementó los niveles de taurina en el organismo. Además, la taurina ejerce un efecto independiente sobre el crecimiento por lo que puede considerarse un aminoácido esencial para *Totoaba macdonaldi*. Por otro lado, la respuesta sobre la composición corporal sugiere una función metabólica distinta de estos dos nutrientes.

2. Capítulo 2

Cuando la metionina se suple a un nivel menor a su requerimiento (subóptimo), se incrementa la expresión del gen *bhm* relacionados a su resíntesis. Aún así, la metionina responde con un mayor crecimiento o eficiencia productiva. Por otro lado, conforme se incrementa la suplementación de metionina, la expresión del gen *mat*, responsable del catabolismo del aminoácido, aumenta. Este incremento se relaciona positivamente con el aumento de peso, ganancia proteica, contenido de colesterol y la expresión de la *hmg CoA* desturasa, enzima responsable de la síntesis de colesterol. Lo anterior sugiere que la metionina es importante para el desempeño productivo mientras que la síntesis del colesterol depende de su catabolismo. Al parecer, la influencia de la metionina sobre el perfil de ácidos grasos parece presentar una relación más compleja para el caso del jurel, por lo que se recomiendan más estudios para comprender mejor dicha relación. Al igual que en *T. macdonaldi,* la suplementación de metionina parece afectar poco la concentración final de taurina en la *S. dorsalis*, de la misma forma la metionina generó un efecto positivo sobre la expresión de *igf-1*.

VII. DISCUSIÓN GENERAL

El diseño experimental aquí utilizado se ajusta a los modelos empleados en ensayos dosis respuesta con la adición de aminoácidos libres, donde la cantidad de metionina a suplementar fue determinada por el requerimiento de varios peces carnívoros marinos, que va en un intervalo de 1.1 a 1.7% (Zhou et al., 2011; Ruchimat et al., 1997). Ya que en este trabajo, la metionina se suplementó al 1%, se puede afirmar que se cubrió desde la subalimentación al exceso. En cambio, la suplementación de taurina de acuerdo con lo previamente reportado para *T. macdonaldi* (Satriyo et al., 2016) no se contempló, ya que el objetivo no fue obtener su requerimiento sino entender la importancia funcional de los nutrientes limitantes. De tal manera que la sustitución de metionina por taurina tenía que ser equivalente al menos en cuanto a peso, ya que se utilizó un diseño factorial para ensayar sólo dos niveles; suplementado y no suplementado.

Respecto a la cisteína, el cual es otro aminoácido azufrado de gran importancia funcional, no se tomó en cuenta como variable. Por tanto, al utilizar de manera constante el nivel y tipo de las fuentes proteicas, se asume que la cantidad de cisteína al ser constante no pudo haber influido en los resultados (Mambrini et al., 1999). Además, una de las principales razones, es el que no existe evidencia de que los peces marinos presenten la capacidad de síntesis a partir de la cisteína como lo ocurre con taurina (Salze and Davis, 2015). No obstante, a pesar de que la cisteína no es un aminoácido limitante principal, sin duda afecta el requerimiento total de azufrados, influyendo en la utilización de la metionina (Zehra and Khan, 2016), por lo que se recomienda en un futuro medir el requerimiento de aminoácidos azufrados, metionina y cisteína, y su respuesta en el crecimiento y metabolismo lipídico.

En este trabajo, el crecimiento asociado a la suplementación de metionina en la *T. macdonaldi* tuvo una magnitud similar a la observada por la suplementación de taurina, lo que hace ambos nutrientes igualmente limitantes. Lo anterior contrasta con la respuesta observada en otros peces omnívoros donde la síntesis de taurina es más cercana a la suficiencia (Michelato et al., 2018, Guimarães et al., 2018). Mientras que la respuesta aquí observada sí se ajusta a lo reportado en otros peces carnívoros (Johnson et al., 2015; Kim et al., 2017). Además del crecimiento somático, la expresión de IGF-1 demuestra que sólo ante la suficiencia de ambos nutrientes se puede alcanzar el estímulo máximo del eje somatotrópico. La importancia de la metionina como aminoácido esencial y proteogénico explica al menos, desde el mecanismo RNAtnc-GNC2 (fig. 3), su efecto como limitante del crecimiento y síntesis proteica. Sin embargo, el efecto de la taurina sobre la expresión de *igf-1* deja varias incógnitas. Al parecer, la taurina al ser un compuesto azufrado que no participa en la síntesis proteica, pero sí en diversos procesos metabólicos, puede impactar de forma directa o indirecta sobre la actividad hormonal y la síntesis proteica. Dentro de estas funciones están su actividad como modulador del funcionamiento mitocondrial (Jong et al., 2012; Hansen et al., 2010), y su participación como osmolito, además de regular la secreción pancreática de insulina (Ito et al., 2015) y su conjugación con el colesterol para formar las sales biliares en el proceso de la digestión (Oda, 2006). Por lo anterior, se sugiere que la taurina es un nutriente puramente esencial, y que si bien deriva de la metionina, para el caso de *T. macdonaldi*, existe una capacidad limitada para sintetizar taurina a partir de metionina, lo cual disminuye el efecto de ahorro proteico como se reporta en especies omnívoras (Michelato et al., 2018).

Los efectos de la combinación de metionina y taurina sobre el crecimiento de *T. macdonaldi* presentaron una sinergia incrementando en un 96.7% el crecimiento con respecto a la dieta sin suplementar (Cap. 1). Incremento que resulta atractivo ya que podría resultar en la formulación con menor concentración de proteína si estos dos compuestos son suplementados. Por otro lado, el incremento en el consumo de alimento al estar presentes ambos nutrientes fue también significativo (51.8%), lo cual parece lógico ya que el aumento en crecimiento incrementa la demanda energética (Klaassen et al., 1992). Aunque el incremento en consumo dista del incremento observado en la dieta suplementada con metionina y taurina (51.8 vs 96.7%), la desviación estándar entre estanques impidió observar diferencias en la conversión alimenticia. Hay que resaltar, que la medición de consumo suele ser un tanto subjetiva cuando se trata de recuperar alimento en estanques con casi un metro de profundidad. Para el caso del índice de eficiencia proteica (PER), ya que está basado también en el consumo del alimento, presentó igualmente variaciones. Sin embargo, aparece únicamente influenciado por la metionina, respuesta similar a lo observado en diversas especies acuáticas (Tulli et al., 2010; Yuangsoi et al., 2016; Rolland et al., 2015).

En la *T. macdonaldi* la síntesis de taurina parece ser marginal, demostrado por su poca capacidad para mantener su concentración a partir del precursor metionina (Capitulo 1). Sin embargo, la enzima de síntesis de la taurina *csad* está presente en *T. macdonaldi*, a diferencia de la cobia (Watson, 2013). De igual manera, el transportador (*taut*) aparece, aunque su expresión varía dependiendo de la concentración de taurina y de otros precursores azufrados como se ha observado en diversas especies animales. Lo anterior indica que dichos mecanismos están presentes en *T. macdonaldi* al igual que en mamíferos. Sin embargo, la actividad de la *csad* podría

ser insuficiente en la *T. macdonaldi* al resultar en una síntesis pobre cuando la taurina es baja. En este sentido se ha propuesto que las enzimas que degradan los aminoácidos azufrados para generar energía tienen una mayor actividad que las enzimas relacionadas a la síntesis de taurina (Knopf et al.,1978). Para afirmar lo anterior en la *T. macdonaldi* más experimentos serán necesarios para conocer la respuesta. La expresión de la enzima de síntesis y transportador de taurina varía dependiendo del órgano donde se mida (riñón e hígado). Estos órganos fueron elegidos ya que ambos son los principales encargados de la regulación de su concentración corporal (Burini et al., 2018). Mientras que en el hígado parece existir una mayor capacidad de estabilizar su concentración (Capítulo 1), en el riñón parece que dicha capacidad está disminuida (Capítulo 1). Lo anterior, coincide como lo que se observa en gatos, donde la tasa de síntesis entre el hígado y riñón difiere significativamente (Park and Rogers, 1999).

En cuanto a la acumulación lipídica en la *T. macdonaldi*, también se observaron diferencias entre la suplementación de metionina y taurina. En el metabolismo del colesterol, la taurina parece ser importante en regular su concentración corporal, lo cual concuerda con la idea de que el taurocolato es la principal forma de excreción en peces carnívoros marinos al formar las sales biliares (Gu et al., 2017). Mientras que la metionina incrementó el contenido del colesterol hepático, lo que genera de alguna forma un efecto opuesto a la taurina, la cual se sintetiza a partir de dicho aminoácido. Es importante mencionar que en animales con capacidad de síntesis de taurina, los efectos sobre el colesterol tienden a mimetizar el efecto de la taurina (Oda, 2006), hecho que no se presenta en esta especie con reducida capacidad de síntesis. Por otro lado, mientras la taurina parece incrementar la ganancia lipídica total en la *T. macdonaldi*, la metionina sólo incrementa la concentración de ciertos ácidos grasos, los cuales podrían estar involucrados

en la síntesis de fosfolípidos, similar a lo observado en otros organismos (Oda, 2006). Lo anterior nos lleva a resaltar un posible papel de la metionina como regulador lipídico, involucrado en la expresión de las enzimas reguladoras de la lipogénesis, que nos llevó al segundo trabajo (Capítulo 2).

En el Capítulo 2 se trabajó con el jurel *S. doralis*, que a diferencia de a *T. macdonaldi* es un pez graso. Las dietas fueron formuladas con restricción en ácidos grasos y colesterol y con diferentes niveles de metionina, manteniendo el requerimiento de taurina constante. En *S. dorsalis* anteriormente se demostró que el colesterol es un nutriente importante que limita el crecimiento (Guerra-Olvera and Viana, 2015), además de que al ser un pez graso, los cambios en depositación lipídica serían más fáciles de observar, por lo que se planteó determinar el efecto de la restricción lipídica sobre el metabolismo de la metionina.

En este experimento se observó un incremento del 73.8% en el crecimiento de los organismos alimentados con la máxima suplementación de metionina (1.7% de la dieta). Incremento que se logró ya que la taurina fue suplementada de acuerdo con su requerimiento (NRC, 2011). A diferencia del experimento anterior, la metionina no logró un mayor incremento que el control sin suplementación, proporcional a lo encontrado con *T. macdonaldi*. También, en este trabajo, el consumo de alimento fue aparentemente menor con la suplementación de metionina, lo cual difiere al experimento anterior (Capítulo 1), resultando en una conversión alimenticia mejor, lo cual concuerda con otros ensayos en diferentes especies de peces marinos (Luo et al., 2005; Ruchimat et al., 1997; Zhou et al., 2006). Dicho incremento en crecimiento se presentó asociado a un incremento en el contenido de proteína en el músculo y en el organismo completo, lo cual influyó sobre la eficiencia proteica (PER). De igual manera, se hace énfasis en la dificultad de

estimar el consumo alimenticio, sobre todo porque la *S. dorsalis* tiende a comer de más y después regurgita alimento con mucho menor estabilidad en el agua. Respecto al metabolismo lipídico la metionina no resultó en un efecto sobre la *srebp-1*, principal regulador de la lipogénesis ni sobre *mtor*. Aunque si se pudo observar una tendencia a incrementarse conforme se aumentó la metionina, no se observaron diferencias, probablemente por la gran variabilidad entre muestras (Capítulo 2). Sin embargo, la expresión de la *hmg*-CoA mostró un efecto significativamente mayor a la concentración alta de metionina, gen que codifica para la expresión la enzima principal de la síntesis de colesterol (Demigné et al., 1995). Lo anterior nos lleva a pensar que el contenido de colesterol está asociado a su síntesis, aunque mayores concentraciones de éste en el cuerpo podrían estar asociados a un exceso en la dieta, o bien una baja excreción del colesterol corporal, similar a lo reportado en mamíferos (Sugiyama et al., 1997; Luo et al., 2005). Además, se observó un incremento en el contenido de colesterol hepático y del organismo completo, coincidiendo con lo observado en la *T. macdonaldi* experimento anterior (Capítulo 1).

Por otro lado, el incremento en la depositación lipídica varió según el nivel de metionina, siendo distinta entre el hígado y músculo, y coincide a lo observado en otras especies (Xu et al., 2019). Sin embargo, genera dudas sobre el papel de la metionina en la síntesis y oxidación lipídica.

Por otro lado, la metionina parece ser utilizada a partir de la segunda suplementación (1.29%), lo cual coincide con lo observado previamente (Barreto et al., 2017) mediante isótopos estables así como lo observado por otros autores en animales de producción pecuaria, en donde reportaron que la metionina se deposita en cantidades bajas (Stradiotti et al., 2016). El incremento lineal de la *mat* con la disminución de la *bhmt* que se estabiliza en sus últimos dos niveles (Capítulo 2), sugiere que la prioridad de la metionina no es como sustrato de la síntesis proteica sino como

sustrato o mediador del metabolismo del colesterol. También, da lugar al efecto de ahorro proteico de la metionina sobre los aminoácidos ramificados valina y leucina al igual que lo reportado en el salmón (Hemre et al., 2016). Efecto que coincide con lo reportado por Torres-Velarde et al. (2018) sobre la influencia directa en la expresión de la *igf-1* y *mtor*, influyendo positivamente en el crecimiento. Sin embargo, aquí la expresión de la *igf-1* se vio limitada, posiblemente por la inversión de energía para la síntesis del colesterol que estaba restringido demostrado por el incremento en *hmg*-CoA.

VIII. CONCLUSIONES GENERALES Y PERSPECTIVAS

Para peces carnívoros como la *T. macdonaldi*, la taurina es un nutriente de igual relevancia que la metionina para obtener el máximo crecimiento, por lo que no se puede prescindir de su suplementación en formulaciones con fuentes proteicas alternativas. La metionina parece aportar muy poco en el mantenimiento de las concentraciones de taurina en la *T. macdonaldi* y aparentemente también en la *S. dorsalis*. La importancia de la metionina podría radicar, además de su función como sustrato en la síntesis proteica, en su capacidad de regular el metabolismo del colesterol a nivel transcripcional, la depositacion de ácidos grasos y la regulación a nivel transcripcional de la *igf-1*, elemento constituyente del eje somatotrópico y modulador de la actividad de la GH en otros tejidos.

Además, la regulación a nivel transcripcional de la *igf-1* podría potencializarse mediante la adición de otros nutrientes que se encuentren en concentraciones subóptimas, como se observó con la taurina en la *T. macdonaldi*.

Para futuras investigaciones se deberían ensayar las posibles interacciones entre la metionina y los componentes lipídicos que regula (como ácidos grasos y colesterol) para comprender el nivel que ejercen sobre el desempeño productivo de los organismos. Así mismo, debido a que la metionina genera diversos compuestos funcionales, la suplementación de éstos en una dieta con niveles subóptimos de metionina permitirá identificar las moléculas responsables de los cambios de composición corporal. Y mediante los cambios en el contenido de compuestos generados por el metabolismo de la metionina, poder definir las rutas con mayor influencia sobre el crecimiento somático. Lo anterior dará lugar a información precisa sobre el requerimiento de aminoácidos

azufrados en peces carnívoros. Por último, para obtener la concentración mínima de aminoácidos necesaria para el máximo desarrollo productivo se deberá comenzar con la implementación de fuentes energéticas alternativas que reduzcan el uso de los aminoácidos como sustratos energéticos.

Referencias

Aguillón-Hernández, O. E., Hernández Hernández, L. H., Shimada Miyasaka, A., and Garduño Lugo, M. 2017. Effects of diets with whole plant-origin proteins added different ratios of taurine:methionine on the growth, macrophage activity and antioxidant capacity of rainbow trout (*Oncorhynchus mykiss*) fingerlings. Veterinary and Animal Science. doi:dx.doi.org/10.1016/j.vas.2017.04.002.

Ajinomoto Eurolysine, S.A.S. 2012. Estimating amino acid requirements through dose-response experiments in pigs and poultry - Protocol and results interpretation - Technical note, February.

Amoah, Y.T.2011. Effect of dietary protein levels on growth and protein utilization in juvenile arctic char (*Salvelinus alpinus*). Aquaculture, Volume 195, Issues 3–4

Andoh, T. 2007. Amino acids are more important insulinotropins than glucose in a teleost fish , barfin flounder (*Verasper moseri*).General and comparative endocrinology, 151, 308–317. doi:10.1016/j.ygcen.2007.01.015

Bae, J.Y., Ok, I.H., Lee, S., Hung, S.S.O., Min, T.S., Bai, S.C. 2011. Re-evaluation of dietary methionine requirement by plasma methionine and ammonia concentrations in surgically modified rainbow trout, (*Oncorhynchus mykiss)*. Asian-Australasian Journal of Animal Science 24:974–981. doi:10.5713/ajas.2011.10189.

Bañuelos-Vargas, I. López, L.M., Pérez-Jiménez, A., Peres, H. 2013. Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on hepatic intermediary metabolism and antioxidant status of totoaba juveniles (*Totoaba macdonaldi*). Comparative Biochemistry and Physiology 170, 18–25.

Barreto-Curiel, F., Abramo, L.R.D. Viana, M.T. 2017. Metabolism of *Seriola lalandi* during Starvation as Revealed by Fatty Acid Analysis and Compound-Specific Analysis of Stable Isotopes. doi:10.1371/journal.PLosOne.0170124.

Ballantyne, J.S. 2001. Amino acid metabolism. In: Nitrogen excretion, Wright P.A., Anderson P.M. (Ed). Fish Physiology Vol. 20. Academic Press: Chicago, pp 77-107.

Berri, C., Metayer-Coustard, S., Lessire, M., Duval, E., Bouvarel, I., Duclos, M., Le Bihan-Duval, E., Tesseraud, S. 2020. Managing poultry meat quality by nutrition, 22th European Symposium on the Quality of Poultry Meat - 16. European Symposium on the Quality of Eggs and Egg Products, World's Poultry Science Association (WPSA). INT., May 2015, Nantes, France. 190 p. hal-02742739

Bower, N.I., Johnston, I.A. 2010. Transcriptional regulation of the IGF signaling pathway by amino acids and insulin-like growth factors during myogenesis in Atlantic salmon. PLoS One. 5. doi:10.1371/journal. PLosOne.0011100.

Brosnan, J.T. 2003. Interorgan amino acid transport and its regulation. Journal of Nutrition 133, 2068S-2072S

Burini, R.C., Borges-Santos, M.D., Moreto, F., Ming-Yu, Y. 2018. Taurine and Oxidative Stress in HIV. HIV/AIDS, 169–182. doi:10.1016/B978-0-12-809853-0.00015-8.

Buttery, P.J., Lindsay, D.B. 1980. Protein Deposition in Animals: Proceedings of Previous Easter Schools in Agricultural Science, Butterworth-Heineman Editors. Nottingham, UK, 315 p.

Caballero-solares, A., Viegas, I., Salgado, M.C., Siles, A.M., Sáez, A., Metón, I., Baanante, I.V., Fernández, F. 2015. Diets supplemented with glutamate or glutamine improve protein retention and modulate gene expression of key enzymes of hepatic metabolism in gilthead seabream (*Sparus aurata*) juveniles. Aquaculture 444, 79–87. doi:10.1016/j.Aquaculture.2015.03.025

Cai, G., Burtle, G. 1996. Methionine Requirement of Channel Catfish Fed Soybean Meal-Corn-Based Diets. Journal of Animal Science 74(3):514-21 doi:10.2527/1996.743514x.

Carter, C.G., Houlihan, D.F. 2001. Protein synthesis. In: Nitrogen excretion, Wright PA, Anderson PM (Ed). Fish Physiology, Vol. 20. Academic Press: Chicago, pp 31-75

Chauvigné, F., Gabillard, J.C., Weil, C., Rescan, P.Y. 2003. Effect of refeeding on IGFI, IGFII, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in Rainbow trout myotomal muscle. General and Comparative Endocrinology 132(2), 209-215

Chesney, R.W., Patters, A.B., Han, X. 2011. Taurine and the kidneys. In: Taurine in Health and Disease, (El Idrissi, A., Shen, C.H., L'Amoreaux, W.J. (Ed). Transworld Research Network, Kerala, pp 75-99.

Cleveland, B.M., Burr, G.S. 2011. Proteolytic response to feeding level in Rainbow trout (*Oncorhynchus mykiss*). Aquaculture 319(1-2), 194-204

Cowey, C.B. 1972. Aspects of protein utilization by fish. Proceedings of the Nutrition Society 34:57–63.

Dai, W., Panserat, S., Plagnes-Juan, E., Seiliez, I., Skiba-Cassy, S. 2015. Amino Acids Attenuate Insulin Action on Gluconeogenesis and Promote Fatty Acid Biosynthesis via mTORC1 Signaling Pathway in trout Hepatocytes, Cellular Physiology and Biochemistry 36(3):1084-1100 doi:10.1159/000430281.

De Almeida Bicudo, Á.J., Possebon-Cyrino, J.E. 2014. Evaluation of methods to estimate the essential amino acids requirements of fish from the muscle amino acid profile. Latin American Journal of Aquatic Research 42:271–275. doi:10.3856/, from: http://www.ncbi.nlm.nih.gov/pubmed/7547838

Dauncey, M.J., Bates, P., 1994. Regulation of porcine insulin-like growth factor I and growth hormone receptor mRNA expression by energy status Regulation of porcine insulin-like growth factor I. American Journal of physiology. 266(5 Pt 1):E776-85. doi:10.1152/ajpendo.

Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M., Kaushik, S.J., 2005. Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*),

Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 142, 19–31. doi:10.1016/j.cbpb.2005.07.005

Dilger, R.N., Baker, D.H. 2008. Excess dietary L-cysteine causes lethal metabolic acidosis in chicks. Journal of Nutrition 138:1628–33.

Duan, C., Duguay S.J., Plisetskaya, E.M. 1993. Insulin-like growth factor I (IGF-I) mRNA expression in Coho salmon, *Oncorhynchus kisutch*: Tissue distribution and effects of growth hormone/prolactin family proteins. Fish Physiology and Biochemistry 11(1-6), 371-379

Edgar, S. E.,Kirk, C.A., Rogers, Q.R., Morris, J.G. 1998. Taurine status in cats is not maintained by dietary cysteinesulfinic acid. Journal of Nutrition 128:751–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9521639

Espe, M., Hevrøy, E.M., Liaset, B., Lemme, A., El-mowafi, A., 2008. Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, *Salmo salar*. 274:132–141. doi:10.1016/j.Aquaculture.2007.10.051.

Espe, M., Veiseth-Kent, E., Zerrahn, J.E., Ronnestad, I., Aksnes, A. 2015. Juvenile Atlantic salmon decrease white trunk muscle IGF-1 expression and reduce muscle and plasma free sulfur amino acids when methionine availability is low while liver sulfur metabolites mostly is affected by treatment. Aquaculture Nutrition DOI: 10.1111/anu.12294

Espe, M., Vikeså, V., Thomsen, T.H., Adam, A.C., Saito, T., Skjærven, K.H. 2020. Atlantic salmon fed a nutrient package of surplus methionine, vitamin B12, folic acid and vitamin B6 improved growth and reduced the relative liver size, but when in excess growth reduced. Aquaculture Nutrition. 26:477–489. doi:10.1111/anu.13010.

Fagbenro, O.A., Balogun, A.M., Fasakin, E.A. 2017. Dietary Methionine Requirement of the African Catfish, *Clarias gariepinus*. Journal of Applied Aquaculture 4438. doi:10.1300/J028v08n04.

FAO. 2004. Protein Sources for the Animal Feed Industry. Food and Agriculture Organization of the United Nations – FAO, Expert Consultation and Workshop Bangkok, (29 April-3 May 2002).

Fereday, A., Gibson, N.R., Cox, M., Pay, P.J., Millward, D.J. 1998. Variation in the apparent sensitivity of the insulin-mediated inhibition of proteolysis to amino acid supply determines the efficiency of protein utilization. Clinic Science. 95, 725-733

Figueiredo-Silva, A.C., Genevie, C., Kaushik, S., Peleteiro, J.B., Valente, L.M. 2010. Modulation of blackspot seabream (*Pagellus bogaraveo*) intermediary metabolic pathways by dispensable amino acids. Amino Acids 39:1401–1416. doi:10.1007/s00726-010-0599

Figueiredo-Silva, A.C., Panserat, S., Kaushik, S., Geurden, I., Polakof, S. 2012. High levels of dietary fat impair glucose homeostasis in rainbow trout. Journal of Experimental Biology 169–178. doi:10.1242/jeb.063933

Fuentes, E.N., Valdés, J.A., Molina, A., Björnsson, B.T. 2013. Regulation of skeletal muscle growth in fish by the growth hormone - insulin-like growth factor system. General and Comparative Endocrinology 192, 136-148

Fuller, M.F., McWilliam, R.T., Wang, T.C., Giles, L.R. 1989. Optimum dietary amino acid pattern for growing pig. British Journal of Nutrition 62:255-267

Funkenstein, B., Silbergeld, A., Cavari, B., Laron, Z. 1989. Growth hormone increases plasma levels of insulinlike growth factor (IGF-I) in a teleost, the Gilthead seabream (*Sparus aurata*). Journal of Endocrinology 120(2), R19-R21

Gabillard, J.C., Yao, K., Vandeputte, M., Guttierez, J., Le Bail, P.Y. 2006. Differential expression of two GH receptor mRNAs following temperature change in Rainbow trout (*Oncorhynchus mykiss*). Journal of Endocrinology 190(1), 29-37

Gaylord, T., Barrows, F. 2009. Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, Oncorhynchus mykiss, feeds. Aquaculture Vol. 287:180-184.

Gaylord, T.G., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E., Shepherd, B. 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 269:514–524. doi:10.1016/j.aquaculture.2007.04.011.

Genevie, F. 2010. Modulation of blackspot seabream (*Pagellus bogaraveo*) intermediary metabolic pathways by dispensable amino acids, Amino Acids. volume 39, pages1401–1416. doi:10.1007/s00726-010-0599-y

Gómez-Requeni, P., Calduch-Giner, J.A., Vega-Rubín de Celis, S., Médale, F., Kaushik, S.J., Pérez-Sánchez, J. 2005. Regulation of the somatotropic axis by dietary factors in Rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition 94(3), 353-361

Gómez-Requeni, P., Mingarro, M., Kirchner, S., Calduch-Giner, J.A., Médale, F., Corraze, G., Panserat, S., Martin, S.A.M., Houlihan, D.F., Kaushik, S.J., Pérez-Sánchez, J. 2003. Effects of dietary amino acid profile on growth performance, key metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream (*Sparus aurata*). Aquaculture 220:749–767. doi:10.1016/S0044-8486(02)00654-3.

Gu, M., Bai, N., Kortner, T.M. 2017. Taurocholate supplementation attenuates the changes in growth performance, feed utilization, lipid digestion, liver abnormality and sterol metabolism in turbot (*Scophthalmus maximus*) fed high level of plant protein. Aquaculture 468:597–604. doi:10.1016/j.Aquaculture.2016.11.022

Guerra-Olvera, F., Viana, M.T. 2015. Effect of dietary cholesterol content on growth and its accumulation in liver and muscle tissues of juvenile yellowtail kingfish (*Seriola lalandi*). Ciencias Marinas 41:143–156.

Guimarães, I.G., Skjærven, K., Moren, M., Espe, M., Hamre, K. 2018. Dietary taurine supplementation in plant protein based diets do not affect growth and reproductive

performance of zebrafish. Aquaculture Research. 2018; 49: 2013– 2022. https://doi.org/10.1111/are.13658

Gunathilaka, G.L.B.E., Kim, M.G., Lee, C., Shin, J., Lee, B.J., Lee, K.J. 2019. Effects of taurine supplementation in low fish meal diets for red seabream (*Pagrus major*) in low water temperature season. Fisheries and Aquatic Science 22:1–10. doi:10.1186/s41240-019-0138-z.

Hansen, S.H., Andersen, M.L., Cornett, C., Gradinaru, R., Grunnet, N. 2010. A role for taurine in mitochondrial function. Journal of Biomedical Science 17 Suppl 1:S23. doi:10.1186/1423-0127-17-S1-S23. Available from: http://www.jbiomedsci.com/content/17/S1/S23

Hayes, K.C., Sturman, J.A. 1981. Taurine in metabolism. Annual Review of Nutrition 1:401–425. doi:10.1146/annurev.nu.01.070181.002153.

Hemre, G., Lock, E., Olsvik, P.A., Hamre, K., Espe, M., Torstensen, B.E., Silva, J., Hansen, A., Waagbø, R., Johansen, J.S., Sanden, M., Sissener, N.H. 2016. Atlantic salmon (*Salmo salar*) require increased dietary levels of B-vitamins when fed diets with high inclusion of plant based ingredients. Peer Journal 1–28. doi:10.7717/peerj.2493

Houlihan, D.F., Agnisola, C., Lyndon, A.R., Gray, C., Hamilton, N.M. 1988. Protein synthesis in a fish heart: responses to increased power output. Journal of Experimental Biology 137, 565-587

Ito, T. N., Yoshikawa, H., Ito, Schaffer, S.W. 2015. Impact of taurine depletion on glucose control and insulin secretion in mice. Journal of Pharmacology Science 129:59–64. doi:10.1016/j.jphs.2015.08.007. Available from: http://dx.doi.org/10.1016/j.jphs.2015.08.007

Jauncey, K., Tacon, A.G.J., Jackson, A.J. 1983. The quantitative essential amino acid requirements of (*Oreochromis mossambicus*), International Symposium on Tilapia in Aquaculture proceedings Publisher, Tel Aviv University, Tel Aviv Israel.

Johnson, R.B., Kim, S.K., Watson, A.M., Barrows, F.T., Kroeger, E.L., Nicklason, P.M., Goetz, G.W., Place, A.R. 2015. Effects of dietary taurine supplementation on growth, feed efficiency, and nutrient composition of juvenile sablefish (*Anoplopoma fimbria*) fed plant based feeds. Aquaculture 445:79–85. doi:10.1016/j.aquaculture.2015.03.030. Available from: http://dx.doi.org/10.1016/j.aquaculture.2015.03.030

Jong, C.J., Azuma, J., Schaffer, S. 2012. Mechanism underlying the antioxidant activity of taurine: Prevention of mitochondrial oxidant production. Amino Acids 42:2223–2232. doi:10.1007/s00726-011-0962-7.

Kim, J.M., Malintha, G.H.T., Gunathilaka, G.L.B.E., Lee, C., Kim, M.G., Lee, B.J., Kim, J.D., Lee, K.J. 2017. Taurine supplementation in diet for olive flounder at low water temperature. Fisheries and Aquatic Sciences 1–8. doi:10.1186/s41240-017-0065-9.

Kim, K.W., Moniruzzaman, M., Kim, K.D., Han, H.S., Yun, H., Lee, S., Bai, S.C. 2016. Effects of dietary protein levels on growth performance and body composition of juvenile parrot fish,

Oplegnathus fasciatus. International Aquatic Research 8:239–245. doi:10.1007/s40071-016-0139-9.

Kimball, S.R., Jefferson, L.S. 2006. Signaling pathways and molecular mechanisms through which branched chain amino acids mediate translational control of protein synthesis. Journal of Nutrition 136(1), 227S-231S

Klaassen, M., Zwaan, B., Heslenfeld, P., Lucas, P., Luijckx, B. 1992. Growth rate associated changes in the energy requirements of tern chicks. Ardea. 80:19–28.

Knopf, K., Sturman, J.A., Armstrong, M., Hayes, K.C. 1978. Taurine: An Essential Nutrient for the Cat. Journal of Nutrition. doi:10.1093/jn/108.5.773.

Langer, S., Fuller, M. F. 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. British Journal of Nutrition. 83:43–8.

Lebret, B., Batonon-Alavo, D.I., Perruchot, M.H., Mercier, Y., Gondret, F. 2018. Improving pork quality traits by a short-term dietary hydroxy methionine supplementation at levels above growth requirements in finisher pigs. Meat Science 145:230–237. doi:10.1016/ 2018.06.040. Doi: /10.1016/j.meatsci.2018.06.040

López, L.M., Flores-Ibarra, M., Bañuelos-Vargas, I., Galaviz, M.A., True, C.D. 2015. Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (*Totoaba macdonaldi*). Fish Physiology and Biochemistry 41:921–936. Doi: /10.1016/j.cbpb.2014.01.003

Luo, Z., Liu, Y., Mai, K., Tian, L., Yang, H., Tan, X., Liu, D. 2005. Dietary l-methionine requirement of juvenile grouper *Epinephelus coioides* at a constant dietary cystine level. Aquaculture. 249:409–418. doi:https://doi.org/10.1016/j.aquaculture.2005.04.030. Available from: http://www.sciencedirect.com/science/article/pii/S0044848605002462

Luquet, P. 1982 Aspects du métabolisme des poissons particulierement importants pour la qualite de léau. In Razionale utilizzo delle risorse idriche in acquacoltura. Atti del Convegno Internazionale Verona, Salone Acquacoltura, 16 Ottobre 1982. Pubblicazione curata dal Centro regionale tutela e sperimentazione Pesca e Acquacoltura dell'E.S.A.V., Italy., pp. 30–38

Madrid, J., Pohlenz, C., Viana, M.T., Lazo, J.P. 2019. Dietary lysine requirement for juvenile, *Totoaba macdonaldi*. Aquaculture. 500:92–98. Doi:10.1016/j.Aquaculture.2018.10.003.

Mambrini, M., Roem, A.J., Cravèdi, J.P., Lallès, J.P., Kaushik, S.J. 1999. Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, Oncorhynchus mykiss. Journal of Animal Science 77:2990–2999. doi:10.2527/1999.77112990x.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G. 2010. Animal Nutrition. 7th Ed. Prentice Hall 576, 692 p.

McMillan, D.N., Houlihan, D.F. 1989. Short-term responses of protein synthesis to re-feeding in rainbow trout. Aquaculture 79(1-4), 37-46

Metón, I., Caseras, A., Cantó, E., Fernández, F., Baanante, I.V. 2000. Liver insulin-like growth factor-I mRNA is not affected by diet composition or ration size but shows diurnal variations in regularly-fed gilthead sea bream (*Sparus aurata*). Journal of Nutrition 130(4), 757-760

Michelato, M., Furuya, W.M., Gatlin, D.M. 2018. Metabolic responses of Nile tilapia *Oreochromis niloticus* to methionine and taurine supplementation. Aquaculture 485:66–72. doi:10.1016/j.aquaculture.2017.11.003.

Miles, R.D., Chapman, F.A. 2014. The Concept of Ideal Protein in Formulation of Aquaculture Feeds 1 Using Ideal Proteins in Aquaculture Feeds: Feeding Excess Protein Is Not Acceptable Lysine: the Reference Amino Acid IFAS Ext. Univ. Florida. 1–3.

Milgen, J.V., Dourmad, J.Y. 2015. Concept and application of ideal protein for pigs. Journal of Animal Science and Biotechnology 6:15. Doi: /10.1186/s40104-015-0016-1

Mingarro, M., Kirchner, S. 2003. Effects of dietary amino acid profile on growth performance, key metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream (*Sparus aurata*), Aquaculture 220, 749–767. doi:10.1016/S0044-8486(02)00654-3

Moore, D.R., Soeters, P.B. 2015. The Biological Value of Protein. Nestle Nutrition Institute Workshop Ser. 82:39–51. doi:10.1159/000382000.

National Research Council (NRC). 2011. Nutrient requirements of fish and shrimp. The National Academy Press. Washington, USA. 392 p. ISBN: 978-0-309-16338-5

Niu, P.D., Pérez-Sánchez, J., Le Bail, P.Y. 1993. Development of a protein binding assay for teleost insulin-like growth factor (IGF)-like: Relationships between growth hormone (GH) and IGF-like in the blood of rainbow trout (Oncorhynchus mykiss). Fish Physiology and Biochemistry 11(1-6), 381-391

Oda, H. 2006. Functions of sulfur-containing amino acids in lipid metabolism. Journal of Nutrition 136:1666S–1669S.

Panserat, S., Kaushik, S., Médale, F. 2013. Rainbow trout as a model for nutrition and nutrient metabolism studies. In: Trout: From Physiology to Conservation. Polakof, S., Moon, T.W. (Ed) Animal Science, Issues and Research, Marine Biology BISAC. 131–154.

Park, T., Rogers, Q.R. 1999. Hepatic and Renal cysteine Sulfinic Acid Decarboxylase Activities in Cats Fed Different Levels of Dietary Protein and Taurine. Journal of Food Science and Nutrition Vol 4, No 1, Pag: 47-51.

Pérez-Sánchez, J., Weil, C., Le Bail, P.Y. 1992. Effects of human insulin-like growth factor-I on release of growth hormone by rainbow trout (*Oncorhynchus mykiss*) pituitary cells. Journal of Experimental Zoology 262(3), 287-290

Pérez-Sánchez, J., Marti-Palanca, H., Kaushik, S.J. 1995. Ration size and protein intake affect circulating growth hormone concentration, hepatic growth hormone binding and plasma

insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (*Sparus aurata*). Journal of Nutrition 125(3), 546-552

Pohlenz, C., Buentello, A., Miller, T., Small, B.C., MacKenzie, D. S., Gatlin, D.M. 2013. Effects of dietary arginine on endocrine growth factors of channel catfish, *Ictalurus punctatus*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 166(2), 215–221.

Reinecke, M. 2006. A Survey on the Insulin and Insulin-like Growth Factor System. In: Fish Endocrinology, Reinecke M., Zaccone G., Kapoor B.G. (Eds). Science Publishers: Plymouth, pp 3-13

Reinecke, M., Collet, C. 1998. The phylogeny of the insulin-like growth factors. International Review of Cytology 183, 1-94

Renaville, R., Hammadi, M., Portetelle, D. 2002. Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology 23, 351-360

Richard, D.M., Frank, A.D. 2011. The Concept of Ideal Protein in Formulation of Aquaculture Feeds. IFAS Ext. Univ. Florida. 1–3.

Rolland, M., Dalsgaard, J., Holm, J., Gómez-Requeni, P., Skov, P.V. 2015. Dietary methionine level affects growth performance and hepatic gene expression of GH-IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets. Comparative Biochemistry and Physiology Part - B Biochemistry and Molecular Biology 181:33–41. doi:10.1016/j.cbpb.2014.11.009.

Ruchimat, T., Masumoto, T., Hosokawa, H., Shimeno, S. 1997. Quantitative methionine requirement of yellowtail (*Seriola quinqueradiata*). Aquaculture 150:113–122. doi:10.1016/S0044-8486(96)01465-2.

Rueda-Lopez, S., Lazo, J.P., Correa-Reyes, G., Viana, M.T. 2011. Effect of dietary protein and energy levels on growth, survival and body composition of juveniles *Totoaba macdonaldi*. Aquaculture 319, 385–390.

Saera-Vila, A., Calduch-Giner, J.A., Pérez-Sánchez, J. 2007. Co-expression of IGFs and GH receptors (GHRs) in gilthead sea bream (*Sparus aurata L*.): Sequence analysis of the GHR-flanking region. Journal of Endocrinology 194, 361-372

Salem, M., Silverstein, J., Rexroad, C.E., Yao, J. 2007. Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). BMC Genomics 8, 1-16

Salze, G.P., Davis, D.A. 2015. Taurine: A critical nutrient for future fish feeds. Aquaculture 437:215–229. Doi: 10.1016/j.aquaculture.2014.12.006.

Salze, G.P., Davis, D.A., Jirsa, D.O., Drawbridge, M.A. 2017. Methionine Requirement for Juvenile White Seabass, *Atractoscion nobilis*, Using Nonlinear Models. Journal of the World Aquaculture Society 48:729–740. Doi: 10.1111/jwas.12413.

Sankian, Z., Khosravi, S., Kim, Y., Lee, S. 2017. Effect of dietary protein and lipid level on growth , feed utilization , and muscle composition in golden mandarin fish *Siniperca scherzeri.* Fisheries and Aquatic Sciences 1–6. doi:10.1186/s41240-017-0053-0

Santiago, C.B. 1985. Amino acid requirements of Nile tilapia. Ph. D. dissertation. Auburn University, Auburn, Alabama.

Satriyo, T.B., Galaviz, M.A., Salze, G., López, L.M. 2017. Assessment of dietary taurine essentiality on the physiological state of juvenile *Totoaba macdonaldi*. Aquaculture Research 48, 5677-5689. Doi:10.1111/are.13391.

Shimada, A. 2005. Nutrición Animal. Ed. Trillas, México 388 p.

Smith, R.W., Houlihan, D.F., Nilsson, G.E., Alexandre, J. 1999. Tissue-specific changes in RNA synthesis in vivo during anoxia in crucian carp. American Journal of Physiology 277, R690-697

Snyder, G.S., Gaylord, T.G., Barrows, F.T., Overturf, K., Cain, K.D., Hill, R.A., Hardy, R. 2012. Effects of carnosine supplementation to an all-plant protein diet for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 338-341, 72-81

Stein, H.H., Fuller, M.F., Moughan, P.J., Sève, B., Mosenthin, R., Jansman, A.J. M., Fernández, J.A., de Lange, C.F.M. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livestock Science 109:282–285. Doi:10.1016/j.livsci.2007.01.019.

Stradiotti, A.C., Bendassolli, J.A., Ducatti, C., Sartori, J.R., Pelícia, V.C., Araujo, P.C., Maruno, M.K., Pezzato, A.C. 2016. Incorporation of Labeled Methionine as a Tissue Tracer in Broiler Chickens. Brazilian Journal of Poultry Science 18(4), 719-724.

Sugiyama, K., Yamakawa, A., Kumazawa, A., Saeki, S. 1997. Methionine content of dietary proteins affects the molecular species composition of plasma phosphatidylcholine in rats fed a cholesterol-free diet. Journal of Nutrition 127:600–607.

Tibaldi, E., Tulli, F. 1999. Dietary threonine requirement of juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture 175:155–166. Doi: 10.1016/S0044-8486(99)00029-0.

Torres-Velarde, J., Llera-Herrera, R., García-Gasca, T., García-Gasca, A. 2018. Mechanisms of stress-related muscle atrophy in fish: An ex vivo approach. Mechanisms of Development 154, 162-169.

Tulli, F., Messina, M., Calligaris, M., Tibaldi, E. 2010. Response of European sea bass (*Dicentrarchus labrax*) to graded levels of methionine (total sulfur amino acids) in soya protein-based semi-purified diets. British Journal of Nutrition 104:664–673. doi:10.1017/S0007114510001029.

Verbrugghe, A., Hesta, M. 2017. Cats and Carbohydrates: The Carnivore Fantasy ?, Veterinary Sciences 1–22. Doi: 10.3390/vetsci4040055

Watson, A.M. 2013. Taurine: An indispensable ingredient in the development of sustainable aquafeeds. University of Maryland. Ph. D. dissertation, Maryland Washington D.C.

Weller, P.A., Dauncey, M.J., Bates, P.C., Brameld, J.M., Buttery P.J., Gilmour R.S. 1994. Regulation of porcine insulin-like growth factor I and growth hormone receptor mRNA expression by energy status Regulation of porcine insulin-like growth factor I. American Journal of physiology 266(5 Pt 1):E776-85. doi:10.1152/ajpendo.

Wood, C.M. 2001. Influence of feeding, exercise, and temperature on nitrogen metabolism and excretion. In: Nitrogen excretion, Wright PA, Anderson PM (Ed). Fish Physiology Vol. 20. Academic Press; Chicago, pp 201-238

Wu, G. 2009. Amino acids: Metabolism, functions, and nutrition. Amino acids 37, 1-17

Wu, G., Bazer, F.W., Dai, Z., Li, D., Wang, J., and Wu, Z. 2014. Amino Acid Nutrition in Animals: Protein Synthesis and Beyond. Annual Review of Animal Bioscience. 2:387–417. Doi: 10.1146/annurev-animal-022513-114113.

Xu, H., Zhang, Q., Wei, Y., Liao, Z., Liang, M. 2019. Dietary methionine increased the lipid accumulation in juvenile tiger puffer *Takifugu rubripes*. Comparative Biochemistry and Physiology Part - B Biochemistry and Molecular Biology 230:19–28. Doi: 10.1016/j.cbpb.2019.01.005.

Yamamoto, T., Surgita, T., Furita., H. 2005. Essential amino acid supplementation to fish meal-based diets with low protein to energy ratios improves the protein utilization in juvenile rainbow trout *Oncorhynchus mykiss*. Aquaculture Vol. 246:379-391.

Yuangsoi, B., Wongmaneeprateep, S., Sangsue, D. 2016. The optimal dietary DL-Methionine on growth performance, body composition and amino acids profile of pangasius catfish (*Pangasius bocourti*). Aquaculture Aquarium Conservation & Legislation Bioflux 9:369–378.

Zehra, S., Khan, M.A. 2016. Total sulphur amino acid requirement and maximum cysteine replacement value for methionine for fingerling *Catla catla* (Hamilton). Aquaculture Research 47: 304-317. doi:10.1111/are.12493

Zhao, Y., Li, J.Y., Jiang, Q., Zhou, X.Q., Feng, L., Liu, Y., Jiang, W.D., Wu, P., Zhou, J., Zhao, J., Jiang, J. 2020. Leucine Improved Growth Performance, Muscle Growth, and Muscle Protein Deposition Through AKT/TOR and AKT/FOXO3a Signaling Pathways in Hybrid Catfish *Pelteobagrus vachelli × Leiocassis longirostris*. Cells 9:327. Doi: 10.3390/cells9020327.

Zhou, Q., Wu, Z., Tan, B., Chi, S., Yang, Q. 2006. Optimal dietary methionine requirement for Juvenile Cobia (*Rachycentron canadum*). Aquaculture 258, 551–557.

Zhou, F., Xiao, J., Hua, Y., Ngandzali, B., Shao, Q. 2011. Dietary l-methionine requirement of juvenile black sea bream (*Sparus macrocephalus*) at a constant dietary cystine level. Aquaculture Nutrition 17: 469-481. doi:10.1111/j.1365-2095.2010.00823.x

Zhou, Z., Wang, X., Wu, X., Gao, Y., Li, X., Dong, Y., Yao, W. 2019. Effects of dietary leucine levels on growth, feed utilization, neuro-endocrine growth axis and TOR-related signaling genes expression of juvenile hybrid grouper (*Epinephelus fuscoguttatus × Epinephelus lanceolatus*). Aquaculture 2019, 504, 172–181.

CAPÍTULO I: Effect of methionine and taurine on the overall performance of *Totoaba macdonaldi*, lipid deposition and taurine tissue concentration

Effect of methionine and taurine on the overall performance of *Totoaba macdonaldi*, lipid deposition and taurine tissue concentration

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Running title: Methionine and taurine effect on marine fish metabolism

Abstract

The effect of methionine and taurine on the overall performance of *Totoaba macdonaldi* was evaluated during a 60-day experimental feeding trial. Methionine (Met) and taurine (Tau) were supplemented each and together under a factorial design, using a Basal diet as control containing a limited amount of Met and Tau (Basal diet, Met, Tau, Met+Tau). One hundred and forty-four juveniles (41±0.5 g) were randomly distributed into 12 tanks (500L) (triplicate groups). At the end, growth, proximal composition, fatty acid profile, cholesterol, taurine content, hepatic gene expression of proteins related to growth, and hepatic and kidney proteins involved in the taurine synthesis and transportation were assessed. A two-way ANOVA analysis revealed that growth rate was positively affected by dietary Met and Tau, whereas their interaction resulted in the highest growth ($p<0.05$). The hepatic IGF-1 expression increased with a similar trend. When dietary Tau was restricted, overexpression of *csad* (cysteine sulphinate decarboxylase) was displayed in the liver tissue, indicating the need for Tau synthesis, whereas in the kidney tissue the expression of *csad* was low. In Tau-based diets, a higher content of fatty acids was obtained in the whole body, while the total cholesterol decreased. However, a different response was noticed when the Met was supplemented. The overall Tau content in fish tissues significantly increased only as a result of Tau supplementation, revealing an insufficient synthesis capacity in *T. macdonaldi*. In conclusion, the Met and Tau are limiting amino acids/derivative for a suitable performance and lipid deposition in *T. macdonaldi*.

Keywords: Amino acids; taurine; methionine; cholesterol; *Totoaba macdonaldi*

1. Introduction

Methionine (Met) is one of the limiting amino acids, being critical in plant-based aquafeeds, ingredients that are poor in this nutrient[(1)]. The importance of Met lies in its condition as a proteogenic amino acid, being essential for most organisms including fish. Besides, Met participates in the synthesis of sulphur compounds of biological importance, such as taurine (Tau), a factor that could modify the primary function of Met on protein synthesis when Tau is deficient. Tau is a derivative sulphonic amino acid resultant from the metabolism of sulphur amino acids[(2)]; present in high concentrations in most animal tissues[(3)]. Tau participates in several essential biological processes, as a neurotransmitter, or as an important osmolyte involved in the homeostasis between the corporal fluids versus the surroundings in the marine environment; as

well as in the synthesis of bile salts, important emulsifiers to digest lipids[4]. The primary synthesis route of Tau is from the trans-sulphuration of Met[4]. However, the amount of Tau synthesized is species specific[5,6], and therefore, it is crucial to determine the physiological role of this nutrient in commercially important species. In rats, up to 70% of the Met in excess can be transformed into Tau; as such, its requirement is very low[5]. Therefore, the synthesis of Tau increases the demand for sulphur amino acids to satisfy their needs when the synthesis pathway is not limited. As such, the requirement of Tau in cats[6] and in the Japanese sole[7] has been demonstrated. In carnivore fish, it has been revealed that Tau should be supplemented even if they can synthesize it; a requirement that increases when plant proteins are used[8]. The Tau requirement in marine fish might be associated with a low synthesis capability, and not necessarily to the lack of sulphur precursors for its synthesis to optimally maintain their primary functions [7]. In this context, the associated proteins with its synthesis, and uptake in tissues acquire great relevance. The genes involved to synthesize Tau (*csad*) and its transporter into the cells (*taut*) are responsible for the Tau tissue concentration[9]. Both genes might be a good indicator for the synthesis capacity and distribution into the body to determine the Tau requirement in a particular tissue and or species. Watson[9], revealed a Tau overexpression in the liver of sablefish (*Anoplopoma fimbria*) and cobia (*Rachycentron canadum*), but not in zebrafish (*Danio rerio*), when Tau was limited. The apparent discrepancy among fish species shows the high variation of synthesis capacity among fish, and the need to study the Tau synthesis capability in the different fish species. When a high synthesis capability is presented, it should be expected a decrease in the synthesis and uptake of Tau by the presence of sulphured amino acids precursors. The liver is considered the primary organ of Tau synthesis. Being the principal organ for the metabolism, higher than that reported in the kidney[10].

The kidney can also produce free Tau, synthesized and found in the glomerular filtrate and cells[11]. Whereas in the liver, the Tau is conjugated with cholesterol to produce the bile salts used as a fat emulsifier which is released in the proximal intestinal section[12]. When high amounts of bile salts are needed, they are usually recycled and re-absorbed in the distal intestine to return to the liver[13], ensuring that Tau levels in the body remain normal. However, carbohydrates in the form of soluble or insoluble fibres are an excellent source of adsorption of bile salts, which are lost through the faeces restricting recycling[14, 13]. Recently, it has been suggested that the microbiota is also responsible for the degradation of the bile salt in the intestinal tract preventing its recycling[14]. Therefore, the presence of carbohydrate could affect the Tau availability levels in

fish, and consequently, influencing the Tau needs in species regardless of their synthesis capability under a sulphur amino acids deficiency. The cholesterol is a compound with great relevance for survival in marine organisms, as well as growth[16]. When limited, the overall growth performance of carnivorous marine fish is restricted, such as in the yellowtail and turbot[17, 18]. However, the cholesterol elimination could cause hepatic disorders such as biliary cholestasis[19] with appalling repercussion over the nutrient absorption.

On the other hand, when Tau is supplemented in some marine fish, the growth rate increases[20]. Nevertheless, still not clear if the maintenance of taurine concentration through the whole-body tissues is directly responsible for growth. Therefore, the objective of this work was to investigate the Met and Tau effects on the overall growth performance of *T. macdonaldi*, and their Tau distribution through the different tissues and fatty acid deposition.

2. Materials and Methods

Feed formulation

Four isonitrogenous and isolipidic diets were formulated to contain different Met and Tau levels in a factorial design. Poultry by-product meal, soybean meal, fish meal, wheat gluten, and gelatine were chosen to formulate 50% crude protein (CP) diets, with a limited amount of Met and Tau in the control Basal diet as recommended for marine fish (NRC, 2011). The Basal diet (BD) was not supplemented at all; the second diet was supplemented with 1% methionine (MET), the third with 1% Taurine (TAU) and the fourth diet with both, methionine and taurine at 1% each (MET+TAU). The amount of Tau and Met supplemented were chosen as previously recommended (21, NRC, 2011). Lysine was supplemented with 0.5 % across dietary treatments to maintain a similar level than that previously reported by Badillo et al.[22] to maximize growth in totoaba. The final amino acid profile, as well as the proximate composition of diets, is presented in Table 1.

The pellets were prepared using a meat grinder after all the ingredients were thoroughly homogenized at 60% humidity. In summary, the starch and gelatine (as binders) were cooked separately, mixed with the other ingredients and blended to produce a homogeneous mixture. The resulting mixture in the form of dough was then cold-pressed through a meat grinder into 2.0 mm diameter strips, from which pellets were cut and dried at 60°C for 24 h. The primary lipid source of the diets was fish oil together with the fat present in some ingredients; the fatty acid profile is shown in Table 2.

Experimental design

A total of 144 juveniles of *Totoaba macdonaldi* donated by the Centro Reproductor de Especies Marinas del Estado de Sonora (CREMES) at Hermosillo, Sonora, México, were transported by road using one cubic meter container with aerated seawater. The juveniles were randomly distributed into 12 plastic tanks containing 500-L marine water (34 ppm; 12 fish per tank), with a total of four treatments with three repetitions each. The water was recirculated through a biofilter (PolyGeyser®; Pneumatic Drop Bead Filter model PG7 International Filter Solutions, TX, USA) coupled to a reservoir. During the feeding trial, the average water quality was 26 °C (± 1.0°C, using 6500 watts heater within a reservoir tank equipped with a sensor), 5 ppm of dissolved oxygen, 0.25 ppm of nitrites and 0 ppm of ammonia. Water loss due to cleaning and evaporation was daily replenished (approximately 5% of the total volume) maintaining the optimum water quality parameters. Before the experiment, the organisms were kept for 15 days for acclimatization and fed a commercial diet (Otohime C2, Marubeni Nisshin Feed Co., Ltd. Tokyo, Japan).

Each diet was randomly assigned to three tanks and fed by hand to apparent satiation four times a day (8:00, 11:00, 14:00 and 17:00 hrs.) for 60 days. Mean individual weight of fish from each experimental unit was determined at initial and day 60 (end of the experiment) by dividing the bulk weight by the total number of fish. The overall performance indices were then, determined according to the following calculations:

*% Weight gain = [(Final weight-Initial weight)*100/ Initial weight]* (1)

*Specific Growth Rate SGR = (ln final weight –ln initial weight)*100/t (in days)* (2)

*FI = Feed intake = (Daily feed intake by fish/ weight by fish)*100* (3)

FCR =Feed conversion ratio = (total amount of feed consumed/weight gain) (4)

Protein Efficiency Ratio PER = g body wet weight increase, / g protein ingested (5)

*TGC (Thermal Growth Coefficient) = [(final weight⅓−initial weight⅓)/(T°*Number of days)]*1000* (6)

*Cf (Condition factor) = (Final weight/Lenght^3)*100* (7)

At the end of the feeding trial, all organisms were humanely killed by hypothermia under the approved protocol from the Institution (UABC). Six fish per experimental unit were taken for proximate composition analysis and fatty acid profile. Besides, three more fish were used to sample liver and kidney tissues for the gene expression.

Proximate composition

The proximal composition of whole fish was estimated from three organisms per tank, which were macerated using a food processor. The samples were then stored in plastic bags at -30 C° until analysis. The feed was pulverized in a micro-pulveriser prior analysis. The moisture content of each sample was determined gravimetrically from the samples (2g) dried to constant weight at 60°C. Total nitrogen content was determined by the micro-Kjeldahl method, and percent crude protein was then calculated as % N x 6.25. Total lipid concentration was estimated using the Soxhlet extraction method with petroleum ether as a solvent, and the crude fat was gravimetrically calculated. Ash content was determined by heating the samples at 550°C for six h. The nitrogen-free extract was calculated by the difference of prior nutrients.

% NFE = 100 - (% crude protein + % total lipid + % ash) (8)

Fatty acid composition

Samples from the diets and fish muscle tissues from each experimental unit (n=3) were homogenized and lipids extracted using dichloromethane-methanol (2:1, v/v) following a modified method by Folch et al. (1957). One percent BHT was used as an antioxidant. After three extractions, the solvent supernatants were pooled, and water was added to separate the phases. The lower phase, containing the lipids (in CH_2Cl_2), was washed with water and then evaporated under N_2 in a pre-weighed vial. The amount of total crude fat was determined gravimetrically. Total lipids were saponified with methanolic solution KOH (0.3N in 90% methanol), dried under N_2 and

weighted using pre-weighed vials. The lipids were then esterified to form methyl esters of fatty acids (FAME) according to Christie (1982) using 14% Boron trifluoride in methanol. The FAME were preserved with 0.01% BHT to avoid oxidation. FAMEs were analysed in an Agilent GC 6850 gas chromatograph equipped with an injector split/splitless, a flame ionization detector (FID) and a capillary column (AGILENT 122-2362E DB-23; 60 m x 0.25 mm, film thickness 25 μm) using nitrogen as the carrier gas. Fatty acids were identified by comparing retention times to those of standards (37 Component FAME Mix, Supelco/Sigma-Aldrich; GLC 87, GLC 96, Nu-Chek Prep.; RM-2, RM-6 and GLC 90, Supelco/Sigma-Aldrich) and well-characterized profiles of samples of marine oils (PUFA1, Supelco/Sigma-Aldrich). Fatty acid profiles are reported as the percentage of FAMEs identified. The software HP ChemStation Data analysis Application model G17001EA E.02.00.493 for Windows was used. With the results of the fatty acid profile, the net fatty acid change as a difference between the initial and final content from each fatty acid as milligram was obtained using the following formula:

$$Net\ change = \frac{Final\ fatty\ acid\ content\ as\ mg}{Final\ weight\ as\ g} - \frac{Initial\ fatty\ acid\ content\ as\ mg}{Initial\ weight\ as\ g}$$

Cholesterol analysis

The cholesterol content analysis was estimated from the non-saponifiable portion of the total lipid extract after the modified Folch methodology (1957). Samples were silanized with hexamethyl-trimethyl pyridine, and then one μL of sample was injected into the Gas Chromatograph specify earlier for the fatty acids (Agilent GC 6850). A capillary column of 100% dimethylpolysiloxane, 30 m, 0.320 mm internal diameter, 0.25 μm film thickness was used. The cholesterol content in the tissues and diets was then calculated from a standard curve using pure Cholesterol at different concentrations.

Taurine content

Individual and independent tissue samples from the whole fish macerated were used for this purpose. In summary, 200 mg of dry samples were individually diluted in 1mL of 1M perchloric acid solutions. Samples were homogenized and centrifuged at 13000 rpm for 2 minutes. After that, the supernatant was transferred into a clean tube and neutralized adding an equal volume of 2M KOH solution. Then, a 50μL aliquot from the solution was used for the analysis.

A commercial kit (Taurine assay kit MET-5071 CELL BIOLABS, INC.) was used for the Tau quantification, containing taurine dioxygenase. The method is based on the enzyme reaction using α-ketoglutarate as the substrate; then, the reaction product (sulphite amino acetaldehyde) was then measured at 405 nm in spectrophotometry. A standard curve with increasing levels of Tau was implemented to calculate the Tau concentration in samples. The final concentration was reported as µmole Tau. Also, a net change of Tau was then calculated using a similar formula than that used for the fatty acids.

Amino acid composition of diets

Individual defatted diet samples were evaluated for AA content using a Waters HPLC equipped with a fluorescence detector (Waters 474 series, Milford, MA, USA). Samples of 10 mg were hydrolysed with 500 µL of 6N HCl containing 0.06% phenol in a closed vial and heated to 110°C for 24 h. Amino acid profiles were determined following Waters AccQ-Tag™ procedure. In summary, hydrolysed samples were dried in a thermal monoblock at 60°C, and one mL of distilled water was added to vials and filtered (0.45µm). Samples were then derivatized using the AccQ-Tag™ procedure. Samples were injected into a chromatograph equipped with a reverse-phase column (3.9 x 150 mm) 4-lm pore size AccQ-Tag™ C18 (Waters) using the water-acetonitrile gradient recommended by the Waters AccQ-Tag™ system. The fluorescence detector was programmed to an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Analyses were run at a constant temperature (37.5 ºC). HPLC signal calibration and standard curves were obtained with amino acid standard solutions containing 25 to 150 pmol of each amino acid, where taurine was added at a similar proportion.

Gene expression

Individual samples from liver and kidney tissues of each experimental unit were used for gene expression. Individually tissues were preserved in RNAlater (Ambion) during the dissection and processed for total RNA extraction using TRIZOL. RNA quantity and quality were measured by gel electrophoresis, and spectrophotometrically (Nanodrop® LITE, Thermo Fisher Scientific INC., Wilmington, USA). Only RNA samples with OD260nm-OD280nm ratios between 1.90 and 2.10 were used for expression quantification.

Total RNA (500 ng) was reverse-transcribed in a 20 μL reaction using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems; Carlsbad CA, USA) in a Verity 96 well thermal cycler (Applied Biosystems). The reverse transcription program consisted of 10 min at 25°C, 120 min at 37°C, 5 min at 85°C and finally kept at 4°C. qRT-PCR reactions were performed with one ng of cDNA, sense and antisense primers (200 nM each, indicated in Table 3) and SYBR® Select Master Mix (Applied Biosystems). The 18S rRNA was used as the internal reference gene. Reactions were conducted in 10 μL, in MicroAmp® Fast Optical 96-well reaction plates (Applied Biosystems) covered with MicroAmp® Optical Adhesive Film (Applied Biosystems).

The relative gene quantification from the liver and kidney tissues was calculated by the ΔΔCT method using an automated threshold and walking baseline for determining the CT values. The PCR conditions were: an initial denaturation and polymerase activation step during 10 min at 95°C; 40 cycles of denaturing for 15 s at 95°C, annealing and extension for 45 s at 60°C; and a final melting curve from 60°C to 95°C for 20 min to check for primer-dimer artifacts. Optimization of qRT-PCR conditions was made on primer annealing temperature (60°C), primer concentration (200 nM) and template concentration (five 1:10 dilution series from 10 ng to 100 ng of input RNA). Nucleotide sequences for 18S, *csad* and *taut*, are available in GenBank under accession numbers, **HM754483**, **KR072487**, and **HQ148721** respectively.

Statistical Analysis

A two-way ANOVA was used to evaluate the data and deemed significant at $P<0.05$ using the statistical software R. The Met and Tau (0 and 1%) levels were the main effects in the statistical model, and when significant changes were found a Tukey multiple comparison analysis ($P<0.05$) was performed. The statistical package lsmeans from the statistical software R was used to obtain the differences within treatments.

3. Results

For growth parameters, both Met, and Tau resulted in a positive effect ($P<0.05$). The two-way ANOVA indicated an independent influence of factors with no interaction (Table 4). Similarly, the feed intake increased by both factors ($P<0.05$). The feed conversion ratio was unaffected by the amino acid supplementation, but the protein efficiency ratio was enhanced by Met

supplementation *(P*<0.05) similar than that observed for the body condition factor (*P*<0.05). The viscerosomatic (VSI) and hepatosomatic index (HSI) was unaffected by the dietary treatments (Table 4).

The *igf-1* expression in the liver was only affected when both, Met and Tau were together (Figure 1). Increments that were not precisely similar than those observed with the somatic growth.

The Tau supplementation revealed a positive effect on the lipid deposition (*P* <0.05) in the whole fish, whereas the protein and ash remained constant (Table 5). However, the fatty acid profile in the muscle tissue was unaffected (Table 6 and 8), whereas changes were observed in the liver. Differences in the proportion of arachidonic acid (20:4n-6; ARA) and docosahexaenoic acid (22:6n-3; DHA) were found (P < 0.05), decreasing when Met was supplemented (Table 7). Regarding the overall hepatic fatty acid profile, a relative decrease in the DHA eicosapentaenoic acid (20:5n-3; EPA) ratio (DHA:EPA ratio) was observed related to Met. When the net gain was calculated, a corresponding increase in saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) was influenced by the presence of Tau, while when Met was supplemented alone resulted in increased levels of monounsaturated fatty acids (MUFA) represented by oleic (18:1n-9) and vaccenic (18:1n-7) acids mainly (Table 11).

The total cholesterol and the relative cholesterol content to the total lipids in the whole fish, significantly decreased when Tau was supplemented (Table 9). On the other hand, the total cholesterol in the liver increased when only Met was supplemented (P<0.05), whereas Tau alone affected the cholesterol concentration related to total fat (P<0.05; Table 10).

The expression of *taut* in the liver tissue resulted in significant interaction with both factors. When Tau or Met was supplemented, an overexpression was estimated. However, when both were present, no higher *taut* expression was registered (P<0.05; Figure 2). In the kidney, the *taut* expression was not influenced by Met or Tau supplementation (Figure 4). The expression of *csad* in the liver tissue was affected by both factors. Whereas in absence, an overexpression was revealed (P<0.05; Figure 3). In contrast, the *csad* expression in the kidney was changed only when higher levels of Tau were present (P<0.05; Figure 5).

The taurine content estimated in the whole fish samples, significantly increased was detected, only when taurine was supplemented (P<0.05), and accordingly the net gain resulted in accumulation (Table 12).

4. Discussion

In the present work, it was demonstrated the role of Met and Tau on the overall performance of totoaba. Both amino acids revealed that could positively affect the growth rate increasing when one or another were supplemented. However, when both were present an even higher growth rate was observed (Table 4) revealing a synergetic, but independent effect. However, when Met was supplemented alone, the expression of the *csda* to synthesize Tau from Met decrease compared to the BS, while the amounts of Tau found in tissues from the whole body was lower than desired, resulting in a negative net change (Table 13). Whereas a higher amount of Tau was found in both cases were Tau was supplemented (TAU, MET+TAU treatments).

All diets were formulated from a Basal diet where Met and Tau were provided in restricted concentrations than that required for the majority of fish carnivore species (25). Whereas two of the experimental diets contained an extra amount of Met and Tau (1% each) and the third diet contained both, (Met and Tau) at 1% each. This manuscript did not intend to find the requirement level of the tested amino acids, while the role of both within growth and metabolism was studied. Nevertheless, with the purpose to provide sufficient precursor and to reach a taurine level to maximize whole body concentration a 1% was chosen besides that found in the BD. Also, the amount of total crude protein was kept as previously recommended for this species (26). Table 1 shows the amino acid profile of diets where it can be appreciated that in general, all diets contained a similar amount of AA unless those used to enrich the experimental diets. While Cys, another sulphur AA as Met, considered an active precursor of Tau was not detected in the analysis, we should then recognize that this AA was found at a similar concentration across all diets without affecting our results.

Most fish nutrition studies within the scope of the present research are focused on Tau requirement and its functionality on growth performance and welfare (27, 28, 29) without estimating the fish species synthesis capacity. While, Satriyo (21) established a Tau requirement for totoaba, based on the growth response when present at different levels, in this work we demonstrated the taurine dependency on its physiological needs versus their synthetic capacity and the availability of its precursors. In general, it is assumed that Tau is a non-essential nutrient, meaning that its synthesis pathway exists in the majority of vertebrate animals (4). Being a sulphur amino acid derivative, it will require the degradation of other sulphur amino acids for their synthesis (4). Met is a sulphur AA with several essential metabolic functions considered as limiting

for growth. However, here it is demonstrated that when Tau is restricted the presence of Met cannot solve their roles in totoaba, even if the gene expression of *csda* decrease. Dissimilar than that reported in other fish species such as tilapia (30) where the synthesis capacity was demonstrated. Tau is used for several physiological processes in the body, including synthesis of bile salts (16), in neurotransmission, osmoregulation, among others (2). The reason for the higher growth rate obtained when only Tau was supplemented it could be due to the Tau physiological properties and the increase in the feed intake (Table 5). Tau has also been previously related to growth (31) but as here it was demonstrated that it cannot be associated with the precursor concentrations due to the reduced capacity to maintain the taurine concentration in tissues. The latter is in contradiction to earlier reports (6,31), that mention that when an organism cannot preserve the taurine concentration in tissues, growth would be affected. Here the growth increase by taurine supplementation appears to be different from Met supplementation; resulting in higher lipid deposition with a lower PER. The fact that MET treatment resulted in a significantly higher PER than that observed with BD and TAU treatments could be attributable to its importance as proteogenic amino acid (32). Also, when both are supplemented (MET+TAU) an even significantly higher PER was noticed corresponding to higher growth, but also significantly higher lipid deposition, without affecting the VSI or HSI. Tau benefits in its supplementation could be related to their various physiological roles in the body, especially its involvement as bile salts precursor, which is in accordance with earlier reports (33).

As a precursor of bile salts, the Tau has a direct effect on the cholesterol excretion via Na-taurocholate salts (Table 10). The cholesterol content in the whole-body totoaba resulted in a significant reduction when Tau was supplemented as previously reported (34). However, in liver tissue, this reduction was not that evident. Nevertheless, when Met was supplemented alone, the cholesterol as mg/g tissue increased significantly compared to the BD. This result is in accordance to previous results observed in rats where a higher amount of cholesterol was detected when Met was offered in excess, associated to the presence of the 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol-7a-hydroxylase expression (35); enzymes that are essential in the novo synthesis of cholesterol.

Regarding the fatty acid profiles obtained in the whole fish fed the different dietary treatments, no differences were observed, while only the DHA:EPA ratio was affected, being significantly lower when both, Met and Tau were present (Table 7). This result could be due to the higher growth rate

and therefore a higher dilution in the tissue since DHA is generally accumulated (36). Whereas in the liver tissue, significant differences were also observed in ARA and DHA values, both being significantly lower when Met was supplemented, alone or together with Tau. These differences could be due to a fat mobilization as a consequence of the S-adenosyl methionine (SAM) activity over the ratio of phosphatidylcholine and phosphatidylethanolamine (37); actions that could affect the lipoproteins conformation (36).

Moreover, calculating the net change of fatty acids (Table 12), as the difference from those present at the beginning and the final amount in tissues, differential retention of fatty acids is observed, possibly influenced by Met and Tau supplementation. When Met and Tau are present, a significant increase in the oleic acid was found, a difference that is even higher when both are present. Whereas, an accumulation of the vaccenic acid (n7) is observed, only when Met was supplemented alone in the diet. The net changes in MUFA as an accumulation when Met and Tau were supplemented alone compared to the BD treatment could be associated to a preferential substrate shift in the β-oxidation pathway involving these fatty acids (36). Additional net changes differences in other fatty acids were noticed (Table 12), however there is a lack of information on this topic in fish nutrition. Insights from mammals research might be valuable and clearly there is need for future research to clarify this matter (38,39).

Regarding the relative expression of IGF-1 in the liver, significantly higher values were revealed when Met and Tau were supplemented together. Whereas under the presence of Tau or Met, the result failed to show differences. The latter could be due to the Met involvement in growth only when Tau was also present. The effect of Tau in the production of liver *IGF-1* has been already reported in some fish species (28), but the physiological process remains unclear. On the other hand, the effect of Met over the liver *IGF-1* expression has been thoroughly described by Rolland (40), regarding the liver IGF-1 expression, is related to an increase in liver GH receptor with different levels of Met in Rainbow trout.

Regarding the Tau synthesis and excretion, the liver and kidney (4) are the primary organs involved. Here it was revealed that in both organs there is Tau synthesis as shown by the expression of the *csda*, being significantly higher in the liver when no Met and Tau are added. However, when Met and Tau are supplemented alone or together, the expression decreased. Meaning that Tau and Met supplementation could be necessary to regulate the taurine concentration in the liver, and at least in the case of liver tissue, Met could help on the Tau demand. However, in the kidney, the

csad expression was only significantly higher when Tau was restricted. These differences show that Tau synthesis is tissue-specific like previously reported in other animals (41). Regarding other fish species, the *csad* shows a similar response than that previously observed in some carnivore fish species to taurine content (9) where only the presence of dietary Tau diminishes the csad expression.

The *TauT* expression in the liver relative to the Tau transport through the body was significantly lower only when Tau were supplemented alone, and the extra supplementation of Met recompense the *TauT* expression. They are explaining the need to export Tau from the liver up to certain level. Whereas in the kidney, no significant differences were registered in *TauT* expression. As such, the latter could be related to the need to increase the Tau transport when it is restricted as it has also been documented in several animals including some fish species (3, 9).

In summary, Met and Tau are limiting amino acids or its derivatives in totoaba, with differentiated function as modulators of growth and lipid metabolism, with a significant influence in the cholesterol content and fatty acid mobilization. Furthermore, the *csad* expression could be used as an indicator of Tau tissue requirement, being mainly responsive to dietary taurine like other carnivorous fishes.

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Figure captions

Fig. 1 Relative gene expression of liver *igf-1* in juveniles of *Totoaba macdonaldi* fed with diets supplemented with taurine and/or methionine, the values are the mean and standard deviation. Different letters mean significant differences by Tuckey multiple contrast ($p<0.05$)

Fig. 2 Relative gene expression of liver Taurine transporter (*taut*) in juveniles of *Totoaba macdonaldi* fed with diets supplemented with taurine and/or methionine, the values are the mean and standard deviation. Different letters mean significant differences by Tuckey multiple contrast ($p<0.05$)

Fig. 3 Relative gene expression of liver cysteine sulphinic acid descarboxylase (*csad*) in juveniles of *Totoaba macdonaldi* fed with diets supplemented with taurine and/or methionine, the values are the mean and standard deviation. Different letters mean significant differences by Tuckey multiple contrast ($p<0.05$)

Fig. 4 Relative gene expression of kidney Taurine transporter (*taut*) in juveniles of *Totoaba macdonaldi* fed with diets supplemented with taurine and/or methionine.

Fig. 5 Relative gene expression of kidney cysteine sulphinic acid descarboxylase (*csad*) in juveniles of *Totoaba macdonaldi* fed with diets supplemented with taurine and/or methionine, the values are the mean and standard deviation. Different letters mean significant differences represent significant differences by two-way ANOVA analysis ($p<0.05$)

References

1 Hardy, R.W., 2008. Farmed fish diet requirements for the next decade and implications for global availability of nutrients. In: Lim, C., Webster, C.D., Lee, C.-S., (Eds.), Alternative Protein Sources in Aquaculture Diets. Boca Raton: CRC Press, New York, NY, pp. 1–15.

2 Burini, R.C., Borges-Santos, M.D., Moreto, F., Ming-Yu, Y., 2018. Taurine and oxidative stress in HIV. In: HIV/AIDS Oxidative Stress Diet Antioxidants (Preedy, V.R., Watson, R.R. Eds). 169-182 pp. doi:10.1016/B978-0-12-809853-0.00015-8.

3 Voss, J.W., Pedersen, S.F., Christensen, S.T., Lambert, I.H., 2004. Regulation of the expression and subcellular localization of the taurine transporter TauT in mouse NIH3T3 fibroblasts. Eur. J. Biochem. 271, 4646–4658.

4 Hayes, K.C., Sturman, J. A., 1981. Taurine in metabolism. Annu. Rev. Nutr. 1, 401–425. doi:10.1146/annurev.nu.01.070181.002153.

5 Daniels, K.M. and Stipanuk, M.H. 1982. The effect of dietary cysteine level on cysteine metabolism in rats. J. Nutr. 11 2:2130-2141.

6 Berson, E. L., Hayes, K. C., Rabin, A. R., Schmidt, S. Y., Watson, G., 1976. Retinal degeneration in cats fed casein. II. Supplementation with methionine, cysteine, or taurine. Invest. Ophthalmol. 15, 52-58.

7 Park, G.S., Takeuchi, T., Yokoyama, M., Seikai, T., 2002. Optimal dietary taurine level for growth of juvenile Japanese flounder Paralichthys olivaceus. Fish. Sci. 68, 824–829. doi:10.1046/j.1444-2906.2002.00498.

8 Espe, M., Hevrøy, E.M., Liaset, B., Lemme, A., El-mowafi, A., Ag, D., Chaussee, R., Wolfgang, D.-H., 2008. Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, Salmo salar. Aquaculture 274, 132–141. doi:10.1016/j.aquaculture.2007.10.051.

9 Watson, A.M., 2013. Taurine: An indispensable ingredient in the development of sustainable aquafeeds. Thesis, University of Maryland. 289p.

10 Chesney, R.W., Patters, A.B., Han, X., 2012. Taurine and the kidneys. In: Idrissi, E.A., L'Amoreaux, W. (Eds.), Taurine and Health Disease. Transworld Research Network, Kerala, India. ISBN: 978-81-7895-520-9.

11 Salze, G.P., Davis, D.A., 2015. Taurine : a critical nutrient for future fish feeds. Aquaculture 437, 215–229. doi:10.1016/j.aquaculture.2014.12.006.

12 Zhou, X., Levin, E.J., Pan, Y., Mccoy, J.G., Sharma, R., Kloss, B., Bruni, R., Quick, M., Zhou, M., 2014. Structural basis of alternating-accsses mechanism in a bile acid transporter. Nature 12811, 1-17. doi:10.1038/nature12811.

13 Kelley, M.J., Ph, D.I., Thomas, J.N., Ph, D., Story, J.A., Ph, D., 1992. Changes in cholesterol accumulation and steroid excretion in response to cellulose, alfalfa or oats in choresterol fed rats, Nutr. Res. 12, 509–518.

14 Horáčkova, Š., Plocková, M., 2017. Importance of microbial defense systems to bile salts and mechanisms of serum cholesterol reduction. Biotechnol. Adv. 36, 682-690. doi:10.1016/j.biotechadv.2017.12.005.

15 Barreto-Curiel, F., Ramirez-Puebla, S.T., Ringø, E., Escobar-Zepeda, A., Godoy-Lozano, E., Vazquez-Duhalt, R., Sanchez-Flores, A., Viana, M.T., 2018. Effects of extruded aquafeed on growth performance and gut microbiome of juvenile Totoaba macdonaldi. Anim. Feed Sci. Technol. 245, 91–103. doi:10.1016/j.anifeedsci.2018.09.002

16 Twibell, R.G., Wilson, R.P., 2004. Preliminary evidence that cholesterol improves growth and feed intake of soybean meal-based diets in aquaria studies with juvenile channel catfish, Ictalurus punctatus. Aquaculture 236, 539–546.

17 Yun, B., Ai, Q., Mai, K., Xu, W., Qi, G., Luo, Y., 2012. Synergistic effects of dietary cholesterol and taurine on growth performance and cholesterol metabolism in juvenile turbot (Scophthalmus maximus L.) fed high plant protein diets. Aquaculture 324–325, 85–91. doi:10.1016/j.aquaculture.2011.10.012.

18 Guerra-Olvera, F., Viana, M.T., 2015. Effect of dietary cholesterol content on growth and its accumulation in liver and muscle tissues of juvenile yellowtail kingfish (Seriola lalandi). Cienc. Mar. 41, 143–156.

19 Nemes, K., Åberg, F., Gylling, H., Isoniemi, H., 2016. Cholesterol metabolism in cholestatic liver disease and liver transplantation: From molecular mechanisms to clinical implications. World J. Hepatol. 8, 924–932. doi:10.4254/wjh.v8.i22.924

20 Lunger, A.N., McLean, E., Gaylord, T.G., Kuhn, D., Craig, S.R., 2007. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (Rachycentron canadum). Aquaculture 271, 401–410. doi:10.1016/j.aquaculture.2007.07.006.

21 Satriyo T. B., Galaviz M. A., Salze G., López L. M., , 2017. Assessment of dietary taurine essentiality on the physiological state of juvenile Totoaba macdonaldi. Aquacult. Res. 48, 5677-5689. doi:10.1111/are.13391.

22 Zapata, D.B., Lazo, J.P., Herzka, S.Z., Viana, M.T., 2016. The effect of substituting fishmeal with poultry by-product meal in diets for Totoaba macdonaldi juveniles. Aquac. Res. 47, 1778–1789. doi:10.1111/are.1263

23 Madrid, J., Pohlenz, C., Viana, M.T., Lazo, J.P., 2019. Dietary lysine requirement for juvenile, Totoaba macdonaldi. Aquaculture 500, 92–98. doi:10.1016/j.aquaculture.2018.10.003

24 Rollin, X., De Saeger, S., Larondelle, Y., Abboudi, T., Bodin, N., Govaerts, B., Detavernier, C., 2009. Protein level affects the relative lysine requirement of growing rainbow trout (Oncorhynchus mykiss) fry. Br. J. Nutr. 102, 37. doi:10.1017/s0007114508158986

25 National Research Council. 2011. Nutrient Requirements of Fish and Shrimp. Washington, DC: The National Academies Press. https://doi.org/10.17226/13039.

26 Rueda-López, S., Lazo, J.P., Correa-Reyes, G., Viana,M.T., 2011. Effect of dietary protein and energy levels on growth, survival and body composition of juvenile Totoaba macdonaldi. Aquaculture 319, 385–390.

27 Aguillón-Hernández, O.E., Hernández-Hernández, L.H., Shimada-Miyasaka, A., Garduño-Lugo, M., 2017. Effects of diets with whole plant-origin proteins added different ratios of taurine:methionine on the growth, macrophage activity and antioxidant capacity of rainbow trout (Oncorhynchus mykiss) fingerlings. Vet. Anim. Sci. 3, 4-9. doi:dx.doi.org/10.1016/j.vas.2017.04.002.

28 Kim, J., Malintha, G.H.T., Gunathilaka, G.L.B.E., Lee, C., Kim, M., Lee, B., Kim, J., Lee, K., 2017. Taurine supplementation in diet for olive flounder at low water temperature 1–8. doi:10.1186/s41240-017-0065-9

29 Gaylord, T., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E., Shepherd, B., 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (Oncorhynchus mykiss). Aquaculture 269, 514–524. doi:10.1016/j.aquaculture.2007.04.011.

30 Michelato, M., Furuya, W.M., Gatlin, D.M., 2018. Metabolic responses of Nile tilapia Oreochromis niloticus to methionine and taurine supplementation. Aquaculture 485, 66–72. doi:10.1016/j.aquaculture.2017.11.003

31 Ito, T., Yoshikawa, N., Ito, H., Schaffer, S.W., 2015. Impact of taurine depletion on glucose control and insulin secretion in mice. J. Pharmacol. Sci. 129, 59–64. doi:10.1016/j.jphs.2015.08.007.

32 Gatlin, D.M., 2002. Food Intake in Fish, Aquaculture. doi:10.1016/S0044-8486(02)00066-2

33 Gu, M., Bai, N., Kortner, T.M., 2017. Taurocholate supplementation attenuates the changes in growth performance, feed utilization, lipid digestion, liver abnormality and sterol metabolism in turbot

(Scophthalmus maximus) fed high level of plant protein. Aquaculture 468, 597–604. doi:10.1016/j.aquaculture.2016.11.022.

34 Yokogoshi, H., Mochizuki, H., Nanami, K., Hida, Y., Miyachi, F., Oda, H., 1999. Nutrient Interactions and Toxicity-Dietary Taurine Enhances Cholesterol Degradation and Reduces Serum and Liver Cholesterol Concentrations in Rats Fed a High-Cholesterol Diet. J. Nutr. 129, 1705–1712.

35 Hirche, F., Schröder, A., Knoth, B., Stangl, G.I., Eder, K., 2006. Effect of dietary methionine on plasma and liver cholesterol concentrations in rats and expression of hepatic genes involved in cholesterol metabolism. Br. J. Nutr. 95, 879–888. doi:10.1079/BJN20061729.

36 Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. Rev. Fish. Sci. 11, 107–184.

37 Poloni, S., Blom, H., Schwartz, I., 2015. Stearoyl-CoA Desaturase-1: Is It the Link between Sulfur Amino Acids and Lipid Metabolism? Biology (Basel). 4(2), 383–396. doi:10.3390/biology4020383.

38 Oda, H., 2006. Functions of sulfur-containing amino acids in lipid metabolism. The J. Nut "The Fith Workshop on the Assessment of Adequate Intake of Dietary Amino Acids". American Society for Nutrition. 1666S-1669S.

39 Toohey, J.I., 2014. Sulfur amino acids in diet-induced fatty liver: a new perspective based on recent findings. Molecules/ 19, 8334-8349.

40 Tall, A.R., 1993. Plasma cholesteryl ester transfer protein. J. Lipid Res. 34(8), 1255–1274.

41 Rolland, M., Dalsgaard, J., Holm, J., Gómez-Requeni, P., Skov, P. V., 2015. Dietary methionine level affects growth performance and hepatic gene expression of GH-IGF system and protein turnover regulators in rainbow trout (Oncorhynchus mykiss) fed plant protein-based diets. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 181, 33–41. doi:10.1016/j.cbpb.2014.11.009.

42 Park T., Rogers Q. R., 1999. Hepatic and Renal cysteine Sulfinic Acid Decarboxylase Activities in Cats Fed Different Levels of Dietary Protein and Taurine. J. Food Sci. Nutr. 4, 47-51.

Table 1. Dietary formulation given as g/Kg, and proximate composition of experimental diets fed to *Totoaba macdonaldi* juveniles. Diets were supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine.

Ingredients	**Treatments**			
	BASAL DIET	**MET**	**TAU**	**MET+TAU**
Poultry by-products meal[a]	237.0	232.0	232.0	229.0
Sardine fish meal[b]	96.0	94.0	94.0	93.0
Gelatin[c]	63.0	61.0	61.0	61.0
Soybean concentrate[d]	248.0	243.0	243.0	240.0
Wheat gluten	50.0	49.0	49.0	48.0
Sardine fish oil[e]	60.0	60.0	60.0	60.0
Methionine[f]	0.0	10.0	0.0	10.0
Taurine[f]	0.0	0.0	10.0	10.0
Corn starch	211.0	215.0	215.0	213.0
Rovimix[g]	25.0	25.0	25.0	25.0
Stay C[g]	2.0	2.0	2.0	2.0
Lysine[h]	5.0	5.0	5.0	5.0
Sodium benzoate	2.0	2.0	2.0	2.0
BHT	0.1	0.1	0.1	0.1
Proximate composition in percentage				
Crude protein	51.2	49.9	51.4	49.4
Crude fat	10.6	10.2	10.2	9.7
Ash	7.7	7.7	7.7	7.6

[a] National Renderers Association, USA

[b] Proteínas marinas y agropecuarias S.A. de C.V. Guadalajara, Jalisco, Mexico

[c] Órganos y semillas San Miguel, Ensenada, Baja California, Mexico

[d] 63% CP; HP300, Nutrivance nutrition advance Midwest-Ag Enterprises, Inc. Minnesota, USA

[e] 68% CP; Monterrey sardine, Mexico

[f] Future foods materias primas, Tlalnepantla, Estado de México, Mexico

[g] DSM nutritional products México S.A. de C.V., Mexico

[h] ADM, Mexico

Table 2. Amino acid profile from experimental diets fed to *Totoaba macdonaldi* juveniles. Diets were supplemented with methionine, taurine and both compare to a Basal diet with a restrictive amount of methionine and taurine.

		Treatments		
Amino acids	**BASAL DIET**	**MET**	**TAU**	**MET+TAU**
ASP	4.27	4.39	4.71	4.22
SER	2.36	2.52	2.57	2.32
GLU	8.85	8.58	9.06	8.06
GLY	4.58	4.35	4.48	4.29
HIS	0.84	0.97	0.86	0.90
ARG	3.30	3.92	3.34	3.26
THR	1.93	1.81	1.71	1.77
ALA	2.94	2.77	2.81	2.75
PRO	4.43	4.10	4.40	4.11
VAL	2.54	2.39	2.59	2.36
MET	**0.80**	**1.51**	**0.65**	**1.97**
TAU	**0.26**	**0.17**	**1.05**	**1.05**
LYS	4.07	3.79	3.80	3.78
ILE	2.37	2.24	2.34	2.11
LEU	4.03	3.95	4.10	3.69
PHE	2.30	2.29	2.23	2.29

Table 3. Dietary fatty acids composition as g/100 g of fat from experimental diets fed to *Totoaba macdonaldi* juveniles. Diets were supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine.

Fatty Acids	BASAL DIET	MET	TAU	MET + TAU
14:0	3.89	3.55	3.73	3.6
16:0	21.68	21.9	22.25	25.4
18:0	2.69	3.54	1.94	2.66
SFAs[a]	28.26.	28.99	27.92	31.67
16:1n-7	8.45	10.46	8.18	8.24
18:1n-9	26.84	23.45	27.46	26.63
18:1n-7	1.5	1.23	1.44	1.4
MUFAs[b]	36.79	35.14	37.08	36.27
18:2n-6	13.34	13.42	13.58	13.19
20:4n-6	2.23	2.54	2.22	2.49
n-6[c]	15.57	15.96	15.8	15.68
20:5n-3	7.97	6.91	6.79	6.93
22:5n-3	0.51	0.68	0.47	0.56
22:6n-3	8.11	6.53	7.09	7.47
n-3[d]	16.59	14.12	14,35	14.96
PUFAs[e]	32.16	30.08	30.15	30.64
C_{18} PUFAs[f]	14.32	14.66	14.64	15.54
LC-PUFAs[g]	18.82	16.66	14,35	17.45
n-3:n-6	1.18	1.008	1.01	0.98
DHA:EPA	1.01	0.94	1.04	1.07
Lipid content[h]	10.6	10.2	10.2	9.7

[a]Saturated fatty acids

[b] Monounsaturated fatty acids

[c] n-3 fatty acids

[d] n-6 fatty acids

[e] Polyunsaturated fatty acids

[f] C_{18} Polyunsaturated fatty acids – sum of all PUFA; includes linolenic acid in addition to individually reported PUFA

[g] Long-chain Polyunsaturated fatty acids – sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds

Table 4. Sequences of the primer pairs used for q-PCR, amplicon size in base pairs (bp), reaction efficiencies (E) and Pearson's coefficient of determination (R^2).

Gene (*symbol*)	**Fwd sequence (5' - 3')**	**Rev sequence (5' - 3')**	**Size (bp)**	**E**	**R^2**
18s	CGGTTCTATTTTGTGGGTTTTC	CTTTCGCTTTCGTCCGTCTT	126	0.98	1.00
Igf1	TCCTGTAGCCACACCCTCTC	GGCCATAGCCTGTTGGTTTA	163	0.97	0.99
taut	TATCATGCTGCTGCTTCTGG	CACATACATGCCACCTTTCG	187	0.98	0.96
csad	TCGCCAAGTACAGCATCAAG	GCAGTCTCCATCAGCACAAA	160	0.95	0.92

Table 5. Production performance of juvenile *Totoaba macdonaldi* fed with diets supplemented with methionine, taurine and both compare to a Basal diet with a restrictive amount of methionine and taurine. Values represent the average ± standard deviation (n=3); SE values and *P* values resulting from two-way ANOVA tests are also provided; means with different letters represent significant differences ($P < 0.05$).

										Methionine	***Taurine***	***Interaction***
Biological Index	**BASAL DIET**	**SD**	**MET**	**SD**	**TAU**	**SD**	**MET + TAU**	**SD**	**PSE**		***P values***	
Initial weight (g)	42.77	1.27	41.66	0.48	41.66	1.1	41.38	1.44	1.3	0.2598	0.259	0.52
Final weight (g)	91.88	6.8^{c}	108.5	7.3^{b}	108.6	4.8^{b}	138.0	1.3^{a}	6.49	P<0.01	P<0.01	0.08
Weight gain (g)	49.11	6.9^{c}	66.92	4.6^{b}	66.94	1.3^{b}	96.64	8^{a}	6.7	P<0.01	P<0.01	0.11
Weight gain (%)	114.91	17.1^{c}	160.9	10.1^{b}	160.6	3^{b}	233.4	22.6^{a}	17.5	P<0.01	P<0.01	0.16
FC	1.30	0.35	1.03	0.01	1.12	0.03	0.97	0.06	0.20	0.0813	0.2751	0.58
TGC	0.58	0.06^{c}	0.75	0.03^{b}	0.75	0.01^{b}	0.99	0.07^{a}	0.06	P<0.01	P<0.01	0.30
SGR (%)	1.39	0.14^{c}	1.71	0.05^{b}	1.71	0.02^{b}	2.18	0.15^{a}	0.12	P<0.01	P<0.01	0.52
FI g/fish	58.3	0.8^{c}	63	3.9^{b}	72	5.3^{b}	88.5	4.43^{a}	2.5	P<0.01	P<0.01	0.22
PER	1.56	0.34^{a}	1.93	0.12^{b}	1.74	0.11^{a}	2.08	0.02^{b}	0.21	0.011	0.17	0.91
Cf	1.53	0.05^{a}	1.71	0.05^{b}	1.61	0.05^{a}	1.72	0.01^{b}	0.05	P<0.01	0.1301	0.23
VSI	3.23	0.27	3.03	0.29	3.19	0.44	3.18	0.17	0.36	0.5828	0.7735	0.60

HSI	1.09	0.17	1.13	0.28	1.01	0.29	1.21	0.12	0.26	0.3814	0.9909	0.55

TGC; Thermic growth coefficient
SGR; Specific growth rate
FI; Feed intake
PER; Protein Efficiency Ratio
Cf; Condition factor
VSI; Viscerosomatic Index
HIS; Hepatosomatic Index
FC; Feed conversion

Table 6. Proximate composition of whole fish *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values represent the average ± standard deviation (n=3); pooled SE (PSE) values and *P* values resulting from two-way ANOVA tests are also provided; different letters represent significant differences ($P < 0.05$).

	Treatments													
%	Initial		Basal Diet		MET		TAU		MET+TAU		**PSE**	***P Values***		
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD		Met	Tau	Interaction
CP	56.7	1.1	60.5	1.9	61.2	0.8	58.8	2.2	59.9	1.5	2.0	0.44	0.23	0.88
CL	10.9	0.8	10.2	0.3^{b}	10.1	1.2^{b}	11.8	1.7^{a}	12.8	1^{a}	1.3	0.54	0.01	0.53
Ash	14.3	1.0	14.8	1.8	14.3	0.9	15.1	1.1	14.5	1.1	1.5	0.10	0.48	0.98

CP, crude protein; CL, crude lipids

Table 7. Whole fish fatty acid profile as g/100 g of fat in *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values represent the average± standard deviation (n=3); pooled SE (PSE) values and P values resulting from two-way ANOVA tests are also provided; different letters represent significant differences ($P < 0.05$).

	Treatments											
Fatty Acids	Basal Diet		MET		TAU		MET+TAU		**PSE**	***P Values***		
	Av	SD	Av	SD	Av	SD	Av	SD		***Methionine***	***Taurine***	***Interaction***
14:0	3.82	0.28	3.60	0.21	3.80	0.18	3.56	0.2	0.25	0.16	0.88	0.64
16:0	19.49	1.2	17.55	1.2	17.35	1.6	19.19	2.4	1.9	0.92	0.15	0.88
18:0	7.17	0.14	3.15	0.18	4.57	0.1	4.42	0.09	0.15	0.69	0.84	0.95
SFAs[a]	30.48	1.4	24.30	1.6	25.72	2.4	27.17	2.3	2.3	0.80	0.14	0.85
16:1n-7	8.17	0.38	11.45	2.5	10.57	1.5	10.09	2.8	2.3	0.29	0.14	0.63
18:1n-9	20.05	0.8	24.90	2.5	22.33	1.9	24.52	4.5	3.2	0.30	0.21	0.57
18:1n-7	2.43	1.6	3.39	6.1	2.71	1.7	2.98	6.4	5.3	0.19	0.45	0.63
MUFAs[b]	30.65	0.33	39.74	6.2	35.60	1.9	37.59	3.8	4.3	0.17	0.49	0.8
18:2n-6	10.39	0.2	10.93	0.6	10.66	0.6	10.90	1.1	0.84	0.54	0.54	0.60
20:4n-6	2.54	0.17	2.43	0.4	2.55	0.3	2.14	0.3	0.38	0.11	0.99	0.80
n-6[c]	12.93	0.5	13.36	1.6	13.21	0.4	13.04	0.6	0.4	0.86	0.96	0.75
20:5n-3	6.96	0.14	7.40	2	8.07	1	6.68	0.2	1.34	0.52	0.22	0.80
22:5n-3	2.24	0.22	1.61	1	1.66	0.4	1.21	1.6	1.12	0.39	0.83	0.38
22:6n-3	9.56	0.26	8.16	2.7	9.96	1.9	8.97	0.9	2.0	0.24	0.89	0.52

n-3[d]	21.14	0.7	18.82	5.4	21.51	2.6	19.33	2.6	3.8	0.27	0.82	0.97
PUFAs[e]	36.78	1.2	33.49	6.8	36.77	3.1	34.18	2.9	4.7	0.24	0.89	0.88
C_{18} PUFAs[f]	12.73	0.7	12.71	0.7	12.48	0.1	13.02	0.1	0.83	0.55	0.94	0.53
LC-PUFAs[g]	21.98	0.7	19.5	5.4	22.68	3.2	19.85	2.7	4.03	0.25	0.81	0.93
n-3:n-6	1.50	0.02	1.36	0.25	1.55	0.1	1.36	0.1	0.19	0.15	0.81	0.85
DHA:EPA	1.37	0.02[a]	1.33	0.07[a]	1.25	0.1[b]	1.09	0.04[b]	0.07	0.06	0.005	0.20
Lipid content (%)	10.2	0.28[a]	10.1	1[a]	11.8	1.7[b]	12.8	1[b]	1.30	0.540	0.013	0.53

[a]Saturated fatty acids

[b] Mono-unsaturated fatty acids

[c] n-3 fatty acids

[d] n-6 fatty acids

[e] Poly-unsaturated fatty acids

[f] C-18 Poly-unsaturated fatty acids– sum of all PUFA; includes linolenic acid in addition to individually reported PUFA

[g] Long chain Poly-unsaturated fatty acids

Table 8. Fatty acid profile in liver tissue of *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values represent the average± standard deviation (n=3); pooled SE (PSE) values and P values resulting from two-way ANOVA tests are also provided; different letters represent significant differences ($P < 0.05$).

										Methionine	*Taurine*	*Interaction*
Fatty Acids	**BASAL DIET**	**SD**	**MET**	**SD**	**TAU**	**SD**	**MET + TAU**	**SD**	**PSE**	***P Values***		
14:0	2.07	0.1	2.74	0.6	2.24	0.2	3.11	1.1	0.79	0.08	0.52	0.80
16:0	20.66	2.4	17.92	1	19.82	2.4	20.36	2.7	2.61	0.42	0.55	0.24
18:0	6.31	0.3	5.01	0.2	4.79	0.09	5.49	0.1	0.24	0.41	0.64	0.37
SFAs[a]	29.03	2.1	25.67	1.9	26.85	2.5	28.95	3.5	3.02	0.81	0.47	0.32
16:1n-7	9.50	1.3	11.77	2.7	10.78	1.5	11.27	2.5	2.33	0.31	0.77	0.51
18:1n-9	17.64	1	18.05	1.6	17.44	1.4	18.84	0.9	1.25	0.64	0.43	0.15
18:1n-7	2.03	1.3	2.43	3.2	2.06	1.2	2.01	1	2.20	0.55	0.65	0.79
MUFAs[b]	29.17	2.6	32.25	0.24	30.28	2.5	32.12	2.1	2.17	0.07	0.69	0.78
18:2n-6	10.57	0.7	10.43	0.7	10.72	1.1	10.68	1	1.11	0.87	0.72	0.93
20:4n-6	4.23	0.4[a]	2.86	0.2[b]	3.60	0.4[a]	2.62	0.1[b]	0.41	P<0.01	0.06	0.38
n-6[c]	14.80	0.4	13.29	1	14.32	1.6	13.29	1.2	1.36	0.28	0.80	0.87
20:5n-3	5.61	1.5	7.60	1.4	6.40	1.5	7.33	1.6	1.79	0.14	0.77	0.57
22:5n-3	2.39	0.3	2.39	0.8	2.08	0.1	2.17	0.4	0.57	0.87	0.38	0.87
22:6n-3	14.12	2.6[a]	10.68	1[b]	13.09	0.8[a]	9.25	1.8[b]	2.03	P<0.01	0.26	0.84
n-3[d]	23.58	0.8	23.53	1	23.27	2.7	21.23	3.1	2.53	0.43	0.33	0.45
PUFAs[e]	40.18	1	39.59	1.9	39.89	4.6	36.73	3.2	3.54	0.31	0.39	0.48
C_{18} PUFAs[f]	12.01	0.9	13.33	0.7	12.6	1.4	12.98	1.3	1.34	0.23	0.86	0.50
LC-PUFAs[g]	26.74	1.3	24.28	2	25.57	2.7	22.16	3.3	2.85	0.07	0.28	0.74
n-3:n-6	1.55	0.05	1.65	0.05	1.56	0.08	1.50	0.26	0.16	0.85	0.40	0.35
DHA:EPA	2.69	1[a]	1.42	0.16[b]	2.11	0.4[a]	1.29	0.3[b]	0.68	0.015	0.32	0.52

Lipid content (%)	27.5	8[b]	40.7	10[b]	56	9.4[a]	56.3	13.1[a]	11.9	0.34	0.011	0.361

[a]Saturated fatty acids

[b] Mono-unsaturated fatty acids

[c] n-3 fatty acids

[d] n-6 fatty acids

[e] Poly-unsaturated fatty acids

[f] C-18 Poly-unsaturated fatty acids– sum of all PUFA;includeslinolenic acid in addition to individually reported PUFA

[g] Long chain Poly-unsaturated fatty acids

Table 9. Fatty acid profile in the muscle tissue of *Totoaba macdonaldi* juveniles fed with diets supplemented with Tau or Met, and both. Values are given as an average ± standard deviation (n=3) as g/100 g of fat. Pooled SE (PSE) values and P values resulting from two-way ANOVA tests are also provided; different letters represent significant differences ($P < 0.05$).

										Methionine	***Taurine***	***Interaction***
Fatty Acids	**BASAL DIET**	**SD**	**MET**	**SD**	**TAU**	**SD**	**MET + TAU**	**SD**	**PSE**	***P Values***		
14:0	2.68	1.3	3.56	0.25	2.43	0.43	2.14	0.27	0.83	0.19	0.92	0.50
16:0	20.41	2	19.19	0.4	20.47	1	19.90	0.4	1.50	0.46	0.93	0.99
18:0	5.17	0.2	4.42	0.2	4.49	0.3	4.70	0.1	0.30	0.99	0.38	0.98
SFAs[a]	28.26	3.4	27.17	0.8	27.39	0.5	26.74	0.4	2.10	0.29	0.98	0.79
16:1n-7	5.89	1.2	10.09	1.7	5.74	1.4	6.10	1.1	1.5	0.42	0.26	0.18
18:1n-9	19.42	1.78	23.37	2.3	21.90	0.18	19.55	0.4	1.73	0.48	0.23	0.63
18:1n-7	2.37	0.6	3.50	3.5	2.49	1.9	2.51	1.2	2.49	0.35	0.25	0.41
MUFAs[b]	27.67	2.1	36.97	3.2	30.14	1.6	28.16	2.4	2.82	0.44	0.37	0.65
18:2n-6	9.62	0.4	10.90	0.5	9.98	0.7	10.28	0.3	0.63	0.46	0.22	0.87
20:4n-6	3.53	0.8	2.14	0.8	3.54	0.2	3.47	0.5	0.77	0.47	0.17	0.70
n-6[c]	13.15	1.1	13.04	0.4	13.52	1.2	13.75	0.9	1.14	0.62	0.46	0.82
20:5n-3	7.87	1.1	6.68	0.5	7.55	0.6	8.42	0.6	0.93	0.39	0.78	0.35
22:5n-3	1.40	0.4	1.21	0.9	1.17	0.1	1.19	0.2	0.62	0.52	0.22	0.56
22:6n-3	17.29	4.5	18.97	2.8	16.29	1.2	17.79	1.4	3.27	0.46	0.65	0.88
n-3[d]	26.1	4.8	27.25	3	29.43	1.1	27.74	2.2	3.60	0.26	0.52	0.80
PUFAs[e]	40.65	5.4	41.92	3.4	44.29	1.9	42.35	2.7	4.21	0.30	0.55	0.86
C_{18} PUFAs[f]	11.14	0.3	11.59	0.4	11.19	0.9	10.72	0.5	0.68	0.21	0.26	0.97
LC-PUFAs[g]	28.74	5.7	29.68	3.7	32.21	1.3	30.41	2.6	4.34	0.36	0.53	0.90

n-3:n-6	1.83	0.1	1.87	0.1	2.07	0.1	2	0.09	0.17	0.24	0.15	0.63
DHA:EPA	2.17	0.3	1.92	0.1	2.32	0.2	2.17	0.04	0.27	0.74	0.44	0.45
Lipid conte (%)	5.36	1.7	4.68	2	4.96	0.4	5.44	1	1.6	0.88	0.56	0.92

[a] Saturated fatty acids

[b] Mono-unsaturated fatty acids

[c] n-3 fatty acids

[d] n-6 fatty acids

[e] Poly-unsaturated fatty acids

[f] C-18 Poly-unsaturated fatty acids– sum of all PUFA; includeslinolenic acid in addition to individually reported PUFA

[g] Long chain Poly-unsaturated fatty acids

Table 10. Cholesterol content as dry basis in whole body of *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values are given as average and standard deviation of means. Pooled SE (PSE) and P values resulting from two-way ANOVA tests are also provided (n=3); Different superscripts letters represent significant differences ($P < 0.05$).

										Methionine	***Taurine***	***Interaction***
	BASAL DIET	**SD**	**MET**	**SD**	**TAU**	**SD**	**MET+ TAU**	**SD**	**PSE**		***P Values***	
Cholesterol (mg/g of tissue)	0.83	0.08^{a}	1.36	0.2^{a}	0.34	0.1^{b}	0.10	0.02^{b}	0.17	0.68	0.035	0.297
Cholesterol (mg/g of fat)	8.19	3.5^{a}	13.2	11^{a}	2.94	5^{b}	0.84	0.9^{b}	7.5	0.71	0.040	0.325
Lipid content (mg/g of tissue)	102	2.8^{a}	101	10^{a}	118	17^{b}	128	10^{b}	13	0.54	0.013	0.526

Table 11. Cholesterol content as dry basis in the liver of *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values represent the average (n=3); SE values and P values resulting from two-way ANOVA tests are also provided. Pooled SE (PSE) values and P values resulting from two-way ANOVA tests are also shown.

										Methionine	***Taurine***	***Interaction***
	BASAL DIET	**SD**	**MET**	**SD**	**TAU**	**SD**	**MET+ TAU**	**SD**	**PSE**		***P Values***	
Cholesterol (mg/g of tissue)	0.734	0.07^{a}	1.17	0.3^{b}	0.61	0.2^{a}	0.78	0.04^{ab}	0.24	0.035	0.070	0.302
Cholesterol (mg/g of fat)	2.71	0.6^{a}	3.16	1.2^{a}	1.15	0.9^{b}	1.44	0.8^{b}	1.09	0.397	0.004	0.84
Lipid content (mg/g of tissue)	275	80^{b}	407	100^{b}	560	94^{a}	563	131^{a}	119	0.34	0.011	0.361

Different superscripts letters represent significant differences ($P < 0.05$).

Table 12. Net change of fatty acids as dry basis of juvenile *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values represent the average (n=3); SE values and P values resulting from two-way ANOVA tests are also provided. Pooled SE (PSE) values and P values resulting from two-way ANOVA tests are also shown.

Fatty acids			**BASAL DIET**	**SD**		**MET**		**SD**		**TAU**		**SD**			**MET+ TAU**	**SD**	**PSE**	***Methionine***	***Taurine***	***Interaction***
																		P Values		
	mg/g inicial	mg/g final	Net change (mg)		mg/g inicial	mg/g final	Net change (mg)		mg/g inicial	mg/g final	Net change (mg)		mg/g inicial	mg/g final	Net change (mg)					
14:0	4.06	3.89	-0.16	0.006^{b}	4.17	3.67	-0.5	0.005^{b}	4.17	4.48	0.31	0.006^{a}	4.19	4.55	0.36	0.009^{a}	0.008	0.57	P<0.001	0.30
16:0	20.69	19.87	-0.81	0.02^{b}	21.24	17.90	-3.34	0.07^{c}	21.24	20.47	-0.77	0.05^{ab}	21.38	24.5	3.18	0.07^{a}	0.072	0.49	0.04	0.07
16:1n-7	8.68	8.33	-0.34	0.01	8.91	11.68	2.77	0.04	8.91	12.47	3.56	0.11	8.97	12.9	3.95	0.09	0.006	0.17	0.07	0.29
18:0	7.61	7.31	-0.30	0.003	7.81	3.22	-4.59	0.002	7.81	5.39	-2.42	0.008	7.87	5.66	-2.21	0.003	0.088	0.13	0.82	0.09
18:1n-9	18.95	20.44	1.49	0.02^{c}	19.54	25.40	5.86	0.05^{b}	19.54	26.35	6.81	0.11^{b}	19.63	31.3	11.75	0.14^{a}	0.111	0.04	0.04	0.99
18:1n-7	1.95	2.47	0.52	0.02^{c}	2.01	3.46	1.45	0.04^{a}	2.01	3.20	1.19	0.27^{b}	2.01	3.81	1.8	0.2^{a}	0.199	0.03	0.12	0.57
18:2n-6	11.03	10.59	-0.43	0.004^{b}	11.32	11.15	-0.17	0.01^{b}	11.32	12.69	1.37	0.03^{a}	11.40	13.9	2.55	0.03^{a}	0.030	0.06	P<0.001	0.41
20:4n-6	2.69	2.58	-0.11	0.004	2.77	2.24	-0.53	0.009	2.77	3.01	0.24	0.01	2.79	2.74	-0.05	0.01	0.014	0.63	0.84	0.59
20:5n-3	7.38	7.09	-0.29	0.003^{b}	7.58	7.55	-0.03	0.02^{b}	7.58	9.53	1.95	0.09^{a}	7.63	8.55	0.92	0.006^{a}	0.055	0.73	0.05	0.36
22:5n-3	2.38	2.28	-0.09	0.005	2.44	1.64	-0.8	0.02	2.44	1.96	-0.48	0.04	2.46	1.55	-0.91	0.05	0.040	0.46	0.77	0.87
22:6n-3	10.15	9.74	-0.40	0.006^{b}	10.42	8.32	-2.1	0.05^{b}	10.42	11.75	1.33	0.12^{a}	10.49	11.4	0.99	0.03^{a}	0.079	0.47	0.05	0.62
Total			-4.17				-4.05				11.95				21.27					
SFA[1]	25.79	24.77	-1.01	0.03^{c}	26.65	24.00	-3.65	0.06^{d}	26.47	26.16	-0.31	0.07^{b}	26.64	0.67	4.44	0.07^{a}	0.075	0.81	0.04	0.67
MUFA[2]	41.33	39.71	-0.62	0.007^{d}	42.72	43.16	0.44	0.05^{c}	42.43	48.45	6.02	0.27^{b}	42.70	24.5	17.12	0.12^{a}	0.176	0.01	0.02	0.37
PUFA[3]	38.04	36.55	-1.49	0.02^{b}	39.32	33.28	-4.87	0.08^{c}	39.05	42.09	3.04	0.3^{a}	39.30	12.9	3.86	0.09^{a}	0.190	0.09	0.001	0.43

Different superscript letters represent significative differences ($P < 0.05$).

[1]Saturated fatty acids

[2] Mono-unsaturated fatty acids

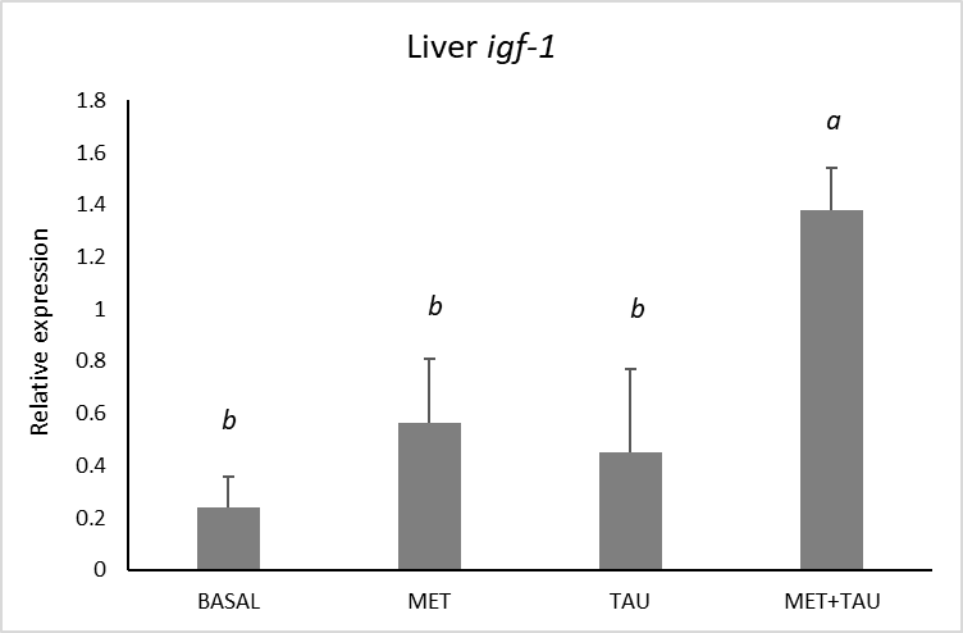
[3] Poly-unsaturated fatty acids

Table 13. Taurine content as dry basis in whole body of *Totoaba macdonaldi* juveniles fed with diets supplemented with methionine, taurine and both compare to a basal diet with a restrictive amount of methionine and taurine. Values pooled SE (PSE) values and P values resulting from two-way ANOVA tests are also provided (n=3).

	BASAL DIET	**SD**	**MET**	**SD**	**TAU**	**SD**	**MET+ TAU**	**SD**	**PSE**	***Methionine***	***Taurine***	***Interaction***
										P Values		
Taurine (mg/g)	1.35	0.6[b]	4.97	0.4[b]	16.16	3.8[a]	12.72	4.2[a]	3.35	0.710	0.017	0.286
Net change (mg)	-11	1.1[c]	-7.7	1.8[c]	4.2	0.7[a]	0.2	1[b]	1.25	0.960	0.008	0.294

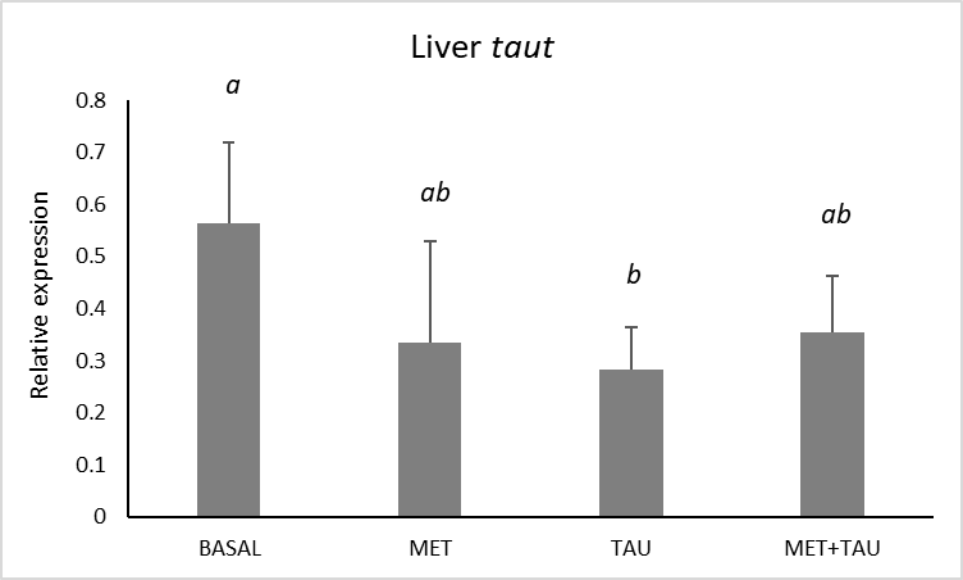
Different superscript letters represent significant differences ($P < 0.05$).

Fig 1



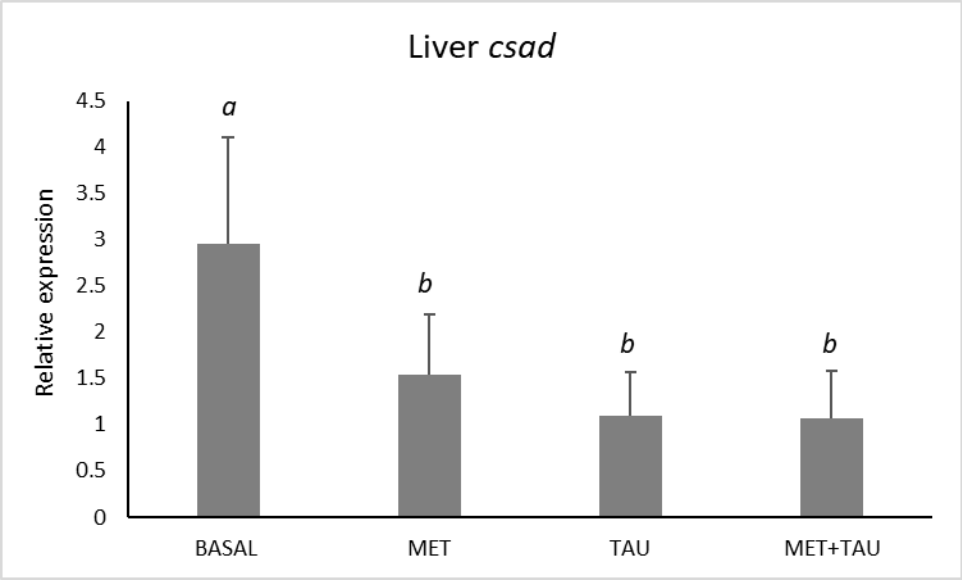
Two-way ANOVA		
Methionine	Taurine	Met-Tau
P<0.001	P<0.001	0.005

Fig 2



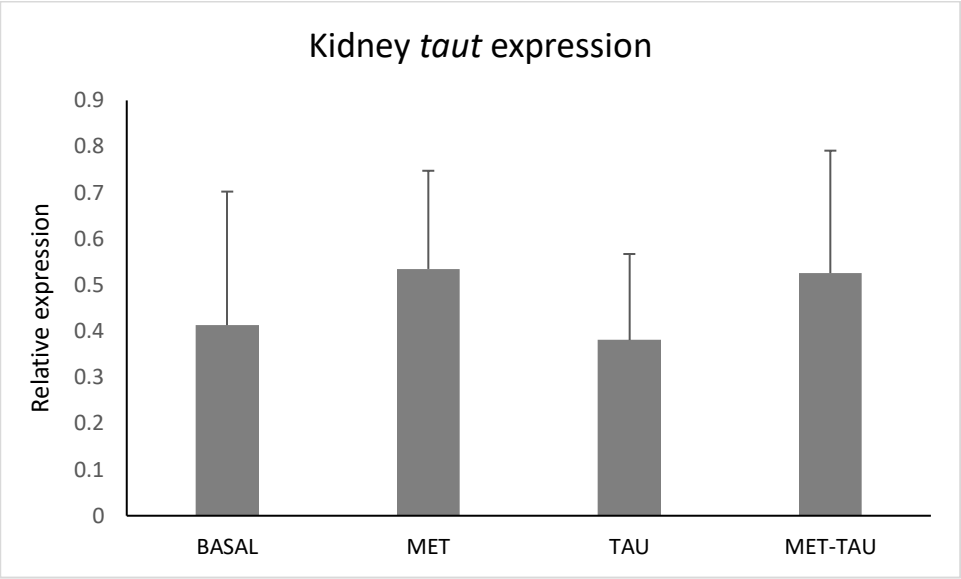
Two-way ANOVA		
Methionine	Taurine	Met-Tau
0.235	0.092	0.030

Fig 3.



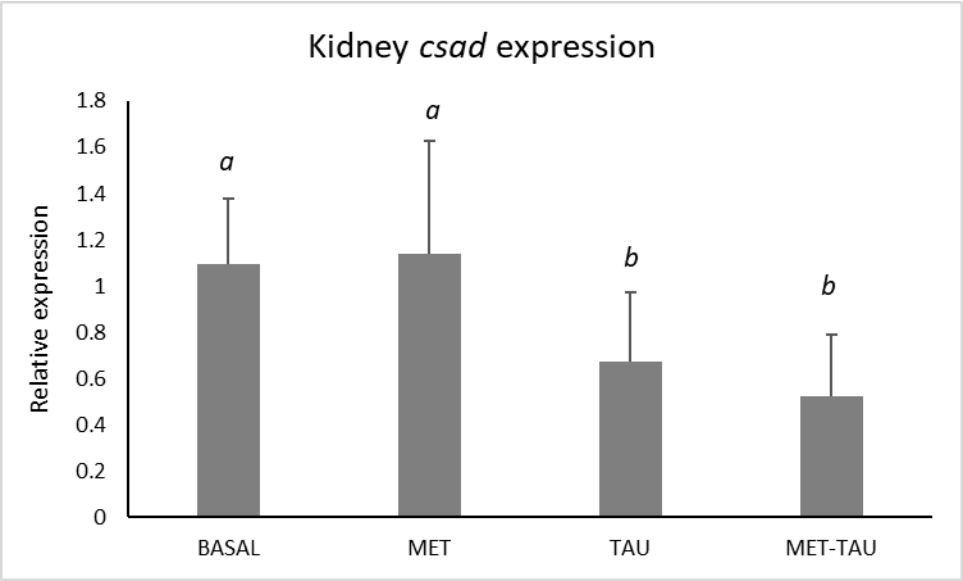
Two-way ANOVA		
Methionine	Taurine	Met-Tau
P<0.14	P<0.079	0.015

Fig 4.



Two-way ANOVA		
Methionine	Taurine	Met-Tau
0.13	0.81	0.89

Fig 5.



Two-way ANOVA		
Methionine	Taurine	Met-Tau
0.65	P<0.001	0.46

CAPÍTULO II: Lipid metabolism in juveniles of Yellowtail, *Seriola dorsalis*, fed different levels of dietary methionine containing a low level of cholesterol: Implication in feed formulation

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Lipid metabolism in juveniles of Yellowtail, *Seriola dorsalis*, fed different levels of dietary methionine containing a low level of cholesterol: Implication in feed formulation

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Abstract

The involvement of dietary methionine in the lipid metabolism of *Seriola dorsalis*, at different levels of dietary methionine (Met), and low content of cholesterol was investigated. Four diets containing different Met levels and a low amount of cholesterol (0.23 g/kg diet) were prepared. One hundred and eighty juveniles (16.2 ± 1.5 g) were randomly distributed into each 12 500-L ponds. After 60 days, the growth in weight (%) significantly increased following the Met supplementation, whereas SGR of all the experimental treatments was higher than the Basal Diet. The expression of the insulin-like growth factor (IGF-1) increased significantly (0.2-fold) and was complemented by significant changes in Mat, BHMT and HMG-CoA, for fish that were in the low and higher Met levels. The cholesterol content in the whole body and liver increased following methionine supplementation, similarly to that observed for crude lipids, in particular in the whole body and liver tissues. However, the relative amount of most fatty acids remained unchanged. Only the oleic acid increased at a higher amount of Met. Therefore, once the dietary requirement of methionine is met, the methionine is used to synthesize cholesterol. It is recommended to supply a higher amount of methionine to spare energy for growth.

KEYWORDS

amino acids, gene expression, growth, lipid metabolism

1 | INTRODUCTION

The ideal protein formulation of diets is a concept based on the total amount of essential (or indispensable) and non-essential (dispensable) amino acids (EAA and NEAA, respectively). Referring to the return into maximum protein retention at a level were all amino acids are equally limiting for growth performance (van Milgen & Dourmad, 2015). Theoretically, the use of dietary protein for growth should be optimized by minimizing its use as an energy source. However, after several attempts to reach the ideal protein formulation levels in fish nutrition studies, inconsistencies among different organisms prevail (NRC, 2011). The lack of commonality could be due to variations in other metabolic functions in addition to growth. Barreto, Focken, D'Abramo, Cuaron, and Viana (2018), Barreto, Focken, D'Abramo, Mata-Sotres, and Viana (2019) using either a similar dietary protein/lipid ratio (Barreto et al., 2018) or different levels of digestible protein failed to increase the level of protein retention at higher protein levels. In both cases, higher growth was achieved when higher available protein was present, but a lower protein efficiency correspondingly occurred.

Amino acids (AA) play an essential role in maintaining homeostasis in gene expression regulation, intracellular protein turnover, nutrient metabolism and oxidative defence (Wu, 2010), where restriction of a particular AA could influence overall performance (Otasevic & Korac, 2016). The intermediate metabolism refers to all metabolites in the intracellular process needed to be converted into cellular components as well as the energy required (Krebs, Camb, & Hamburg, 1937). In fish, it has been proposed that ingested

carbohydrates are not involved in the specific dynamic action (Fu & Xie, 2004). Therefore, identification of all limiting nutrients to complement the stoichiometric theory of "ideal protein formulation" is essential (van Milgen & Dourmad, 2015). The efficiency of use of one or another nutrient depends on relative digestibility capacity in association with their preferred routes for intermediate metabolism. According to Kaushik and Seilez (2010), there is no clear distinction between the AAs needed for maintenance or growth. Therefore, the measurement of the levels of expression of genes is an accurate way to measure metabolic routes.

Methionine (Met) is one of the limiting amino acids and one of the two amino acids containing sulphur, apart from cysteine, being limiting for growth. Recently, Madrid, Pohlenz, Viana, and Lazo (2019) estimated the Met requirement for *Totoaba macdonaldi* to be 0.76 g/kg via a back calculation from the quantified lysine requirement by using the whole-body Met concentration in whole fish and considering the Lys concentration as 100%.

Even if Met is an essential amino acid, it can be synthesized through the trimethyl glycine (betaine) as a methyl donor via the betaine–homocysteine methyltransferase (BHMT) reaction (Yang, Wang, Yang, Xu, & Gong, 2017). In addition, Met has a principal role in intermediate metabolism as a methyl donor, whereby Met can also be converted into S-methyl methionine (SAM), needed for the synthesis of phosphatidylethanolamine into phosphatidylcholine. This process has a regulatory function in the metabolism of cholesterol in the liver (Hirche, Schröder, Knoth, Stangl, & Eder, 2006). However, to synthesize SAM, the methionine adenosyltransferase (MAT) is crucial (Ramani & Lu, 2017). Therefore, the Met requirement for growth could depend on whether a particular intermediary metabolism route is favoured or needed, apart from a role in growth, to produce a metabolite in the body. Therefore, under a dietary low cholesterol level, or cholesterol-free, the dietary methionine requirement may increase in order to sustain the growth rate.

The role of Met in lipid metabolism has been described (Oda, 2006), where its restriction results in a decrease in fatty acid deposition in liver and tissues in several species as rats, pigs and humans (Morita et al., 1997; Poloni, Blom, & Schwartz, 2015; Zhou et al., 2016). The same response has been identified for tiger pufferfish (Xu, Zhang, Wei, Liao, & Liang, 2019), rainbow trout (*Oncorhynchus mykiss*) (Rolland et al., 2016), *Salmo salar* (Espe, Rathore, Du, Liaset, & El-Mowafi, 2010) and cobia (*Rachycentron canadum*) (Wang et al., 2016). Craig and Moon (2013) revealed that Met restriction in rainbow trout (*Oncorhynchus mykiss*), the reduction of total lipid contents, could indicate either a reduction in lipogenic capacity or an increase in fat oxidation, where carbohydrates may play an important role. However, the later results have not been supported by a methionine restriction that resulted in an increase in fat accumulation in the liver but a decrease in muscle tissue in rainbow trout (Latimer, Cleveland, & Biga, 2018).

The role of Met in the synthesis of cholesterol and phospholipids has been reported in plasma and tissues of rats and fish (Craig & Moon, 2013). *Seriola dorsalis* is highly efficient in synthesizing cholesterol when fed diets with low cholesterol levels (Guerra-Olvera and Viana, mistakenly reported as *S. lalandi*). Under the low cholesterol levels, a depressed growth rate resulted, with an increased following the presence of dietary cholesterol (Guerra-Olvera & Viana, 2015). According to Maita, Maekahwa, Satoh, Futami, & Sotoh (2006), fish-free diets fed to *S. quinqueradiata* resulted in lower growth and lower endogenous cholesterol, a concentration that increased accordingly, and the taurine was supplemented. The cholesterol is synthesized through the hydroxymethyl-glutaryl-CoA reductase (HMG-CoA; Illingworth & Tobert, 2001), whereas the lipogenesis is controlled by the sterol regulatory element-binding protein 1 (SREBP-1) (Smulan et al., 2016). Both these activities require energy for their synthesis.

Most experiments related to Met requirement are designed to measure growth as a response variable. Sometimes, the expression of the insulin growth factor 1 (IGF1) is given to show the growth stimulation, while rapamycin receptor (mTOR) as the growth coordinator to explains optimum growth conditions (Torres-Velarde, Llera-Herrera, García-Gasca, & García-Gasca, 2018; Vélez et al., 2017). However, metabolic routes are not always investigated, thereby introducing the uncertainty of the methionine requirement under different metabolic and nutritional conditions. Therefore, studies to determine the Met requirements should be complemented with an understanding of lipid metabolism, estimating the overall performance in association with the gene expression for the enzymes involved in growth, Met synthesis and lipid metabolism.

Today, there is an enormous concern about formulation of diets for aquaculture species under the conditions of use of a limited amount of fish meal and fish oil. Fish oil contains a comparatively large amount of cholesterol (NRC, 2011), whereas vegetable oils do not contain cholesterol, and crude lipids from animal origin usually contain less than that found in marine oils (NRC, 2011). Therefore, the role of Met and its possible role in the synthesis of cholesterol under dietary limitations needs to be investigated.

The present work focused on achieving an understanding of the different routes of Met metabolism using experimental diets containing different levels of Met, from its apparent requirement stated by Madrid et al. (2019) to excess, under the conditions of limited dietary cholesterol. Effects on the metabolism of the fatty acids, cholesterol and the overall performance of *S. dorsalis* were measured in relationship to an analysis of the different genes involved.

2 | MATERIALS AND METHODS

2.1 | Formulation and preparation of diets

Four diets were prepared to contain four different levels of methionine from 7.5 to 17.9 g/kg (Basal Diet, 7.5; L-Met, 10.0; M-Met, 12.9; H-Met, 17.9 g Met/kg). The diets intentionally contained a comparatively low amount of fishmeal (93 g/kg) to restrict the amount of cholesterol derived from this ingredient. Lipid sources were beef tallow and poultry by-product meal (PBM) also contributing to less cholesterol (200 mg/kg) than what where be derived from a marine source (NRC, 2011). According to Guerra-Olvera and Viana (2015), the

TABLE 1 Ingredient composition of diets (g/kg) formulated to contain different amount of methionine to feed juveniles of *Seriola dorsalis* for 60 days

	Experimental Treatments			
Ingredients	**Basal Diet**	**L-MET**	**M-MET**	**H-MET**
Poultry meal [a]	229	229	229	229
Fish meal [b]	93	93	93	93
Gelatin [c]	61	61	61	61
Soy protein concentrate [d]	269	269	269	269
Beef tallow [e]	80	80	80	80
DHA-Nature [f]	14	14	14	14
Taurine [g]	5	5	5	5
Lysine [h]	5	5	5	5
Rovimix[i]	25	25	25	25
Stay C[i]	2	2	2	2
Benzoate [j]	0.1	0.1	0.1	0.1
BHT [j]	2	2	2	2
Maizena™ [k]	215	212	210	205
Methionine [l]	0	2.5	5	10
Proximate composition on dry matter basis (g/kg)				
Crude protein	428	427	416	419
Crude lipid	105	106	100	104
Ash	67	66	67	66
NFE	400	401	407	411

Note: NFE (g/kg) = 100% − (crude protein + crude lipid + ash).

[a]Pet food grade from Proteínas Marinas y Agropecuarias SA de CV, Guadalajara, Jalisco, México.

[b]Sardine fishmeal from Proteínas Marinas y Agropecuarias SA de CV, Guadalajara, Jalisco, México.

[c]Progel Mexicana SA de CV, Léon, Guanajuato, México.

[d]Alimentos COLPAC, Hermosillo, Sonora, México.

[e]Industrial de Grasas y Derivados, Tijuana, Baja California, México.

[f]ADM, México, containing 24%DHA and 7%EPA.

[g]Taurine Future foods, Tlanepantla, Estado de México, México.

[h]L-Lysine from ADM México

[i]DSM Nutritional Products México SA de CV, Guadalajara, Jalisco, Mexico, contains in g kg ρ-aminobenzoic acid 1.45; biotin 0.02; myo-inositol 14.5; nicotinic acid 2.9; Ca-Pantothenate 1.0; pyridoxine-HCl 0.17; riboflavin 0.73; thiamine-HCl 0.22; menadione 0.17; α-tocopherol 1.45; cyanocobalamine 0.0003; calciferol 0.03; L-ascorbyl-2-phosphate-Mg 0.25; folic acid 0.05; choline chloride 29.65; retinol 0.015; NaCl 1.838; MgSO4.7H2O 6.85; NaH2PO4.2H2O 4.36; KH2PO4 11.99; Ca(H2PO4)2.2H2O 6.79; Fe-citrate 1.48; Ca-lactate 16.35; AlCl3.6H2O 0.009; ZnSO4.7H2O 0.17; CuCl2 0.0005; MnSO4.4H2O 0.04; KI 0.008; CoCl2 0.05 and Stay–C (vitamin C, ascorbic acid 0.75).

[j]Interquímica SA de CV, Atizapán de Zaragoza, México.

[k]Maizena™, Unilever Food Solutions, México.

[l]Methionine, Future foods, Tlanepantla, Estado de México, México.

cholesterol requirement for *S. dorsalis* ranges from 0.56 to 0.82 g/kg of diet, which is more than double that a supplied in the experimental diets. Sources of DHA and EPA were provided via the addition of 14 g/kg DHA-Nature™ (containing 24 g/kg and 7 g/kg of DHA and EPA, respectively). All experimental feeds were manufactured at the Instituto de Investigaciones Oceanológicas (UABC) according to in-house protocols. Briefly, the main ingredients were ground to 0.8 millimetres (Pulvex 2000), sifted (Kemutek-Gardner K300) and mixed using a vertical cutter-mixer (Robot Coupe) until a homogenized mixture was achieved. Micronutrients (a premix of vitamins and minerals, together with antioxidants and antimicrobials and the DHA/EPA ingredient mixture) were mixed for a minute (Robot Coupe R-60) and then incorporated to the bulk ingredient mixture. Beef tallow was added and thoroughly mixed. Then, water was added, and the whole blend was mixed for approximately 10 min. After that, the dough was immediately pelleted using a commercial-grade food grinder and dried at 60°C until < 90% moisture was achieved (around 24 hr). All feeds were kept refrigerated (4°C) throughout the feeding trial. The ingredient composition of diets and amino acid and fatty acid profiles are presented in Tables 1, 2 and 3, respectively.

TABLE 2 Amino acid profile of experimental diets (g/kg diet) formulated to contain different amount of methionine to feed juveniles of *Seriola dorsalis* for 60 days

	Experimental treatments			
AA	**Basal Diet**	**L-MET**	**M-MET**	**H-MET**
Essential AA				
HIS	10.8	10.8	10.4	10.5
ARG	32.2	32.1	31.3	31.5
THR	16.9	17.9	17.4	17.3
VAL	20.2	20.3	20.8	20.6
MET	7.5	10.0	12.9	17.9
LYS	10.5	10.5	10.8	10.7
ILE	6.0	6.0	6.1	6.1
LEU	31.2	31.3	32.1	31.9
PHE	18.1	18.8	19.3	19.2
TAU	6.1	6.2	6.3	6.3
Non-essential AA				
ASP	10.8	10.8	10.5	10.6
SER	24.0	23.9	23.3	23.5
GLU	34.6	34.6	33.6	33.8
GLY	32.2	32.1	25.3	31.5
ALA	57.1	57.0	55.5	55.9
PRO	30.6	30.5	29.7	30.0
TYR	12.2	12.0	11.9	12.0
CYS	Tr	Tr	Tr	Tr

Abbreviation: Tr, trace amount.

2.2 | Experimental design and sampling

One hundred and eighty juvenile *Seriola dorsalis* (16.2 ± 0.7 g) (15 per experimental unit) in a completely randomized design were distributed into each of 500-L available water ponds (750-L capacity), in total for four treatment and three repetitions each. Each tank was connected to a recirculation system consisting of a six cubic feet biofilter in line with a protein skimmer and a UV lamp (40 watts). Natural marine water (35 ppm) was recirculated using a 1.5HP Jacuzzi water pump (Sweetwater, SHE2.4; Miami). The system also consisted of a reservoir (1000L). A 5% water was renewed per day to compensate the water loss for evaporation or through the sediment removal. The water temperature of the system was maintained at 26.0 ± 1°C by using 6.5 kW heaters and a sensor within the reservoir. Water samples were collected twice a week to measure the concentration of ammonia, nitrites, nitrates, pH and carbonate using a commercial kit for marine water (Aquarium Pharmaceutical, Inc.). The fish were acclimatized for two weeks and fed a standard diet according to Badillo-Zapata, Lazo, Herzka, & Viana (2016), after that, fed for 60 days, a total of 6% of their body weight or at an apparent satiation divided among four feedings per day (8:00, 11:00, 14:00 and 17:00 hr.). Mean individual weight of fish from each experimental unit was determined at the initiation and termination of the experiment by dividing the bulk weight by the total number of fishes for each tank, and performance response indices were determined according to the following calculations:

$$\%\text{Weight gain} = \left[(\text{Final weight} - \text{Initial weight}) * 100/\text{Initial weight}\right] \quad (1)$$

$$\text{SpecificGrowth Rate, SGR} = (\ln \text{final weight} - \ln \text{initial weight}) * 100/t \quad (2)$$

$$\text{Feed conversion FC} = (\text{Total amount of feed consumed}/\text{final body wet weight gain}) \quad (3)$$

$$\text{Protein Efficiency Ratio, PER} = (\text{g body wet weight increase}/\text{g protein ingested}) \quad (4)$$

$$\text{Hepatosomatic index (HSI)} = 100 \times (\text{liver weight}/\text{whole} - \text{body weight}) \quad (5)$$

$$\text{Viscerosomatic index (VSI)} = 100 \times (\text{viscera weight}/\text{whole} - \text{body weight}) \quad (6)$$

$$\text{Condition factor, Cf} = \left(\text{Final weight}/\text{Lenght}^3\right) * 100$$

At the end of the feeding trial, all fish were not fed for 14 hr and then humanely sacrificed by hypothermia under the approved protocol from the Institution (UABC). Six fish per replicate or experimental unit (n = 3) were removed and pooled for chemical composition and fatty acid analyses. An additional three more fish per replicate were used for dissection and removal of individual muscle and liver tissues to obtain fatty acid profiles and storage in plastic bags at −80°C for future analysis. For the gene expression, only liver tissues were sampled and immediately stored in RNAlater (Ambion) at −80°C.

TABLE 3 Fatty acid profile (% of total) of experimental diets formulated to contain different amount of methionine to feed juveniles of *Seriola dorsalis* for 60 days

	Experimental Treatments			
Fatty acids	Basal Diet	L-MET	M-MET	H-MET
14:0	5.02	4.92	4.97	4.97
15:0	0.35	0.34	0.35	0.35
16:0	23.48	23.02	23.52	23.25
16:1	3.44	3.37	3.00	3.40
18:0	13.97	14.69	13.60	13.83
18:1n9	35.61	35.88	36.37	36.24
18:1n7	1.32	1.30	1.81	1.31
18:2n6	10.72	10.51	10.18	10.62
18:3n6	0.96	0.94	1.07	0.95
18:3n3	0.14	0.14	0.16	0.14
20:1n9	0.32	0.31	0.36	0.32
20:4n6	0.45	0.44	0.55	0.44
20:5n3	0.38	0.37	0.37	0.37
22:6n3	3.85	3.77	3.67	3.81
Total	100	100	100	100
SFA	42.82	42.97	42.44	42.40
MUFA	40.69	40.86	41.54	41.27
PUFA	16.82	16.48	16.36	16.65
n3/n6	2.78	2.78	2.81	2.78
SAT/IN	0.745	0.749	0.733	0.732

2.3 | Chemical analysis

To analytically determine the chemical composition of tissues and proximate composition of diets, the AOAC procedures were follows. Three fish (whole) per tank were removed and macerated using a food processor. The samples were then stored in plastic bags at −30°C. A 2g of sample of each experimental feed was macerated in a micropulverized prior analysis. The moisture content of each sample was gravimetrically calculated from the samples dried to constant weight at 60°C. Total nitrogen content was determined by the micro-Kjeldahl method, and crude protein was then calculated as % N × 6.25. Total lipid concentration was estimated using the Soxhlet extraction method with petroleum ether as a carrier solvent, and the crude lipid was gravimetrically calculated. Results are reported as g/kg in dry weight. Ash content was gravimetrically determined by heating the samples to 550°C for 6 hr. All methods were conducted according to AOAC (1995) described methodology.

2.4 | Fatty acid composition

Samples of fish tissues (whole fish, liver and muscle) for each experimental unit (n = 3) in triplicate were homogenized and lipids

TABLE 4 Sequences of the primer pairs used for q-PCR, amplicon size in base pairs (bp), reaction efficiencies (E) and Pearson's coefficient of determination (R^2)

Gene (symbol)	Fwd sequence (5'–3')	Rev sequence (5'–3')	Size (bp)	E	R^2
mat	GGTGGCCAAATCTCTGGTAA	CTTCTTCACGATGTCCAGCA	154	0.91	1.00
bhmt	GATGAAGCATGTGCTGGAGA	TGGAGCATCGCTGTTACAAG	150	1.03	0.98
hmg	TGTGGCTGGAACTTTGACTG	GACCACTGTGCTGAAGACGA	201	0.94	1.00
srebp−1	CTCCCAGTCCTTTGCTATCG	AGTGCTCAACGGTGGGATAC	152	0.90	0.99
mtor	ACC TTTGACGAGGCTGAGAA	TGGGTGATCTCCTCCATCTC	164	0.95	0.99
igf−1	TCTTCAAGAGTGCGATGTGC	GGCCATAGCCTGTTGGTTTA	189	0.93	1.00
actb	TGCGTG ACATCAAGGAGAAG	AGGAAGGAAGGCTGGAAGAG	175	1.00	0.99

extracted using dichloromethane–methanol (2:1, v/v) following a modified method by Folch, Lees, and Sloane-Stanley (1957). One per cent BHT was added as an antioxidant. After three extractions, the solvent supernatants were pooled, and water was added to separate the phases. The lower phase, containing the lipids (in CH_2Cl_2), was washed with water and evaporated under N_2 in a preweighed vial, and the amount of total crude lipid was determined gravimetrically. Total lipids were saponified with methanolic solution KOH (0.3N in 90% methanol), dried under N_2 and weighed using preweighed vials. The lipids were then esterified to form methyl esters of fatty acids (FAME), according to Christie (1993) using 14% Boron trifluoride in methanol. The FAME samples were preserved with 0.01% BHT to avoid oxidation. FAMEs were analysed in an Agilent GC 6850 gas chromatograph equipped with an injector split/splitless, a flame ionization detector (FID) and a capillary column (AGILENT 122-2362E DB-23; 60 m × 0.25 mm, film thickness 25 μm) using nitrogen as the carrier gas. Fatty acids were identified by comparing retention times to those of standards (37 Component FAME Mix, Supelco/Sigma-Aldrich; GLC 87, GLC 96, Nu-Chek Prep.; RM-2, RM-6 and GLC 90, Supelco/Sigma-Aldrich) and well-characterized profiles of samples of marine oils (PUFA1, Supelco/Sigma-Aldrich). Fatty acid profiles are reported as the percentage of FAMEs identified. The software HP ChemStation Data analysis Application model G17001EA E.02.00.493 for Windows was used for the quantification and calculations of fatty acids.

2.5 | Cholesterol analysis

The cholesterol content of tissue and the experimental diets was estimated from the non-saponifiable portion of the total lipid extract after the modified Folch et al. (1957) methodology. Samples were silanized with hexamethyl–trimethyl pyridine, and then, one μL of the sample was injected into the gas chromatograph as earlier stated for the fatty acids (Agilent GC 6850). A capillary column of 100% dimethylpolysiloxane, 30 m, 0.320 mm internal diameter and 0.25 μm film thickness was used. Estimates of cholesterol content were determined based on a standard curve that was generated using pure cholesterol (C8667_Merck) at different concentrations.

2.6 | Amino acid analysis

The AA content of individual samples of the lipid extracted experimental diets was evaluated for AA content using a Waters HPLC equipped with a fluorescence detector (Waters 474 series). Samples of 10 mg were hydrolysed with 500 μL of 6N HCl and 0.06% phenol in a closed vial and heated to 110°C for 24 hr. Amino acid profiles were determined following Waters AccQ-Tag™. In summary, hydrolysed samples were dried in a thermal Monoblock at 60°C, and one mL of distilled water was added to vials and filtered (0.45μm). Samples were then derivatized using the AccQ-Tag™ procedure followed by injection into a chromatograph equipped with a reverse-phase column (3.9 × 150 mm) 4-lm AccQ-Tag™ C-18 (Waters). The water–acetonitrile gradient recommended by the Waters AccQ-Tag™ system was used. The fluorescence detector was programmed to an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Analyses were conducted at a constant temperature (37.5°C). HPLC signal calibration and standard curves were obtained with amino acid standard solutions containing 25 to 150 pmol of each amino acid, and a similar amount of taurine was added.

2.7 | Gene expression

Individual samples from the liver of the sampled fish from each experimental unit were used to evaluate the expression of specific genes (Table 4). Tissues were individually preserved in RNAlater (Ambion) during the dissection and processed for total RNA extraction using TRIzol® (Thermo Fisher Scientific). RNA quantity and quality were measured by gel electrophoresis and spectrophotometrically (Nanodrop® LITE, Thermo Fisher Scientific INC). Only RNA samples with OD260nm-OD280nm ratios between 1.90 and 2.10 were used for expression quantification.

Total RNA (500 ng) was reverse-transcribed in a 20-μL reaction using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) in a Verity 96-well thermal cycler (Applied Biosystems). The reverse transcription programme consisted of 10 min at 25°C, 120 min at 37°C, 5 min at 85°C and finally maintained at 4°C. qRT-PCRs were performed with one ng of cDNA, sense and antisense

primers (200 nM each: Table 4) and SYBR® Select Master Mix (Applied Biosystems).

The relative gene quantification from the liver samples tissues was calculated by the ΔΔCT method (Livak & Schmittgen, 2001) using an automated threshold and walking baseline for determining CT values. The PCR conditions were as follows: an initial denaturation and polymerase activation step during 10 min at 95°C; 40 cycles of denaturing for 15 s at 95°C, annealing and extension for 45 s at 60°C; and a final melting curve from 60°C to 95°C for 20 min to check for primer–dimer artefacts. Optimization of qRT-PCR conditions was achieved on primer annealing temperature (60°C), primer concentration (200 nM) and template concentration (five 1:10 dilution series from 10 ng to 100 ng of input RNA). β-actin (*actb*) was used as the internal reference gene (GenBank acc. no HM754483). GenBank accession numbers for the studied genes are as follows: XM_023406500 for *mat*, XM_023401708 for *bhm*, AB218826 for *hmg*, XM_023423288 for *srebp-1*, XM_023407710for *mtor* and AB439208 for *igf-1* (Table 4).

2.8 | Statistical analysis

Statistical analysis was performed using the software STATISTICA 8.0™ (StatSoft, Inc). All different analysis was done using three independent replicated provided from each experimental unit.

One-way ANOVA was used to determine whether significant differences existed among the array of performance indices, somatic indices, proximal composition, whole body, liver and muscle fatty acid profile, cholesterol content and gene expression levels. If significant differences were found, then a post hoc Tukey rank test was performed. For all cases, statistical significance was set at $p < .05$.

3 | RESULTS

The supplementation of methionine resulted in a significant increase in growth with no significant difference between fish fed the M-MET and H-Met diets and a significant increase in the H-MET diet versus the M-MET diets (Table 5). The FC results mirrored the weight gain results, whereas no significant difference in PER was found among the three experimental diets supplemented with methionine. The crude protein content of the whole fish, muscle and liver resulted in significant differences among fish fed the experimental diets supplemented with methionine (Table 6), where the content was always higher in fish fed higher Met levels. Similarly, the crude lipid content in all sampled tissues was significantly higher at higher Met inclusion. For the muscle tissue and whole fish, the concentrations in M-MET treatment were significantly higher than those for all the other dietary treatments (Table 6).

The fatty acid profiles of whole fish, muscle and liver tissues revealed significant differences for 18:0 and 18:2n6 fatty acids only (Table 7). For 18:0 (stearic acid), percentages for the L-MET and M-MET dietary treatments were significantly higher and the percentage of 18:2n6 for the M-MET treatment was significantly higher those of the BASAL and L-MET dietary treatments. In the

TABLE 5 Production performance of juvenile *Seriola dorsalis* fed for 60 days with four experimental diets formulated to contain different levels of methionine. Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$

	Experimental treatments			
Biological indices	**Basal Diet**	**L-MET**	**M-MET**	**H-MET**
Initial weight	15.7 ± 0.6	15.6 ± 0.9	16.2 ± 0.6	15.9 ± 0.6
Final weight	50.7 ± 2.38^{d}	65.6 ± 2.59^{c}	72.0 ± 1.34^{b}	76.5 ± 2.0^{a}
Feed consumption (% of fish weight)	7.1 ± 0.3^{a}	6.7 ± 0.08^{ab}	6.0 ± 0.02^{b}	6.1 ± 0.1^{b}
Weight gain (g)	34.8 ± 2.4^{c}	49.9 ± 2.6^{b}	56.6 ± 1.3^{ab}	60.5 ± 2.0^{a}
Weight gain (%)	218.5 ± 23.2^{c}	316.8 ± 3.9^{b}	368.1 ± 1.3^{ab}	379.9 ± 22.2^{a}
SGR	2.1 ± 0.1^{b}	2.6 ± 0.01^{a}	2.8 ± 0.01^{a}	2.8 ± 0.08^{a}
PER	1.2 ± 0.13^{b}	1.5 ± 0.09^{ab}	1.6 ± 0.04^{a}	1.8 ± 0.14^{a}
FC	2.0 ± 0.23^{a}	1.6 ± 0.1^{ab}	1.3 ± 0.03^{b}	1.4 ± 0.1^{b}
Total length (cm)	16.28 ± 0.45 ^{b}	18.47 ± 1.38 ^{a}	18.45 ± 1.30 ^{a}	18.46 ± 1.14 ^{a}
Cf	1.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
IVS (%)	7.4 ± 1.3	7.8 ± 1.8	7.9 ± 1.6	7.1 ± 1.4
IHS (%)	1.4 ± 0.3	1.4 ± 0.0	1.5 ± 0.2	1.3 ± 0.2

Note: Fifteen fish per experimental unit were weight, and each average was taken as a repetition ($n = 3$).

SGR = Specific growth rate ((ln final weight –ln initial weight)*100/t)

PER = Protein efficiency ratio (g body wet weight increase,/ g protein ingested)

FC = Feed conversion (total amount of feed consumed/weight gain)

Cf = Condition factor (Final weight/Lenght^{3})*100)

Abbreviations: HSI, Hepatosomatic index; VSI, Viscerosomatic index.

TABLE 6 Chemical composition (mg/g tissue dry weight) of juvenile *Seriola dorsalis* fed for 60 days with different experimental diets formulated to contain four levels of methionine

	Experimental treatments			
	Basal Diet	L-MET	M-MET	H-MET
Whole fish				
Crude protein	145.2 ± 0.23^{b}	159.2 ± 13.8^{ab}	191.4 ± 9.57^{a}	187.2 ± 8.7^{a}
Crude lipid	19.3 ± 1.19^{b}	17.6 ± 2.68^{b}	30.7 ± 1.4^{a}	20.8 ± 0.89^{b}
Ash	37.7 ± 2.64	36.9 ± 1.22	40.2 ± 2	35.8 ± 1.5
Moisture	758 ± 11	755 ± 13	710 ± 16	740 ± 13
Muscle				
Crude protein	218.2 ± 11^{b}	232.5 ± 15.1^{ab}	253.1 ± 9.3^{a}	236.8 ± 3.5^{a}
Crude lipid	18.8 ± 1.48^{b}	19 ± 2.8^{b}	22.8 ± 1.5^{a}	17.1 ± 0.5^{b}
Ash	18.2 ± 0.89	16.1 ± 1.13	17.9 ± 1.81	16.8 ± 1.1
Moisture	701 ± 10	716 ± 8	695 ± 7	719 ± 9
Liver				
Crude protein	307.2 ± 7.6^{b}	392.6 ± 21.6^{ab}	380.8 ± 28.9^{ab}	418.9 ± 27.6^{a}
Crude lipid	173.2 ± 10^{b}	299 ± 12.8^{a}	276 ± 38^{a}	299 ± 17^{a}
Moisture	230 ± 9^{a}	192 ± 10^{ab}	188 ± 8^{b}	185 ± 6^{b}

Note: 3 fish per experimental unit were pooled in triplicate samples. Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$.

TABLE 7 Whole-body fatty acid profile (% of total fatty acids) of juvenile *Seriola dorsalis* fed for 60 days with different experimental diets formulated to contain four levels of methionine

	Experimental treatments			
Fatty acids	Basal Diet	L-MET	M-MET	H-MET
14:0	3.25 ± 0.33	3.35 ± 0.12	3.02 ± 0.15	3.20 ± 0.05
15:0	0.27 ± 0.02	0.29 ± 0.04	0.28 ± 0.01	0.26 ± 0.03
16:0	18.13 ± 0.49	17.30 ± 0.14	17.59 ± 0.36	17.22 ± 0.08
16:1	3.74 ± 0.07	3.54 ± 0.18	3.55 ± 0.17	3.55 ± 0.34
18:0	6.44 ± 0.41^{b}	8.01 ± 0.88^{a}	8.89 ± 1.00^{a}	6.99 ± 0.58^{b}
18:1n9	39.93 ± 0.64	39.99 ± 1.57	38.52 ± 2.65	39.80 ± 0.60
18:1n7	1.48 ± 0.09	1.66 ± 0.27	1.67 ± 0.35	1.54 ± 0.39
18:2n6	13.67 ± 0.24^{b}	13.37 ± 0.11^{b}	14.33 ± 0.42^{a}	13.87 ± 0.10^{ab}
18:3n6	0.79 ± 0.09	0.80 ± 0.04	0.83 ± 0.05	0.79 ± 0.03
18:3n3	0.17 ± 0.04	0.20 ± 0.01	0.16 ± 0.01	0.17 ± 0.02
20:1n9	0.50 ± 0.02	0.46 ± 0.01	0.48 ± 0.02	0.47 ± 0.03
20:4n6	1.40 ± 0.28	1.33 ± 0.19	1.30 ± 0.15	1.40 ± 0.06
20:5n3	0.55 ± 0.06	0.51 ± 0.06	0.54 ± 0.04	0.56 ± 0.06
22:5n3	1.758 ± 0.22	1.7505 ± 0.36	1.573 ± 0.4	1.831 ± 0.21
22:6n3	7.35 ± 0.76	6.94 ± 0.35	6.71 ± 0.72	7.75 ± 0.21
SFA	28.09 ± 0.31	28.95 ± 0.30	29.78 ± 0.38	27.67 ± 0.19
MUFA	45.65 ± 0.21	45.65 ± 0.51	44.22 ± 0.80	45.36 ± 0.34
PUFA	25.69 ± 0.24	24.90 ± 0.16	25.44 ± 0.26	26.37 ± 0.10
n3/n6	0.62 ± 0.25	0.61 ± 0.33	0.55 ± 0.27	0.64 ± 0.38
SAT/IN	0.39 ± 0.03	0.41 ± 0.02	0.43 ± 0.02	0.39 ± 0.02

Note: Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$.

muscle tissue, both the L-MET and M-MET dietary treatments had significantly higher percentages of 18:3n6 relative to the Basal Diet and the M-MET dietary treatment had significantly lower percentages of 22:5n3 and 22:6n3 fatty acids, relative to all other dietary treatments (Table 8). In the liver, a significant decrease in DHA was observed in the L-MET and M-MET dietary treatments, whereas a significant increase of oleic acid (18:1n9) was found in M-MET relative to the Basal Diet (Table 9).

TABLE 8 Muscle fatty acid profile (% of total fatty acids) of juvenile *Seriola dorsalis* fed for 69 days with experimental diets formulated to contain different amount of methionine

	Experimental Treatments			
Fatty acids	Basal Diet	L-MET	M-MET	H-MET
14:0	2.27 ± 0.13	2.62 ± 0.19	2.33 ± 0.06	2.38 ± 0.22
15:0	0.28 ± 0.01	0.34 ± 0.03	0.25 ± 0.01	0.25 ± 0.04
16:0	16.35 ± 0.27	16.40 ± 0.14	16.24 ± 0.26	15.77 ± 0.29
16:1	2.87 ± 0.06	3.09 ± 0.11	2.99 ± 0.09	2.66 ± 0.26
18:0	7.90 ± 0.36	7.90 ± 0.56	8.19 ± 0.49	8.86 ± 0.56
18:1n9	36.85 ± 1.82	36.88 ± 2.79	38.81 ± 0.93	35.50 ± 1.80
18:1n7	2.34 ± 0.33	2.28 ± 0.09	2.02 ± 0.20	2.32 ± 0.01
18:2n6	14.80 ± 0.50	14.84 ± 0.49	15.61 ± 0.24	15.25 ± 0.54
18:3n6	0.75 ± 0.02^{b}	0.90 ± 0.04^{a}	0.85 ± 0.02^{a}	0.80 ± 0.03^{ab}
18:3n3	0.20 ± 0.02	0.20 ± 0.04	0.16 ± 0.02	0.15 ± 0.02
20:1n9	0.47 ± 0.03	0.50 ± 0.05	0.49 ± 0.01	0.45 ± 0.05
20:4n6	1.53 ± 0.20	1.33 ± 0.19	1.13 ± 0.03	1.32 ± 0.15
20:5n3	0.73 ± 0.09	0.82 ± 0.17	0.67 ± 0.02	0.69 ± 0.07
22:5n3	2.18 ± 0.13^{a}	2.15 ± 0.24^{a}	1.79 ± 0.02^{b}	2.45 ± 0.07^{a}
22:6n3	9.77 ± 0.59^{a}	8.94 ± 1.22^{ab}	7.77 ± 0.12^{b}	10.39 ± 1.30^{a}
SFA	26.80 ± 0.19	27.26 ± 0.23	27.01 ± 0.21	27.26 ± 0.28
MUFA	42.53 ± 0.56	42.75 ± 0.76	44.31 ± 0.31	40.93 ± 0.53
PUFA	29.96 ± 0.22	29.18 ± 0.34	27.98 ± 0.07	31.05 ± 0.31
n3/n6	0.75 ± 0.16^{a}	0.71 ± 0.33^{a}	0.59 ± 0.09^{b}	0.79 ± 0.29^{a}
SAT/IN	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.03	0.38 ± 0.02

Note: Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$.

TABLE 9 Liver fatty acid profile (% of total fatty acids) of juvenile *Seriola dorsalis* fed for 60 days of experimental diets formulated to contain different levels of methionine

	Experimental treatments			
Fatty acids	Basal Diet	L-MET	M-MET	H-MET
14:0	2.17 ± 1.06	2.97 ± 0.42	2.05 ± 1.05	2.46 ± 0.95
15:0	0.28 ± 0.09	0.30 ± 0.06	0.23 ± 0.05	0.23 ± 0.07
16:0	15.96 ± 2.79	17.41 ± 0.23	15.48 ± 3.10	15.42 ± 2.90
16:1	2.62 ± 0.95	2.89 ± 0.02	2.86 ± 0.55	2.64 ± 0.43
18:0	7.73 ± 0.33	9.58 ± 3.28	9.29 ± 1.18	9.25 ± 0.64
18:1n9	38.04 ± 1.60^{b}	42.51 ± 3.30^{ab}	44.43 ± 3.04^{a}	41.78 ± 0.68^{ab}
18:1n7	2.07 ± 0.58	1.35 ± 0.15	2.02 ± 0.92	1.52 ± 0.37
18:2n6	13.91 ± 0.39	13.42 ± 0.35	13.53 ± 0.26	14.15 ± 0.46
18:3n6	0.66 ± 0.08	0.62 ± 0.01	0.66 ± 0.06	0.69 ± 0.05
18:3n3	0.15 ± 0.04	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.03
20:1n9	0.57 ± 0.10	0.54 ± 0.17	0.68 ± 0.07	0.63 ± 0.01
20:4n6	2.00 ± 1.19	1.23 ± 0.001	1.27 ± 0.40	1.34 ± 0.10
20:5n3	0.62 ± 0.25	0.44 ± 0.03	0.45 ± 0.12	0.48 ± 0.05
22:5n3	2.7885 ± 0.32	1.005 ± 0.46	1.608 ± 0.55	1.67 ± 0.61
22:6n3	10.66 ± 2.99^{a}	5.12 ± 0.53^{b}	5.23 ± 1.46^{b}	6.12 ± 0.40^{ab}
SFA	26.14 ± 1.07	30.26 ± 1.00	27.05 ± 1.35	27.36 ± 1.14
MUFA	43.30 ± 0.81^{b}	47.29 ± 0.91^{ab}	49.99 ± 1.15^{a}	46.57 ± 0.37^{ab}
PUFA	30.79 ± 0.75^{a}	21.94 ± 0.20^{b}	22.87 ± 0.41^{b}	24.57 ± 0.24^{b}
n3/n6	0.86 ± 0.31^{a}	0.44 ± 0.41^{b}	0.48 ± 0.42^{b}	0.52 ± 0.26^{ab}
SAT/IN	0.35 ± 0.03	0.44 ± 0.05	0.37 ± 0.05	0.38 ± 0.10

Note: Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$.

TABLE 10 Cholesterol content on diets, and the whole body and liver of juvenile given as mg/kg obtained after *Seriola dorsalis* was fed for 60 days the experimental diets formulated with different methionine levels under cholesterol restriction

	Experimental treatments			
	Basal Diet	L-MET	M-MET	H-MET
Diets	0.24	0.23	0.21	0.21
Whole body	0.29 ± 0.03^{b}	0.49 ± 0.16^{b}	1.03 ± 0.08^{a}	1.19 ± 0.3^{a}
Liver	0.39 ± 0.05^{b}	0.66 ± 0.18^{b}	0.67 ± 0.09^{b}	1.18 ± 0.14^{a}

Note: Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$.

The cholesterol content in the whole body was significantly higher for the M-MET and H-MET dietary treatments, whereas in the liver, a significantly higher amount was only found in fish fed H-MET diets (Table 10).

The amino acid profile obtained in the whole fish after the experiment was significantly higher only for the Val and Leu concentrations (M-MET and H-MET dietary treatments), whereas the concentration of Met did not vary significantly among the experimental treatments (Table 11).

Concerning the gene expression levels, a tendency to increase towards higher MET levels was observed in *mtor* and *srebp-1*, but there were no significant differences among dietary treatments. Likewise, a tendency to increase accordingly to Met supplementation was observed and a significant increase in H-MET inclusion was found in *mat* (120.56 ± 3.29), all three dietary treatments containing methionine for *hmg-CoA* and H-MET relative to the BASAL and L-MET dietary treatment for *igf-1*. A significant decrease in expression level as Met increase was found in *bhmt* in M-MET (0.61 ± 0.12) and H-MET (0.63 ± 0.12) (Figure 1).

TABLE 11 Whole-body amino acid profile (g/kg) of juvenile *Seriola dorsalis* fed with different levels of methionine

	Experimental treatments			
AA	Basal Diet	L-MET	M-MET	H-MET
Essential AA				
HIS	23.9 ± 3.9	33.1 ± 0.7	29.2 ± 3.9	28.2 ± 1.2
ARG	51.3 ± 2.2	66.6 ± 3.1	55.4 ± 10.2	63.6 ± 2.9
THR	30.6 ± 0.15	34.2 ± 4.4	31.7 ± 1.6	35.5 ± 4.7
VAL	29.1 ± 0.9^{b}	29.0 ± 1.0^{b}	32.8 ± 1.4^{a}	33.8 ± 0.2^{a}
MET	13.9 ± 1.8	15.9 ± 4.3	14.4 ± 3.4	15.8 ± 0.4
LYS	46.0 ± 1.7	41.5 ± 9.2	58.1 ± 9.4	64.8 ± 8.8
ILE	26.0 ± 1.2	27.6 ± 1.4	29.5 ± 1.6	29.2 ± 1.8
LEU	49.0 ± 0.7^{c}	50.6 ± 0.1^{bc}	53.8 ± 1.0^{ab}	56.5 ± 2.1^{a}
PHE	33.0 ± 0.35	44.2 ± 7.9	35.3 ± 6.4	33.6 ± 3.3
TAU	13.6 ± 3.2	15.5 ± 1.2	17.5 ± 0.9	12.8 ± 2.7
Non-essential AA				
ASP	36.8 ± 0.7	32.3 ± 7.9	47.8 ± 8.8	47.1 ± 5.6
SER	31.2 ± 1.4	33.7 ± 0.2	37.0 ± 5.1	35.1 ± 0.5
GLU	61.9 ± 1.0	56.4 ± 9.8	55.7 ± 30.6	76.7 ± 5.7
GLY	62.0 ± 6.6	74.5 ± 6.0	61.8 ± 2.2	71.9 ± 5.2
ALA	33.2 ± 2.5	30.0 ± 1.9	32.7 ± 7.2	43.4 ± 0.8
PRO	39.9 ± 1.6	37.6 ± 1.5	41.8 ± 1.2	42.9 ± 2.7
CYS	Tr	Tr	Tr	Tr
TYR	27.6 ± 2.5	37.0 ± 7.7	31.2 ± 4.4	31.0 ± 1.8

Note: Values in the same row with different superscripts are statistically different $p < .05$; $n = 3$.

Abbreviation: Tr, trace amount.

4 | DISCUSSION

The present diets were formulated to have the same dietary level, while the content of Met was varied (from 7.5 to 17.9 g/kg feed), all with low cholesterol level but similar among the experimental diets. Even if cholesterol can be synthesized by most fish, according to earlier experiences (Guerra-Olvera & Viana, 2015), reduced growth would result due to a restrictive content of cholesterol in diets, probably for energy expenditure on its synthesis. In the present work, no fish oil was used, containing a low amount of fish meal, resulting in low cholesterol content (around 0.2 mg/kg), whereas different levels of Met resulted in similar growth among treatments. The latter is contrary to most works related to the Met effect on nutrition physiology (Dabrowski & Guderley, 2002; Gao et al., 2019; Latimer et al., 2018), where apparent differences were reported. Even if there is no evidence to suggest that in those studies, the level of cholesterol in the experimental diets was at a restrictive level, fish oil, which contains a high amount of cholesterol, was used in the diets, assuming that cholesterol content was provided in sufficient amount (NRC, 2011). In this work, the low amount of cholesterol was compensated by its synthesis from methionine, until methionine reaches its needs for growth. The fact that it is also confirmed with the IGF-1 expression, which resulted in a significantly higher (0.2-fold) at higher Met, whereas the mTOR resulted in an increase expression (but not significantly) according to the dietary content of Met. For the present work, the branch amino acids Val and Leu increased in fish tissue, particularly for the M-MET and H-MET dietary treatments. Our observation has support from the results of the investigation of Torres-Velarde et al. (2018), where those AAs were demonstrated to have a direct influence on the expression of IGF-1 and mTOR. The lack of a stronger response in the IGF-1 in our present work could be a result of the energy required for cholesterol synthesis, as shown by the expression of HMG-CoA. Here, the cholesterol content in tissues was significantly increased in response to increasing levels of dietary methionine, M-MET and H-MET for the whole body and H-MET for liver, possible reducing the energy directed for growth. The lack of significant difference in

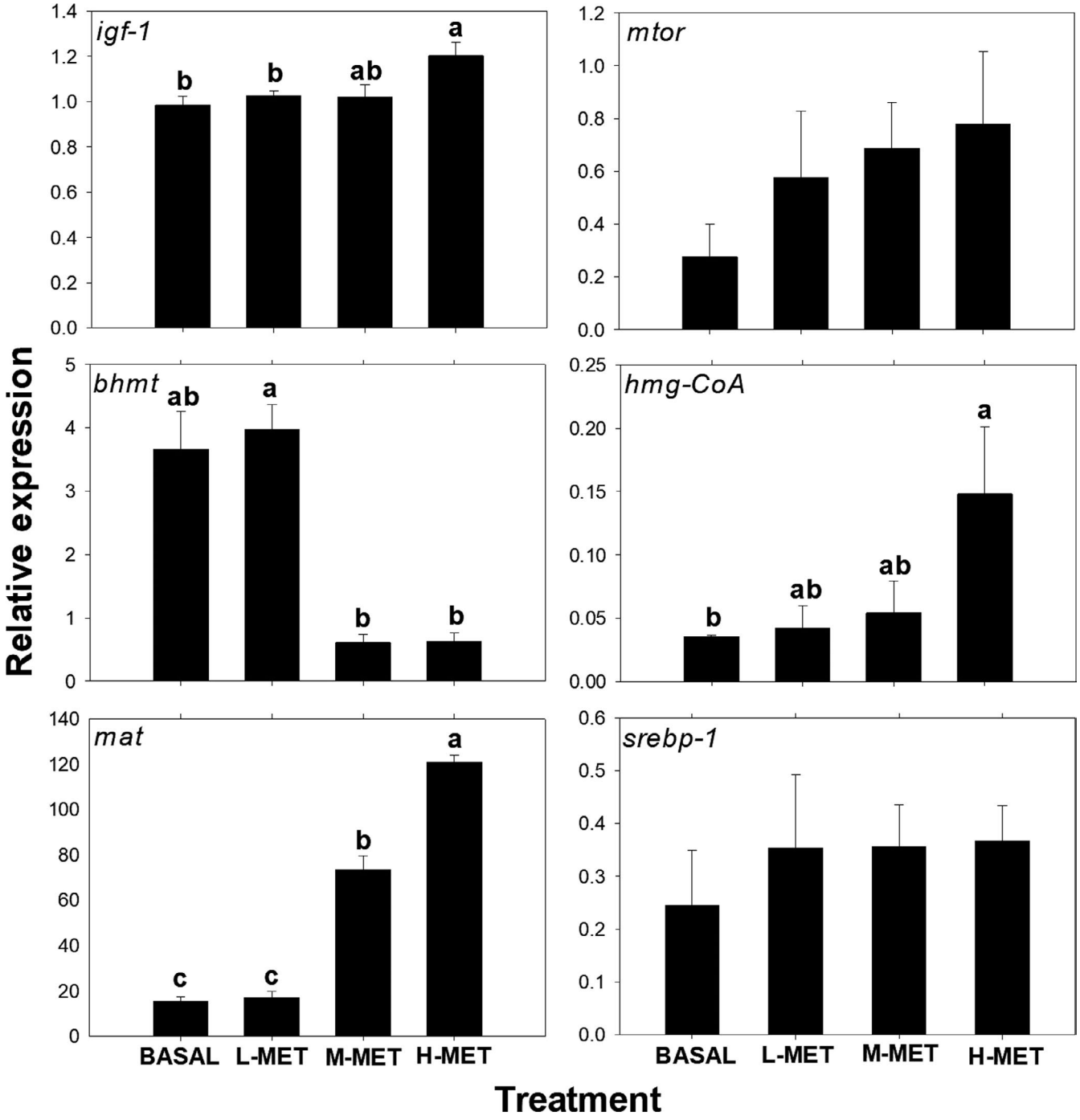

FIGURE 1 Relative gene expression from liver tissue of juvenile *Seriola dorsalis* fed experimental diets containing different levels of methionine; Basal Diet, 7.5 Met; L-Met, 10.0; M-Met, 12.9; H-Met, 17.9 g/kg. The genes tested were as follows: A) methionine adenosyltransferase (MAT); B) betaine–homocysteine methyltransferase (BHMT); C) hydroxy-methyl-glutaryl-coenzyme A reductase (HMG-CoA); D) sterol regulatory element-binding protein (SREBP-1); target of rapamycin (TOR); E) insulin-like growth factor-1 (IGF-1)

mTOR expression lacks an explanation based on what would be anticipated, despite the data showing an increasing trend in expression. A reduction among individuals through increased sample size may have revealed significant differences among dietary treatments.

In general, the fat deposition in the liver was significantly influenced by the increased Met supplementations from 173 to 299 mg/g tissue. The accumulation of lipids in the liver when Met is not restricted is a result reported for most previous investigations with fish and mammals (Craig & Moon, 2013; Espe et al., 2010; Rolland et al., 2016; Wang et al., 2016). In contrast, Latimer et al. (2018) reported a decrease in lipid accumulation at higher levels of Met. This observation could be due to differences in the physiological status of fish with the use of soybean meal as the primary protein being a source of health impairment (Blafuss, Gaylord, Sealey, & Powel, 2019).

The lipid accumulation in the muscle tissue, and whole body, in our investigation, was significantly higher only in the M-MET treatment, which is in contrast to most works reported (Craig & Moon, 2013; Espe et al., 2010; Rolland et al., 2016; Wang et al., 2016). However, our research effort was designed for discerning the priority in the different metabolic pathways where Met participates, using different levels of Met under the low presence of cholesterol. Nevertheless, by the gene expression from the different routes involved, the IGF-1 only was significantly higher in the H-MET treatment by 0.2-fold. So even if the growth rate was directly affected by Met supplementation, this result indicates that when all other metabolites are supplied as needed, growth should be even higher.

Also, the MAT expression significantly increased in M-MET and H-MET treatments, precisely the opposite than that observed with BHMT. Once again, this is an inconsistency because, under the present conditions, the dietary Met requirement should be that registered in the M-MET (12.9 g/kg diet). According to Smulan et al. (2016), SREBP-1 is the key coordinator for the lipogenesis of triglycerides. However, in the present work, this gene did not show significant differences among treatments. As a result, no differences in the crude lipid content in the whole body and muscle tissue were observed, whereas only in the M-MET treatment higher content was detected. The fatty acid accumulation resulted in slight differences in a few of the total fatty acids. In the whole body, the stearate and linoleic fatty acid increased in the L-MET and M-MET treatments, followed by a decrease to the same level than that observed in the Basal Diet in H-MET (Table 7). The change in the proportion of stearate and linoleic acid appears to be associated with fat deposition, possibly due to a reduction in lipolysis more than the lipogenesis in the L-MET treatment. Wang et al. (2016) revealed changes in fatty acid composition related to dietary Met supplementation in cobia (*Rachycentron canadum*). Those authors showed an increase in fat deposition in the liver from 6.2 to 10.2 g Met/kg diet, and further, a decrease was observed up when Met was supplied in excess 14.2 g Met/kg diet. However, Zhou et al. (2016), in a review manuscript dealing with Met restriction in pigs and rats, raises the importance of reducing the Met to decrease the lipogenesis.

In the present work, the cholesterol content in the whole body was significantly higher at higher Met supplementation (M-MET and H-MET). In contrast, in the liver, only the H-MET resulted in significantly higher accumulation. An effect that somehow is directly related to Met, where Met is prioritized for growth, and once the Met reach its requirement for growth, then the cholesterol biosynthetic pathway is initiated. According to Madrid et al. (2019), the Met requirement in totoaba should be 0.79 g/kg; however, according to our results, Met requirement should be higher. Our cumulative results suggest that the dietary Met requirement for *S. dorsalis* falls within the range of higher than 10.0 but less than 12.9 g/kg. Moreover, even for the L-MET treatment where the HMG-CoA was low (0.05), cholesterol started to accumulate in the different tissues, where the significantly highest expression was observed for the H-MET treatment. In agreement with Maita et al. (2006), the cholesterol biosynthesis is regulated by the HMG-CoA reductase, as here reported. However, here we found that before this activity, Met needs to be present as required to sustain growth. Therefore, it is recommended to supply cholesterol in fishmeal-free diets to spare protein for growth.

Among the LC-PUFAs, the DHA and EPA are generally not synthesized in marine fish (Morais, Monroig, Zheng, Leaver, & Tocher, 2009), or at least it has yet to be detected in *S. dorsalis*. Therefore, the relative reduction of DHA in those fish with higher growth could be related to a dilution effect in muscle tissues where there is an energy sparing effect.

The continuous growth along treatments means that growth, even under Met restriction, as its synthesis takes on significance. While the cholesterol synthesis, when is restricted, preceded until Met has reached the requirement. According to Guerra-Olvera and Viana (2015), the cholesterol requirement for *S. dorsalis* should be between 2.8 to 4.1 g/kg fat and 0.56 to 0.82 g/kg of diet. And despite the cholesterol restriction, the cholesterol was accumulated in the muscle of *S. dorsalis* up to 1.48 in muscle and 2.5 mg/g in liver tissues, at higher levels than that observed in the present study. Nonetheless, Guerra-Olvera and Viana (2015) revealed less accumulation of cholesterol following the dietary cholesterol addition. Despite the cholesterol accumulation under a restriction of dietary cholesterol, in that work, it was concluded that cholesterol synthesis could hamper the growth due to the energy cost of synthesis. For that experiment, gene expression was not analysed.

This study would have yielded more information about response if another diet containing little or no cholesterol had been included in the experimental design, and if the cholesterol content of fish tissue had been determined before the initiation of the experiment. Nonetheless, methionine exerts a direct influence on the growth of *S. dorsalis*, despite the restriction of dietary cholesterol. The energy used to synthesize cholesterol could restrict the growth rate potential, thereby explaining the low IGF-1 changes, as well as the mTOR. We conclude that under the dietary cholesterol restriction, the methionine requirement for *S. dorsalis* probably falls within the range of higher than 10.0 but <12.9 g/kg. This range is outside the reported methionine requirement for species of marine fish (range of 8 to 9 g/kg, NRC, 2011; Madrid et al., 2019). It appears that with the cholesterol restriction, more dietary methionine is needed to "compensate" for the cholesterol restriction. In our study, at a dietary level of 12.9 g/kg of methionine, no significant increases in growth occur while increases in cholesterol in the body tissue continue to be observed. Accordingly, in fish diets without fishmeal and fish oil ingredients, there is an obvious need to ensure that sufficient dietary cholesterol is provided to reduce the energy expenditure required for its synthesis.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

AOAC (1995). *Official Methods of Analysis of AOAC International*, Vol. *1*. Arlington, VA: Association of Official Analytical Chemists International.

Badillo-Zapata, D., Lazo, J. P., Herzka, S. Z., & Viana, M. T. (2016). The effect of substituting fishmeal with poultry by-product meal in diets for Totoaba macdonaldi juveniles. *Aquaculture Research*, *47*, 1778–1789.

Barreto-Curiel, F., Focken, U., D'Abramo, L. R., Cuaron, J. A., & Viana, M. T. (2018). Use of isotopic enrichment to assess the relationship among dietary protein levels, growth and nitrogen retention in juvenile *Totoaba macdonaldi*. *Aquaculture*, *495*, 794–802. https://doi.org/10.1016/j.aquaculture.2018.06.001

Barreto-Curiel, F., Focken, U., D'Abramo, L. R., Mata-Sotres, J., & Viana, M. T. (2019). Assessment of amino acid requirements for *Totoaba macdonaldi* at different levels of protein using stable isotopes and a non-digestible protein source as a filler. *Aquaculture*, *503*, 550–561. https://doi.org/10.1016/j.aquaculture.2019.01.038

Blafuss, P. C., Gaylord, T. G., Sealey, W. M., & Powel, M. S. (2019). Effects of high-soy diet on S100 gene expression in liver and intestine of rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, *86*, 764–771. https://doi.org/10.1016/j.fsi.2018.12.025

Christie, W. W. (1993). Preparation of ester derivatives of fatty acids for chromatographic analysis. In W. W. Christie (Ed.), *Advances in lipid methodology*, Vol. *2* (pp. 69–111). Dundee: Oily Press.

Craig, P. M., & Moon, T. W. (2013). Methionine restriction affects the phenotypic and transcriptional response of rainbow trout (*Oncorhynchus mykiss*) to carbohydrate-enriched diets. *British Journal of Nutrition*, *109*(3), 403–414. https://doi.org/10.1017/S0007114512001663

Dabrowski, K., & Guderley, H. (2002). Intermediary metabolism. In J. E. Halver & R. W. Hardy (Eds.), *Fish nutrition* (pp. 309–365). San Diego, CA: Academic Press.

Espe, M., Rathore, R. M., Du, Z. Y., Liaset, B., & El-Mowafi, A. (2010). Methionine limitation results in increased hepatic FAS activity, higher liver 18: 1 to 18: 0 fatty acid ratio and hepatic TAG accumulation in Atlantic salmon, *Salmo salar*. *Amino Acids*, *39*(2), https://doi.org/10.1007/s00726-009-0461-2

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *276*, 497–507.

Fu, S. J., & Xie, X. J. (2004). Nutritional homeostasis in carnivorous southern catfish (*Silurus meridionalis*): Is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *Comparative Biochemistry and Physiology A*, *139*, 359–363. https://doi.org/10.1016/j.cbpb.2004.10.003

Gao, Z., Wang, X., Tan, C., Zhou, H., Mai, K., & He, G. (2019). Effect of dietary levels on growth performance, amino acid metabolism and intestinal homeostasis in turbot (*Scophthalmus maximus L.*). *Aquaculture*, *498*, 335–342.

Guerra-Olvera, F., & Viana, M. T. (2015). Effect of dietary cholesterol content on growth and its accumulation in liver and muscle tissues of juvenile yellowtail kingfish (*Seriola lalandi*). *Ciencias Marinas*, *41*, 143–156. https://doi.org/10.7773/cm.v41i2.2514

Hirche, F., Schröder, A., Knoth, B., Stangl, G. I., & Eder, K. (2006). Effect of dietary methionine on plasma and liver cholesterol concentrations in rats and expression of hepatic genes involved in cholesterol metabolism. *British Journal of Nutrition*, *95*(5), https://doi.org/10.1079/bjn20061729

Illingworth, D. R., & Tobert, J. A. (2001). HMG-CoA reductase inhibitors. *Advances in Protein Chemistry*, *56*, 77–114. https://doi.org/10.1016/s0065-3233(01)56003-9

Kaushik, S. J., & Seiliez, I. (2010). Protein and amino acid nutrition and metabolism in fish: Current knowledge and future needs. *Aquaculture Research*, *41*, 322–332. https://doi.org/10.1111/j.1365-2109.2009.02174.x

Krebs, H. A., Camb, M. A., & Hamburg, M. D. (1937). The intermediate metabolism of carbohydrates. *The Lancet*, *25*, 736–738. https://doi.org/10.1016/S0140-6736(00)88690-0

Latimer, M. N., Cleveland, B. M., & Biga, P. R. (2018). Dietary methionine restriction: Effects on glucose tolerance, lipid content and micro-RNA composition in the muscle of rainbow trout. *Comparative Biochemistry and Physiology C*, *208*, 47–52. https://doi.org/10.1016/j.cbpc.2017.10.012

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402–408.

Madrid, J., Pohlenz, C., Viana, M. T., & Lazo, J. P. (2019). Dietary lysine requirement for juvenile, Totoaba macdonaldi. *Aquaculture*, *500*, 92–98. https://doi.org/10.1016/j.aquaculture.2018.10.003

Maita, M., Maekahwa, J., Satoh, K., Futami, K., & Sotoh, S. (2006). Disease resistance and hypocholesterolemia in yellowtail Seriola quinqueradiata fed a non-fishmeal diet. *Fishery Science*, *72*, 513–519. https://www.springer.com/journal/12562

Morais, S., Monroig, Ó., Zheng, X., Leaver, M. J., & Tocher, D. R. (2009). Highly unsaturated fatty acid synthesis in Atlantic salmon: Characterization of Elovl5- and Elovl2-like elongases. *Marine Biotechnology*, *11*, 627–639. https://doi.org/10.1007/s10126-009-9179-0

Morita, T., Oh-hashi, A., Takei, K., Ikai, M., Kasaoka, S., & Kiriyama, S. (1997). Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. *Journal of Nutrition*, *127*, 470–477. https://doi.org/10.1093/jn/127.3.470

National Research Council (NRC) (2011). *Nutrient Requirements of Fish and Shrimp* (392 pp). Washington, DC: Editorial National Academics Press.

Oda, H. (2006). Functions of sulfur-containing amino acids in lipid metabolism. *Journal of Nutrition*, *1*(2), 1666s. https://doi.org/10.1093/jn/136.6.1666S

Otasevic, V., & Korac, B. (2016). Amino acids: Metabolism. In B. Caballero P.M. Finglas & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 149–155). Cambridge, MA: Academic Press.

Poloni, S., Blom, H., & Schwartz, I. (2015). Stearoyl-CoA desaturase-1: is it the link between sulfur amino acids and lipid metabolism? *Biology (Basel)*, *4*, 383–396. https://doi.org/10.3390/biology4020383

Ramani, K., & Lu, S. C. (2017). Methionine adenosyltransferases in liver health and diseases. *Liver Research*, *1*, 103–111. https://doi.org/10.1016/j.livres.2017.07.002

Rolland, M., Skov, P. V., Larsen, B. K., Holm, J., Gómez-Requeni, P., & Dalsgaard, J. (2016). Increasing levels of dietary crystalline methionine affect plasma methionine profiles, ammonia excretion, and the expression of genes related to the hepatic intermediary metabolism in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology B*, *198*, 91–99. https://doi.org/10.1016/j.cbpb.2016.04.006

Smulan, L. J., Ding, W., Freinkman, E., Gujja, S., Edwards, Y. J. K., & Walker, A. K. (2016). Cholesterol-independent SREBP-1 maturation is linked to ARF1 inactivation. *Cell Reproduction*, *16*, 1–10. https://doi.org/10.1016/j.celrep.2016.05.086

Torres-Velarde, J., Llera-Herrera, R., García-Gasca, T., & García-Gasca, A. (2018). Mechanisms of stress-related muscle atrophy in fish: An *ex vivo* approach. *Mechanisms of Development*, *154*, 162–169. https://doi.org/10.1016/j.mod.2018.07.002

Van Milgen, V. J., & Dourmad, J. Y. (2015). Concept and application of ideal protein for pigs. *J. Animal Science and Biotechnology*, *6*, 15. https://doi.org/10.1186/s40104-015-0016-1

Vélez, E. J., Lutfi, E., Azizi, S. H., Perelló, M., Salmerón, C., Riera-Codina, M., … Gutiérrez, J. (2017). Understanding fish muscle growth regulation to optimize aquaculture production. *Aquaculture*, *467*, 28–40. https://doi.org/10.1016/j.aquaculture.2016.07.004

Wang, Z., Mai, K., Xu, W., Zhang, Y., Liu, Y., & Ai, Q. (2016). Dietary methionine level influences growth and lipid metabolism via GCN2 pathway in cobia (*Rachycentron canadum*). *Aquaculture*, *454*, 148–156. https://doi.org/10.1016/j.aquaculture.2015.12.019

Wu, G. (2010). Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, *1*, 31–37. https://doi.org/10.3945/an.110.1008.1

Xu, H., Zhang, Q., Wei, Y., Liao, Z., & Liang, M. (2019). Dietary methionine increased the lipid accumulation in juvenile tiger puffer Takifugu rubripes. *Comparative Biochemistry and Physiology B*, *230*, 19–28. https://doi.org/10.1016/j.cbpb.2019.01.005

Yang, Z., Wang, Z. Y., Yang, H. M., Xu, L., & Gong, D. Q. (2017). Effects of dietary methionine and betaine on slaughter performance, biochemical and enzymatic parameters in goose liver and hepatic composition. *Animal Feed Science and Technology*, *228*, 48–58. https://doi.org/10.1016/j.anifeedsci.2017.04.003

Zhou, X., He, L., Wan, D., Yang, H., Yao, K., Wu, G., … Yin, Y. (2016). Methionine restriction on lipid metabolism and its possible mechanisms. *Amino Acids*, *48*, 1533–1540. https://doi.org/10.1007/s00726-016-2247-7

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