UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS DEL MAR Y LIMNOLOGÍA

Efecto de los carbohidratos y lípidos en la dieta sobre el crecimiento, eficiencia alimenticia, enzimas digestivas y del metabolismo intermediario en el hígado del robalo blanco *Centropomus undecimalis* (Bloch, 1792) y pargo canané *Ocyurus chrysurus* (Bloch, 1791)

TESIS
(POR ARTÍCULO CIENTÍFICO)

QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS

PRESENTA:
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DEDICATORIA

Dedico esta tesis a mi madre…

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CAPÍTULO 1

Estructura general de la tesis: Introducción general, hipótesis y objetivos

Resumen

El presente capítulo tiene la finalidad de mostrar la estructura de la presente tesis, proporciona una introducción general de las habilidades y limitaciones fisiológicas de los peces para utilizar los carbohidratos y lípidos como fuente de energía, así como una breve descripción del estado general del robalo blanco *Centropomus undecimalis* y el pargo canané *Ocyurus chrysurus*. Esta tesis se encuentra conformada por cinco capítulos en total. El **primer capítulo** lleva como título "**Estructura general de la tesis**" y contiene información general de los diferentes temas. El **segundo capítulo** tiene como objetivo evaluar la habilidad de *C. undecimalis* y *O. chrysurus* para utilizar los carbohidratos en el alimento a través de un estudio comparativo y se titula: "**Efecto de los carbohidratos en el alimento sobre el crecimiento, la eficiencia alimentaria y el metabolismo de la glucosa en juveniles de róbalo común (*C. undecimalis*) y pargo canané (*O. chrysurus*)**". En este capítulo se utilizaron indicadores zootécnicos (p. ej. crecimiento y consumo de alimento), así como herramientas bioquímicas (p. ej. análisis bromatológicos, actividad *in vitro* de enzimas digestivas y del metabolismo intermediario en el hígado) y moleculares (expresión de genes). El **tercer capítulo** se titula "**Evaluación de la relación proteína: lípidos sobre el crecimiento, la eficiencia alimentaria y la respuesta metabólica en juveniles de pargo cola amarilla (*O. chrysurus*, Bloch, 1791)**". El **cuarto capítulo** es titulado" **Efectos fisiológicos y metabólicos del efecto de ahorro de proteína por lípidos en el alimento en juveniles de róbalo blanco *C. undecimalis* (Bloch, 1792)**". Tanto el tercero y cuarto capítulo, utilizaron indicadores zootécnicos (p. ej. crecimiento y consumo de alimento) y herramientas bioquímicas (p. ej. análisis bromatológicos y actividad *in vitro* de enzimas digestivas y del metabolismo intermediario en el hígado). Y finalmente, el **quinto capítulo** comprende una discusión general y conclusiones.

Abstract

This chapter aims to show the structure of this thesis, provide a general overview of the physiological skills and limitations of fish to use carbohydrates and lipids as an energy source, as well as a brief description of the general status of common snook *Centropomus undecimalis* and yellowtail snapper *Ocyurus chrysurus*. This thesis consists of five chapters in total. The **first chapter** name is "**General structure of the thesis**" and contains general information. The **second chapter** aimed to evaluate the ability of *C. undecimalis* and *O. chrysurus* to use dietary carbohydrates through a comparative study and is name: " **Effect of dietary carbohydrates on growth performance, feed efficiency and glucose metabolism in common snook (*Centropomus undecimalis*) and yellowtail snapper (*Ocyurus chrysurus*) juveniles**". This chapter used growth performance indicators (e.g. food growth and consumption), as well as biochemical analyses (e.g. proximal composition, *in vitro* activity of digestive enzymes and intermediate metabolism in the liver) and molecular (gene expression). The **third chapter** is name " **Evaluation of protein: lipid ratio on growth, feed efficiency and metabolic response in juvenile yellowtail snapper (*Ocyurus chrysurus*, Bloch, 1791)**". The **fourth chapter** was titled " **Physiological and metabolic protein-sparing effects of dietary lipids on common snook *Centropomus undecimalis* (Bloch, 1792) juveniles**"). Both the third and fourth chapters used growth performance indicators (e.g. food growth and consumption) and biochemical analyses (e.g. proximal composition and *in vitro* activity of digestive enzymes and intermediate metabolism in the liver). Finally, the **fifth chapter** includes a general discussion and conclusions.

Introducción general

La proteína es por lo general el mayor componente dietético en la formulación de alimentos balanceados para peces en la acuicultura, particularmente para las especies con hábitos carnívoros (Tacon, 2004; Tacon & Metian, 2008). La demanda de proteína en los alimentos balanceados por los peces varía entre 24 y 70%, dependiendo de la etapa de vida, nivel trófico, salinidad y temperatura del agua (Oliva-Teles, Couto, Enes, & Peres, 2020). En peces, la proteína además de ser necesaria para propósitos plásticos (crecimiento, mantenimiento, reparación de tejido), síntesis de enzimas, hormonas y otros metabolitos, cumplen funciones energéticas (NRC, 2011; Wilson, 2002). La proteína es considerada el sustrato primario de los peces para la obtención de energía, aproximadamente el 80% de la producción total de adenosín trifosfato (ATP) en peces proviene del catabolismo de proteína (Cowey & Walton, 1988; Jia, Li, Zheng, & Wu, 2017). El uso de la proteína con fines energéticos por los peces en la acuicultura es indeseable debido a su alto costo en comparación con otros componentes dietéticos altamente energéticos como son los lípidos y carbohidratos (NRC, 2011; Sargent, Tocher, & Bell, 2002; Wilson, 1994). Por otra parte, la liberación de amonio, producto directo del catabolismo de la proteína, impacta negativamente el medio ambiente, debido a que es uno de los principales componentes nitrogenados responsables de la eutrofización del agua (Van Waarde, 1983). El catabolismo de proteína con fines energéticos es inevitable en peces, pero puede reducirse con un nivel de inclusión apropiado de energía digestible no proteica reemplazado por carbohidratos y lípidos, un efecto comúnmente referido como ahorro de proteína (Kaushik & Seiliez, 2010; NRC, 2011).

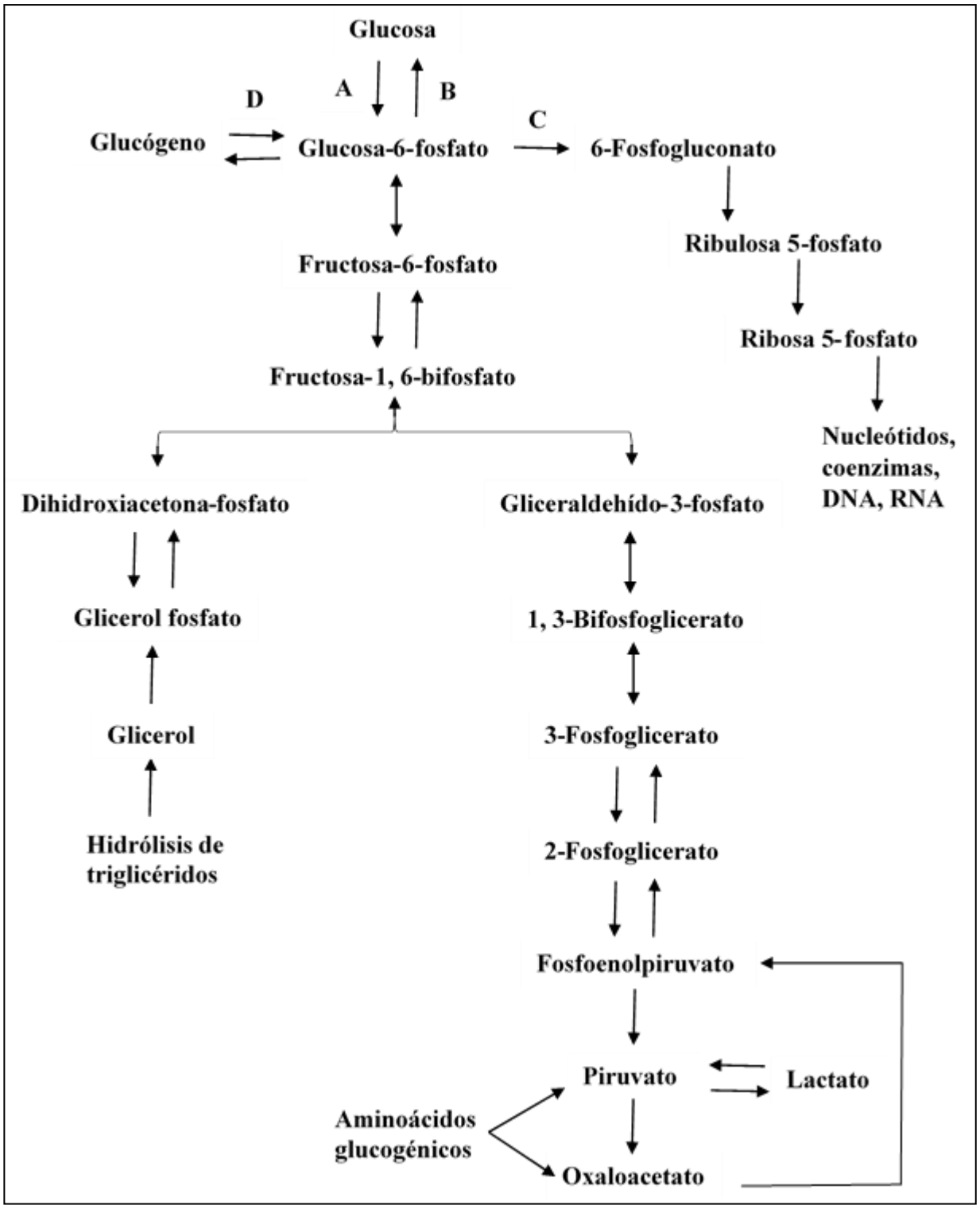

Figura 1. Rutas metabólicas: glucólisis (**A**), gluconeogénesis (**B**), pentosa fosfato (**C**) y síntesis de glucógeno (**D**).

1. Generalidades del uso de carbohidratos por peces

Los carbohidratos son la fuente de energía dietética más barata en vertebrados terrestres (Wilson, 1994). En peces, no existe un requerimiento de carbohidratos debido a su eficiente habilidad para sintetizar glucosa a partir de precursores de origen proteínico y lipídico (glicerol, piruvato, aminoácidos y lactato) (NRC, 2011). Por otra parte, en algunos peces se ha observado que un nivel de inclusión adecuado de carbohidratos en la formulación de alimentos balanceados reduce el catabolismo de proteína con fines energéticos (ahorro de proteína), lo cual a su vez promueve la retención de proteína con fines de crecimiento (NRC, 2011). Sin embargo, la habilidad de los peces para utilizar los carbohidratos es limitada con respecto a los vertebrados terrestres (Panserat, Rideau, & Polakof, 2014), estos experimentan prolongados periodos de hiperglicemia después de consumir carbohidratos, especialmente las especies con hábitos carnívoros (Bergot, 1979; Enes, Peres, Almeida, Couto, & Oliva-Teles, 2011; Hutchins, Rawles, & Gatlin, 1998; Lee, Kim, & Lall, 2003; Panserat *et al*., 2001), a pesar de poseer un sistema homeostático de glucosa activo (Polakof, Panserat, Soengas, & Moon, 2012). Las bases fisiológicas de la aparente intolerancia de glucosa en los peces es un tema polémico complicado por la extrema diversidad de hábitos alimenticios, hábitats, características anatómicas y fisiológicas (Kamalam, Medale, & Panserat, 2017).

Varias hipótesis han sido propuestas para tratar de explicar la baja habilidad de algunos peces para utilizar la glucosa de origen dietético en comparación con otros vertebrados: 1) una baja potencia de la glucosa como secretagogo de insulina en comparación con los aminoácidos (Mommsen & Plisetskaya, 1991); 2) una baja fosforilación de glucosa en tejidos sensibles a la insulina como lo es el musculo blanco (pobre transporte de glucosa) (Navarro *et al*., 1999; West, Arthur, Suarez, Doll, & Hochachka, 1993); 3) la ausencia de inhibición de la producción de glucosa endógena (gluconeogénesis) (Enes, Panserat, Kaushik, & Oliva-Teles, 2009; Panserat *et al*., 2001). Polakof *et al*. (2012) sugieren que la relativa habilidad de los peces para utilizar los carbohidratos con respecto a otros vertebrados se encuentra estrechamente relacionado con su estilo de vida ectotérmico (una baja tasa metabólica y un metabolismo adaptado a largos periodos de ayuno).

2.1. Habilidad de los peces para utilizar carbohidratos

La relativa habilidad de los peces para utilizar los carbohidratos como fuente de energía varía ampliamente entres especies, pero depende de sus hábitos alimenticios (Stone, 2003; Wilson, 1994). Los peces con hábitos herbívoros y omnívoros pueden tolerar porcentajes de inclusión de carbohidratos en el alimento entre 20 y 42%, sin efectos adversos en su crecimiento o supervivencia, mientras que los porcentajes de inclusión de carbohidratos en especies con hábitos carnívoros puede varía entre 6 y 32% (Kamalam *et al*., 2017). Los peces con hábitos herbívoros y omnívoros en comparación con los carnívoros presentan una mayor actividad de las enzimas α amilasa, sacarasa y maltasa (Harpaz & Uni, 1999; Hidalgo, Urea, & Sanz, 1999), mayor tasa de absorción de glucosa en el intestino (Buddington, Chen, & Diamond, 1987), mayores receptores de insulina en el musculo esquelético (Parrizas, Planas, Plisetskaya, & Gutierrez, 1994), mejor regulación de la síntesis de glucosa endógena (gluconeogénesis) (Enes, Panserat, Kaushik, & Oliva-Teles, 2006, 2008; Figueiredo-Silva *et al*., 2013; Panserat *et al*., 2001) y una mayor velocidad de respuesta en la inducción de la enzima hepática glucoquinasa (GK) o hexoquinasa IV (HK IV) después de consumir carbohidratos (Boonanuntanasarn *et al*., 2018; Panserat *et al*., 2001; Ren *et al*., 2015), lo que posiblemente explica el mejor control de la glucosa postprandial en las especies herbívoras y omnívoras, respecto a las carnívoras (Furuichi & Yone, 1981; Panserat *et al*., 2000).

2.2. Enzima GK en la homeostasis de la glucosa en peces

La enzima GK o HK IV al igual que la HK I, HK II y HK III, cataliza la primera reacción de la ruta de glucólisis (fosforilación de glucosa a glucosa-6 fosfato), pero la GK a diferencia del resto de isoenzimas se caracteriza por su baja afinidad por la glucosa (K_m 10 mM) y por no ser inhibida por su producto (glucosa-6 fosfato) (Iynedjian, 2009). La enzima GK juega un papel central como sensor de glucosa en la regulación de homeostasis de la glucosa (Panserat *et al*., 2014). La glucosa-6 fosfato, producto de la activación de la enzima GK, es utilizada en la ruta de glucólisis, pentosa fosfato o la síntesis de glucógeno (glucogénesis) (Figura 1).

En la ruta de glucólisis, la glucosa-6 fosfato es transformada a través de una serie de reacciones a piruvato y adenosín trifosfato (ATP), esta ruta es regulada por la actividad de tres enzimas claves: hexoquinasas (HKs), fosfofructoquinasa-1 (PFK-1) y piruvato quinasa (PK) (Figura 1). La

enzima fosfofructoquinasa-1 (PFK-1) cataliza la conversión de fructosa 6-fosfato a fructosa-1, 6-fosfato. La enzima PK cataliza la conversión de fosfoenolpiruvato a piruvato y la última etapa de glucólisis (Rui, 2014). Por otra parte, la glucosa-6 fosfato por estimulación alostérica, inactiva la enzima glucógeno fosforilasa (degradación de glucógeno) y activa la enzima glucógeno sintasa (síntesis de glucógeno) (Figura 1). El almacenamiento de glucógeno funciona como reserva para la producción de glucosa-6 fosfato, sustrato metabólico para la vía de glucólisis (Agius, 2008). La ruta de la pentosa fosfato, es una ruta metabólica alterna a la vía de glucólisis que al igual que la síntesis de glucógeno, contribuye al mantenimiento de la glucosa postprandial a partir de la molécula glucosa-6 fosfato (Agius, 2008) (Figura 1). En la ruta de la pentosa fosfato, la glucosa-6 fosfato se utiliza para la síntesis de ribosa para la formación de nucleótidos, así como también para la formación de nicotinamida adenina dinucleótido fosfato reducido (NADPH), necesario en la síntesis de ácidos grasos, el cual es formado por acción de las enzimas glucosa-6-fosfato deshidrogenasa (G6PDH) y 6-fosfogluconato deshidrogenasa (6PGDH) (Mayes & Bender, 2003).

En vertebrados, la enzima GK se expresa en una gran cantidad de tejidos, pero principalmente en el hígado (Li, Xu, Zhang, Jiang, & Liu, 2016; Panserat *et al*., 2014; Zhou *et al*., 2018). En peces, la enzima hepática GK puede ser inducida bioquímica y molecularmente por largos o cortos periodos de alimentación con carbohidratos, como ocurre en mamíferos (Panserat *et al*., 2000; Tranulis, Dregni, Christophersen, Krogdahl, & Borrebaek, 1996); sin embargo, la velocidad de inducción de esta enzima por los carbohidratos varía ampliamente entre grupos. En peces, la inducción de la enzima hepática GK ocurre 1 hora después de consumir carbohidratos en las especies con hábitos herbívoros y omnívoros, en las especies carnívoras ocurre después de 4-6 horas (Boonanuntanasarn *et al*., 2018; Caseras, Metó, Fernández, & Baanante, 2000; Panserat *et al*., 2001; Ren *et al*., 2015), mientras que en mamíferos (ratas) ocurre en cuestión de minutos (15 minutos) (Khu, Goncharova, & Rubtsov, 2015). La diferencia en el tiempo de activación de la enzima hepática GK entre mamíferos y peces, es atribuido al estilo de vida ectotérmico de los peces (baja tasa metabólica) (Polakof *et al*., 2012).

A pesar de que los peces con hábitos omnívoros poseen una mayor habilidad para utilizar los carbohidratos respecto a las especies carnívoras, las especies omnívoras exhiben una menor expresión bioquímica y molecular de la enzima hepática GK en comparación con las especies

carnívoras (Capilla *et al*., 2004; Panserat *et al*., 2000). Panserat *et al*. (2014) sugiere que la inducción de la enzima hepática GK no es un factor limitante en la utilización de los carbohidratos por los peces, al menos para las especies de hábitos omnívoros (Boonanuntanasarn *et al*., 2018; Capilla *et al*., 2004). Por otra parte, la relativa habilidad de los peces con hábitos carnívoros para metabolizar carbohidratos se encuentra directamente relacionado con la inducción de la enzima hepática GK. Por ejemplo, en el mero rojo (*Epinephelus morio*) se reportó una dependencia positiva de las enzimas G6PDH y 6PGDH hacia la regulación metabólica de la enzima GK en el hígado por los carbohidratos en el alimento (Castillo, Alvarez-González, Cuzon, Suárez, & Gaxiola, 2018; Castillo, Callejas, *et al*., 2018). Por otra parte, en la dorada (*Sparus aurata*) (Couto, Enes, Peres, & Oliva-Teles, 2008) y lubina Europea (*Dicentrarchus labrax*) (Enes, Sanchez-Gurmaches, Navarro, Gutiérrez, & Oliva-Teles, 2010) se observó una dependencia positiva en la concentración de glucógeno en el hígado (glucogénesis) y la actividad de la enzima hepática PK con respecto a la regulación metabólica de la enzima GK por los carbohidratos en el alimento.

2.3. Enzima GK en peces con hábitos carnívoros

En mamíferos, la enzima hepática GK es activada por una alta proporción insulina/glucagón, lo cual ocurre inmediatamente después de consumir carbohidratos, pero es inhibida por una baja proporción insulina/glucagón, lo cual ocurre en periodos de ayuno (Enes *et al*., 2009). En peces con hábitos carnívoros, la activación de la enzima hepática GK depende del porcentaje de inclusión de carbohidratos en el alimento. Por ejemplo, en especies como la dorada (Enes *et al*., 2008), el dentó común (*Dentex dentex*) (Pérez-Jiménez *et al*., 2015), la lobina negra (*Micropterus salmoides*) (Lin, Shi, Mu, Chen, & Luo, 2018), la lubina europea (Enes *et al*., 2006) y el mero rojo (Castillo, Alvarez-González, *et al*., 2018; Castillo, Callejas, *et al*., 2018), se ha observado que un nivel de inclusión de carbohidratos en el alimento entre 18-20% resultó en una inducible expresión bioquímica de la enzima hepática GK respecto a porcentajes de inclusión de carbohidratos inferiores a este valor. Zhou *et al*. (2018) reportaron en el pompano dorado (*Trachinotus ovatus*) una máxima expresión bioquímica y molecular de la enzima hepática GK en un nivel de inclusión de carbohidratos en el alimento de 28%, respecto a 0 y 12%. Por otra parte, Couto, Enes, Peres, & Oliva-Teles (2008) en la dorada y Pérez-Jiménez *et al*. (2015) en el dentón común, reportaron una disminución en la expresión bioquímica de la enzima hepática GK cuando

utilizaron porcentajes de inclusión de carbohidratos en la dieta, superiores a 20, 30 y 24%, respectivamente. Enes, Sanchez-Gurmaches, Navarro, Gutiérrez, & Oliva-Teles (2010) reportaron en la lubina europea que un nivel de inclusión de carbohidratos en el alimento de 30% estimuló la secreción de insulina, respecto a un nivel de inclusión de carbohidratos de 0 y 10%. Lo anterior sugiere que el efecto de los carbohidratos (glucosa dietética) como secretagogo de insulina y activación de la enzima hepática GK, ocurre en porcentaje de inclusión alrededor del 20% de carbohidratos en el alimento, al menos en las especies aquí citadas.

2.4. Gluconeogénesis en peces carnívoros

La gluconeogénesis, es la ruta metabólica responsable de la síntesis *de novo* de glucosa a partir de precursores como el glicerol, piruvato, aminoácidos y lactato (NRC, 2011) (Figura 1). En vertebrados carnívoros, la homeostasis de glucosa esta específicamente relacionado con la gluconeogénesis (Moon, 1988; Suarez & Mommsen, 1987). La regulación de esta ruta metabólica depende de la actividad de tres enzimas claves: fosfoenolpiruvato carboxiquinasa (PEPCK), fructosa-1,6-bisfosfatasa (FBPasa) y glucosa-6-fosfatasa (G6Pasa). La enzima PEPCK cataliza la conversión de oxalacetato a fosfoenolpiruvato, la primera etapa de gluconeogénesis. La enzima FBPasa cataliza la conversión de fructosa-1,6-bifosfatasa a fructosa-6-fosfatsa. La enzima G6Pasa cataliza la conversión de glucosa-6-fosfato a glucosa, la última etapa de gluconeogénesis (Rui, 2014).

Una de las hipótesis que se han planteado para explicar la relativa baja capacidad de los peces para utilizar los carbohidratos, particularmente de aquellos con hábitos carnívoros, es parcialmente atribuido a la persistente producción de glucosa endógena, esto independientemente de la ingesta de carbohidratos, un desbalance entre la gluconeogénesis y la glucólisis (Enes *et al*., 2009). Por ejemplo, en peces como la trucha arcoíris (*Oncorhynchus mykiss*) (Panserat *et al*., 2001), lubina Europea (Enes *et al*., 2006) y dorada (Enes *et al*., 2008), a pesar de exhibir una adaptación de la enzima hepática GK a los carbohidratos en el alimento (20-24%), las actividades de las enzimas hepáticas claves G6Pasa y FBPasa permanecieron constantes en comparación a un alimento control (0% carbohidratos). Sin embargo, en el salmón atlántico (*Salmo salar*) se reportó una relación positiva entre la actividad de la enzima hepática GK y el nivel de inclusión de carbohidratos en el alimento (0, 5, 10, 20, 30%), mientras que la actividad de la enzima

hepática FBPasa mostró una relación negativa, pero fue considerada despreciable para los autores (Borrebaek & Christophersen, 2000).

En la última década, el interés por explorar el efecto de los carbohidratos en el metabolismo intermediario en peces ha crecido, particularmente en aquellas especies con hábitos carnívoros, los cuales, debido a su importancia económica, son considerados una prometedora propuesta para la acuicultura. Por ejemplo, en el mero rojo, se observó una regulación nutricional en la actividad de las enzimas hepáticas involucradas en la glucólisis (GK y PK) y gluconeogénesis (FBPasa) por la presencia de carbohidratos en el alimento (Castillo *et al*., 2018; Castillo *et al*., 2018). Así mismo, Ma *et al*. (2019) reportaron en la lobina negra, que la presencia de carbohidratos en el alimento reguló la actividad de las enzimas hepáticas de la glucólisis (GK y PK) y gluconeogénesis (PEPCK, FBPasa y G6Pasa). Por otro lado, en la cobia (*Rachycentron canadum*) la presencia de carbohidratos en la dieta reguló a nivel molecular la expresión de enzima PK y PEPCK; sin embargo, a nivel bioquímico sólo la enzima PEPCK fue regulada, la enzima PK permaneció constante, independiente del nivel de inclusión de carbohidratos en el alimento (12, 22 y 34%) (Li *et al*., 2019). Los resultados obtenidos en los estudios mencionados sugieren que algunas especies de peces con hábitos carnívoros son capaces de regular o inhibir en cierto punto la producción de glucosa endógena en presencia de carbohidratos en el alimento.

Generalidades del uso de lípidos por peces

En peces, los lípidos cumplen diversas funciones, entre las cuales destacan las hormonales, estructurales y energéticas, además son fuente de ácidos grasos esenciales (AGE) (Leaver *et al*., 2008). La deficiencia de AGE en el alimento genera problemas de salud en los peces (Oliva-Teles, 2012). El requerimiento de AGE en los peces varia ampliamente entre especies por la extrema diversidad de hábitos alimenticios, hábitats, características anatómicas y fisiológicas, pero algunas generalidades en función de la temperatura del agua han sido descritas. Por ejemplo, los peces que habitan zonas frías requieren altas cantidades de estos AGE, sobretodo del tipo omega 3 para mantener la flexibilidad y permeabilidad de las membranas celulares, mientras que los peces de zonas tropicales requieren AGE del tipo omega 3 y 6 (Webster & Lim, 2001). Por otra parte, algunos peces dulceacuícolas son capaces de elongar y desaturar ácidos grasos del tipo omega 3 de 18 carbonos, específicamente el ácido linolenico (18:3), así como, de 20 y 22 carbonos: el ácido eicosapentaenoico (20:5) y docosahexaenoico (22:6), respectivamente

(Sargent *et al*., 2002). Bowyer *et al*. (2013) a través de una extensa revisión bibliográfica reportaron en función de la temperatura tres clasificaciones en el requerimiento de lípidos en peces: (i) especies de agua fría (≤ 20°C); (ii) especies templadas (20-25°C); (iii) especies de aguas cálidas (≥ 25°C). En las primeras observaron un requerimiento general de lípidos en la dieta de 35-40%, en las segundas de 15-20%, mientras que en las terceras fue de 10-14%.

En peces, los lípidos juegan un importante role como fuente de energía, en particular en aquellas especies con hábitos carnívoros, donde los carbohidratos juegan un menor papel como fuente de energía debido a su baja abundancia en su dieta natural (Sargent *et al*., 2002). La obtención de energía por los peces a partir del catabolismo de lípidos ocurre por la β oxidación de los AG y por gluconeogénesis (Li *et al*., 2016; Nanton *et al*., 2003; Peng *et al*., 2014; Stubhaug *et al*., 2007).

La β oxidación de AG ocurre en la mitocondria y los perixomas de la célula, principalmente en el hígado. En la β oxidación, los AG son convertidos a acetil-CoA, este proceso es catalizado por un grupo de enzimas acetil-CoA sintetasas. Los AG que son utilizados como sustratos en la β oxidación sufren la remoción de un par de átomos de carbono por oxidación, hasta que el AG se descompone por completo. En el proceso de oxidación de los AG, además de obtener moléculas de acetil-CoA, también se obtiene poder reductor en forma de nicotin adenin dinucleótido reducido (NADH) y flavín adenín dinucleótido reducido ($FADH_2$). Las moléculas de acetil-CoA son oxidadas para la obtención de ATP, mientras que las coenzimas reducidas NADH y $FADH_2$ pueden ingresar a la cadena respiratoria (Schulz, 1991).

La obtención de energía de los lípidos por gluconeogénesis ocurre a través del glicerol (Figura 1). El glicerol se obtiene por hidrólisis de los triglicéridos, reacción que es catalizada por lipasas. Posteriormente el glicerol es convertido a fosfato de dihidroxiacetona, el cual es convertido a glicerol 3-fosfato por la enzima triosa fosfato isomerasa para posteriormente ingresar a la ruta de gluconeogénesis para la síntesis *de novo* de glucosa (Suarez & Mommsen, 1987).

3.1. Habilidad de los peces para utilizar lípidos

El uso de lípidos como fuente de energía por los peces ha sido ampliamente documentado. En general, un incremento en el contenido de lípidos en el alimento reduce el catabolismo de proteínas con propósitos energéticos en los peces, lo cual a su vez promueve la retención de

proteína con fines de crecimiento (Guo *et al*., 2019; Lee & Kim, 2009; Li *et al*., 2016; Li *et al*., 2016; Wang *et al*., 2005); sin embargo, altos porcentajes de inclusión de lípidos en el alimento genera problemas negativos en el crecimiento y promueve la deposición de lípidos de reserva en el pez (Peres & Oliva-Teles, 1999; Regost *et al*., 2001). Por otra parte, un decremento en la energía digestible del alimento a través de la reducción de los niveles de proteína con un incremento concomitante de energía digestible suplido por lípidos genera en algunos peces una extrema eficiencia en el uso de la proteína para el crecimiento, lo cual presiona a los peces a utilizar los lípidos como sustratos energéticos para cubrir sus requerimientos de energía (Abdo de la Parra, Rodríguez-Ibarra, Hernández, C., Hernánez, K., González-Rodríguez, Martínez-Rodríguez, & García-Ortega, 2010; Catacutan, Pagador, & Teshima, 2001; Wang *et al*., 2017; Wang *et al*., 2018).

4. Descripción de las especies

5. Robalo blanco *Centropomus undecimalis*

5.1. Taxonomía

La familia *Centropomidae* se encuentra constituida por 12 especies (Figura 2), representadas por un solo género *Centropomus*, la cual está confinada en la zona tropical y subtropical de América. Habita ambientes costeros, salobres y dulceacuícolas, en el Atlántico Occidental se encuentran las especies: *C. undecimalis, C. mexicanus, C. paralellus, C. poeyi, C. pectinatus* y *C. ensiferus;* por otra parte, en el Pacifico Oriental encontramos a *C. viridis, C. unionensis, C. robalito, C. medius, C. armatus* y *C. nigrescens* (Rivas, 1986)

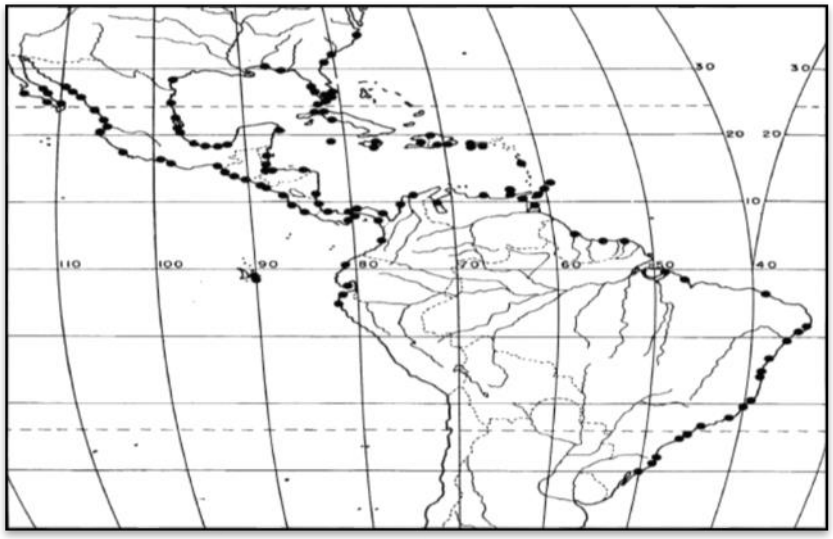

Figura 2. Distribución Geográfica del género *Centropomus* en el continente Americano (Rivas, 1986).

A continuación, se desglosa la descripción taxonómica de la familia *Centropomidae* y género *Centropomus* (Nelson, 2006):

Phylum	Chordata
Subphylum	Craniata
Superclace	Gnatostomata
Clase	Actinopterygii
Subclase	Neopterygii
Dvisión	Teleostei
Orden	Perciforme
Suborden	Percoidei
Familia	Centropomidae
Género	*Centropomus*
Especie	*C. undecimalis* (Bloch, 1792)

5.2. Ecología

El robalo blanco se distribuye geográficamente desde Carolina del Norte, Estados Unidos hasta Rio de Janeiro, Brasil, incluyendo las Bahamas, el Golfo de México y algunas islas de las Antillas. Su abundancia y distribución tiene una estrecha relación con la presencia de ambientes costeros, salobres y dulceacuícolas donde la fluctuación de salinidad va de 0 hasta 45 ppm (Figura 3 y 4), la salinidad es un factor importante en su distribución (Rivas, 1986).

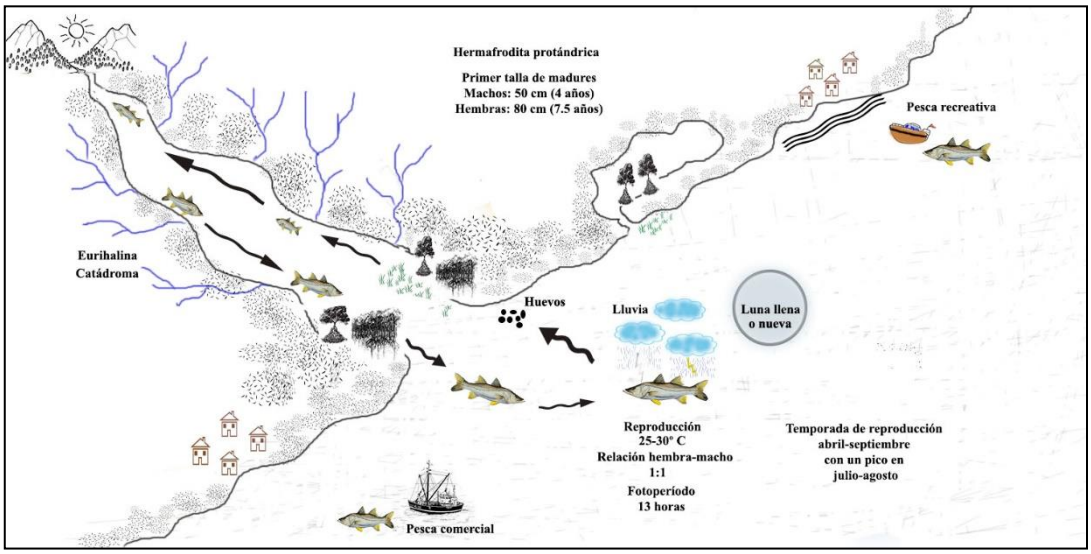

Figura 3. Ciclo de vida del robalo blanco *C. undecimalis.*

Es un depredador visual, de hábitos carnívoros con tendencia piscívora (McMichael *et al*., 1989), se considera un depredador oportunista debido a su alimentación en el medio natural, el cual comprende 48 taxones dentro de los cuales encontramos organismos pelágicos, demersales y bentónicos (Blewett *et al*., 2006). Es un forrajero de comportamiento crepuscular-nocturno, posee una adaptación morfológica ocular que le permite detectar a sus presas en poca presencia de luz, debido a que posee una visión de tipo escotópica (Eckelbarger *et al*., 1980).

Es una especie hermafrodita protándrica (Taylor *et al*., 1996), diádroma, eurihalina (Perez-Pinzón & Lutz, 1991) y estenoterma (Gilmore *et al*., 1978). La zona de reproducción de esta especie es en las costas y la época en la cual se lleva a cabo en el Golfo de México es en el mes de Abril a Septiembre con un pico de mayor actividad en Junio y Agosto cuando las temperaturas alcanzan los 25-30ºC; similar ocurre en las costas de Florida, EUA (Taylor *et al*., 1998; Lorán-Núñez *et al*., 2012). La talla de primera madures para los machos es de aproximadamente 50 cm (4 años) y

para hembras es de 80 cm (7.5 años), iniciando la reversión sexual macho-hembra después de los primeros desoves (Perera-García *et al*., 2011; Taylor *et al*., 2000).

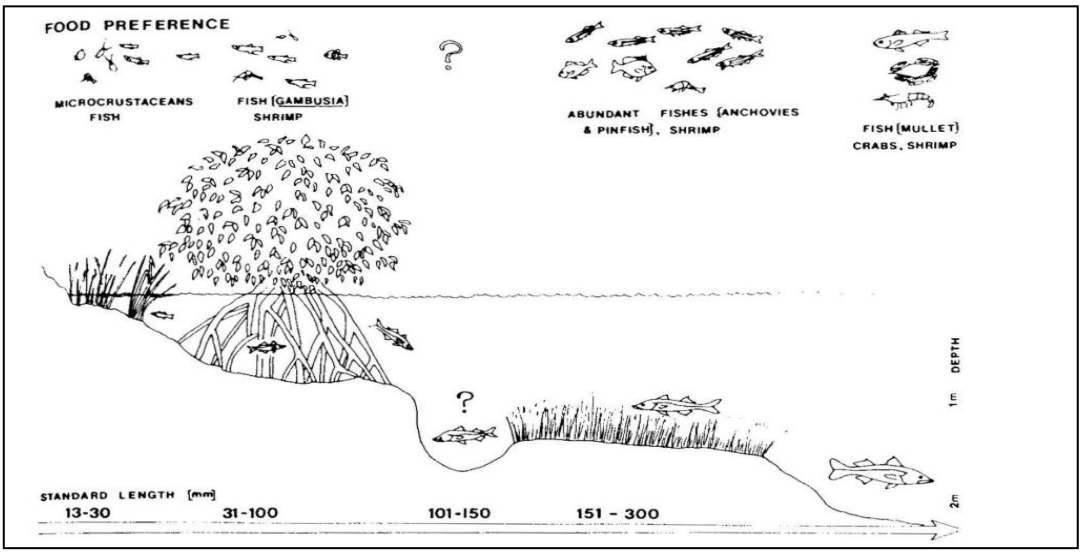

Figura 4. Distribución horizontal y alimentación de robalo blanco *C. undecimalis* (Gilmore *et al*., 1983).

En el medio natural, los huevos una vez fecundados permaneces cerca de las costas hasta su eclosión (pelágicos), una vez que los juveniles presentan comportamientos pelágicos, fisiológicamente son más tolerantes a los cambios de salinidad (Tsuzuki *et al*., 2007), lo que les permite ocupar diversos ambientes acuáticos durante su primer año de vida, los juveniles tempranos (11-156 mm) ocupan ambientes salobres y dulceacuícolas, donde su alimentación está compuesta por peces y camarones; los juveniles (10-174 mm) en zonas de marismas consumen principalmente crustáceos y peces; los juveniles (150-300 mm) en praderas marinas prefieren peces y camarones en su dieta (Gilmore *et al*., 1983b; McMichael *et al*., 1989).

5.3. Reproducción en cautiverio y desarrollo larvario

Noyola Regil *et al*. (2015) reportan en juveniles de robalo blanco temperaturas preferenciales entre los 27 y 29 °C. En cautiverio, robalo blanco alcanza la maduración sexual y desarrollo gonadal ocurren, sin embargo, la fase de maduración final de los ovocitos en la gónada femenina no finaliza, esto evita que se lleve a cabo el desove natural. La maduración final de los ovocitos no se lleva a cabo debido al bloqueo en la secreción de la hormona luteinizante por parte de la

glándula pituitaria (Mylonas *et al*., 1997). La reproducción en cautiverio de robalo banco se ha logrado con el uso de hormona liberadora de gonadotropina (Ibarra-Castro *et al*., 2011), ésta hormona estimula la secreción de la hormona luteinizante por parte de la glándula pituitaria, permitiendo la maduración final de ovocitos y posteriormente el desove (Mylonas & Zohar, 2000).

Las larvas eclosionan 48 horas después de la fertilización y 36 horas después de la eclosión, el saco vitelino es completamente absorbido, en ese momento las larvas ya presentan boca e intestino funcionales (Ibarra-Castro *et al*., 2011). El desarrollo de larvas a juvenil comprende un periodo de aproximadamente 35 días (16.4 mm LT) (Ibarra-Castro *et al*., 2011; Lau & Shafland, 1982), por otra parte, Jimenez-Martinez *et al*. (2012) reportan en el día 35 post-eclosión, una máxima actividad de la enzima pepsina, lo que indica un estómago funcional.

5.4. Pesquerías

En México, las capturas de robalo blanco se reportan en conjunto con el robalo prieto (*C. poeyi*) y chucumite (*C. parallelus*), los estados con mayor captura son Campeche (33.7 %) Tabasco (28.1 %) y Veracruz (31.2 %), los estados con las menores capturas son Tamaulipas (3.3 %), Quintana Roo (1.3 %) y Yucatán (2.4 %) (SAGARPA, 2017), es una de las pesquerías rivereñas más importantes en México. La pesca de robalos se encuentra reglamentada desde 1993, ese año se estableció el primer periodo de veda en Soto La Marina en el estado de Tamaulipas hasta Barra de Chachalacas en el estado de Veracruz, del 15 de mayo al 30 de junio y del 1 de julio al 15 de agosto del mismo año, desde Barra de Chachalacas a Barra de Tonalá en el estado de Veracruz (SAGARPA, 2014). Posteriormente, en los estados de Tabasco y Campeche en el año 2007, la NOM-037-PESC-2004 (SAGARPA, 2007), estableció para el sistema lagunar compuesto por los humedales de Usumacinta, en los municipios de Jonuta, Emiliano Zapata y Balancán en el estado de Tabasco, Ciudad del Carmen y Palizadas en el estado de Campeche, las especificaciones de captura para robalo son: "Redes de enmalle con luz de malla mínima de 140 mm (5.5 pulgadas) e hilo de monofilamento poliamida (PA) con diámetro de 1.7 mm, como máximo o de multifilamento poliamida (PA) en los números comerciales del 15 o 18". En los estados de Tabasco, Campeche, Yucatán y Quintana Roo no existe un periodo de veda establecido en la pesquería de robalos. Por otra parte, la NOM-017-PESC-1994 establece en el caso particular de la pesca recreativa de robalos, permite un volumen máximo de captura por persona de cinco ejemplares por día, considerando las medidas de manejo existentes y periodos de veda (SAGARPA, 2007a). A pesar de que existe un plan de manejo sobre la pesquería de robalos, Caballero-Chávez (2012) indica que desde el 2001 las poblaciones de robalo blanco se encuentran sobreexplotadas.

5.5. Nutrición

Los requerimientos nutricionales en robalo blanco han sido de poco interés para los investigadores, los estudios que se han llevado a cabo se han enfocado en establecer el requerimiento de proteína en diferentes condiciones de salinidad, estableciendo un requerimiento de proteína dietética de aproximadamente 53% en diferentes condiciones de salinidad (Gracia-López *et al*., 2003; Souza *et al*., 2011; Tucker, 1987). Lemus *et al*. (2017) a través de ensayos de

digestibilidad *in vitro* e *in vivo* recomienda utilizar harinas protiblend y subproductos de ave como sustitutos de proteína alterna a la harina de pescado en la formulación de alimentos balanceados para juveniles de robalo blanco. Arenas-Pardo (2015) estableció en juveniles de robalo blanco un requerimiento de proteína dietética de 57% en 35 ups y 52% en 3 ups, remplazando totalmente la harina de pescado como fuente de proteína por fuentes de proteína alternas (harina de subproductos de ave y protiblend). Investigaciones sobre los requerimientos nutricionales en otros miembros del género *Centropomus* son limitadas. Recientemente, en *C. viridis* se reportó que un reemplazo del 15-45% de harina de pescado por harina de soya, no afecta el crecimiento de esta especie (Arriaga-hernández *et al*., 2021).

El barramundi (*Lates calcarifer*) es una especie de gran interés para la acuicultura, los requerimientos nutricionales de esta especie han sido ampliamente estudiados. Barramundi, anteriormente se encontraba taxonómicamente clasificado dentro del género *Centropomus*, pero posteriormente fue reclasificado al género *Lates* (Rivas, 1986). Catacutan & Coloso, (1995) reportaron en barramundi que una disminución en la cantidad de energía digestible suplido por proteína acompañado con un incremento en la energía digestible suplido por lípidos en la dieta (5 a 10%), permitió reducir la demanda de proteína de 50% a 42%, sin efectos adversos en el crecimiento. Por otra parte, Catacutan & Coloso (1997) en esta misma especie, reportaron que un incremento en el nivel de carbohidratos en la dieta de 15 a 20% en la dieta mejoró el factor de conversión alimenticia (FCA) y la tasa de eficiencia proteica (TEP).

6. Pargo canané *Ocyurus chrysurus.*

6.1. Taxonomía

La familia Lutjanidae está conformada por ciento y cinco especies, setenta géneros, los cuales están organizados en cuatro subfamilias. Su distribución geográfica comprende el océano Atlántico, Índico y Pacifico. Habitan zonas tropicales y subtropicales, se les encuentra en condiciones de salinidad dulce acuícolas, salobres y marinas.

A continuación, se desglosa la clasificación taxonómica de la familia Lutjanidae y el género *Ocyurus* (Nelson, 2006).

Phylum	Chordata
Subphylum	Craniata
Superclace	Gnatostomata
Clase	Actinopterygii
Subclase	Neopterygii
Dvisión	Teleostei
Orden	Perciforme
Suborden	Percoidei
Familia	Lutjanidea
Género	*Ocyurus*
Especie	*O. chrysurus* (Bloch, 1791)

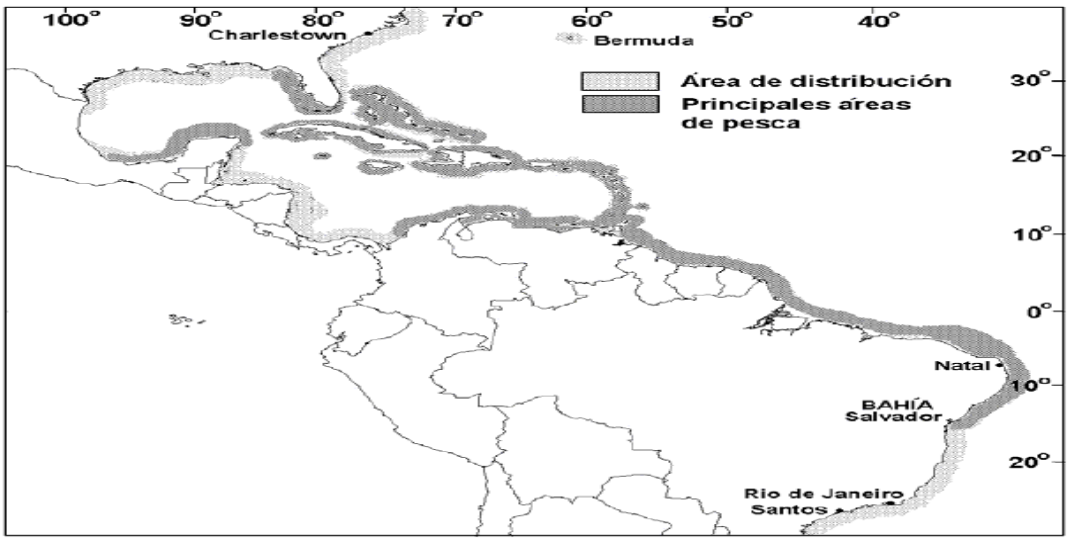

Figura 5. Distribución geográfica de pargo canané *O. chrysurus* (Autor desconocido).

6.2. Ecología

El pargo canané se distribuye geográficamente desde Massachusetts, Estados Unidos hasta el sureste de Brasil, es una especie de importancia comercial en el Caribe (Cummings, 2004) (Figura 5). Durante su ciclo de vida habita diferentes ambientes costeros, tamaños de tallas de 2.5 cm hasta 25 cm ocupan zonas de manglares y pastos marinos (Nagelkerken & Van der Velde, 2004) (Figura 6), por otra parte, tamaños de tallas de 25 cm hasta 35 cm ocupan zonas de arrecifes, donde alcanzan su madurez sexual y llevan a cabo su reproducción (De La Morinière *et al*., 2002; Verweij *et al*., 2008).

Es considerado un depredador de tipo zooplanctivoro y zoobentivoro, su presa principal está compuesta por peneidos, los peces son considerados como presas secundarias dentro de sus hábitos alimenticios (Rincón-Sandoval *et al*., 2009) (Figura 7). El pargo canané es una especie gonocórica, catalogado como un desovador asincrónico, la primera talla de maduración sexual promedio en las hembras y machos es de 24.5 cm de longitud total, aproximadamente 2 a 3 años de edad, su reproducción se lleva a cabo en los meses de enero a septiembre, con mayor actividad en abril y mayo, en las costas de Yucatán (Garcia *et al*., 2003; Trejo-Martínez *et al*., 2011). 2009).

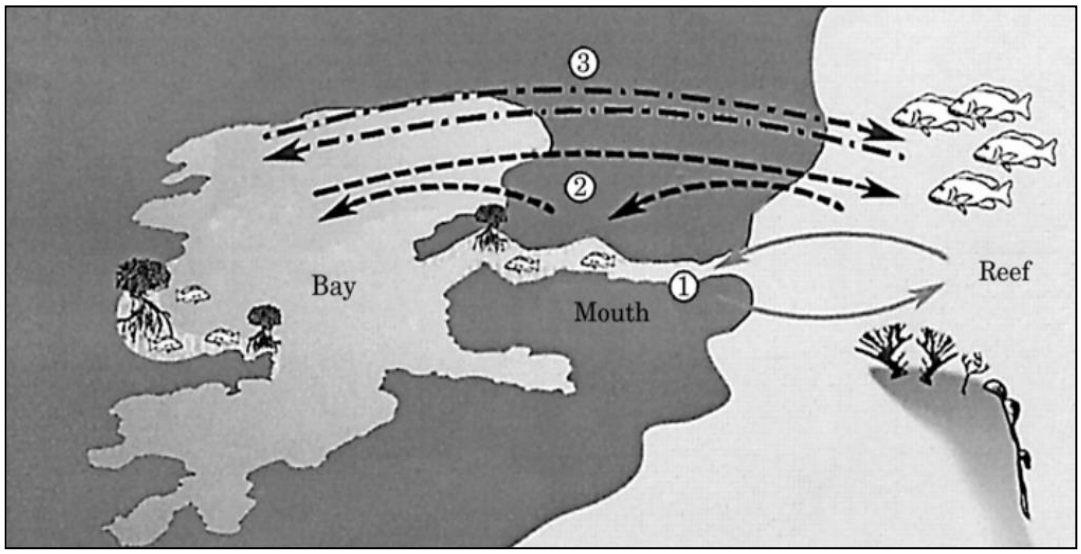

Figura 6. Ciclo de vida del pargo canané. Migración del ciclo de vida posterior al asentamiento, número 2 para el pargo canané (De La Morinière *et al*., 2002).

6.3. Reproducción en cautiverio y desarrollo larvario

Noyola Regil *et al*. (2015) reportan en juveniles de pargo canané temperaturas preferenciales entre los 25 y 27 °C. La reproducción de pargo canané en cautiverio se ha llevado a cabo de manera natural (Gutiérrez-Sigeros *et al*., 2018; Turano *et al*., 2000) y por inducción hormonal, utilizando gonadotropina coriónica humana (hCG) (Soletchnik *et al*., 1989). Los huevos de pargo canané son de tipo pelágicos, la eclosión de los huevos fertilizados se lleva a cabo entre las 18 y 24 horas, en una temperatura de 27 °C. Las reservas del saco vitelino son consumidas 48 horas post-eclosión, la transformación de larvas a juveniles se completa en un periodo de tiempo de 14 a 28 días en 27 °C (Faulk *et al*., 2007; Gutiérrez-Sigeros *et al*., 2018; Riley *et al*., 1997), sin embargo, Ahumada-Hernández *et al*. (2014) sugieren que el pargo canané alcanza su desarrollo de larva a juvenil hasta el día 34 post-eclosión, debido a una máxima actividad de la enzima pepsina, indicador de una eficiente digestión de proteínas y un completo desarrollo del estómago.

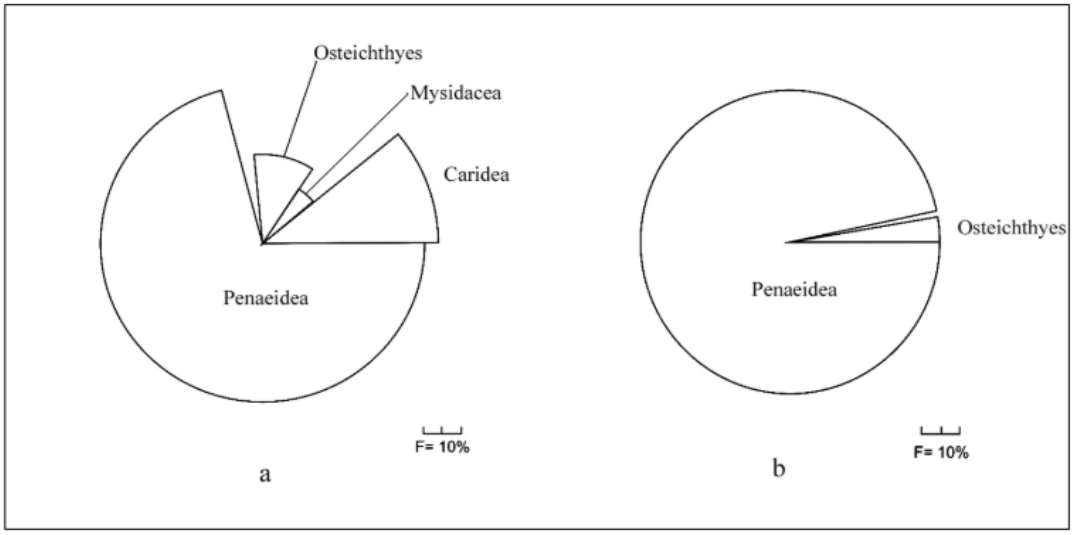

Figura 7. Representación gráfica del régimen alimenticio de pargo canané por temporada climática, a) fría (noviembre-abril) y b) cálida (mayo-octubre) en la costa de Yucatán (Rincón-Sandoval *et al*., 2009).

6.4. Pesquerías

Por otra parte, el pargo canané se encuentra en la lista roja de especies amenazadas de la Unión Internacional para la Conservación de la Naturaleza (IUCN por sus siglas en ingles), catalogado como "Data deficient" (Lindeman *et al*., 2016). En México no existe un plan de manejo pesquero para esta especie. Brulé *et al*. (2008) señalan que el complejo mero-pargo, entre ellas el pargo canané, constituyen el componente principal de la pesquería de escama del estado de Yucatán. El pargo canané es considerado como pesca incidental o de acompañamiento en las pesquerías de otras especies marinas (SAGARPA, 2017), no obstante, esta pesquería se encuentra en una fase de explotación creciente (Arcos-Huitrón & Arreguín-Sánchez, 2011; Brulé *et al*., 2008), lo anterior es alarmante debido a la ausencia de una regulación de pesca del recurso.

6.5. Nutrición

Existen muy pocos estudios enfocados en determinar los requerimientos nutricionales en pargo canané. Faulk *et al*. (2007) observaron que el uso de *Isochrysis galbana* y *Nannochloris oculata* como alimento de rotífero y artemia, los cuales a su vez son utilizados como alimento en pargo canané durante el periodo larval, no cubren eficientemente los requerimientos de ácidos grasos altamente insaturados, por lo que recomiendan enriquecer rotíferos y artemia con emulsiones

ricas en ácido araquidónico, docosahexaenoico y eicosapentaenoico. Barreto-Altamirano (2016) propuso el uso de harina de ave y canola como fuentes de proteína alternas a la harina de pescado en la formulación de alimentos balanceados para juveniles de pargo canané, debido a la elevada hidrólisis y digestibilidad aparente obtenidos en ensayos de digestibilidad *in vitro* e *in vivo*, respectivamente. Enriquez-Reyes (2018) estableció el requerimiento de proteína de juveniles de pargo canané entre 53 y 58 %, utilizando harina de canola y ave como sustituto parcial de la harina de pescado.

Integrantes de la familia Lutjanidae como el pargo cordero (*Lutjanus analis*), el pargo lunarejo (*L. guttatus*) y pargo de manglar (*L. argentimaculatus*) son de gran interés para la acuicultura. Los estudios relacionados en la nutrición de los pargos anteriormente mencionados se han enfocado principalmente en evaluar la habilidad de estas especies para utilizar las proteínas y lípidos en la dieta. Watanabe, Ellis, & Chaves (2001) reportaron en el pargo cordero que un nivel de lípidos en la dieta entre 6 y 9% es ideal para un eficiente crecimiento utilizando un nivel de proteína de 40%. Catacutan *et al*. (2001) reportaron que un nivel de proteína de 42.5% con 6 o 12% de lípidos en la dieta en pargo de manglar proporcionó un similar rendimiento en cuanto a crecimiento y eficiencia alimenticia (FCA y TEP), respecto a un nivel de proteína de 50% con 6 y 12% lípidos. Similarmente, Abdo de la Parra *et al*. (2010) reportaron en el pargo lunarejo que un nivel de proteína de 45% en la dieta con 9, 12 y 15% lípidos proporcionó un rendimiento zootécnico y alimenticio similar a un nivel de 50% proteína; sin embargo, un nivel de proteína de 40% redujo el crecimiento de esta especie e incrementó los valores de FCA.

7. Justificación e importancia

La acuicultura, posiblemente es el sector de producción de alimentos de crecimiento más acelerado que existe, actualmente representa casi el 50 % de los productos pesqueros mundiales destinados a la alimentación (FAO, 2018). Por otra parte, la acuicultura se cataloga como una estrategia que permite disminuir la presión pesquera de los recursos naturales que se encuentran destinados a ésta actividad, permitiendo la recuperación de sus poblaciones.

El pargo canané y robalo blanco son especies económicamente importantes desde un punto de vista pesquero y recreativo en el Este del continente de América (Brulé *et al*., 2008; Caballero-Chávez, 2012). Sin embargo, el robalo blanco es considerado como una pesquería en

sobreexplotación a pesar de contar con un plan de manejo (Caballero-Chávez, 2012), por otra parte, el pargo canané es un recurso en fase de explotación creciente (Brulé *et al*., 2008), pero no cuenta con un plan de manejo, lo que es alarmante. Estas especies han sido catalogadas como candidatos para la acuicultura, se adaptan fácilmente al cautiverio, al consumo de alimento inerte y su reproducción es posible llevarla en condiciones de cautiverio (Ibarra-Castro *et al*., 2011; Gutiérrez-Sigeros *et al*., 2018; Tucker, 1987; Turano *et al*., 2000). Sin embargo, uno de los componentes más importante en el establecimiento de una tecnología de cultivo, es la formulación de un alimento balanceado, elaborada a partir de una selección y proporción apropiada de ingredientes que permitan ser aprovechados eficientemente por los peces. La literatura existente sobre los requerimientos nutricionales del pargo canané y robalo blanco son escasos, las investigaciones solo se han enfocado en determinar su requerimiento de proteína en el alimento, el cual ha sido establecido en un valor entre 50-58% (Arenas, 2015; Concha-Frías *et al*., 2018; Enríquez, Gaxiola, & Pacheco, 2017; Gracia-López *et al*., 2003; Tucker, 1987).

La proteína es el ingrediente más costoso en la formulación de alimentos balanceados en peces, además su producción depende de las pesquerías de otras especies de peces, las cuales se encuentran en el límite de su máximo rendimiento sostenible (Tacon, 2004; Tacon & Metian, 2008). Por otra parte, la liberación de amonio, producto directo del catabolismo de la proteína, impacta negativamente el ambiente, debido a que es uno de los principales componentes nitrogenados responsables de la eutrofización del agua (Van Waarde, 1983). Por lo tanto, desde una perspectiva económica y ambiental, es necesario llevar a cabo investigaciones sobre la capacidad del pargo canané y el robalo blanco para utilizar carbohidratos y lípidos como sustratos energéticos, lo que permitirá entender aspectos sobre su fisiología nutritiva y a su vez desarrollar estrategias nutricionales para optimizar el uso de proteína en la formulación de alimentos balanceados para estas especies.

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9. Hipótesis

i. La presencia de carbohidratos en el alimento como fuente de energía digestible en juveniles de pargo canané (*Ocyurus chrysurus*) y el robalo blanco (*Centropomus undecimalis*) promoverá la expresión de la enzima glucocinasa (hexoquinasa IV), reorganizará el metabolismo digestivo e intermediario hepático a favor del metabolismo de este nutriente, lo cual reducirá el uso de la proteína con fines energéticos y promoverá la retención de la proteína para el crecimiento.

ii. El incremento del nivel de lípidos en el alimento como fuente de energía digestible en juveniles de pargo canané (*O. chrysurus*) y el robalo blanco (*C. undecimalis*) reorganizará el metabolismo digestivo e intermediario hepático a favor del metabolismo de este nutriente, lo cual reducirá el uso de la proteína con fines energéticos y promoverá la retención de la proteína para el crecimiento.

10. Objetivo general

Determinar el efecto de la presencia de carbohidratos y el incremento de lípidos en el alimento como fuente de energía digestible sobre el desempeño zootécnico, eficiencia alimenticia, indicadores fisiológicos, composición proximal, enzimas digestivas y enzimas claves del metabolismo intermediario del hígado en juveniles de pargo canané (*O. chrysurus*) y el robalo blanco (*C. undecimalis*).

11. Objetivos particulares

i. Evaluar el efecto de la presencia de carbohidratos en la dieta (20% versus 0%) sobre el desempeño zootécnico, eficiencia alimenticia, indicadores fisiológicos, composición proximal, actividad de enzimas digestivas (pepsina, proteasas alcalinas totales y α amilasa) y enzimas claves del hígado involucradas en la ruta de glucólisis (glucoquinasa, GK [actividad y expresión de RNA mensajero]; hexoquinasas, HK; piruvato quinasa, PK), pentosa fosfato (glucosa-6-fosfato deshidrogenasa, G6PDH; 6-fosfogluconato deshidrogenasa, 6PGDH), gluconeogénesis (glucosa-6-fosfatasa, FBPasa) y catabolismo de aminoácidos (Alanina aminotransferasa, ALT) en juveniles de pargo canané (*O. chrysurus*) y el robalo blanco (*C. undecimalis*), a través de un estudio comparativo.

ii. Evaluar el efecto de dos niveles de lípidos (6 y 12%) y proteína (40 y 50%) en el alimento sobre el desempeño zootécnico, eficiencia alimenticia, indicadores fisiológicos, composición proximal, actividad de enzimas digestivas (proteasas alcalinas totales, tripsina, quimotripsina y lipasas) y enzimas claves del hígado involucradas en la ruta de glucólisis (PK), pentosa fosfato (G6PDH y 6PGDH), gluconeogénesis (FBPasa) y catabolismo de aminoácidos (ALT) en juveniles de pargo canané (*O. chrysurus*).

iii. Evaluar el efecto de dos niveles de lípidos (6 y 12%) y proteína (40 y 50%) en el alimento sobre el desempeño zootécnico, eficiencia alimenticia, indicadores fisiológicos, composición proximal, actividad de enzimas digestivas (proteasas alcalinas totales, tripsina, quimotripsina y lipasas) y enzimas claves del hígado involucradas en la ruta de glucólisis (PK), pentosa fosfato (G6PDH y 6PGDH), gluconeogénesis (FBPasa) y catabolismo de aminoácidos (ALT) en juveniles de robalo blanco (*C. undecimalis*).

12. Esquema general de la tesis

A continuación, se presenta la estructura general de la tesis. Se define el número de capítulos, así como su respectivo título.

Capítulo 1. Estructuración general de la tesis.

Capítulo 2. Efecto de los carbohidratos de la dieta sobre el rendimiento del crecimiento, la eficiencia alimentaria y la regulación del metabolismo de la glucosa en juveniles de *Centropomus undecimalis* (Bloch, 1792) y *Ocyurus chrysurus* (Bloch, 1791).

Capítulo 3. Evaluación de la relación proteína: lípidos en el crecimiento, la eficiencia alimentaria y la respuesta metabólica en juveniles de pargo cola amarilla (*Ocyurus chrysurus*, Bloch, 1791).

Capítulo 4. Efectos fisiológicos y metabólicos de los lípidos de la dieta en la conservación de proteínas en los juveniles de róbalo *Centropomus undecimalis* (Bloch, 1792).

Capítulo 5. Discusión general y conclusiones.

CAPÍTULO 2

Artículo 1

Sometido a: *Aquaculture*

Effect of dietary carbohydrates on growth performance, feed efficiency and glucose metabolism in common snook (*Centropomus undecimalis*) and yellowtail snapper (*Ocyurus chrysurus*) juveniles

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Abstract

In recent years, interest in commercial farming of the common snook *Centropomus undecimalis* and yellowtail snapper *Ocyurus chrysurus* has been increasing in the Atlantic West; however, these species are carnivorous with a high protein requirement in the diet. The effect of dietary carbohydrates on growth performance, feed efficiency, glycemic response, digestive and key liver enzymes of intermediary metabolism for catadromous *C. undecimalis* and marine *O. chrysurus* were investigated and the capability of these two species to utilize carbohydrates was compared. Two diets were formulated to contain 51.6% digestible protein, 7.9% digestible lipid and 0% corn starch (Control diet, C) or 20% corn starch (High carbohydrates diet, HC). The results showed that no effect of dietary carbohydrates on growth performance was found in both species. Feed efficiency, protein efficiency ratio and liver glycogen were higher in *C. undecimalis* fed the HC diet, but this does not happen in *O. chrysurus*. Intestinal amylase activity was induced by the HC diet in both species. Blood glucose peaked in *C. undecimalis* six hours after being fed with the HC diet (7.8 mmol/L), whereas in *O. chrysurus* the peak was reached until nine hours (14.1 mmol/L). Hepatic glucokinase activity was induced by the HC diet in both species; however, at the molecular level, GK mRNA expression was higher in *C. undecimalis* fed the HC diet, but for *O. chrysurus*, no effect of the HC diet on GK mRNA expression was observed. Also, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities increased only in *C. undecimalis* fed the HC diet. Pyruvate kinase activity increased and fructose-1, 6-bisphosphatase decreased in both species fed the HC diet. Alanine aminotransferase activity was unaffected by the experimental diets, but *O. chrysurus* showed higher activity than *C. undecimalis*. Data shown indicated that both fish species were able to adapt to the presence of carbohydrates in the diet by reorganizing digestive and hepatic glucose metabolism, but *C. undecimalis* demonstrated a greater capacity to utilize and storage carbohydrates than *O. chrysurus.* Fish with similar food habits, but different life cycles showed different capacities to utilize carbohydrates.

Resumen

En años recientes, el interés por cultivar robalo blanco *Centropomus undecimalis* y pargo canané *Ocyurus chrysurus* ha incrementado en la costa del Atlántico Este; sin embargo, estas especies son carnívoras con un requerimiento alto de proteína en el alimento. Por esta razón, en el presente estudio, se investigó el efecto de la presencia de carbohidratos en el alimento en el pez catádromo *C. undecimalis* y la especie marina *O. chrysurus* a través de un estudio comparativo, sobre la respuesta del rendimiento de crecimiento, la eficiencia alimenticia, respuesta glucémica, enzimas digestivas y del metabolismo intermediario. Dos dietas fueron formuladas para contener 51.6% de proteína y 7.9% de lípidos digeribles con 0% de almidón de maíz (alimento control, C) o 20% (alimento rico en carbohidratos, HC). Los resultados mostraron que en ambas especies no se encontró ningún efecto de los carbohidratos de la dieta sobre el rendimiento del crecimiento. La eficiencia alimenticia, el índice de eficiencia proteica y el glucógeno hepático fueron más altos en *C. undecimalis* alimentados con HC, lo que no sucedió en *O. chrysurus*. La actividad de la amilasa intestinal fue inducida por el alimento HC en ambas especies. La glucosa en sangre alcanzó su punto máximo en *C. undecimalis* seis horas después de haber sido alimentado con HC (7,8 mmol / L), mientras que en *O. chrysurus* el pico se alcanzó hasta las nueve horas (14,1 mmol / L). La actividad de la glucoquinasa hepática fue inducida por HC en ambas especies; sin embargo, a nivel molecular, la expresión de RNAm de GK fue mayor en *C. undecimalis* alimentado con HC, pero en *O. chrysurus*, no se observó ningún efecto de HC sobre la expresión de RNAm de GK. Además, las actividades de glucosa-6-fosfato deshidrogenasa y 6-fosfogluconato deshidrogenasa aumentaron sólo en *C. undecimalis* alimentado con HC. La actividad de la piruvato quinasa aumentó y la fructosa-1, 6-bisfosfatasa disminuyó en ambas especies alimentadas con HC. La actividad de alanina aminotransferasa no se vio afectada por las dietas experimentales, pero *O. chrysurus* mostró una actividad más alta que *C. undecimalis*. Los datos mostrados indicaron que ambas especies de peces se adaptaron a la presencia de carbohidratos en el alimento por reorganización del metabolismo de la glucosa digestiva y hepática, pero *C. undecimalis* demostró una mayor capacidad para utilizar y almacenar carbohidratos que *O. chrysurus*. Especies de peces con hábitos alimenticios similares (carnívoros), pero con ciclos de vida diferentes, mostraron diferentes capacidades para utilizar los carbohidratos en el alimento.

Aquaculture

Effect of dietary carbohydrates on growth performance, feed efficiency and glucose metabolism in common snook (Centropomus undecimalis) and yellowtail snapper (Ocyurus chrysurus) juveniles

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- Feed efficiency improved by dietary carbohydrates in *C. undecimalis* but not in *O. chrysurus*.
- Dietary carbohydrates effected digestive enzymes activities in both fish species.
- Dietary carbohydrates effected key glucose metabolic enzymes in both fish species.
- Hepatic GK plays a crucial role in glucose metabolism between *O. chrysurus* and *C. undecimalis*.

Effect of dietary carbohydrates on growth performance, feed efficiency and glucose metabolism in common snook (*Centropomus undecimalis*) and yellowtail snapper (*Ocyurus chrysurus*) juveniles

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ABSTRACT

In recent years, interest in commercial farming of the common snook *Centropomus undecimalis* and yellowtail snapper *Ocyurus chrysurus* has been increasing in the Atlantic West; however, these species are carnivorous with a high protein requirement in the diet. The effect of dietary carbohydrates on growth performance, feed efficiency, glycemic response, digestive and key liver enzymes of intermediary metabolism for catadromous *C. undecimalis* and marine *O. chrysurus* were investigated and the capability of these two species to utilize carbohydrates was compared. Two diets were formulated to contain 51.6% digestible protein, 7.9% digestible lipid and 0% corn starch (Control diet, C) or 20% corn starch (High carbohydrates diet, HC). The results showed that no effect of dietary

carbohydrates on growth performance was found in both species. Feed efficiency, protein efficiency ratio and liver glycogen were higher in *C. undecimalis* fed the HC diet, but this does not happen in *O. chrysurus*. Intestinal amylase activity was induced by the HC diet in both species. Blood glucose peaked in *C. undecimalis* six hours after being fed with the HC diet (7.8 mmol/L), whereas in *O. chrysurus* the peak was reached until nine hours (14.1 mmol/L). Hepatic glucokinase activity was induced by the HC diet in both species; however, at the molecular level, GK mRNA expression was higher in *C. undecimalis* fed the HC diet, but for *O. chrysurus*, no effect of the HC diet on GK mRNA expression was observed. Also, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities increased only in *C. undecimalis* fed the HC diet. Pyruvate kinase activity increased and fructose-1, 6-bisphosphatase decreased in both species fed the HC diet. Alanine aminotransferase activity was unaffected by the experimental diets, but *O. chrysurus* showed higher activity than *C. undecimalis*. Data shown indicated that both fish species were able to adapt to the presence of carbohydrates in the diet by reorganizing digestive and hepatic glucose metabolism, but *C. undecimalis* demonstrated a greater capacity to utilize and storage carbohydrates than *O. chrysurus*. Fish with similar food habits, but different life cycles showed different capacities to utilize carbohydrates.

KEY WORDS: *Centropomus undecimalis*; *Ocyurus chrysurus*; Dietary carbohydrates; Glucose metabolism; Carnivorous fish species.

1. Introduction

Fish, particularly carnivorous species require high-protein diets to obtain amino acids for protein synthesis and for energy purposes (Jia *et al*., 2017; Oliva-Teles *et al*., 2020; Walton and Cowey, 1982). However, the use of proteins as dietary source of energy by fish is considered undesirable because it usually represents the most expensive dietary component, and protein catabolism by-products account for the major source of nitrogen waste, one of the main factors responsible for water pollution (Tacon and Metian, 2008; Waarde, 1983). Thus, from an economic and environmental perspective, it is recommended to reduce the catabolism of amino acids for energy purposes (protein sparing effect) by increasing the use of non-protein energy sources such as carbohydrates or lipids (Bowyer *et al*., 2013; Tacon, 2004).

Carbohydrate is usually the least expensive form of dietary energy for animal production (Wilson, 1994). Fish do not have a specific requirement for dietary carbohydrates, although the major mechanisms involved in their digestion and metabolism are found in them (Enes *et al*., 2009; Krogdahl *et al*., 2005); however, in some species, the inclusion of carbohydrates in the diet may reduce catabolism of protein for energy purposes and increase protein retention for growth (Cui *et al*., 2010; Hung *et al*., 1989; Hutchins *et al*., 1998; Ma *et al*., 2018). The ability of fish to use dietary carbohydrates as energy source varies widely among species. Generally, herbivores and omnivores fish have higher capacity to use carbohydrates than carnivorous species (Furuichi *et al*., 1986; Panserat *et al*., 2000; Polakof *et al*., 2012), just as freshwater and warmwater appear to be better able to seem use carbohydrates than marine and coldwater (Kamalam *et al*., 2017). In general, it has been stablished that a dietary inclusion level of 20% carbohydrates is the maximum recommended for carnivorous fish, and 40% for herbivores or omnivores species (Stone, 2003; Wilson, 1994).

The catadromous common snook *Centropomus undecimalis* and marine yellowtail snapper *Ocyurus chrysurus* are distributed throughout the tropical and subtropical western Atlantic Ocean from the United States of America to Brazil (De La Morinière *et al*., 2002; Gilmore *et al*., 1983). These species are high-market value and support an economically important commercial and recreational fishing industry in the Atlantic West (Caballero-Chávez, 2012; McClellan and Cummings, 1998). In recent years, interest in commercial farming of these fish species in American countries has been increasing (Alvarez-Lajonchère and Ibarra-Castro, 2013). *C. undecimalis* and *O. chrysurus* have been described as carnivorous species (Ahumada-Hernández *et al*., 2014; Concha-Frias *et al*., 2016; Jimenez-Martinez *et al*., 2012). To date, studies of the nutritional requirements of *C. undecimalis* and *O. chrysurus* are limited. Previous studies reported that the optimum protein requirement of both fish species ranged from 50% to 55% (Concha-Frías *et al*., 2018; Enriquez, 2018; Gracia-López *et al*., 2003; Tucker, 1987). In addition, alternative protein sources to fishmeal have been proposed only for *C. undecimalis* (Lemus *et al*., 2017; Silvão and Nunes, 2017). However, to our knowledge, no studies have been conducted to evaluate the capability of *C. undecimalis* and *O. chrysurus* to utilize dietary carbohydrates as energy source.

Therefore, the objetive of this study was to evaluate the effect of dietary carbohydrates on growth performance, feed efficiency, glycemia response, digestive enzymes (pepsin, alkaline proteases and amylase) and key liver enzymes of intermediary metabolism of *C. undecimalis* and *O. chrysurus* juveniles. Given the similarity in terms of thermal physiology (Noyola Regil *et al*., 2015) and nature of the eating habits (Blewett *et al*., 2006; Rincón-Sandoval *et al*., 2009), but different cycle life of both species (De La Morinière *et al*., 2002; Gilmore *et al*., 1983), a comparative study was carried out to increase knowledge and understand changes in the nutritional response and potential of the mechanisms involved in glucose metabolism to obtain information about their nutrition.

2. Materials and methods

Experimental diet

Two diets with a similar content of digestible protein (51%) and lipid (8%) (Abdo de la Parra *et al*., 2010; Catacutan and Coloso, 1995) were formulated to contain 20% starch (High carbohydrates diet, HC) or no starch (Control diet, C). To adjust the total energy of diet C, 20% wheat bran was used (Table 1). It was used as control because it contains starch resistant to digestion (Xie *et al*., 2008) and also non-digestible protein that requires a pH of 12.5 for hydrolysis (Balandrán-Quintana *et al*., 2015), such that fiber and minerals make the principal dietary contribution. The ingredients were sieved through a 250-µm mesh and then mixed for 30 min in a common blender. The dough was extruded at 120°C and broken down to a size of 3-mm in particle diameter using a Bonnot-Model 2EXTWPKR (Ohio, USA) extruder. Next, the dough was dried via ventilation at 60°C for three hours, and the feed was sealed in vacuum-packed bags and stored at -20°C until use.

Experimental animals and experimental design

Juvenile *C. undecimalis* and *O. chrysurus* were obtained from the Marine Fish Reproduction Laboratory at Unidad Multidisciplinaria de Docencia e Investigación, Universidad Nacional Autónoma de México (UNAM), Sisal, Yucatán, Mexico, using the methodology described by Gutiérrez-Sigeros *et al*. (2018) and Ibarra-Castro *et al*. (2011), respectively. The feeding trial was performed in a thermoregulated recirculating water system (Resun CW 1000 chillers, Guangdong Risheng Group Co. LTD, Shenzheng, China)

that maintained the temperature of eight rectangular fiberglass tanks with a 100-L water capacity (73-cm length, 44-cm width and 33-cm height). At the beginning of trial, 13 juvenile *C. undecimalis* of four-month old (mean ± standard deviation (SD) = 5.3 ± 0.2 g initial wet weight and 9.4 ± 0.1 cm total length) and 10 juvenile *O.* chrysurus of five-month old (11.6 ± 0.2 g initial wet weight and 10.1 ± 0.2 cm SD) were placed in each tank; four replicate groups of fish were used to test each randomly assigned diet. Tanks were supplied with continuous air and seawater flow (5.0 L/min). During the experimental period, water temperature was 28.1 ± 0.5°C SD; 36 ± 0.5 ups salinity; 8.1 ± 0.3 pH; 0.01 ± 0.0 mg/L ammonia concentration; and 4.5 ± 0.2 mg L^{-1} dissolved oxygen concentration. Before the experiment, the fish were acclimated to the experimental diet and conditions for two weeks. The fish were fed ad libitum thrice a day (09:00 h, 13:30 h and 18:00 h) (García-Galano *et al*., 2003). Uneaten feed was collected and then dried until it reached a constant weight (AOAC, 1990).

Sampling and Glycemic measurement

Three fish were randomly sampled from each tank six hours after the morning meal at the end of the 75-day trial. The fish were anesthetized with 0.1 mg/mL clove oil according to Castillo *et al*. (2018), then the fish were killer according to the recommendations of the Helsinki Convention and the Official Mexican Norm NOM-062-ZOO-1999 (SAGARPA, 1999). The liver, stomach, pyloric cecum and intestine were immediately frozen in liquid nitrogen and stored at -80°C for later use. The liver was divided into three parts to assess glycogen, metabolic enzymes and mRNA expression.

For blood glucose measurement, 42 fish for each dietary treatment were individualized. The fish were food-deprived for 24 h and then food was supplied. Three fish per treatment were sampled at 0, 3, 6, 9, 12, 18 and 24 h. Blood was collected from the caudal artery using a BD Ultra-Fine™ syringe (BD, New Jersey, US) that was previously dipped in heparin; glucose was tested using a FreeStyle Optimo Neo device (Abbott, New Jersey, USA). At 0, 6, 12 and 24 h, the fish were euthanized by an anesthesia overdose as previously mentioned. Then, the liver was frozen in liquid nitrogen and stored at -80°C until analysis.

Proximate analysis, growth and feed efficiency

Crude protein content was determined by flash combustion; then N_2 was quantified by thermal conductivity detection using a CHNS elemental analyzer (Costech ECS-4010, Valencia, USA); N_2 was converted to protein using a conversion factor of 6.38 (Lynch and Barbano, 1999). Crude lipid levels were determined using the hexane extraction method with a Goldfish system (Nielsen and Carpenter, 2017). Moisture content was determined after the sample was oven-dried and brought to a constant dry weight (60°C for 120 h). Ash content was determined by combusting the sample in a muffle furnace at 550°C for six hours (AOAC, 1990). Glycogen content was determined according to the method described by Carroll *et al*. (1956).

To evaluate fish performance, the following parameters were calculated: Survival rate (SR as percentage) = (final number/initial number of live fish) *100; weight gain (WG as wet weight) = (final weight – initial weight); specific growth rate (SGR as percentage) = [ln (final mean body wet weight – initial mean body wet weight)/days of culture] *100; feed efficiency (FE) = WG/dry feed intake; protein efficient ratio (PER) = WG/ dry protein intake.

Digestive enzyme activities

The stomach was homogenized (dilution 1:5) with an Ultra Turrax IKA T18 homogenizer (North Chase, Wilmington, USA) in 100 mmol/L glycine-HCl ice-cold at pH 3, whereas the pyloric cecum and intestine were homogenized (dilution 1:2) in 30 mmol/L tris-HCl + 12.5 mmol/L CaCl2 ice-cold at pH 7.5 and centrifuged at 1670 g for 30 min at 4°C (5420R-Eppendorf, DE). The resulting supernatant was separated to measure pepsin activities in stomach, and alkaline proteases, alpha amylase in the pyloric cecum and intestine.

Pepsin activity in the stomach was measured using hemoglobin (0.5%) in 100 mmol/L glycine-HCl at pH 2 (Anson, 1938). Total alkaline protease activities were evaluated using Hammarsten-grade casein (0.05%) in 100 mmol/L tris-HCl and 10 mmol/L $CaCl_2$ at pH 9 with the method reported by Kunitz (1947) and modified by Walter (1984). One unit of enzyme activity was defined as 1 μg of tyrosine released per min at 280 nm.

Alpha amylase activity was measured using soluble starch (2%) in 100 mM/L citrate phosphate and 50 mmol/L NaCl at pH 7.5 with the Somoyi-Nelson procedure described by

Robyt and Whelan (1968). One unit of amylase activity was defined as 1 μmol of maltose released per min at 600 nm.

Hepatic key intermediary enzyme activities

Fish livers were homogenized (dilution 1:5) in ice-cold buffer (50 mmol/L tris-HCl, 0.5 mmol/L phenylmethyl sulfonyl fluoride (PMSF), 4 mmol/L ethylenediaminetetraacetic acid (EDTA), 50 mmol/L sodium fluoride (NaF), 250 mmol/L sucrose and 500 mmol/L dithiothreitol (DTT) at pH 7.5) and centrifuged at 1670 g at 4°C for 30 min. The resulting supernatant was separated to assess PK, FBPase, G6PDH and 6PGDH activities.

Pyruvate kinase (PK, EC 2.7.1.40) activity was evaluated in a 0.25 mL reaction containing 250 mmol/L glycyl-glycine, pH 7.4, 10 mmol/L $MgCl_2$, 100 mmol/L KCl, 0.15 mmol/L NADH, 2.8 mmol/L phosphoenolpyruvate, and 21 U/mL lactate dehydrogenase. Adenosine diphosphate (ADP) was added at the end of the experiment (Bonamusa *et al*., 1992). Fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11) activity was evaluated in a 0.2 mL reaction containing 85 mmol/L imidazole-HCl, pH 7.7, 5 mmol/L $MgCl_2$, 0.5 mmol/L NADP, 12 mmol/L 2-mercaptoethanol, 0.05 mmol/L fructose-1,6-diphosphate, 25 U/mL phosphoglucose isomerase, and 0.48 U/mL glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.43) was evaluated in a 0.2 mL reaction containing 77 mmol/L imidazole HCl, pH 7.7, 5 mmol/L $MgCl_2$, 1 mmol/L NADP, and 10 mmol/L glucose 6-phosphate. 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) activity was evaluated in a 0.2 mL reaction containing 83 mmol/L imidazole HCl, pH 7.7, 3 mmol/L $MgCl_2$, 0.5 mmol/L NADP, and 2 mmol/L gluconate 6-phosphate (P7877-SIGMA-ALDRICH, St. Louis, MO, USA) (Bonamusa *et al*. 1992). Alanine aminotransferase (ALT, EC 2.6.1.2) was measured using a commercial kit (Linear Chemical assay kit ALT/GPT BR opt.).

Additionally, a frozen liver sample was homogenized (dilution 1:5) in ice-cold buffer (80 mmol/L tris-HCl, 5 mmol/L EDTA, 2 mmol/L DDT, 1 mmol/L PMSF and 1 mmol/L KCl, and 1 mmol/L sucrose, pH 7.6) and centrifuged at 1670 g at 4°C for 30 min to evaluate the hexokinase (HKs, EC 2.7.1.1) and glucokinase (GK, EC 2.7.1.2) activities. The supernatant was filtered through a Sephadex G-25 column (G25150-SIGMA-ALDRICH, St. Louis, MO, USA) (Caseras *et al*., 2000). For the analysis of HKs/GK activities, low K_m HK and

high K_m GK were measured using 1 and 100 mmol/L glucose, respectively. The determination of GK activity needed correction by measuring the glucose dehydrogenase (GDH, EC 1.1.1.47) activity (Tranulis *et al*., 1996).

Low K_m HK activity was evaluated in a 0.2 mL reaction containing 100 mmol/L tris-HCl, 7.5 mmol/L $MgCl_2$, 100 mmol/L KCl, 2.5 mmol/L DDT, 2 mmol/L NADP, 0.48 U/mL glucose-6-phosphate dehydrogenase, 2 mmol/L ATP and 1 mmol/L glucose. High K_m GK activity was evaluated in a 0.2 mL reaction containing 100 mmol/L tris-HCl, 7.5 mmol/L $MgCl_2$, 100 mmol/L KCl, 2.5 mmol/L DDT, 2 mmol/L NADP, 0.48 U/mL glucose-6-phosphate dehydrogenase, 2 mmol/L ATP and 100 mmol/L glucose. Glucose dehydrogenase was evaluated in a 0.2 mL reaction containing a 100 mmol/L tris-HCl, 7.5 mmol/L $MgCl_2$, 100 mmol/L KCl, 2.5 mmol/L DDT, 2 mmol/L NADP and 100 mmol/L glucose (Borrebaek *et al*., 1993; Tranulis *et al*., 1996).

Enzyme assays were monitored spectrophotometrically by observing the changes in NADH/NAD and NADP/NADPH absorbance at 340 nm at 37°C (Borrebaek *et al*., 1993; Tranulis *et al*., 1996). All enzymatic activities were reported as mg of soluble protein (specific activity). Protein concentration was determined according to Bradford (1976) using a commercial protein of bovine serum albumin as standard (B2064, SIGMA-ALDRICH, St. Louis, MO, USA).

Primer design and RNA expression of hepatic glucokinase

GK primers were designed using published sequences of a conserved region of the GK homologous gene enzymes from different fish species obtained from the GenBank database [www.ncbi.nlm.nih.gov/genbank] using free-access Bio-tools software [http://biotools.nubic.northwestern.edu/OligoCalc.htm]. The GK oligonucleotides were designed with the following features: length of 20-21 nucleotides, an alignment temperature of 55°C and 40-60% cytosine-guanine content (Table 2).

The RNA extractions and preparation of homogenates were performed by pooling three fish per replicate using the TRIzol reagent (TRIzol©, ThermoFisher Scientific, Waltham, MA, USA). Then, the extracted RNA was quantified using a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) at 260-280 nm; RNA samples were treated

with DNAseI© (ThermoFisher Scientific, Waltham, MA, USA) to remove any DNA contamination. cDNA was generated with a commercial kit (Hig-capacity cDNA Reverse Transcription Kit, ThermoFisher Scientific, Waltham, MA, USA). A quantitative polymerase chain reaction (qPCR) analysis of mRNA gene expression was performed using an IQ5 fluorometric thermocycler (BIO-RAD®, Hercules, CA, USA) with SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA). Real-time PCR efficiencies were determined for each primer pair using standard curves obtained from serial dilution of the cDNA pool. Relative expression of the GK gene was calculated with a comparative $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008), and the 18S rRNA gene was used as internal control (Leung and Woo, 2012).

Statistical analysis

Data were shown as the mean ± standard error (SE). The data of postprandial blood glucose and hepatic GK activity were analyzed with Analysis of Variance (ANOVA) test utilizing time, diet and specie as factors. The rest of the analyses were performed with two-way ANOVA, when the interaction of carbohydrate level and species kind resulted significant, a Tukey´s test was applied. Prior to these analyses, normality and homogeneity of residuals were verified. The analyses were performed with R statistical (Team, 2019).

3. Results

Growth performance, feed utilization and liver glycogen

SR, WG and SGR were not significantly affected by dietary carbohydrate level in both species (Table 3). *O. chrysurus* showed higher WG than *C. undecimalis*, but a lower SGR ($p < 0.05$). FE and PER were higher in *C. undecimalis* fed the HC diet than the others groups. Among these groups, FE and PER were significantly lower in *O. chrysurus* fed the C and HC diets than in *C. undecimalis* fed the C diet. Liver glycogen content was significantly higher in *C. undecimalis* fed the HC diet than in the other groups ($p < 0.05$) (Figure 1).

Digestive enzymes and glycemic response

Proteolytic enzyme (pepsin and alkaline proteases) activities were higher in the C than the HC diet ($p < 0.05$) groups (Table 2 and 3). Amylase activity was not affected by the

carbohydrate level in the diet in the pyloric cecum (Figure 3A), but in intestine the activity of amylase was higher in fish fed the HC diet ($p < 0.05$) (Figure 3B). *O. chrysurus* showed higher proteolytic and amylase activities than *C. undecimalis* ($p < 0.05$).

Glucose level in blood of *C. undecimalis* fed the HC diet peaked at 6:00 h (7.8 mmol/L) after feeding (Figure 4A), then decreased; whereas *O. chrysurus* fed the HC diet peaked at 9:00 h (14.1 mmol/L) after feeding, then decreased ($p < 0.05$).

Liver enzymes

GK activity was higher in fish fed the HC diet and peaked at 6:00 h after feeding, then decreased (Figure 4B and 5). The GK mRNA expression was higher in *C. undecimalis* fed HC diet ($p < 0.05$), while the rest of groups were similar to each other (Figure 5). HK activity was significantly higher in *O. chrysurus* fed the HC diet than in the other groups (Figure 5). Among these groups, HK activity was lower in *C. undecimalis* fed diet HC than in the *O. chrysurus* fed diet C, except for *C. undecimalis* fed diet C ($p < 0.05$). G6PDH and 6PGDH activities were higher in *C. undecimalis* fed the HC diet than the others groups ($p < 0.05$) (Figure 6). PK activity increased in the fish group fed HC diet, while activity of FBPase decreased ($p < 0.05$) (Figure 5 and 7). ALT activity was higher in *O. chrysurus* than *C. undecimalis* ($p < 0.05$) (Figure 7).

4. Discussion

The results of this comparative study showed a great variety of physiological response to dietary carbohydrates between *C. undecimalis* and *O. chrysurus* with potential consequence on their capacity to use them.

Growth performance, feed utilization efficiency and liver glycogen

Previous studies have shown that an inclusion level of 20% of carbohydrate in the diet can improve growth performance in some carnivorous fish species (Cui *et al*., 2010; Hung *et al*., 1989; Hutchins *et al*., 1998). The results of this study showed that compared with the control (C), no growth improvement (WG and SGR) was observed due to incorporating 20% carbohydrate in the diet (HC) for both species. However, *C. undecimalis* showed better feed and protein utilization efficiencies (FE and PER) when fed HC compared with C, indicating a clear protein sparing effect of dietary carbohydrates, as previously reported

in carnivorous *Dentex dentex* (Pérez-Jiménez *et al*., 2015) and *Dicentrarchus labrax* (Enes *et al*., 2006). The incorporation of carbohydrates in the diet did not affect *O. chrysurus* feed efficiency. The differences in carbohydrate utilization between *C. undecimalis* and *O. chrysurus*, both carnivorous species, may be related to dietary composition of their natural habitats (Blewett *et al*., 2006; Rincón-Sandoval *et al*., 2009) since *C. undecimalis* is catadromous (Gilmore *et al*., 1983) and *O. chrysurus* is marine species (De La Morinière *et al*., 2002).

In line with our observations, the SGR values in both fish species are within the range reported in previous studies (Concha-Frías *et al*., 2018; Enríquez *et al*., 2017; Gracia-López *et al*., 2003). On the other hand, the superior WG in *O. chrysurus* compared to *C. undecimalis* may be attributed to the differences in initial weight and stocking density. However, the high SGR values shown in *C. undecimalis* indicated that this specie has faster growth rate, which is consistent to its greater eficiency to assimilate food (FE and PER), resultanting in more available energy for growth.

Digestive enzymes

The adaptive response of fish digestive enzymes to the nutrient content in the diet can be considered as an indicator of their digestive potential (Lundstedt *et al*., 2004). In this study, the nutritional response of digestive enzymes by dietary carbohydrate levels showed the same tendency in both carnivorous species. The activities of proteolytic enzymes (pepsin and alkaline proteases) decreased in the fish group fed HC diet. In contrast to this study, some authors have reported that the increase in dietary carbohydrate content induced an increase in the activity of these enzymes in some carnivorous fish (Liu *et al*., 2020; Pérez-Jiménez *et al*., 2009), while in others no effect had been found (Guerrero-Zárate *et al*., 2019; Ren *et al*., 2011). The low proteolytic activity recorded in *O. chrysurus* and *C. undecimalis* fed HC diet suggested that protein digestion could have been negatively affected by carbohydrate inclusion in the diet, as previously reported in other studies for *Oncorhynchus mykiss* (Storebakken *et al*., 1998) and *Sparus aurata* (Couto *et al*., 2012; Enes *et al*., 2008).

For amylase, adaptability to dietary carbohydrate level was observed although the activity was only recorded in the intestine. The nutritional adaptation of amylase activity to dietary

carbohydrates has been also observed in other carnivorous fish, such as *Epinephelus coioides* (Liu *et al*., 2020), *Rachycentron canadum* (Ren *et al*., 2011) and *S. aurata* (Fountoulaki *et al*., 2005). On the other hand, the non-inducible effect of dietary carbohydrates on amylase at the pyloric cecum in this study differed from that reported in *D. dentex* (Pérez-Jiménez *et al*., 2009). In fish, the distribution of amylase production through the gastrointestinal tract depends on pancreas tissue diffusion, which greatly varies among species (Chesley, 1925). Thus, it was not surprising that carbohydrate digestion of *O. chrysurus* and *C. undecimalis* occurred mainly in the intestine than in the pyloric cecum. Nonetheless, dragging digestive fluid secreted in the pyloric cecum to the intestine by the effect of chyme displacement cannot be discarded.

In general, amylase digestive potential -different from that of proteolytic enzymes- has been found to depend on the natural diet of each fish species; herbivorous and omnivorous species have shown more amylase activity than carnivorous fish (Hidalgo *et al*., 1999). Likewise, those digestive variations found within each feeding group were considered a reflection of each individual digestive strategy, which could have been associated to habitat and environmental condition (Chakrabarti *et al*., 1995; Fernández *et al*., 2001). In this study, *O. chrysurus* showed greater proteolytic and amylase activity than those of *C. undecimalis*. These results indicated that *O. chrysurus* has a digestive system better adapted to protein and carbohydrate digestion than *C. undecimalis*.

Glycemic response

Glucose homeostasis regulation is directly related to the response of the hormonal and enzymatic mechanisms involved in carbohydrate metabolism of each species (Furuichi and Yone, 1981; Polakof *et al*., 2012). In this study, the glycemic response was consistent with the dietary carbohydrate content in both species, as previously reported for other carnivorous fish (Castillo *et al*., 2018a; Hutchins *et al*., 1998; Lee *et al*., 2003). However, the glycemic pattern differed between the two species. In *C. undecimalis*, the blood glucose level reached a maximum of 7.8 mmol/L six hours after being fed HC diet, which was consistent with that found in the carnivorous *Dicentrarchus labrax* fed with the same level of corn starch (Enes *et al*., 2011). Whereas glucose absorption in *O. chrysurus* persisted until reaching a maximum value of 14.1 mmol/L nine hours after being fed. Considering

the high amylase potential compared to the low glucose uptake speed recorded in *O. chrysurus*, a deficient adaptive response of the mechanisms should have been involved in glucose absorption (Buddington *et al*., 1997). In the same way, the high and prolonged levels of glucose in blood indicated that *O. chrysurus* has a poor glycemic control compared with other carnivorous species.

Liver enzymes

The liver plays a central role in the regulation of numerous intermediary metabolism processes in response to nutritional status (Walton and Cowey, 1982). Hepatic GK plays a key role as glucose sensor in glucose homeostasis regulation, and it is the first and limiting step in excess glucose storage (Iynedjian, 2009). In this study, hepatic GK expression of both molecular and enzymatic levels were induced in fish fed HC (Figure 3). The result in this study was consistent with previous research studies in carnivorous fish species, such as *Salmo salar* (Tranulis *et al*., 1996), *S. aurata, O. mykiss* (Panserat *et al*., 2000), *D. labrax* (Enes *et al*., 2006) and *Trachinotus ovatus* (Zhou *et al*., 2018). Nevertheless, it is important to emphasize that the GK mRNA expression and activity in *C. undecimalis* liver was greater than *O. chrysurus* (Figure 2B and 3B). These data could explain the poor glycemia control and lack of liver glycogen storage observed in *O. chrysurus*. However, the absence of significant differences in GK activity between the species recorded after six hours also suggested that the low glucose phosphorylation by GK can be related with an effect of insulin resistance (Párrizas *et al*., 1994) or as a consequence of low glucose-stimulated insulin secretion (Mommsen and Plisetskaya, 1991).

HK catalyzes the conversion of glucose to glucose 6-phosphate, but in contrast to GK, this enzyme allows glycolysis to be carried out under fasting conditions. In fish, the nutritional regulation of hepatic HK by dietary carbohydrates is controversial whereas no significant effect on HK activity was reported in *S. aurata, O. mykiss* (Panserat *et al*., 2000) and *D. labrax* (Enes *et al*., 2006). On the contrary, in other species, such as *E. coioides* (Liu *et al*., 2020), *Perca fluviatilis* (Borrebaek and Christophersen, 2000) and *Salmo gairdneri* (Fideu *et al*., 1983) a significant increase of HK activity by dietary carbohydrates was shown. In this study, HK activity increased in *O. chrysurus* when fed the carbohydrate-rich diet, whereas no significant effect was observed in *C. undecimalis*. It is also interesting to

observe that the HK enzymatic expression was higher in *O. chrysurus* than *C. undecimalis*. These results indicated that HK enzyme in *O. chrysurus* was under nutrition regulation; however, it is difficult to propose a physiological purpose to the HK activity increase, considering that the GK enzyme was also nutritionally induced by dietary carbohydrates in this species.

PK catalyzes the last step of glycolysis and controls the rate of pyruvate and ATP formation. Several authors have shown that no specific modulatory effect of dietary carbohydrates has been observed on hepatic PK nutritional activity regulation in some carnivorous fish species (Borrebaek and Christophersen, 2000; Enes *et al*., 2008; Li *et al*., 2019). The data showed hepatic PK activity adaptability to dietary carbohydrates in both fish species, indicating that PK enzyme contributed to the postprandial glucose utilization in *O. chrysurus* and *C. undecimalis*. In addition, an induction of hepatic PK activity by dietary carbohydrates was also observed in *E. morio* (Castillo *et al*., 2018a) and *Micropterus salmoides* (Lin *et al*., 2018; Ma *et al*., 2018).

The pentose phosphate pathway is an alternative to glycolysis, which contributes to maintain postprandial glucose homeostasis from glucose 6-phophate oxidation (Panserat *et al*., 2009). In this study, G6PDH and 6PGDH, both key enzymes of the pentose phosphate pathway showed an activity enhancement in *C. undecimalis* fed the carbohydrate-rich diet, as previously reported in other carnivorous fish (Enes *et al*., 2008; Lin *et al*., 1977; Liu *et al*., 2020). However, the activity of these enzymes was unaffected in *O. chrysurus* when they were fed the same diet. Accordingly, in *E. morio*, a positive dependence of the hepatic enzymes G6PDH and 6PGDH was recorded towards the metabolic regulation of hepatic GK by dietary carbohydrates (Castillo *et al*., 2018a, 2018b). Therefore, current data suggest that the lack of induction of the hepatic G6PDH and 6PGDH enzymes observed in *O. chrysurus* was a consequence of the low expression of hepatic GK at both molecular and enzymatic levels.

Amino acids are assumed as the main gluconeogenic substrate for fish (Jia *et al*., 2017; NRC, 2011) In this study, the hepatic ALT activity, one of the major aminotransferase enzymes in the amino acid catabolism was not affected by the presence or absence of digestible carbohydrates in the diet. In contrast to this study, hepatic ALT activity was

depressed in *S. aurata* (Enes *et al*., 2008) and *Seriola quinqueradiata* (Shimeno *et al*., 1996) after being fed with a carbohydrate-rich diet. These data suggested that the amino acid catabolism was an important metabolic pathway for *O. chrysurus* and *C. undecimalis* to obtain energy, as reported in the same manner for the carnivorous *D. labrax* by other authors (Enes *et al*., 2006). Interestingly, a significantly higher hepatic ALT activity was observed in *O. chrysurus*, which indicated that the carnivorous *O. chrysurus* has a greater metabolic dependence on protein than *C. undecimalis*.

Regarding gluconeogenesis, some fish have shown that regardless of the carbohydrate intake level, a high and persistent activity is observed in the key enzymes involved in the gluconeogenic pathway (FBPase, G6Pase and PEPCK) (Enes *et al*., 2006; Liu *et al*., 2020; Panserat *et al*., 2001). In this study, hepatic FBPase activity decreased as dietary carbohydrates level increased, indicating that the mechanisms involved in gluconeogenic pathway control in *C. undecimalis* and *O. chrysurus* are strongly sensitive to dietary glucose phosphorylation. These results agree with those previously reported in the same manner for other carnivorous fish, such as *R. canadum* (Li *et al*., 2019) and *E. morio* (Castillo *et al*., 2018a). On the other hand, the unaffected hepatic ALT activity with respect to low FBPase activity in both fish groups fed HC diet suggested that an alternative gluconeogenic substrate to the amino acids was metabolically depressed by the carbohydrate supplement in the diet, such glycerol (Rui, 2014).

As a whole the effects of high carbohydrates diet (HC) in glucose metabolism in juvenile *C. undecimalis* and *O. chrysurus*, such as stimulation of digestive and glycolytic potential, and suppression of gluconeogenic potential suggest that these species are able to adapt dietary carbohydrates. During the last decades, researchers have shown increased interest in exploring new strategies to improve the use of carbohydrates in the diet by fish through nutritional programming. For example, in *O. mykiss* was observed that a nutritional early stimulation by dietary carbohydrates leads to persistent changes (or enhancement) at juvenile stage in genes and enzymes involved in carbohydrate digestion, glucose transport and metabolism (Geurden *et al*., 2014, 2007; Liu *et al*., 2017). The immediate question is to address the possibility of enhancing glucose metabolic pathways in juvenile or adult stages

of *C. undecimalis* and *O. chrysurus* by a specific nutritional programming with dietary carbohydrates at early-life stages in both fish species.

5. Conclusion

To our knowledge, this study evaluated for the first time in *C. undecimalis* and *O. chrysurus* the parameters and mechanisms related to glucose metabolism in response to the presence and absence of digestible carbohydrates in the diet. These data indicated that *C. undecimalis* was able to metabolize digestible carbohydrates efficiently while *O. chrysurus* showed poor ability. Both fish species were able to adapt to the presence of carbohydrates in the diet by reorganizing digestive and hepatic glucose metabolism, but apparently hepatic GK played a crucial role in the utilization and storage of dietary carbohydrates between *O. chrysurus* and the *C. undecimalis*. The knowledge gathered from this study may be helpful for nutritionists to formulate feed based on more sustainable and cheaper feedstuffs. However, additional studies should pay attention to the relationships between the optimal level of carbohydrate sources, particularly in *O. chrysurus*.

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List of tables

Table 1 Formulation and proximate analysis of the experimental diets.

Ingredients (% dry weight)	Diet	
	C	HC
Fish meal[a]	52.0	52. 0
PbP prime meal [a]	20.0	20. 0
Corn starch[b]	0	20. 0
Wheat bran*	20. 0	0
Fish oil[b]	1. 0	1. 0
DSM vitamin and mineral pre-mix[c]	2. 0	2. 0
Pregelatinized gelatin	5. 0	5. 0
Proximate analysis (% dry weight)		
Dry matter	97.5	97.2
Crude protein	54.1	51.6
Crude lipid	8.1	7.9
Ash	14.7	13.3
Gross energy kJ/g	17.6	17.0

*Wheat bran was used as control diet because it contains digestion-resistant starch (Xieje *et al*. 2008) and also non-digestible protein due to a requirement for pH = 12.5 for digestion (Balandran-Quintana *et al*. 2015), so it was used to adjust the total energy of the diets. Proximate composition: 14% protein, 4% lipid and 86% carbohydrate.

[a]Proteínas Marinas y Agropecuarias S.A. de C.V.

[b]Productos Químicos de Yucatán S.A. de C.V., Mérida, Yucatán, Mexico.

[c]Donated by DMS (tropical fish), Nutritional Products Mexico S.A. de C.V.: A, D_3, E, K_3, B_1, B_2, B_{12}, folic acid, B_6, pantothenic acid, niacin, biotin, choline C, iron, manganese, sodium, copper, iodine, zinc, cobalt, selenium, molybdenum, calcium, phosphorus, magnesium, sodium, potassium, carophyll®, lysine, threonine, calcium carbonate, methionine, tryptophan, sodium lasalocid, monensin, tylosin, salinomycin, avoparcin, flavomycin arsanilic acid, amprolium, zinc bacitracin, virginiamycin, calcium propionate,

silicic acid, lignosulfonate, lecithin, the preservatives butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA); probiotics, glutase keta, lipase, proteases, amylase, cellulase and *Acemita tocuyama*.

Table 2 Nucleotide sequences of the primers used to amplify glucokinase (GK) and 18S r RNA genes (Leung and Woo, 2012).

Primer	Forward/reverse sequence
GK	5′-AATGTTGTGGGTTTACTCAG-′3
	5′-CCAACAATCATCCCAACTTC-′3
18S rRNA	5′-CTTGGATGTGGTAGCCGTTT-′3
	5′-GGATGCGTGCATTTATCAGA-′3

Table 3. Growth performance, feed efficiency and survival rate of juvenile *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. Values represent the mean ± standard error (SE) ($N = 4$).

Species	*C. undecimalis*		*O. chrysurus*		*p-value*[1]		
Diet	C	HC	C	HC	Diet	Species	Interaction
WG[2]	15.3 ± 0.8	14.7 ± 0.4	18.4 ± 1.8	18.3 ± 1.5	NS	0.01 (*O. ch* > *C. un*)	NS
SGR	1.70 ± 0.0	1.68 ± 0.0	1.26 ± 0.1	1.25 ± 0.1	NS	0.01 (*C. un* > *O. ch*)	NS
FE	0.69 ± 0.0^{b}	0.81 ± 0.0^{a}	0.43 ± 0.0^{b}	0.44 ± 0.0^{b}	0.00	0.00	0.00
PER	1.32 ± 0.0^{b}	1.56 ± 0.0^{a}	0.82 ± 0.0^{c}	0.86 ± 0.0^{c}	0.00	0.00	0.00
SR	100 ± 0.0	100 ± 0.0	97.5 ± 0.2	97.5 ± 0.2	NS	NS	NS

[1]Means with different superscripts in the same rows indicate significant interaction between diet and species ($p < 0.05$).

[2]Abbreviations: WG, weight gain; SGR, specific growth rate; FE, feed efficiency; PER, protein efficient ratio; SR, survival rate; *O. ch*, *O. chrysurus*; *C. un*, *C. undecimalis*.

Figure legends

Figure 1. Liver glycogen in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. Values represent the mean ± standard error (SE) ($N = 4$). Columns with different superscripts indicate significant interaction between diet and species ($p < 0.05$).

Figure 2. Specific activity of stomach pepsin in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. Values represent the mean ± standard error (SE) ($N = 4$). Columns with different superscripts indicate significant interaction between diet and species ($p < 0.05$).

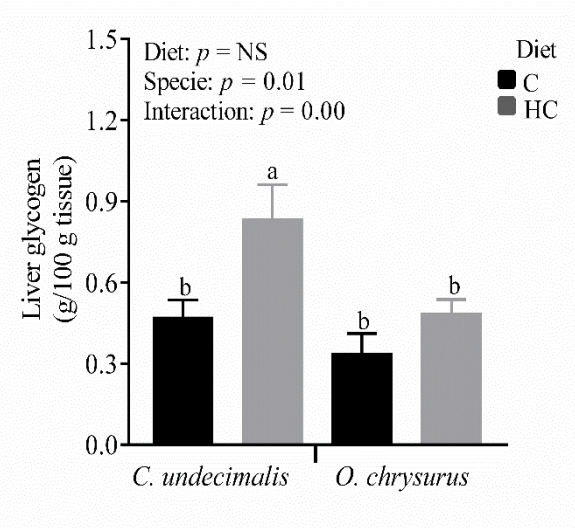
Figure 3. Specific activities of digestive enzymes in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. (**A**) The pyloric cecum. (**B**) Intestine. Values represent the mean ± standard error (SE) ($N = 4$). Columns with different superscripts indicate significant interaction between diet and species ($p < 0.05$).

Figure 4. Postprandial blood glucose (**A**) and specific activities of hepatic glucokinase-GK (**B**) in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. ■: *C. undecimalis* fed with HC diet; ●: *C. undecimalis* fed with C diet; ▼: *O. chrysurus* fed with HC diet; ▲: *C. chrysurus* fed with C diet. Values represent the mean ± standard error (SE) ($N = 3$). Individual means or groups of means with different superscripts indicate significant interaction between diet and species ($p < 0.05$).

Figure 5. Specific activities and mRNA expression of key hepatic enzymes involved in glycolysis (glucokinase (GK), hexokinase (HK) and pyruvate kinase (PK)) pathway in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. Values represent the mean ± standard error (SE) ($N = 4$). Columns with different superscripts indicate significant interaction between diet and species ($p < 0.05$).

Figure 6. Specific activities of key hepatic enzymes involved in the pentose phosphate pathway in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. Values represent the mean ± standard error (SE) (N = 4). Columns with different superscripts indicate significant interaction between diet and species ($p < 0.05$).

Figure 7. Specific activities of key hepatic enzymes involved in gluconeogenesis and amino acid catabolism pathways in *C. undecimalis* and *O. chrysurus* fed diets with (HC) or without (C) carbohydrates. Values represent the mean ± standard error (SE) (N = 4). Columns with different superscripts indicate significant interaction between diet and species ($p < 0.05$).



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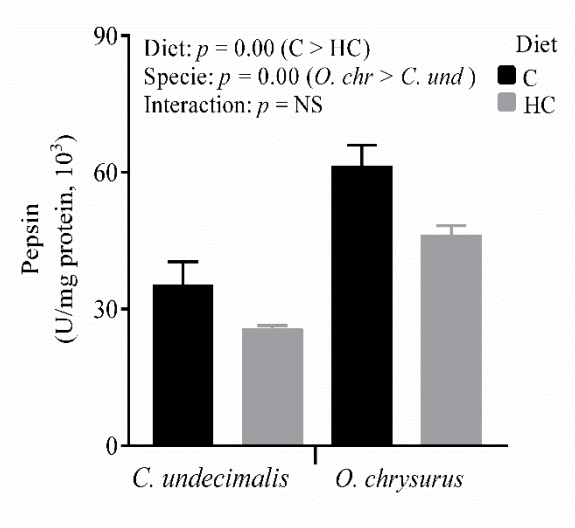
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Specie: p = 0.00 (O. chr > C. und)
Interaction: p = NS
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C
HC
Pepsin
(U/mg protein, 10³)
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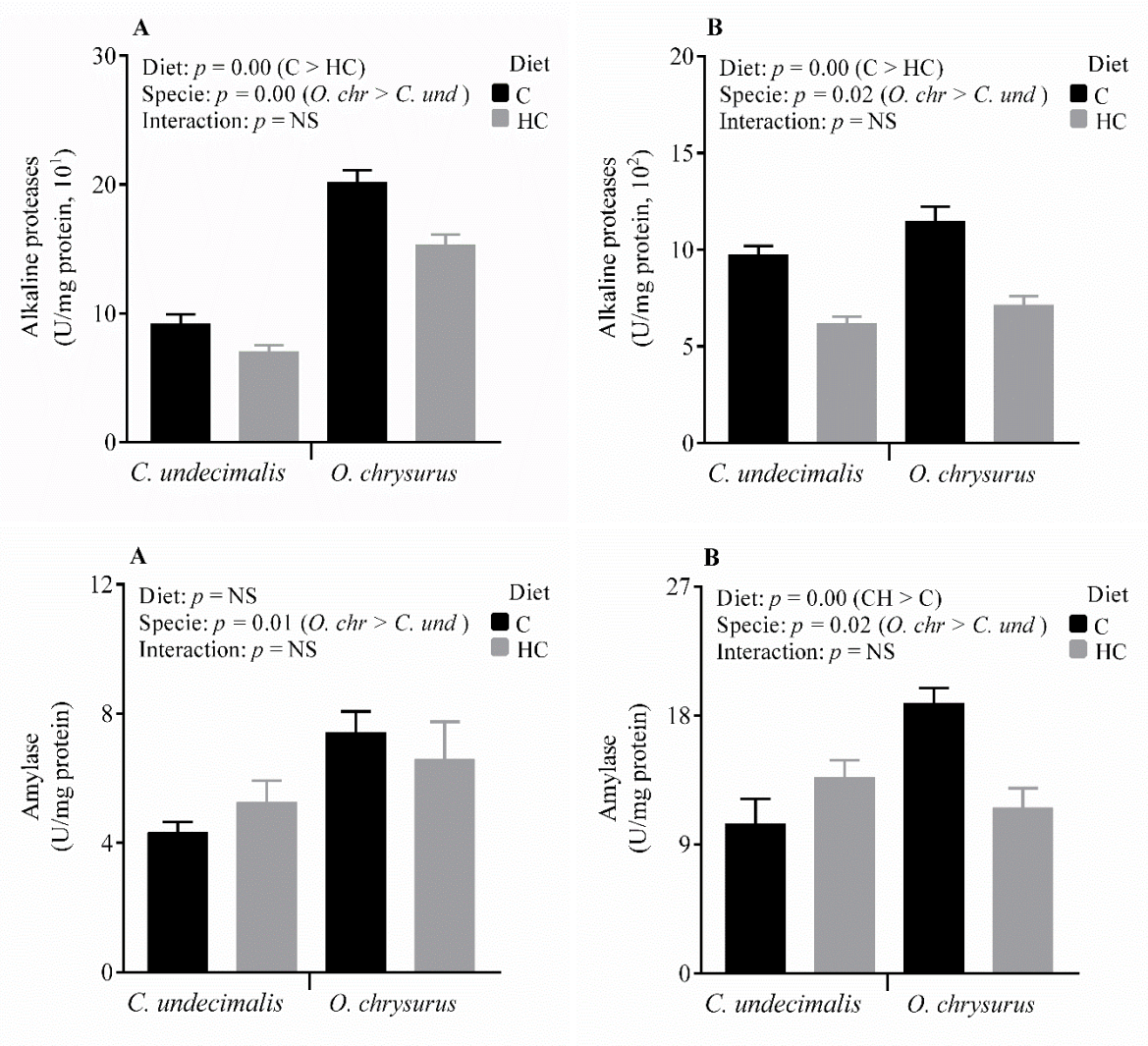
60
30
0
C. undecimalis
O. chrysurus

Figure 2. TIF



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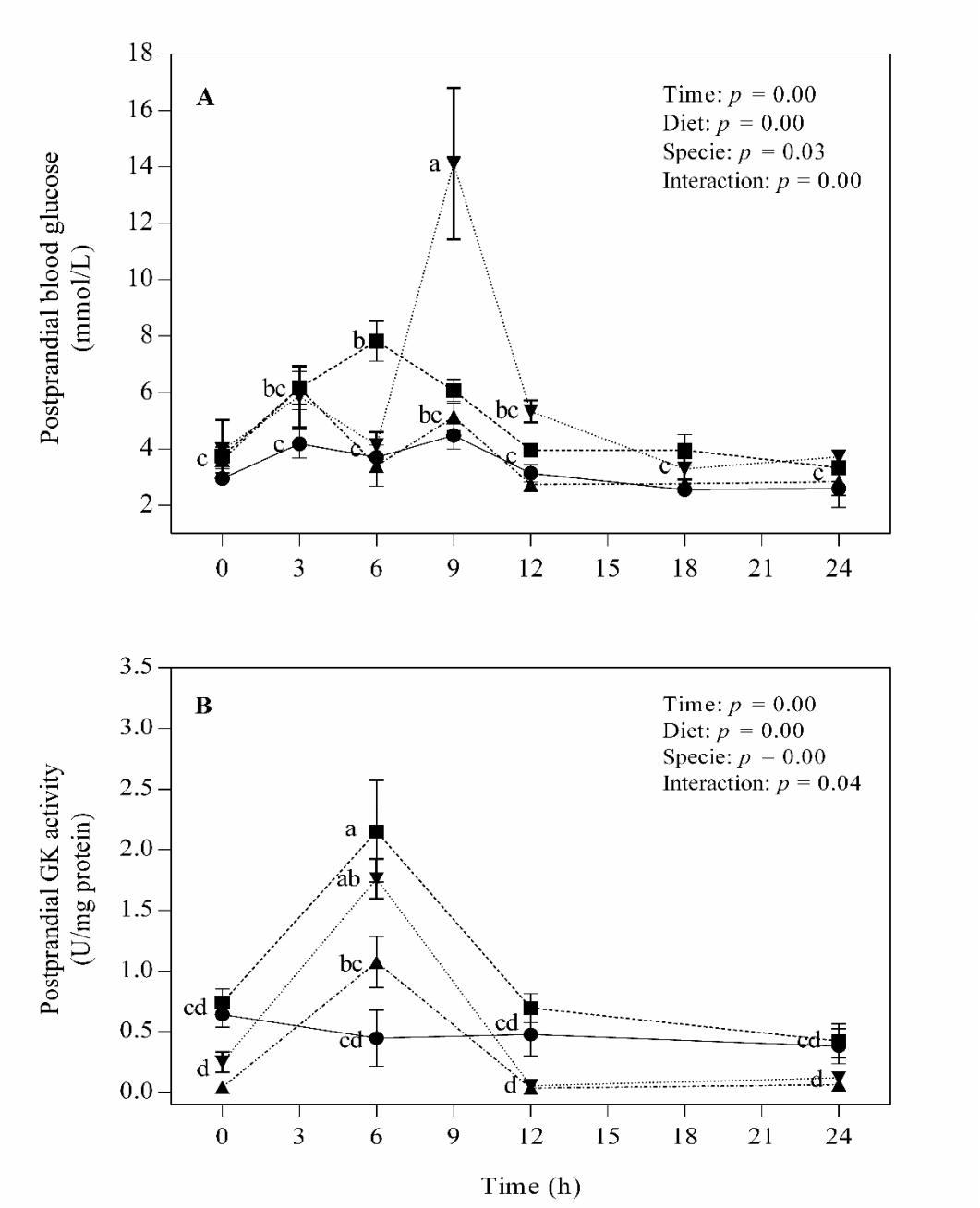

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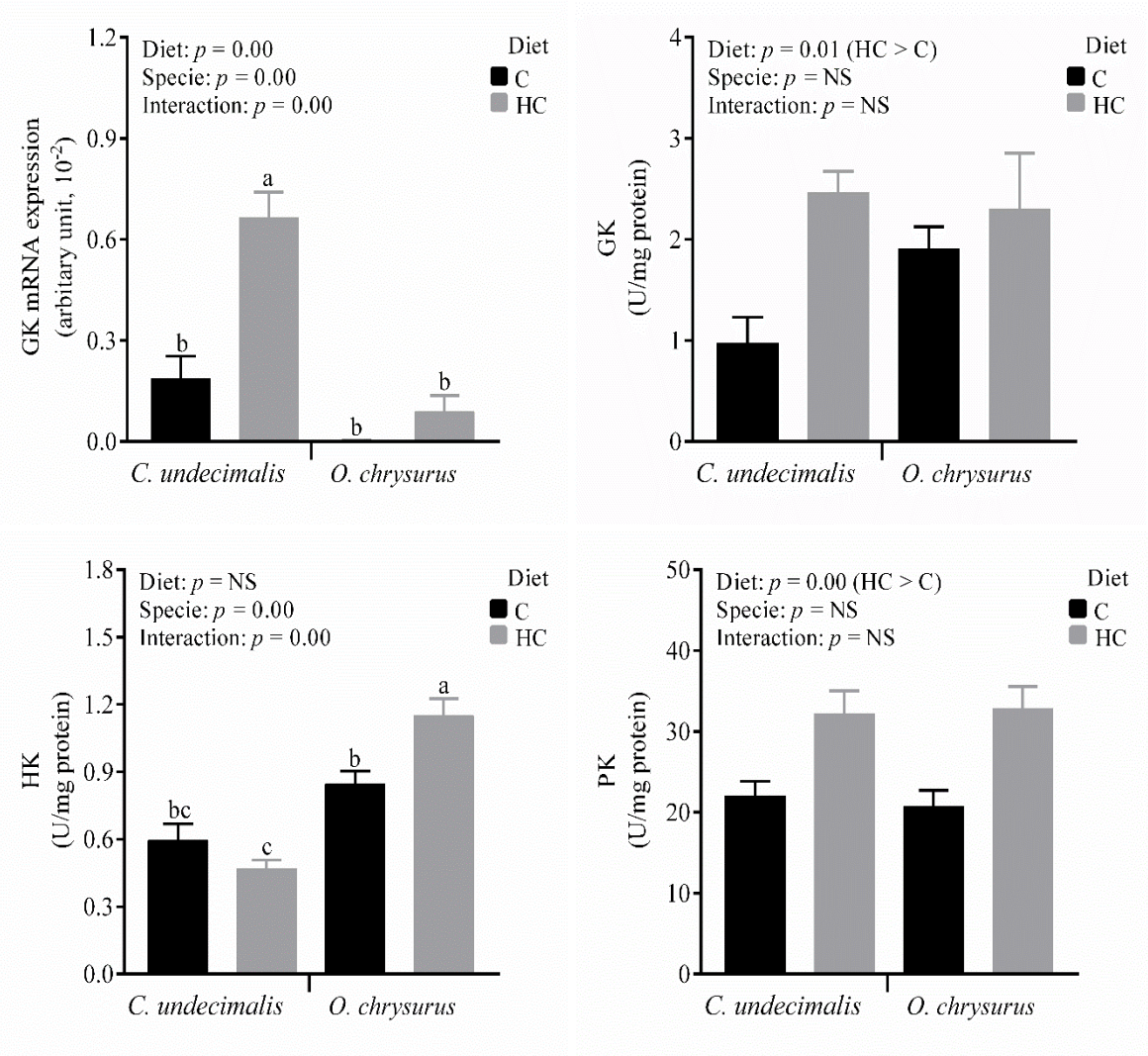

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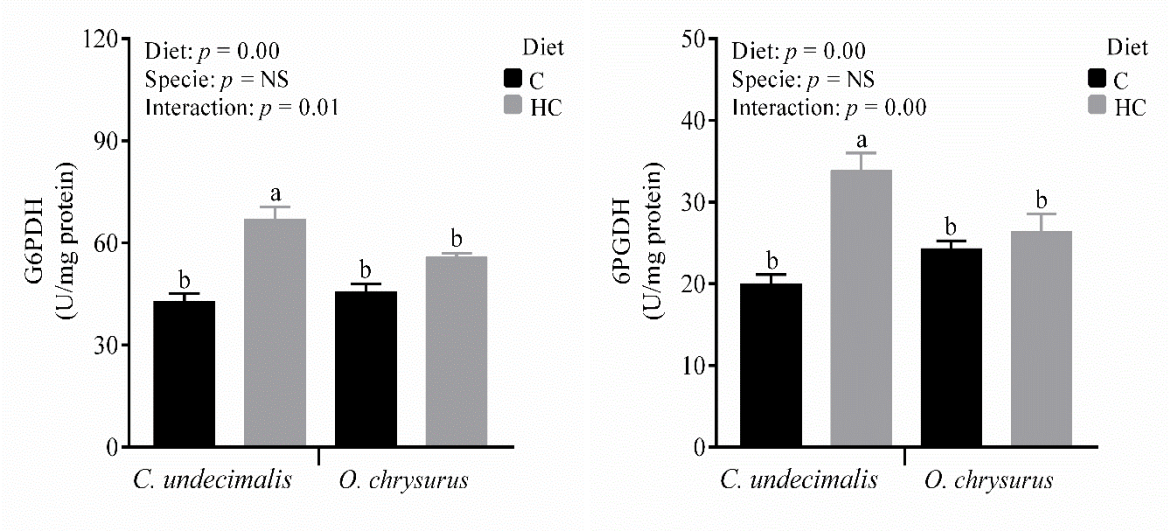

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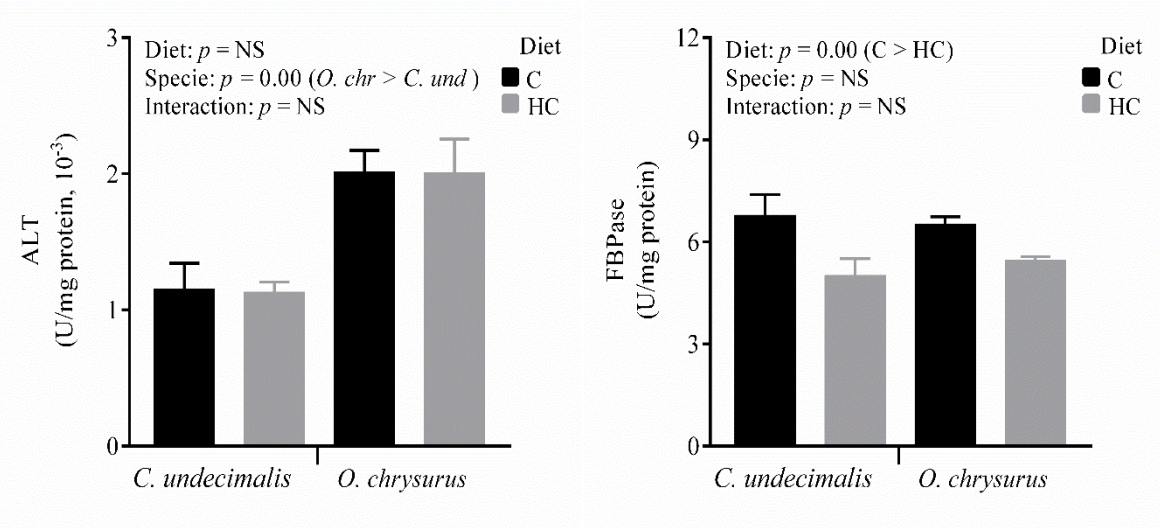
69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(U/mg protein, 10^{-3})
Diet: p = NS
Specie: p = 0.00 (*O. chr* > *C. und*)
Interaction: p = NS
Diet
C
HC
3
2
1
0
C. undecimalis
O. chrysurus
FBPase
(U/mg protein)
Diet: p = 0.00 (C > HC)
Specie: p = NS
Interaction: p = NS
Diet
C
HC
12
9
6
3
0
C. undecimalis
O. chrysurus

Figure 7.TIF

CAPÍTULO 3

Artículo 2

Sometido a: *Latin American Journal of Aquatic Research*

Evaluation of protein: lipid ratio on growth, feed efficiency and metabolic response in juvenile yellowtail snapper (*Ocyurus chrysurus*, Bloch, 1791)

Short title: protein: lipids in yellowtail snapper

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(Received *XXXX*; accepted *XXXX*)

Abstract

This study was conducted evaluate the ability of juveniles *Ocyurus chrysurus* (13.7 ± 0.45 g) to utilize lipid as energy source on growth, feed efficiency, body composition, digestive and hepatic enzyme activities. Four diets of two protein levels (40 and 50%) with two lipid levels (6 and 12%) and 2% of digestible carbohydrates were formulated. Fish were fed for 60 days to apparent satiation at a stocking density of 10 fish per tank (100 L). Growth gain of fish fed 50% dietary protein was higher than of fish fed 40% dietary protein ($P < 0.05$). However, feed efficiency was significantly higher at 12% than 6% of dietary lipid. Whole body lipid and glycogen in the liver significantly increased with dietary lipid content. Alkaline protease activity trend to increase as dietary protein increased. Trypsin activity increased significantly as dietary lipid decreased, whereas chymotrypsin activity showed the opposite trend ($P < 0.05$). Bile salt-dependent lipase activity trend to increase as dietary energy decreased. Glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH) and fructose-1, 6-biphosphatase (FBPase) significantly increased with increasing dietary lipid levels. However, alanine aminotransferase (ALT) activity increased with dietary protein content. By the other side, pyruvate kinase (PK) activity increased with both dietary protein and lipid content. The results of this study indicate that high dietary lipid (12%) improved the feed efficiency, but did not reduce dietary protein demand in juveniles *O. chrysurus*.

Resumen

Este estudio se llevó a cabo para evaluar la capacidad de los juveniles de *Ocyurus chrysurus* (13,7 ± 0,45 g) para utilizar lípidos como fuente de energía en el crecimiento, la eficiencia alimentaria, la composición corporal y las actividades enzimáticas digestivas y hepáticas. Se formularon cuatro dietas con dos niveles de proteínas (40 y 50%), dos niveles de lípidos (6 y 12%) y 2% de carbohidratos digeribles. Los peces se alimentaron durante 60 días hasta la aparente saciedad a una densidad de población de 10 peces por tanque (100 L). La ganancia de crecimiento de los peces alimentados con un 50% de proteína dietética fue mayor que la de los peces alimentados con un 40% de proteína dietética (P <0.05). Sin embargo, la eficiencia alimenticia fue significativamente mayor al 12% que al 6% de los lípidos de la dieta. Los lípidos corporales y el glucógeno en el hígado aumentaron significativamente con el contenido de lípidos de la dieta. La actividad de la proteasa alcalina tiende a aumentar a medida que aumenta la proteína de la dieta. La actividad de la tripsina aumentó significativamente a medida que disminuyeron los lípidos de la dieta, mientras que la actividad de la quimotripsina mostró la tendencia opuesta (P <0,05). La actividad de la lipasa dependiente de sales biliares tiende a aumentar a medida que disminuye la energía alimentaria. La glucosa-6-fosfato deshidrogenasa (G6PDH), la 6-fosfogluconato deshidrogenasa (6PGDH) y la fructosa-1, 6-bifosfatasa (FBPasa) aumentaron significativamente con el aumento de los niveles de lípidos en la dieta. Sin embargo, la actividad de la alanina aminotransferasa (ALT) aumentó con el contenido de proteínas de la dieta. Por otro lado, la actividad de la piruvato quinasa (PK) aumentó con el contenido de proteínas y lípidos de la dieta. Los resultados de este estudio indican que un alto contenido de lípidos en la dieta (12%) mejoró la eficiencia de la alimentación, pero no redujo la demanda de proteínas en la dieta en los juveniles de *O. chrysurus.*

135

136 Sisal, Yucatán, February 16th 2021

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140

141 Dra. Gabriela Gaxiola:

142

143 We have reached a decision regarding your submission to Latin American Journal of Aquatic

144 Research, **"Evaluation of protein: lipid ratio on growth, feed efficiency and metabolic**

145 **response in juvenile yellowtail snapper (Ocyurus chrysurus, Bloch, 1791)".**

146

147 Our decision is to: Accepted

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149

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Evaluation of protein: lipid ratio on growth, feed efficiency and metabolic response in juvenile yellowtail snapper (*Ocyurus chrysurus*, Bloch 1791)

Short title: protein: lipids in yellowtail snapper

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Abstract

This study was conducted evaluate the ability of juveniles *Ocyurus chrysurus* (13.7 ± 0.45 g) to utilize lipid as energy source on growth, feed efficiency, body composition, digestive and hepatic enzyme activities. Four diets of two protein levels (40 and 50%) with two lipid levels (6 and 12%) and 2% of digestible carbohydrates were formulated. Fish were fed for 60 days to apparent satiation at a stocking density of 10 fish per tank (100 L). Growth gain of fish fed 50% dietary protein was higher than of fish fed 40% dietary protein ($P < 0.05$). However, feed efficiency was significantly higher at 12% than 6% of dietary lipid. Whole body lipid and glycogen in the liver significantly increased with dietary lipid content. Alkaline protease activity trend to increase as dietary protein increased. Trypsin activity increased significantly as dietary lipid decreased, whereas chymotrypsin activity showed the opposite trend ($P < 0.05$). Bile salt-dependent lipase activity trend to increase as dietary energy decreased. Glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH) and fructose-1, 6-biphosphatase

(FBPase) significantly increased with increasing dietary lipid levels. However, alanine aminotransferase (ALT) activity increased with dietary protein content. By the other side, pyruvate kinase (PK) activity increased with both dietary protein and lipid content. The results of this study indicate that high dietary lipid (12%) improved the feed efficiency, but did not reduce dietary protein demand in juveniles *O. chrysurus*.

Keywords: *Ocyurus. chrysurus*, Lutjanidae, dietary lipid, protein-sparing effect, carnivorous fish.

Introduction

Protein is the main component in fish feed formulation and the most expensive dietary component (Oliva-Teles et al. 2020). In fish, protein is not only important for growth (protein synthesis), but also plays a central role in energy balance (ATP production) (Jia et al. 2017), especially when the protein supply in the diet is excessive or / and the energy level from non-protein sources is insufficient (NRC 2011). Therefore, it is important to develop diets with an adequate protein: energy balance (Catacutan et al. 2001, Kim et al. 2004, Abdo de la Parra et al. 2010).

Lipids are the main non-protein energy source for fish and also provide essential fatty acids, which play an important role in growth and health condition (Tocher 2003). For energy purposes, an adequate inclusion of lipid levels in the diet can help reduce the catabolism of dietary protein in fish (Shimeno et al. 1996, Lee et al. 2002, Guo et al. 2019). Conversely, excessive and / or deficient dietary lipid levels could adversely affect growth performance, increase factor conversion ratio (FCR), and lead to fat deposition (Peres & Oliva-Teles 1999, Tuan & Williams 2007). On the other hand, the ability of fish to use dietary lipid as non-protein energy source can be influenced by the content of dietary carbohydrates, which can also serve as sources of non-protein energy (Shimeno et al. 1981, Zhou et al. 2016, Guerrero-Zárate et al. 2019).

Yellowtail snapper (*Ocyurus chrysurus*, Bloch 1791) (Pisces: Lutjanidae) is a highly valued tropical carnivorous marine fish in the east coast of America (McClellan & Cummings 1998, Brulé et al. 2008, Cladis et al. 2014). *O. chrysurus* is considered a good candidate for aquaculture due this specie has the advantages of spawns without the use of hormones, is tolerant of handling, and accepts artificial feed (Watanabe et al. 1998, Turano et al. 2000, Gutiérrez-

Sigeros et al. 2018). Thus, it is necessary to develop commercial diet for this specie. Previous reports revealed that *O. chrysurus* have a high dietary protein requirement (≥50%) (Enríquez et al. 2017). To date, no studies concerning the ability of *O. chrysurus* to utilize dietary lipids as energy sources have been carried out. Therefore, the purpose of the present study was to evaluate the effect of different dietary protein and lipid levels with a minimal dietary digestible carbohydrates inclusion level (2%) on growth performance, feed utilization, proximate composition, digestive and hepatic enzyme activities.

Methodology

Four experimental diets were formulated to contain either 40 and 50% crude protein combined with either 6 and 12% fat (Table 1). The ingredients were sieved through a 250-µm mesh and then mixed for 30 min in a common blender. The dough was extruded and broken down to a size of 3-mm in particle diameter using a Bonnot-Model 2EXTWPKR (Ohio, USA) extruder. Next, the dough was dried via ventilation at 60 °C for 3 h, and the feed was sealed in vacuum-packed bags and stored at -20 °C until use.

Experimental procedures

O. chrysurus juveniles were obtained from Marine Fish Reproduction Laboratory at Unidad Multidisciplinaria de Docencia e Investigación Sisal (Yucatán, México) following the methodology described by Gutiérrez-Sigeros et al. (2018). The trial was performed in a thermoregulated recirculating water system (Resun CW 1000 chillers, Guandong Risheng. Co LTD, China) operated with 12 rectangular fiberglass tanks with a 100 L water capacity (73 cm x 44 cm x 33 cm). Ten fish were placed in each tank (13.7 ± 0.45 g initial weight and 10.5 ± 0.25 cm total length), and 3 replicate groups of fish were used to test each diet. Each diet was randomly assigned. Tanks were supplied with continuous air and seawater flows (5.0 L min^{-1}). During the experimental period, the water temperature was 28.1 ± 0.6°C, salinity was 36 ± 0.4 g L^{-1}, pH was 8.2 ± 0.1, ammonia concentration was 0.01 ± 0.0 mg L^{-1} and dissolved oxygen was 4.1 ± 0.4 mg L^{-1}. Before the experiment, the fish were acclimated to the experimental diet for 2 weeks. The fish were fed 2 times a day (10:00 and 18:00 h) at *ad libitum*. Uneaten feed was collected half an hour after each feeding and then dried (AOAC 1997).

Sample collection

The growth trial lasted 60 days. The fish were anesthetized with 0.1 mg/mL clove oil according to Castillo et al. (2018) then the fish were killer (according to of recommendations of NOM-062-ZOO-1999, from México) (SAGARPA 1999). Immediately, the pyloric caeca, intestine, liver and mesenteric fat were sampled. The liver and mesenteric fat were used to determine the hepatosomatic and fat mesenteric indices. The pyloric caeca, intestine and liver were frozen in liquid nitrogen and stored at -80°C. The liver was divided into two parts to assess glycogen and metabolic enzymes. Three fish per tank were sampled and pooled to assess the whole body proximate composition.

Growth performance

At the end of the experiment, survival (S, %), weight gain (WG, g), specific growth rate (SGR, % day^{-1}), feed intake (FI, g fish), feed conversion ratio (FCR), protein efficiency ratio (PER) and hepatosomatic index (HSI) were calculated as follow:

$$S = \left(\frac{\text{final number of live fish}}{\text{initial number of live fish}}\right) \times 100$$

$$WG = \text{final mean body wet weight (g)} - \text{initial mean body wet weight (g)}$$

$$SGR = \left(\frac{\text{Ln[final mean body wet weight (g)} - \text{initial mean body wet weight (g)]}}{\text{time (days)}}\right)$$

$$FI = \left(\frac{\text{dry food consumer per tank (g)}}{\text{number of live fish per tank}}\right)$$

$$FCR = \left(\frac{FI}{WG}\right)$$

$$PER = \left(\frac{WG}{\text{dry protein intake (g)}}\right)$$

$$HSI = \left(\frac{\text{wet weight liver (g)}}{\text{wet body weight (g)}}\right) \times 100$$

Proximate analysis

Crude protein content was determined by flash combustion, then N_2 was quantified by thermal conductivity detection using a CHNS elemental analyzer (Costech ECS-4010, Valencia, US), N_2 was converted to protein using a conversion factor of 6.38 (Lynch & Barbano 1999). Crude lipid levels were determined using the hexane extraction method with a Goldfish system (Nielsen & Carpenter 2017). The moisture content was determined after the sample was oven-dried and brought to a constant dry weight (60°C for 120 h). The ash content was determined by combusting the sample in a muffle furnace at 550°C for 6 hr (AOAC 1997). The glycogen content was determined using the method reported by Carroll et al. (1956).

Digestive enzyme activity

The pyloric caeca and intestine were homogenized with an Ultra Turrax IKA T18 homogenizer (North Chase, Wilmington, US) in 30 mM L^{-1} tris-HCl + 12.5 mM L^{-1} $CaCl_2$ at pH 7.5, and centrifuged at 1670 g for 30 min at 4°C (5420R-Eppendorf, Germany).

Total alkaline protease activities were evaluated using Hammarsten-grade casein (0.05%) in 100 mM L^{-1} tris-HCl and 10 mM L^{-1} $CaCl_2$ at pH 9 with the method reported by Kunitz (1947) and modified by Walter (1994). One unit of enzyme activity was defined as 1 µg of tyrosine released per min at 280 nm.

Trypsin activity was assayed using N-a-benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA) 3.5 mM L^{-1} as substrate in 100 mM L^{-1} tris at pH 8 following the method of Erlanger et al. (1961). Chymotrypsin was evaluated using N-succinyl- ala–ala-pro-phe p-nitroanilide (SAAPNA) 1 mM L^{-1} as substrate in 100 mM/L tris at pH 8 following the method of DelMar et al. (1979). One unit of enzyme was defined as 1 µM of p-nitroanilide released per min using a coefficient of molar extinction of 8800 at 410 nm.

Bile salt-dependent lipase activity was measured using the method described by Gjellesvik et al. (1992) with 4-nitrophenyl octanoate (1 mM L^{-1}) in 500 mM/L tris and 6 mM/L sodium taurocholate at pH 7.4. One unit of lipase activity was defined as 1 µM L^{-1} of nitrophenol hydrolysed per min using a molar extinction coefficient of 16300 at 415 nm.

Hepatic key intermediary enzyme activities

The livers were homogenized (dilution 1:5) in ice-cold buffer (50 mM L^{-1} tris-HCl, 0.5 mM L^{-1} phenylmethyl sulfonyl fluoride (PMSF), 4 mM L^{-1} ethylenediaminetetraacetic acid (EDTA), 50

298 mM L^{-1} sodium fluoride (NaF), 250 mM L^{-1} sucrose and 500 mM/L dithiothreitol (DTT, at pH
299 7.5) and centrifuged at 1670 g for 30 min at 4°C. The resulting supernatant was separated to
300 assess PK, FBPase, G6PDH and 6PGDH activities. Pyruvate kinase (PK) activity was evaluated
301 in a 0.25 mL reaction containing 250 mM L^{-1} glycyl-glycine, pH 7.4, 10 mM L^{-1} $MgCl_2$, 100
302 mM L^{-1} KCl, 0.15 mM L^{-1} NADH, 2.8 mM L^{-1} phosphoenolpyruvate, and 21 U mL^{-1} lactate
303 dehydrogenase. ADP was added at the end of the experiment (Bonamusa et al. 1992). Fructose
304 1,6 bisphosphatase (FBPase) activity was evaluated in a 0.2 mL reaction containing 85 mM L^{-1}
305 imidazole-HCl, pH 7.7, 5 mM L^{-1} $MgCl_2$, 0.5 mM L^{-1} NADP, 12 mM L^{-1} 2-mercaptoethanol,
306 0.05 mM L^{-1} fructose-1,6-diphosphate, 25 U mL^{-1} phosphoglucose isomerase, and 0.48 U mL^{-1}
307 glucose-6-phosphate dehydrogenase. Activity of glucose-6-phosphate dehydrogenase (G6PDH)
308 was evaluated in a 0.2 mL reaction containing 77 mM L^{-1} imidazole HCl, pH 7.7, 5 mM L^{-1}
309 $MgCl_2$, 1 mM L^{-1} NADP, and 10 mM L^{-1} glucose 6-phosphate. 6-Phosphogluconate
310 dehydrogenase (6PGDH) activity was evaluated in a 0.2 mL reaction containing 83 mM L^{-1}
311 imidazole HCl, pH 7.7, 3 mM L^{-1} $MgCl_2$, 0.5 mM L^{-1} NADP, and 2 mM L^{-1} gluconate 6-
312 phosphate (Bonamusa et al. 1992). Alanine aminotransferase (ALT) was measured using a
313 commercial kit (Linear Chemical assay kit ALT/GPT BR opt.).
314 Enzyme activity was measured using a microplate spectrophotometer (BioTek, Vermont, US) at
315 37°C. Enzyme assays were monitored spectrophotometrically by observing the changes in the
316 absorbance of NADH/NAD and NADP/NADPH at 340 nm (Borrebaek et al. 1993, Tranulis et
317 al. 1996). Digestive and metabolic enzyme activities were reported as mg of soluble protein
318 (specific activity). Protein concentration was determined according to Bradford (1976) using a
319 Sigma protein assay kit with bovine serum albumin as standard

320 *Statistical analysis*

321 Data are presented as the mean ± standard error (SE). The rest of analysis were performed with
322 two-way ANOVA test ($P < 0.05$), when the interaction of protein and lipid level resulted
323 significant a Tukey´s test was applicate. Prior to theses analysis, the normality and homogeneity
324 of residuals were verified. The analyses were performed with R statistical (Core Team 2019).

325

Results

The growth performance and feed efficiency of *O. chrysurus* fed with different protein: lipid ratios for 60 days is shown in Table 2. Survival was not significantly affected by experimental diets. FW, WG and SGR improved significantly as protein level increased from 40% to 50%, but no significant differences were observed to lipid level. FI, PER and FCR were affected by dietary lipid level ($P < 0.05$), but not dietary protein level ($P > 0.05$). FI and FCR increased significantly as dietary lipid level decreased; whereas, PER showed an opposite trend.

Whole fish composition, liver glycogen, hepatosomatic index are listed at Table 3. Moisture and ash contents of whole-body were significantly affected by both protein and lipid levels ($P < 0.05$). Moisture content increased significantly as protein level increased and lipid level decreased; whereas, ash content showed an opposite trend ($P < 0.05$). Protein content of whole body was significantly influenced by dietary protein level, but not by dietary lipid level. The content of protein of fish fed 40% dietary protein was significantly higher than that of fish fed 50% dietary protein ($P < 0.05$). Whole body lipid and liver glycogen contents were significantly affected by dietary lipid level, and tended to increase with increasing dietary lipid level ($P < 0.05$). HSI did not differ among experimental fish group.

Digestive enzymes activities in the pyloric caeca and intestine of *O. chrysurus* are presented in Table 4 and Table 5, respectively. A significant interaction between dietary protein and lipid levels was observed in alkaline proteases and lipase activities in both digestive organs ($P < 0.05$). In the pyloric caeca, alkaline protease activity was significantly higher for fish fed the 50:12 diet than the rest of dietary treatments. In intestine, the alkaline protease activity of fish fed diet 50:12 was significantly higher than that of fish fed the 40:12 diet, but not significantly different from those of fish fed the 40:6 and 50:6 diets. For lipase, fish fed the 40:6 diet showed significantly higher activity than the rest of dietary treatments in both digestive organs, whereas that of fish fed the 40:12 diet showed the lower lipase activity ($P < 0.05$). Trypsin and chymotrypsin activities were significantly affected by dietary lipid level, but not by dietary protein level. Trypsin activity increased significantly as dietary lipid level increased; whereas, chymotrypsin activity showed an opposite trend ($P < 0.05$).

Hepatic metabolic enzymes activities are shown in Table 6. PK activity was significantly affected by the dietary levels of protein and lipid, and increased with increasing dietary protein

and lipid levels. G6PDH, 6PGDH and FBPase activities were influenced by dietary lipid level, but not by dietary protein level. The activities of the G6PDH, 6PGDH and FBPase enzymes of the fish fed 12% dietary lipids were significantly higher than those of the fish fed 6% lipids. ALT activity was significantly affected by dietary protein level, and increased as protein level increased.

Discussion

This study examined the ability of *O. chrysurus* to utilize dietary lipid as energy source. This was achieved through four experimental diets with two graded levels of protein (40% and 50%) and lipid (6% and 12%) each, which containing a minimal level of digestible carbohydrates (2% corn starch).

Growth, feed efficiency and biochemical composition

Dietary protein content decrement with or without a concomitant increase of non-protein energy content (lipid or carbohydrates) provides an extremely efficient improvement in protein utilization without impacting growth in numerous species of fish (NRC 2011). However, in this study, SGR and WG were not affected by the level of lipid in the diet. The lack of protein optimization for growth performance by dietary lipid increasing effect observed in this study is in line with other species of the same family, such as mutton snapper (*Lutjanus analis*) (Watanabe et al. 2001), spotted rose snapper (*Lutjanus guttatus*) (Abdo de la Parra et al. 2010), red porgy (*Pagrus pagrus*) (Schuchardt et al. 2008), mangrove red snapper (*Lutjanus argentimaculatus*) (Catacutan et al. 2001) and red snapper (*Lutjanus campechanus*) (Miller et al. 2005). According to these data, dietary lipid appears to be provide a poor impact on improvement efficiency of protein utilization on growth performance in Lutjanidae species.

Dietary energy ranged from 13.6 to 18.6 KJ g^{-1}, and the maximum growth (SGR and WG) was observed in the diets 50:6 and 50:12, which containing 16.6 and 18.6 KJ g^{-1}, respectively. On the other hand, diet 40:12 contained a similar energy content that diet 50:6 (16.4 and 16.6 KJ g^{-1}, respectively), but fish fed with the diet 40:12 showed a lower growth (SGR and WG) compared to fish fed with the diet 50:6. These data suggest that the lower growth of the *O. chrysurus* registered in the 40:12 diet was due to a deficiency in protein and not energy. In *L. argentimaculatus* (Catacutan et al. 2001), *P. pagrus* (Schuchardt et al. 2008) and *L. guttatus*

(Abdo de la Parra et al. 2010), it was also observed that a dietary protein level of 40% induced poor growth.

In this study, PER and FCR improved with increasing the inclusion of lipids in the diet from 6% to 12%. In contrast to the present results, no differences in PER or FCR with dietary lipid level increasing from 6% to 15% in *L. analis* (Watanabe et al. 2001), 6% to 12% in *L. argentimaculatus* (Catacutan et al. 2001), 9% to 15% in *L. guttatus* (Abdo de la Parra et al. 2010) and 8% to 14% in red snapper (Miller et al. 2005). This data suggest that *O. chrysurus* has a relative higher ability to utilize dietary lipid as energy source than other snapper species.

In the present study, whole body lipids increased with dietary lipid content, as previously reported in malabar grouper (*Epinephelus malabaricus*) (Tuan & Williams 2007) and rockfish (*Sebastes schlegeli*) (Lee et al. 2002). The protein composition of the whole body is a relevant indicator to determinate the optimal dietary protein requirement in fish. For example, Abbas et al. (2005) in *L. argentimaculatus* and Mohanta et al. (2008a) in silver barb (*Puntius gonionotus*), found a maximum whole body protein content at the minimum dietary protein level for maximum growth for these species, then it decreased to higher dietary protein levels. In this study, the protein content of the whole body decreased when dietary protein level increased from 40% to 50%. These data suggest that the protein requirement for *O. chrysurus* could be less than 50%, but greater than 40%. On the other hand, whole body moisture content incresed with dietary protein levels and decreased with dietary lipid levels. The decrease of body moisture content could be explicated to the increase in the content of proteins and lipids in the whole body at the level of protein and lipid in the diet, respectively. In addition, the whole body ash content increased with decreased protein and increased lipid levels in the diet, which agrees with the findings of other workers in several other fishes (Alam et al. 2008, Mohanta et al. 2008b).

Digestive enzymes

The ability of a fish to use a diet successfully in terms of growth and energy, probably depends in principle on its set of digestive enzymes and their response to the composition and digestibility of the nutrients in a given diet (Lemieux et al. 1999, Furné et al. 2005, Papoutsoglou & Lyndon 2006). Thus, an inducible effect on the activity of alkaline protease by the increase in dietary protein content has been documented in fish species, such as Asian sea bass (*Lates*

414 *calcarifer*) (Eusebio & Coloso 2002) and hybrid catfish (*Clarias batrachus* × *C. gariepinus*)
415 (Giri et al. 2003), as well as by increasing dietary lipids in European sea bass (*Dicentrarchus*
416 *labrax*) (García-Meilán et al. 2016). This could explain the higher alkaline protease activity
417 observed in fish fed the 50:12 diet, which contained the highest levels of protein and lipid in this
418 study

419 Trypsin and chymotrypsin are considered the main constituents of alkaline protease enzymes and
420 the most important enzymes in the process of protein digestion (Rungruangsak-Torrissen et al.
421 2006, Sunde et al. 2004). In the present study, trypsin and chymotrypsin activities were not
422 affected by dietary protein content, suggesting that *O. chrysurus* is well suited to protein
423 digestion regardless of its concentration in the diet, as reported by Lundstedt et al. (2004) for
424 pintado (*Pseudoplatystoma corruscans*). However, the activity of trypsin and chymotrypsin was
425 affected at the lipid level. For trypsin, the increase of dietary lipid resulted in a decrease in its
426 activity, as previously observed in *D. labrax* (García-Meilán et al. 2016). This fact could be due
427 to the action of cholecystokinin (CCK), a potent hormone that regulates pancreatic enzymes
428 secretion. Dietary lipid represent an important stimulus for the release of CCK in fish (Li et al.
429 2016, Murashita et al. 2008). Einarsson & Davies (1996) and Tillner et al. (2013) reported an
430 antagonistic effect of CCK on trypsin secretion in Atlantic salmon (*Salmo salar*) and larvae of
431 Atlantic cod (*Gadus morhua*), respectively. Therefore, it is probably that the decrease in trypsin
432 activity can be attributed to the effect of CCK due to increased lipids in the diet. This remains a
433 speculative statement. An improvement in digestion and utilization of protein cannot be
434 discarded due to trypsin increased in 6% lipid in the diet (Krogdahl et al. 1994). In contrast to
435 trypsin, chymotrypsin activity increased with dietary lipid, indicating that these enzymes were
436 affected in a different way by dietary lipids in *O. chrysurus*. One possible explanation for these
437 results is that the effect of CCK on chymotrypsin secretion could be opposite to observed for
438 trypsin (Wicker & Puigerver 1987). However, further research is needed to prove this fact. In
439 addition, the disproportionate behavior of trypsin and chymotrypsin activities with respect to
440 alkaline proteases suggests that other constitutive alkaline proteases were affected by dietary
441 lipid and protein contents.

442 Pancreatic bile salt-dependent lipase have a high substrate specificity for triacylglycerols
443 containing long chain polyunsaturated fatty acids, which are abundant in the marine food chain

(Gjellesvik et al. 1992). Therefore, this enzyme could be considered to principal lipase in marine fish (Kurtovic et al. 2009). Gómez-Requeni et al. (2013) found that in silverside (*Odontesthes bonariensis*) an adaptive response of bile salt-dependent lipase to fish oil inclusion in the diet. In this study, bile salt-dependent lipase activity trend to decreased with dietary lipid level increase; however, is interesting note to that fish fed the lowest energy diet showed a significantly high bile salt-dependent lipase activity and vice versa, suggesting that the digestive performance of this enzyme was primarily modulated by energy than by lipid content in the diet.

Liver enzymes

Although amino acids are the preferential energy substrate for fish (Jia et al. 2017), it has been shown that an increase of non-protein energy (lipid or carbohydrates) in the diet can reduce dietary amino acid catabolism in some fish (Fernández et al. 2007, Enes et al. 2008, Wang et al. 2018). In the present study, the amino acid catabolism enzyme ALT was not affected by the change in dietary lipid content, whereas an increase of dietary protein level resulted in an upregulation of ALT activity. These data suggest that the capacity of *O. chrysurus* to catabolize amino acids depends of protein availability instead gross energy from the diet, as was found in milkfish (*Chanos chanos*) (Coloso et al. 1988).

As for the gluconeogenesis pathway, there was not effect of dietary protein content on the activity of *O. chrysurus* hepatic FBPase. In contrast, FBPase activity increased with dietary lipid content. Kirchner et al. (2003) in rainbow trout (*Oncorhynchus mykiss*), hepatic gluconeogenesis (FBPase activity) did depress with dietary lipid content increase due an effectively oxidation of them. The increased of FBPase activity with dietary lipid level found in this study suggests that lipids were driven by *O. chrysurus* to gluconeogenesis pathway (Rito et al. 2019), as previously observed in largemouth bass (*Micropterus salmoides*) (Guo et al. 2019) and tropical gar (*Atractosteus tropicus*) (Guerrero-Zárate et al. 2019). These findings match with the higher feed efficiency (FI, PER and FCR) found for *O. chrysurus* fed high dietary lipid level.

Besides to key gluconeogenesis enzyme FBPase, the activity of the key enzyme of glycolysis PK of *O. chrysurus* was also nutritionally stimulated by dietary lipid increased; however, there was no consistent effect of increasing PK activity over unaffected FBPase activity when dietary protein level increased. Imbalances between glycolysis and gluconeogenesis pathway has been

also reported for numerous fish species (Coloso et al. 1988, Castillo et al. 2018, Guerrero-Zárate et al. 2019, Liu et al. 2020). The upregulation of PK shown in this study could be associated to glucose budget. Although that the ALT activity was higher for fish fed 50% dietary lipid, it was not reflected on FBPase performance, particularly at 50:6 diet. However, the high dietary protein dependence of *O. chrysurus* shown in this study (growth), it is possible suppose an active ATP production by oxidation of amino acids at 50% dietary protein level (Walton & Cowey 1982).

G6PDH and 6PGDH are key enzymes of the pentose phosphate pathway involved in the production of NADPH necessary for fatty acid synthesis (Tocher, 2003). In this study, G6PDH and 6PGDH activity did not change by dietary protein content, whereas an increase of dietary lipid content resulted in an activity enhancement for both enzymes. These data indicate that lipogenesis was stimulated by a high dietary lipid content, which also was reflected to high whole body lipid. Lipogenesis as well glycogen synthesis are adaptive mechanisms of homeostasis glucose (Enes et al. 2009). Therefore, the high stimulation of lipogenesis and hepatic glycogen content at 12% dietary lipids indicate that endogenous glucose synthesis rate was higher than its oxidation rate, as was described by Guo et al. (2019) for *M. salmoides*.

The result from the present study indicate that an increase in dietary lipid level from 6% to 12% enhanced liver gluconeogenesis, glycolytic and lipogenic pathways, but did not depress amino acid catabolism in juvenile *O. chrysurus*. The high dietary lipid level (12%) improved feed efficiency, but did not reduce protein demand in the diet in juvenile *O. chrysurus*.

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List of tables

Table 1. Formulation and proximate analysis of the experimental diets (% dry matter) (40-6: diet with 40% protein and 6% lipids; 40-12: diet with 40% protein and 12% lipids; 50:6: 50% protein and 6% lipids; 50-12: 50% protein and 12% lipids).

Ingredients (%)	40-6	40-12	50-6	50-12
Fish meal[1]	46.5	46.5	57.0	57.0
Poultry by product meal PRIME[1]	8.0	8.0	10.0	10.0
Soy protein concentrate[2]	12.0	12.0	15.0	15.0
Corn starch[3]	2.0	2.0	2.0	2.0
Fish oil[1]	1.35	7.2	0.09	6.0
Premix[4]	2.0	2.0	2.0	2.0
Filler (talc)[5]	22.1	16.3	7.91	2.0
Wheat bran[6]	5.0	5.0	5.0	5.0
Carboxymethylcellulose[c]	1.0	1.0	1.0	1.0
Proximate analysis (%)				
Dry matter	98.8	97.8	98.7	98.2
Crude protein	40.1	39.8	50.1	50.9
Lipid	6.7	12.2	6.1	12.7
Ash	35.1	29.3	21.1	16.3
Gross energy (kJ g^{-1})[7]	13.6	16.4	16.6	18.6
Protein: energy ratio (mg kJ^{-1})	29.4	24.3	30.1	27.3
Lipids: energy ratio (mg kJ^{-1})	4.9	7.4	3.7	6.8

[1]Proteínas Marinas y Agropecuarias S.A. de C.V. Jalisco, México.
[2]Vimifos, S.A. de C.V. Jalisco, México.
[3]MaltaCleyton S.A. de C.V. Yucatán, México.
[4]DMS Peces Tropicales de Engorda, Nutritional Products México S.A. de C. V. Composition: vitamin A, vitamin D3, vitamin E, vitamin K3, vitamin B1, vitamin B2, vitamin B12, folic acid, vitamin B6, pentothenic acid, niacin, biotin, choline, vitamin C, iron, manganese, sodium, copper, iodine, zinc, cobalt, selenium, molybdenum, calcium, phosphorus, magnesium, sodium, potassium, carophyll, lysine, threonine, calcium carbonate, methionine, tryptophan, sodium lasolacid, monensin, tylosin, salinomycin, avoparcin, flavomycin arsanilic, amprolium, zinc bacitracin, virginiamicin, calcium propinate, silicic acid, lignosulfanate, lecithin, BHT, BHA, probiotics, glutase keta, lipase, proteases, amylases, cellulases and acemita.

[5]Talc was used as filler (Dias et al. 1998), it is an inorganic substance almost insoluble powder in aqueous solution, the transfer across biological membranes is unlikely, this substance is not toxic to aquatic life (ECHA 2020).

[6]Wheat bran was used as fiber because it contains digestion-resistant starch (Xie et al. 2008) and also non digestible protein, due it a requirement for a pH = 12.5 for digestion (Balandrán-Quintana et al. 2015). Proximate composition: 14% protein, 4% lipid and 86% carbohydrate.

[7]Energy was calculated using a calorimetric bomb (PARR, Moline, IL, US).

Abbreviations: 40-6, diet with 40% protein and 6% lipids; 40-12, diet with 40% protein and 12% lipids; 50:6, 50% protein and 6% lipids; 50-12, 50% protein and 12% lipids

766 Table 2. Growth performance of yellowtail snapper fed diets containing different protein: lipid ratio. Values are shown as means ±
767 SEM (n = 3).

Diet name[3]	CP[1] (%)	CL (%)	FW (g)	WG (g)	SGR (%)	FI (g fish^{-1})	PER	FCR	S (%)
40:6	40	6	20.1 ± 0.1	6.1 ± 0.2	0.66 ± 0.0	20.8 ± 0.4	0.74 ± 0.1	3.41 ± 0.3	100± 0.0
40:12	40	12	19.5 ± 0.4	6.3 ± 0.6	0.77 ± 0.1	17.1 ± 1.1	0.92 ± 0.1	2.76 ± 0.2	96.6 ± 3.3
50:6	50	6	20.5 ± 1.3	6.9 ± 0.9	0.74 ± 0.1	22.2 ± 0.9	0.74 ± 0.2	3.26 ± 0.4	96.6 ± 3.3
50:12	50	12	23.4 ± 1.2	9.8 ± 1.1	0.96 ± 0.1	17.3 ± 1.4	1.13 ± 0.0	1.77 ± 0.1	96.6 ± 3.3
Means of main effects[2]									
	40		20.0 ± 0.2^{B}	6.2 ± 0.3^{B}	0.68 ± 0.0^{B}	18.9 ± 0.9	0.83 ± 0.1	3.10 ± 0.2	98.3 ±1.6
	50		21.9 ± 1.0^{A}	8.3 ± 0.9^{A}	0.85 ± 0.1^{A}	19.7 ± 1.3	0.88 ±0.2	2.52 ± 0.4	96.6 ± 2.1
		6	20.3 ± 0.6	6.5 ± 0.5	0.69 ± 0.0	21.5 ± 0.5^{Y}	0.68 ± 0.1^{Z}	3.33 ± 0.2^{Y}	98.3 ±1.6
		12	21.7 ± 0.9	8.0 ± 0.9	0.82 ± 0.1	17.2 ± 0.8^{Z}	1.03 ± 0.1^{Y}	2.67 ± 0.2^{Z}	96.6 ± 2.1
Two-way ANOVA[4]									
Dietary protein			*	*	*	NS	NS	NS	NS
Dietary lipid			NS	NS	NS	**	**	**	NS
Interaction			NS	NS	NS	NS	NS	NS	NS

768 [1] CP denotes crude protein and CL denotes crude lipid. The number bellowing the letters denotes the level (%) of protein and lipid
769 formulated for each test diet.

770 [2] Single capital letters indicate significant difference between main effect means (Dietary protein = A > B; Dietary lipid = Y > Z).

771 [3] Diet names represent the average values of three tanks per treatment.

772 [4] Significance terms: NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$.

773 Abbreviations: IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; S, survival; FI, food intake; PER,
774 protein efficiency ratio; FCR, feed conversion ratio

Table 3. Whole body composition (wet matter), liver glycogen and hepatosomatic index of yellowtail snapper fed diets containing different protein: lipids ratio. Values are shown as means ± SEM (n = 3).

Diet name[3]	CP[1] (%)	CL (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Liver glycogen (g/100g liver)	HSI
40:6	40	6	69.9 ± 0.3	18.3 ± 0.2	3.55 ± 0.0	5.04 ± 0.1	0.22 ± 0.02	1.59 ± 0.1
40:12	40	12	68.4 ± 0.3	18.0 ± 0.1	5.23 ± 0.2	5.14 ± 0.1	0.25 ± 0.02	1.55 ± 0.1
50:6	50	6	71.2 ± 0.2	16.7 ± 0.2	3.75 ± 0.2	4.89 ± 0.0	0.22 ± 0.01	1.34 ± 0.1
50:12	50	12	69.2 ± 0.2	16.8 ± 0.2	5.83 ± 0.1	4.95 ± 0.0	0.28 ± 0.01	1.52 ± 0.0
Means of main effects[2]								
	40		69.2 ± 0.4^{B}	18.2 ± 0.1^{A}	4.39 ± 0.3	5.09 ± 0.1^{A}	0.24 ± 0.01	1.57 ± 0.1
	50		70.2 ± 0.5^{A}	16.7 ± 0.1^{B}	4.79 ± 0.4	4.92 ± 0.0^{B}	0.25 ± 0.02	1.43 ± 0.1
		6	70.6 ± 0.3^{Y}	17.5 ± 0.4	3.65 ± 0.1^{Z}	4.97 ± 0.1^{Z}	0.22 ± 0.01^{Z}	1.46 ± 0.1
		12	68.8 ± 0.2^{Z}	17.4 ± 0.3	5.53 ± 0.1^{Y}	5.04 ± 0.2^{Y}	0.26 ± 0.01^{Y}	1.54 ± 0.1
Two-way ANOVA[4]								
Dietary protein			**	***	NS	**	NS	NS
Dietary lipid			***	NS	***	***	*	NS
Interaction			NS	NS	NS	NS	NS	NS

[1] CP denotes crude protein and CL denotes crude lipid. The number bellowing the letters denotes the level (%) of protein and lipid formulated for each test diet.

[2] Single capital letters indicate significant difference between main effect means (Dietary protein = A > B; Dietary lipid = Y > Z).

[3] Diet names represent the average values of three tanks per treatment.

[4] Significance terms: NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Abbreviations: HSI, hepatosomatic index.

Table 4. Specific activities (in U/mg of protein) of digestive enzymes in the pyloric caeca of yellowtail snapper fed diets containing different protein: lipids ratio. Values are shown as means ± SEM (n = 3).

Diet name[3]	CP[1] (%)	CL (%)	Alkaline proteases[5]	Trypsin	Chymotrypsin	Lipase
40:6	40	6	16.1 ± 0.6^{b}	14.9 ± 3.1	5.77 ± 0.2	6.90 ± 0.8^{a}
40:12	40	12	15.7 ± 1.1^{b}	3.28 ± 0.5	6.36 ± 0.5	1.68 ± 0.2^{bc}
50:6	50	6	17.4 ± 1.0^{b}	18.6 ± 2.4	5.06 ± 0.2	3.62 ± 0.2^{b}
50:12	50	12	24.2 ± 2.3^{a}	3.57 ± 0.2	8.19 ± 0.9	0.54 ± 0.0^{c}
Means of main effects[2]						
	40		$15.9 \pm 0.5^{B.}$	9.08 ± 2.9	6.07 ± 0.2	4.29 ± 1.2^{A}
	50		20.8 ± 1.9^{A}	11.1 ± 3.5	6.63 ± 0.8	2.08 ± 0.7^{B}
		6	16.7 ± 0.6	16.7 ± 1.9^{Y}	5.42 ± 0.2^{Z}	5.26 ± 0.8^{Y}
		12	20.0 ± 2.2	3.42 ± 0.3^{Z}	7.28 ± 0.6^{Y}	1.11 ± 0.3^{Z}
Two-way ANOVA[4]						
Dietary protein			**	NS	NS	***
Dietary lipid			NS	***	**	***
Interaction			*	NS	NS	*

[1] CP denotes crude protein and CL denotes crude lipid. The number bellowing the letters denotes the level (%) of protein and lipid formulated for each test diet.

[2] Single capital letters indicate significant difference between main effect means (Dietary protein = A > B; Dietary lipid = Y > Z). Single lower letters indicate significant difference between diets at $p < 0.05$ by Tukey´s test (a > b).

802 [3] Diet names represent the average values of three tanks per treatment.

803 [4] Significance terms: NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

804 [5] Alkaline proteases activities are expressed at 10^{-2} U mg protein^{-1}. Trypsin, chymotrypsin and lipase activities are expressed at 10^{-4} U

805 mg

806

Table 5. Specific activities (in U/mg of protein) of digestive enzymes in intestine of yellowtail snapper fed diets containing different protein: lipids ratio. Values are shown as means ± SEM (n = 3).

Diet name[3]	CP[1] (%)	CL (%)	Alkaline proteases[5]	Trypsin	Chymotrypsin	Lipase
40:6	40	6	17.6 ± 2.6^{ab}	18.3 ± 2.6	6.29 ± 0.7	8.16 ± 0.1^{a}
40:12	40	12	12.4 ± 0.7^{b}	2.79 ± 0.1	7.09 ± 0.5	1.95 ± 0.1^{c}
50:6	50	6	19.1 ± 0.4^{ab}	23.2 ± 1.6	5.57 ± 0.9	4.48 ± 0.1^{b}
50:12	50	12	29.5 ± 4.9^{a}	5.75 ± 1.7	11.02 ± 0.0	1.01 ± 0.2^{d}
Means of main effects[2]						
	40		14.9 ± 1.7^{B}	10.5 ± 3.6	7.09 ± 0.5	5.06 ± 1.4^{A}
	50		24.3 ± 3.2^{A}	14.5 ± 4.0	8.29 ± 0.0	2.74 ± 0.7^{B}
		6	18.4 ± 1.2	20.7 ± 1.8^{Y}	5.93 ± 0.5^{Z}	6.32 ± 0.8^{Y}
		12	20.9 ± 4.4	4.27 ± 1.0^{Z}	9.46 ± 0.9^{Y}	1.48 ± 0.2^{Z}
Two-way ANOVA[4]						
Dietary protein			*	NS	NS	***
Dietary lipid			NS	***	**	***
Interaction			*	NS	NS	***

[1] CP denotes crude protein and CL denotes crude lipid. The number bellowing the letters denotes the level (g kg^{-1}) of protein and lipid formulated for each test diet.

[2] Single capital letters indicate significant difference between main effect means (Dietary protein = A > B; Dietary lipid = Y > Z). Single lower letters indicate significant difference between diets at $p < 0.05$ by Tukey´s test (a > b).

[3] Diet names represent the average values of three tanks per treatment.

[4] Significance terms: NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

[5] Alkaline proteases activities are expressed at 10^{-2} U mg protein^{-1}. Trypsin, chymotrypsin and lipase activities are expressed at 10^{-4} U mg

Table 6. Specific activities (in U/mg of protein) of intermediary metabolic enzymes in liver of yellowtail snapper fed diets containing different protein: lipids ratio. Values are shown as means ± SEM (n = 3).

Diet name[3]	CP[1] (%)	CL (%)	PK	G6PDH	6PGDH	FBPase	ALT[5]
40:6	40	6	4.00 ± 0.2	22.4 ± 0.3	12.1 ± 0.4	6.34 ± 0.1	5.57 ± 0.2
40:12	40	12	10.9 ± 0.5	23.8 ± 0.4	13.9 ± 0.5	8.32 ± 0.5	5.54 ± 0.3
50:6	50	6	10.4 ± 0.9	22.1 ± 0.5	12.6 ± 0.7	6.41 ± 0.1	7.55 ± 0.1
50:12	50	12	14.9 ± 0.5	24.4 ± 1.4	16.1 ± 0.8	8.10 ± 0.4	8.05 ± 0.0
Means of main effects[2]							
	40		7.46 ± 1.5^{B}	23.1 ± 0.4	13.0 ± 0.5	7.34 ± 0.5	5.55 ± 0.2^{B}
	50		12.7 ± 1.1^{A}	23.2 ± 0.8	14.4 ± 0.9	7.26 ± 0.4	7.80 ± 0.1^{A}
		6	7.21 ± 1.5^{Z}	22.2 ± 0.3^{Z}	12.3 ± 0.4^{Z}	6.38 ± 0.4^{Z}	6.54 ± 0.5
		12	12.9 ± 0.9^{Y}	24.1 ± 0.6^{Y}	15.1 ± 0.6^{Y}	8.21 ± 0.5^{Y}	6.81 ± 0.6
Two-way ANOVA[4]							
Dietary protein			***	NS	NS	NS	***
Dietary lipid			***	*	*	***	NS
Interaction			NS	NS	NS	NS	NS

[1] CP denotes crude protein and CL denotes crude lipid. The number bellowing the letters denotes the level (g kg^{-1}) of protein and lipid formulated for each test diet.
[2] Single capital letters indicate significant difference between main effect means (Dietary protein = A > B; Dietary lipid = Y > Z).
[3] Diet names represent the average values of three tanks per treatment.
[4] Significance terms: NS = $P > 0.05$; * = $P < 0.05$; *** = $P < 0.001$

861 [5] ALT activities are expressed at 10^{-2} U mg protein^{-1}.

862 Abbreviations: PK, Pyruvate kinase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase;

863 FBPase, fructose-1, 6-biphosphatase; ALAT, alanine aminotransferase.

864

CAPÍTULO 4

Artículo 3

Sometido a: *Aquaculture Nutrition*

Physiological and metabolic protein-sparing effects of dietary lipids on common snook *Centropomus undecimalis* (Bloch, 1792) juveniles

Running title: Protein sparing effect of lipid in common snook

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Abstract

Carnivorous common snook (*Centropomus undecimalis*) has potential for aquaculture, but its protein requirement in diet is 500 g kg^{-1} and the energetic role of lipids in this species has not been explored. Thus, this study investigated the ability of juvenile common snook (8.7 ± 1.2 g) to use dietary lipids as an energy source. The fish were fed four practical diets with two protein levels (400 and 500 g kg^{-1}) associated with two lipid levels (60 and 120 g kg^{-1}) for 12 weeks. Growth performance significantly improved with increasing dietary lipid levels, but no significant difference was observed at the protein level. Fish fed the 400:60 (protein: lipid level) diet exhibited significantly higher feed conversion ratios than those fed 400:120, 500:60, and 500:120 diets, but fish fed the 400:120 diet had significantly higher protein efficiency ratios. Juvenile common snook showed the ability to modulate digestive protease and lipase secretion when dietary protein or lipid levels were modified. The activity of alanine aminotransferase decreased as the dietary lipid level increased ($p < 0.05$), while fructose-1, and 6-bisphosphatase activities were not significantly affected by dietary protein or lipid levels. Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and pyruvate kinase activities increased as dietary protein and lipid levels increased ($p < 0.05$). The results of this study indicate that an increase in dietary lipid level has a protein-sparing effect, and that the diet containing 400 g kg^{-1} protein with 120 g kg^{-1} lipid is suitable for growth and effective protein utilisation in common snook.

Resumen

El carnívoro róbalo común (*Centropomus undecimalis*) tiene potencial para la acuicultura, pero su requerimiento de proteínas en la dieta es de 500 g kg^{-1}, y el papel energético de los lípidos en esta especie aún no se ha explorado. Por lo tanto, este estudio investigó la capacidad de juveniles de róbalo común (8,7 ± 1,2 g) para utilizar los lípidos de la dieta como fuente de energía. Los peces fueron alimentados con cuatro dietas prácticas con dos niveles de proteína (400 y 500 g kg-1) asociados con dos niveles de lípidos (60 y 120 g kg-1) durante 12 semanas. El rendimiento del crecimiento mejoró significativamente con el aumento de los niveles de lípidos en la dieta, pero no se observaron diferencias significativas a nivel de proteínas. Los peces alimentados con la dieta 400: 60 (proteína: nivel de lípidos) exhibieron índices de conversión alimenticia significativamente más altos que los alimentados con las dietas 400: 120, 500: 60 y 500: 120, pero los peces alimentados con la dieta 400: 120 tuvieron índices de eficiencia proteica significativamente más altos. El róbalo común juvenil mostró la capacidad de modular la secreción de proteasa y lipasa digestiva cuando se modificaron los niveles de proteínas o lípidos de la dieta. La actividad de la alanina aminotransferasa disminuyó a medida que aumentaba el nivel de lípidos en la dieta ($p < 0,05$), mientras que las actividades de fructosa-1 y 6-bisfosfatasa no se vieron afectadas significativamente por los niveles de proteínas o lípidos en la dieta. Las actividades de glucosa-6-fosfato deshidrogenasa, 6-fosfogluconato deshidrogenasa y piruvato quinasa aumentaron a medida que aumentaban los niveles de proteínas y lípidos de la dieta ($p < 0,05$). Los resultados de este estudio indican que un aumento en el nivel de lípidos en la dieta tiene un efecto ahorrador de proteínas, y que la dieta que contiene 400 g kg^{-1} de proteína con 120 g kg^{-1} de lípido es adecuada para el crecimiento y la utilización efectiva de proteínas en el róbalo común.

Sisal, Yucatán, February 14th 2021

Dra. Gabriela Gaxiola
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Dear Dr Gaxiola:

It is a pleasure to accept your manuscript entitled **"Physiological and metabolic protein-sparing effects of dietary lipids on common snook Centropomus undecimalis (Bloch, 1792) juveniles"** in its current form for publication in Aquaculture Nutrition.

Thank you for your fine contribution. On behalf of the Editors of Aquaculture Nutrition, we look forward to your continued contributions to the Journal.

DOI: 10.1111/anu.13250 (Received in February 17th 2021).

Sincerely,

Professor Zhen-Yu Du

Editor, Aquaculture Nutrition

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947 Physiological and metabolic protein-sparing effects of dietary lipids on common snook
948 *Centropomus undecimalis* (Bloch, 1792) juveniles

949

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962

963 **Abstract**

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when dietary protein or lipid levels were modified. The activity of alanine aminotransferase decreased as the dietary lipid level increased ($p < 0.05$), while fructose-1, and 6-bisphosphatase activities were not significantly affected by dietary protein or lipid levels. Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and pyruvate kinase activities increased as dietary protein and lipid levels increased ($p < 0.05$). The results of this study indicate that an increase in dietary lipid level has a protein-sparing effect, and that the diet containing 400 g kg^{-1} protein with 120 g kg^{-1} lipid is suitable for growth and effective protein utilisation in common snook.

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Keywords: *Centropomus undecimalis*, dietary protein, dietary lipid, digestive enzymes, metabolic enzymes, protein-sparing effect

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Introduction

Proteins are usually the main constituent of fish feeds and the most expensive dietary component (Oliva-Teles, Couto, Enes, & Peres, 2020). In fish, proteins contribute not only to growth (protein synthesis) but also energy production (amino acid catabolism) (Jia, Li, Zheng, & Wu, 2017). The use of dietary protein as a source of energy by fish is considered undesirable because of its high cost. Additionally, ammonia release, associated with amino acid catabolism, negatively impacts the environment because nitrogen is one of the main nutrients responsible for water eutrophication (Cowey, 1995). Catabolism of dietary amino acids by fish is reduced when other energy-yielding nutrients, such as lipids and carbohydrates, are found in adequate amounts in the diet (Guerrero-Zárate et al., 2019; Sagada et al., 2017; Welengane, Sado, & Bicudo, 2019). However, excessive energy can lead to the deposition of excess lipids in the flesh, reducing feed intake (Lee, Jeon, & Lee, 2002; Peres & Oliva-Teles, 1999; Tuan & Williams, 2007). Conversely, a low non-protein energy content in the diet may reduce fish growth due to the use of protein as a source of dietary energy (Catacutan, Pagador, & Teshima, 2001; Kim, Lim, Kang, Kim, & Son, 2012; Kim, Wang, Choi, Park, & Bai, 2004). Therefore, protein and non-protein energy in the diet should be kept in balance. As nutrients, lipids are an important energy source in fish, especially carnivorous species, where carbohydrates play little role as energy. The

common snook (*Centropomus undecimalis*) has been described as a carnivorous species (Concha-Frías et al., 2016; Jimenez-Martinez et al., 2012; Machado et al., 2013) that is a high-market-value species with high aquaculture potential in the tropical and subtropical east of the American continent (Alvarez-Lajonchère & Ibarra-Castro, 2013; Caballero-Chávez, 2012; Tucker, Matthew, Landau, & Faulkner, 1985). This specie is one of the largest body size members of the genus *Centropomus* (Tringali, Bert, Seyoum, Bermingham, & Bartolacci, 1999). Common snooks have the advantages of fast growth (Tucker, 1987), low feed conversion ratio (Alvarez-Lajonchère & Ibarra-Castro, 2013), and they mature and spawn in captivity (Ibarra-Castro et al., 2011). Regarding the nutritional requirements, previous reports have revealed that common snook has a protein requirement in the diet of 500 g kg^{-1} (Concha-Frías et al., 2018; Gracia-López, García-Galano, Gaxiola-Córtes, & Pacheco-Campos, 2003; Tucker, 1987). Additionally, in a recent study, Lemus et al. (2018) proposed a selection of marine and terrestrial ingredients as an alternative source to fishmeal protein for common snook feeds. To date, no studies have been performed on the ability of common snooks to utilise lipids as energy sources. Therefore, the purpose of this study was to evaluate the effect of different dietary protein and lipid levels on common snook growth performance, feed utilisation, and digestive and hepatic enzyme activities.

Methodology

Diets

Four experimental diets were formulated to contain either 400 or 500 g kg^{-1} crude protein combined with either 60 or 120 g kg^{-1} crude lipid (Table 1). The ingredients were sieved through a 250-µm mesh and then mixed in a blender for 30 min. The dough was extruded and broken down to pieces 3-mm in particle diameter using a Bonnot-Model 2EXT-W/PKR (Bonnot Company, Akron, Ohio, USA) extruder. Next, the dough was dried via ventilation at 60°C for 3 h, and the feed was sealed in vacuum-packed bags and stored at -20°C until use.

Experimental procedures

Common snook juveniles were obtained from the Marine Fish Reproduction Laboratory at Unidad Multidisciplinaria de Docencia e Investigación de Sisal, Yucatán, México, following the methodology described by Ibarra-Castro et al. (2011). Trials were performed in a

thermoregulated recirculating water system (Resun CW 1000 chillers, Guandong Risheng. Co., Ltd., ROC) operated with 12 rectangular 100-L fibreglass tanks (73 cm × 44 cm × 33 cm). Eleven fish were selected and randomly placed in each tank (mean ± standard deviation [SD] = 8.7 ± 1.2 g initial wet weight [ww], and 10.8 ± 05 cm total length). Three replicate groups of fish were used to test each randomly assigned diet. Tanks were supplied with continuous air and seawater flow (5.0 L min^{-1}). During the experimental period, water parameters were 28.4 ± 0.4°C temperature, 36 ± 0.9 g L^{-1} salinity, 8.2 ± 0.1 pH, 0.01 ± 0.0 mg L^{-1} ammonia concentration, and 4.2 ± 0.3 mg L^{-1} dissolved oxygen. Before the experiment, the fish were acclimated to the experimental diet for two weeks and fed twice a day (10:00 and 18:00 h) at 40 g kg^{-1} of their total biomass (García-Galano et al., 2003). The feed ration was adjusted every 15 days in relation to the increase in fish weight. Uneaten feed was collected half an hour after each feeding and then dried (AOAC, 1997).

Sample collection

The growth trial lasted 12 weeks. Three fish were randomly sampled from each tank 6 h after the morning meal. The fish were anesthetised with 0.1 mg mL^{-1} clove oil according to Castillo, Alvarez, Cuzon, Suárez, & Gaxiola (2018); then, the fish were killed humanely according to the recommendations of the Helsinki Convention and the Official Mexican Norm NOM-062-ZOO-1999 (SAGARPA, 1999). Immediately, the pyloric caeca, intestine, liver, and mesenteric fat were sampled. Both liver and mesenteric fat were used to determine the hepatosomatic index (HIS). The pyloric caeca, intestine, and liver were frozen in liquid nitrogen and stored at -80°C. Three fish per tank were sampled and pooled to assess the whole-body proximate composition.

Growth performance

At the end of the experiment, survival rate (S), wet weight (ww) weight gain (WG), specific growth rate (SGR), feed intake (FI), protein intake (PI), lipid intake (LI), energy intake (EI), protein efficiency ratio (PER), feed conversion ratio (FCR), condition factor (CF), and HSI were calculated as follows:

$$S = (final\ number\ of\ fish / initial\ number\ of\ fish) \times 100;$$

$$WG = ww\ final\ fish\ weight - ww\ initial\ fish\ weight;$$

$$SGR = [\ln(ww\ final\ fish\ weight - ww\ initial\ fish\ weight)/days\ of\ culture\ ] \times 100$$

$$FI = food\ intake\ per\ tank / number\ of\ fish\ per\ tank$$

$$PI = FI \times percent\ of\ protein\ per\ diet$$

$$LI = FI \times percentage\ of\ lipid\ per\ diet;$$

$$EI = (FI \times energy\ content\ per\ diet)/days;$$

$$PER = WG\ /\ FI;$$

$$FCR = dray\ FI\ /\ GW;$$

$$CF = (ww\ body\ weight \times 100)/body\ length^3.$$

$$HSI = (ww\ liver\ weight \times 100\ )/ww\ weight\ of\ fish.$$

Proximate analysis

Crude protein content was determined by flash combustion; then, N_2 was quantified by thermal conductivity detection using a CHNS elemental analyser (Costech ECS-4010, Valencia, USA); N_2 was converted to protein using a conversion factor of 6.38 (Lynch & Barbano, 1999). Crude lipid levels were extracted with hexane using a Goldfish system (Nielsen & Carpenter, 2017). The moisture content was determined after the sample was oven-dried and brought to a constant dry weight (60°C for 120 h). Ash content was determined by combustion of the sample in a muffle furnace at 550°C for 6 h (AOAC, 1997).

Digestive enzyme activity

The pyloric caeca and intestine were homogenised with an Ultra Turrax IKA T18 homogeniser (North Chase, Wilmington, U.S.A.) in 30 mmol/L Tris-HCl (1186-53-1-Fisher Scientific, Hampton, NH, USA) + 12.5 mmol/L $CaCl_2$ (223506-Sigma-Aldrich, St. Louis MO, USA) at pH 7.5 and centrifuged at 1670 g at 4°C for 30 min (5420R-Eppendorf, DE, USA). Total alkaline protease activities were evaluated using casein (0.05%) (9000-71-9-Fisher Scientific, Hampton, NH, USA) in 100 mmol/L Tris-HCl and 10 mmol/L $CaCl_2$ at pH 9 with the method reported by Kunitz (1947) and modified by Walter (1994). One unit of enzyme activity was defined as 1 mmol/L of tyrosine released per min using a molar extinction coefficient of 1280 at 280 nm. Trypsin activity was assayed using N-a-benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA, B4875-Sigma-Aldrich, St. Louis, MO, USA) 3.5 mmol/l as substrate in 100 mmol/l

Tris (T1503-Sigma-Aldrich) at pH 8 (Erlanger, Kokowsky, & Cohen, 1961). Chymotrypsin was evaluated using N-succinyl- ala-ala-pro-phe p-nitroanilide (SAAPNA, S7388-Sigma-Aldrich, St. Louis, MO, USA) 1 mmol/l as substrate in 100 mmol/l Tris at pH 8 (DelMar, Largman, Brodrick, & Geokas, 1979). One unit of enzyme was defined as 0.001 mM of p-nitroanilide released per min using a molar extinction coefficient of 8800 at 410 nm. Lipase activity was measured with 4-nitrophenyl octanoate (21742-Sigma-Aldrich) (1 mmol/L) in 500 mmol/L Tris and 6 mmol/L sodium taurocholate (86339; Sigma-Aldrich, St. Louis, MO, USA) at pH 7.4 (Gjellesvik, Lombardo, & Walther, 1992). One unit of lipase activity was defined as 0.001 mmol/L of nitrophenol hydrolysed per min using a molar extinction coefficient of 16300 at 415 nm.

Hepatic metabolic enzyme activities, glycogen and triglycerides content

The livers were homogenised (dilution 1:5) in ice-cold buffer (50 mmol/L Tris-HCl, 0.5 mmol/L phenylmethyl sulfonyl fluoride (PMSF, P7612-Sigma-Aldrich, St. Louis, MO, USA), 4 mmol/L ethylenediaminetetraacetic acid (EDTA, E9884-Sigma-Aldrich), 50 mmol/L sodium fluoride (NaF, 215309- Sigma-Aldrich, 250 mmol/L sucrose, and 500 mmol/L dithiothreitol (DTT, 43819-Sigma-Aldrich) at pH 7.5) and centrifuged at 1670 g at 4°C for 30 min. The resulting supernatant was separated to assess PK (pyruvate kinase), FBPase (fructose-1, and 6-bisphosphatase), G6PDH (glucose-6-phosphate dehydrogenase), 6PGDH (6-phosphogluconate dehydrogenase) and ALT (alanine aminotransferase) activities. PK (EC 2.7.1.40) activity was evaluated in a 0.25 mL reaction containing 250 mmol/L glycyl-glycine (G1002-Sigma-Aldrich), pH 7.4, 10 mmol/L $MgCl_2$ (M2676-Sigma-Aldrich), 100 mmol/L KCl (P3911-Sigma-Aldrich), 0.15 mmol/L NADH (N8129-Sigma-Aldrich), 2.8 mmol/L phosphoenolpyruvate (P7127-Sigma-Aldrich), and 21 U/mL lactate dehydrogenase (L2500-Sigma-Aldrich). ADP (A5285-Sigma-Aldrich) was added at the end of the experiment (Bonamusa, Garcia de Frutis, Fernandez, & Baanante, 1992). FBPase (EC 3.1.3.11) activity was evaluated in a 0.2 mL reaction containing 85 mmol/L imidazole-HCl (I2399-Sigma-Aldrich), pH 7.7, 5 mmol/L $MgCl_2$, 0.5 mmol/L NADP (93205-Sigma-Aldrich), 12 mmol/L 2-mercaptoethanol (M3148- Sigma-Aldrich), 0.05 mmol/L fructose-1,6-diphosphate (47810-Sigma-Aldrich), 25 U/mL phosphoglucose isomerase (F2668-Sigma-Aldrich), and 0.48 U/mL glucose-6-phosphate dehydrogenase (G7877-Sigma-Aldrich).

G6PDH (EC 1.1.1.43) was evaluated in a 0.2 mL reaction containing 77 mmol/L imidazole HCl, pH 7.7, 5 mmol/L $MgCl_2$, 1 mmol/L NADP, and 10 mmol/L glucose 6-phosphate (G7879-Sigma-Aldrich). 6PGDH (EC 1.1.1.44) activity was evaluated in a 0.2 mL reaction containing 83 mmol/L imidazole HCl, pH 7.7, 3 mmol/L $MgCl_2$, 0.5 mmol/L NADP, and 2 mmol/L gluconate 6-phosphate (P7877-Sigma-Aldrich) (Bonamusa et al., 1992). ALT (EC 2.6.1.2) was measured using a linear chemical assay kit (ALT/GPT BR opt., Barcelona, ES).

Liver enzyme assays were monitored spectrophotometrically by observing changes in the absorbance of NADH/NAD and NADP/NADPH per min at 340 nm for 10 min (Borrebaek, Waagbø, Christophersen, Tranulis, & Hemre, 1993; Tranulis, Dregni, Christophersen, Krogdahl, & Borrebaek, 1996). All enzyme activities were measured at 37°C (Bonamusa et al., 1992; Concha-Frías et al., 2018; Gjellesvik, Lombardo, & Walther, 1992). Digestive and metabolic enzyme activities were reported as mg of soluble protein (specific activity). Protein concentration was determined according to Bradford (1976) using a Sigma-Aldrich protein assay kit with bovine serum albumin as a standard.

Liver glycogen content was determined using the method reported by Carroll, Longley, & Roe (1956). Triglycerides were analysed by the enzymatic colorimetric method using a commercial kit (ELITechGroup, Paris, France).

Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). Growth assessment of the common snook was performed with a linear mixed model, as shown below in Eq. (1).

$$Weight_{ij} = \alpha_i + Time_{ij} + Time_i^2 + Time_{ij} \times Protein_i \times Lipid_i + e_{ij}$$

where j is the tank corresponding to weight i, and $tank_j$ is the random slope, which is assumed to be normally distributed with mean 0 and variance σ^2. The data exploration and model analysis were carried out following the protocol described by Zuur, Ieno, Walker, Saveliev, & Smith (2005) and Zuur, Ieno, Walker, Savelieve, & Smith (2009), respectively. The package nlme (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2019) in R software (Team, 2019) was used to fit the models. Akaike information criterion (AIC) and Bayesian information criterion (BIC) values and likelihood ratio (L. ratio) tests were used to compare the difference between the log likelihoods

of the models and fitted by restricted maximum likelihood. A random slope was included for the tank (AIC = 302.84, L. ratio = 113.24; $p < 0.001$) and structure variance ($\varepsilon_{ij} \sim \sigma^2[\text{Time}_{ij}]^{2\delta}$) determined a parameter constant $\delta = 1.97$, in which this variability value increased in weight over time (AIC = 275.9; L. ratio = 28.94; $p < 0.001$). The correlation structure was determined ($h[k,\emptyset]=\emptyset^k$) where k is the absolute difference in time units between weight measurements, e.g. time lag and $\emptyset$ represents the correlation between two weight measurements separated by two weeks estimated at 0.83 (AIC = 251.40; L. ratio = 26.50; $p < 0.001$). Model assumptions were validated by visual inspection of residual plots versus fitted values and the residuals for temporal dependency. The package nlme (Pinheiro et al., 2019) in R software (Team, 2019) was used to fit the models.

The rest of the analysis was performed with a two-way ANOVA test ($p < 0.05$); when protein and lipid interactions were significant, a Tukey´s test was applied. Prior to these analyses, normality (Shapiro-Wilk) and homogeneity (Bartlett) residual tests were verified. The analyses were performed using R statistical software.

Results

At the end of the trial, the survival rate of every experimental group was 100%. Growth rate, FW, WG, and SGR were significantly and positively affected by increased lipid levels in the diet (Figure 1, Table 2, and Table 3), but no significant effect was observed by dietary protein level.

Table 4 shows FI, PI, LI, EI, PER, and FCR results. FI and LI were significantly and positively affected by increased lipid levels in the diet, but no significant effect was observed due to dietary protein level. PI increased as dietary protein and lipid levels ($p < 0.05$) increased. The interaction between dietary protein and lipid levels was significant for EI, PER, and FCR. Fish fed with the 500:120 (protein:lipid level) diet showed a significantly higher EI value and those fed with the 400:600 diet showed the lowest EI value. The PER value was significantly higher in fish fed with the diet 400:120, whereas those fish fed with the 400:60 diet showed the significantly highest value of FCR.

Whole body composition and condition indices of fish

Final whole-body composition and condition indices are shown in Tables 5 and 6. The interaction between dietary protein and lipid levels was significant for fish whole-body moisture, protein, and ash. In the 400:60 diet, whole-body moisture was significantly lower than the rest of dietary treatments, but it showed a significantly higher whole-body protein value. Whole-body ash of the fish fed with the 400:120 diet showed a significantly lower value than the other diets; no significant differences were found among the rest of the experimental diets. Liver glycogen and CF were not significantly affected by the experimental diets. HSI and triglycerides were significantly and positively affected by increasing levels of dietary lipids, however no significant effect was observed due to dietary protein level and the interaction between dietary protein and lipid levels.

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Digestive enzymes

Digestive enzyme activities of the pyloric caeca and intestine are shown in Tables 7 and 8. Alkaline protease activity was not affected by diet in the pyloric caeca ($p > 0.05$). However, in the intestine, alkaline protease activity was significantly and positively affected by increasing levels of dietary protein, however an opposite effect on the activity of this enzyme was observed when dietary lipid level increased from 60 to 120 g kg^{-1}. The interaction between dietary protein and lipid levels was significant for trypsin, chymotrypsin, and lipase activities in both the pyloric caeca and intestine. Diet 400:120 showed significantly higher trypsin and lipase activities, whereas the diets of 500:60 and 500:120 showed the lowest activities for these enzymes ($p < 0.05$). Chymotrypsin activity was significantly higher in the 400:60 diet than in the rest of the experimental diets; however, no significant differences were observed in the activity of chymotrypsin among the rest of the diets.

The activities of key liver enzymes are shown in Table 9. FBPase activity was not affected by the experimental diets ($p > 0.05$). ALT activity was significantly and negatively affected by

increasing levels of dietary lipids from 600 to 1200 g kg^{-1}. No significant effect was found on the dietary protein level and the interaction term on ALT activity. The activities of PK, G6PDH, and 6PGDH enzymes were significantly and positively affected by increasing both dietary protein and lipid levels.

Discussion

To gain a better understanding of the ability of the common snook to use lipids as an energy source, this study measured changes in growth performance, feeding efficiency, and activities of digestive protease and lipase enzymes, as well as hepatic enzymes including glycolysis, lipogenesis, amino acid catabolism, and gluconeogenic enzymes. This result was achieved through four experimental diets with two graded protein (400 and 500 g kg^{-1}) and lipid (60 and 120 g kg^{-1}) levels each, which contained a minimal level of digestible carbohydrates (20 g kg^{-1} corn starch).

In this study, increasing dietary lipid content from 60 to 120 g kg^{-1} improved the growth performance of the common snook, which indicated that the increasing dietary energy level by lipids provided a more efficient utilisation of dietary protein for the growth of the common snook. The protein-sparing effects of lipids have been reported in several fish species, such as catfish (*Pseudobagrus fulvidraco*) (Kim & Lee, 2005), hybrid tambatinga (♀*Colossoma macropomum* × ♂*Piaractus brachypomus*) (Welengane et al., 2019), rockfish (*Sebastes schlegeli*) (Lee et al., 2002), and snakehead fish (*Channa argus*) (Sagada et al., 2017). Furthermore, in this study, a decrease in protein level in common snook from 500 to 400 g kg^{-1} at 120 g kg^{-1} lipid level in the diet did not affect growth performance and FCR, but improved PER, which indicated a more efficient dietary protein utilisation for growth. These data suggest that a dietary protein level of 400 g kg^{-1} with 120 g kg^{-1} lipid could meet the needs for optimal growth of the common snook. However, Concha-Frías et al. (2018) previously observed relatively lower growth (based on SGR) in common snook (3.15 g initial weight) fed 400 g kg^{-1} protein with 151 g kg^{-1} lipid and 300 g kg^{-1} wheat and corn meal, compared to 500 g kg^{-1} protein with 154 g kg^{-1} lipid and 140 g kg^{-1} wheat and corn meal. The differences between studies could have been attributed to those in dietary formulation, particularly digestible carbohydrate inclusion as a dietary non-protein energy source, which were supplied in a minimal percentage in the test diets of this study (20 g kg^{-1} corn starch). In general, the dietary carbohydrate level recommended for

carnivorous fish is ≤ 200 g kg^{-1} (Wilson, 1994), and if it exceeds fish tolerance, their growth may decrease (Ma et al., 2019; Wang et al., 2016).

On the other hand, common snook has shown the capability of adjusting to voluntary feed intake to meet the needs for maximal growth (García-Galano, Pérez, Gaxiola, & Sánchez, 2003), as previously reported in other fish species, such as European sea bass (*Dicentrarchus labrax*) (Boujard et al., 2004), rainbow trout (*Oncorhynchus mykiss*) (Boujard et al., 2004), and seabream (*Sparus aurata*) (Santinha, Medale, Corraze, & Gomes, 1999). This result could explain the significantly higher FI observed as dietary lipid level increased.

An increase in lipid and protein levels in the diet in common snook induced lipid deposition in the whole body. These trends seem to be closely related to dietary energy content. The positive relationship between dietary energy content and whole-body lipid deposition has also been demonstrated in other fish species, such as the malabar grouper (*Epinephelus malabaricus*) (Tuan & Williams, 2007), olive flounder (*Paralichthys olivaceus*) (Kim et al., 2004), and rockfish (*Sebastes schlegeli*) (Lee et al., 2002). Meanwhile, moisture, protein, and ash in the whole body were affected by the interaction of dietary protein and lipid levels; however, no clear tendency was observed.

HSI and CF are indicators used to assess the nutritional status of fish. In this study, HSI increased by 12% with an increase in dietary lipid levels from 60 to 120 g kg^{-1}. The changes in the HSI reflect alterations in glycogen and lipid deposition in fish liver (Guerrero-Zárate et al., 2019; Guo et al., 2019). However, data in this study showed that liver glycogen deposition was not affected by protein or lipid dietary levels, but liver triglycerides increased with dietary lipid content, indicating that the HSI was affected by lipid deposition, as reported in Manchurian trout (*Brachymystax lenok*) (Chang, Niu, Jia, Li, & Xu, 2018), meagre (*Argyrosomus regius*) (Chatzifotis et al., 2010), and turbot (*Psetta maxima*) (Regost et al., 2001) after consuming lipid-rich diets for a long time. On the other hand, several studies have shown that there is a strong linkage between CF and whole-body lipid content in fish (Lambert & Dutil, 1997; Li, Liu, Jiang, Zhu, & Ge, 2010). CF was not significantly affected by either dietary protein or lipid level in this study, indicating that the increased whole-body lipid content of common snook fed high dietary protein and lipid levels did not alter the body mass with respect to length.

The digestive response of a fish to nutrient composition in the diet reflects its digestive capacity and probably determines the effectiveness of a given diet in optimising fish growth (Papoutsoglou & Lyndon, 2006; Tsuzuki, Sugai, Maciel, Francisco, & Cerqueira, 2007; Woo & Kelly, 1995). In this study, alkaline protease activity was unresponsive to diet in the pyloric caeca, while in the intestine it decreased with increased lipids and decreased protein levels in the diet. This heterogeneous distribution of alkaline protease activity along the digestive section has also been reported in other species of fish (Almeida, Lundstedt, & Moraes, 2006; Rodiles et al., 2012). Digestive variation of alkaline protease might have been associated with the specific function of digestive structure (Pérez-Jiménez et al., 2009). Although no data were available on the distribution of pancreatic tissue in the common snook or any other member of the genus *Centropomus*, Borges et al. (2010) reported that in the fat snook (*Centropomus parallelus*), protein absorption occurs only in the rectum. Machado et al. (2013) found no histological and histochemical differences in the digestive tract between both common and fat snooks. In this sense, the lack of differences in alkaline protease activity in the pyloric caeca lead to the assumption that this organ is well suited for protein digestion regardless of its concentration in the diet. In contrast, a decrease in alkaline protease activity in the intestine could be a response to an absorption process of proteases by intestinal cells in the posterior region rather than digestive adaptation of this enzyme to dietary composition (García-Meilán et al., 2016; Sire & Vernier, 1992).

Trypsin and chymotrypsin are considered the major constituents of alkaline proteases because of their key role in the protein digestion process (Rungruangsak-Torrissen, Moss, Andresen, Berg, & Waagbø, 2006). In this study, trypsin and chymotrypsin activities showed a homogeneous distribution along the pyloric caeca to the intestine, suggesting an adaptative digestive response of these enzymes to dietary composition (Almeida et al., 2006; Lundstedt, Melo, & Moraes, 2004). Trypsin and chymotrypsin activities increased when dietary protein level decreased from 500 to 400 g kg^{-1}, however, a diet with 120 g kg^{-1} lipid induced higher activity of trypsin, whereas 60 g kg^{-1} lipid in diet induced higher chymotrypsin activity. Considering the absence of significant differences in common snook growth at the protein level, lower protein content may have triggered an increase in trypsin and chymotrypsin activities to digest and utilise the available protein more effectively (El-Saidy, Dabrowski, & Bai, 2000; Krogdahl, Lea, & Olli, 1994; Olli, Hjelmeland, & Krogdahl, 1994; Rodiles et al., 2012). On the other hand, a

disproportional increase in trypsin and chymotrypsin activities at 400 g kg^{-1}, protein with respect to lipid content in diet, suggests that lipids play a regulatory effect on the secretion and activation mechanisms of these enzymes, as well as the protein digestion process when protein content is limited to the diet (Murashita, Fukada, Rønnestad, Kurokawa, & Masumoto, 2008), which requires further investigation.

Usually, carnivorous fish consume fat-rich food; thus, the presence of lipase in their digestive tract is justified (Chakrabarti, Gani, Chaki, Sur, & Misra, 1995). Lipase adaptation to high-fat diets has been observed in some carnivorous fish, such as the orange-spotted grouper (*E. coioides*) (Liu et al., 2020), sea bass (García-Meilán et al., 2016), and snakehead fish (Sagada et al., 2017). In contrast, Guerrero-Zárate et al. (2019) found no adaptation of lipase in tropical gar fed with a graded level of dietary lipids (62.4–183 g kg^{-1} diet). In this study, a linear relationship between lipase activity and lipid level was found at 400 g kg^{-1} protein level, but at 500 g kg^{-1} protein level, lipase activity was unresponsive to lipid content. These data suggest that lipid utilisation to the digestive level by common snook is determined by the protein content in the diet.

Hepatic ALT is one of the major degrading enzymes in amino acid catabolism (Walton & Cowey, 1982). In this study, hepatic ALT activity decreased as dietary lipid increased, confirming the protein-sparing effect of lipids. Similar results were also observed in yellowtail (*Seriola quinqueradiata*) (Shimeno, Hosokawa, & Takeda, 1996) and rainbow trout (Kirchner, Kaushik, & Panserat, 2003). On the contrary, the activity of hepatic ALT was not affected by the dietary protein level, indicating that protein catabolism was the same irrespective of the dietary protein level. This observation is not in line with data reported by other authors, who described a positive relationship between amino acid degradation by hepatic ALT and dietary protein intake (Fernández et al., 2007; Gaye-Siessegger, Focken, & Becker, 2006; Wang et al., 2018). The unresponsiveness of the hepatic ALT enzyme to the different protein levels in this study may have been related to the fact that protease trypsin and chymotrypsin activities were enhanced by lower dietary protein levels.

Regarding gluconeogenesis, the results of this study showed that the activity of the hepatic gluconeogenic enzyme FBPase was not affected by dietary protein or lipid levels. Changes in

gluconeogenic potential have been used to emphasise the role of dietary proteins or lipids as gluconeogenic substrates in fish (Cowey, Cooke, Matty, & Adron, 1981). For example, hepatic FBPase enzyme activity has been reported to correlate positively with dietary lipid level in largemouth bass (*Micropterus salmoides*) (Guo et al., 2019) and in tropical gar (Guerrero-Zárate et al., 2019). In addition, Pérez-Jiménez, Guedes, Morales, & Oliva-Teles (2007) and Kirchner et al. (2003) found a linear relationship between FBPase enzyme activity and dietary protein level in European sea bass and rainbow trout, respectively. The unresponsive activity of hepatic FBPase found in this study suggested that (1) common snook can control glucose synthesis regardless of dietary protein or lipid content; and (2) in contrast to decreased amino acid catabolism (hepatic ALT) with lipid level, the possibility of an additive effect of lipids on the gluconeogenesis pathway cannot be excluded.

In this study, experimental diets were formulated to contain a minimal level of digestible carbohydrates (20 g kg^{-1} corn starch). Interestingly, the PK and pentose phosphate pathways (G6PDH and PGDH) increased as dietary protein and lipid levels were increased. No regulatory factor in FBPase activity was detected among the dietary treatments (Table 5). Walton and Cowey (1982) pointed out that the role of liver glycolysis in fish is most likely to supply precursors for the biosynthetic process rather than pyruvate for oxidation, because the oxidation of amino acids is the main energy production route with some contribution from β-oxidation of fatty acids. In this sense, our results suggest that the snook fed high dietary protein and lipid levels utilised glucose for the generation of metabolic precursors through glycolysis (pyruvate), as well as the pentose phosphate pathway with specific molecules such as reduced NADPH necessary for the synthesis of unsaturated fatty acids and ribose 5-phosphate for the synthesis of nucleotides. In addition, Qin et al. (2020) concluded that the enhancement of G6PDH activity and the accumulation of NADPH play an essential role in protecting cells from oxidative stress. This scenario is consistent with the higher growth and whole-body lipid content observed in these treatments.

In conclusion, this study indicates that an increase in dietary lipids led to a protein sparing effect in common snook. Increasing lipid levels from 60 to 120 g kg^{-1} improved fish growth, however it led to the deposition of excess lipids in the whole body. Even though dietary protein requirements of common snook have been established as 500 g kg^{-1} in diet, the results in this

study regarding growth and feed efficiency were not negatively affected by a decrease in dietary protein from 500 to 400 g kg^{-1} at 120 g kg^{-1} lipid level with 20 g kg^{-1} corn starch and thus this diet may be suitable for effective protein utilisation in the common snook.

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Data availability statement

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

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List of tables

Table 1. Formulation and proximate analysis of the experimental diets (% dry matter): 400:60 (400 g kg^{-1} protein and 60 g kg^{-1} lipid); 400:120 (400 g kg^{-1} protein and 120 g kg^{-1} lipid); 500:60 (500 g kg^{-1} protein and 60 g kg^{-1} lipid); and 500:120 (500 g kg^{-1} protein and 120 g kg^{-1} lipid).

Ingredients (g kg^{-1})	400:60	400:120	500:60	500:120
Fish meal[a]	465	465	570	570
Poultry by product meal PRIME[a]	80	80	100	100
Soy protein concentrate[b]	120	120	150	150
Corn starch[c]	20	20	20	20
Fish oil[a]	13.5	72	0.9	60
Premix[e]	20	20	20	20
Filler (talc)[f]	221.5	163	79.1	20
Wheat bran[g]	50	50	50	50
Carboxymethylcellulose[c]	10	10	10	10
Proximate analysis (%)				
Dry matter	988	978	937	982
Crude protein	401	398	501	509
Lipid	67	122	61	127
Ash	351	293	211	163
Gross energy (kJ g^{-1})[h]	136	164	166	186
Protein: energy ratio (mg kJ^{-1})	294	243	301	269
Lipids: energy ratio (mg kJ^{-1})	49	74	37	68

[a]Proteínas Marinas y Agropecuarias S.A. de C.V. Jalisco, MX.

[b]Vimifos, S.A. de C.V. Jalisco, MX.

[c]MaltaCleyton S.A. de C.V. Yucatán, MX.

[d]Productos Químicos de Yucatán S.A. de C.V. Yucatán, MX.

[e]DMS Peces Tropicales de Engorda, Nutritional Products México S.A. de C. V. Composition: vitamin A, vitamin D3, vitamin E, vitamin K3, vitamin B1, vitamin B2, vitamin B12, folic acid, vitamin B6, pentothenic acid, niacin, biotin, choline, vitamin C, iron, manganese, sodium, copper, iodine, zinc, cobalt, selenium, molybdenum, calcium, phosphorus, magnesium, sodium,

potassium, carophyll, lysine, threonine, calcium carbonate, methionine, tryptophan, sodium lasolacid, monensin, tylosin, salinomycin, avoparcin, flavomycin arsanilic, amprolium, zinc bacitracin, virginiamicin, calcium propinate, silicic acid, lignosulphonate, lecithin, BHT, BHA, probiotics, glutase keta, lipase, proteases, amylases, cellulases, and acemita.

[f]Talc is used as a filler (Dias, Huelvan, Dinis, & Métailler, 1998); it is an inorganic substance that is almost insoluble in aqueous solution; transfer across biological membranes is unlikely, and this substance is not toxic to aquatic life (ECHA, 2020).

[g]Wheat bran was used as fibre because it contains digestion-resistant starch (Xie, Cui, Li, & Tsao, 2008) and also non digestible protein because it is a requirement for pH = 12.5 for digestion (Balandrán-Quintana, Mercado-Ruiz, & Mendoza-Wilson, 2015). Proximate composition: 14% protein, 4% lipid, and 86% carbohydrate.

[h]Energy was calculated using a calorimetric bomb (PARR, Moline, IL, USA).

Table 2. Estimated regression parameters in growth (mean ± standard error of the mean [SEM]) of the common snook fed diets containing different protein:lipid ratios.

Diet name[‡]	CP[†] (g kg^{-1})	CL (g kg^{-1})	Slope (g day^{-1})
400:60	400	60	0.22 ± 0.01
400:120	400	120	0.29 ± 0.02
500:60	500	60	0.27 ± 0.02
500:120	500	120	0.29 ± 0.02
Means of main effects[§]			
	400		0.26 ± 0.01
	500		0.28 ± 0.02
		60	0.24 ± 0.01[Z]
		120	0.29 ± 0.02[Y]
Parameters	Estimates	SEM	p-value
Intercept	8.58	0.16	***[¶]
Time	0.10	0.02	***
Protein	-0.06	0.23	NS
Lipid	-0.12	0.22	NS
Time	0.0013	0.0001	***
Time : Protein	0.04	0.02	NS
Time : Lipid	0.08	0.02	**
Protein : Lipid	0.10	0.32	NS
Time : Protein : Lipid	-0.05	0.03	NS
AIC[¶¶]	251.40		
BIC	281.52		
δ	1.97		
ø	0.83		
σ	0.0039		

[†]CP = crude protein; CL = crude lipid. The superscript number denotes the level (g kg^{-1}) of proteins and lipids formulated for each test diet.

§Single superscript capital letters indicate significant difference between main effect means (Dietary lipid = Y > Z); single subscript small letters indicate significant difference between diets at $p < 0.05$ by Tukey´s test (a > b).

‡Diet names represent the average values of three tanks per treatment.

¶Significance terms: NS = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

¶¶δ, variance structure parameter; ∅, correlation structure parameter; σ residual standard error.

Table 3. Growth performance (mean ± standard error mean [SEM]) of the common snook fed diets containing different protein:lipid ratios.

Diet name‡	CP† (g kg^{-1})	CL (g kg^{-1})	FW (g)	WG (g)	SGR (%)	S (%)
400:60	400	60	27.7 ± 0.56	19.1 ± 0.45	1.43 ± 0.01	100
400:120	400	120	36.7 ± 0.87	28.1 ± 1.01	1.79 ± 0.05	100
500:60	500	60	34.7 ± 1.93	26.0 ± 1.95	1.70 ± 0.07	100
500:120	500	120	38.3 ± 4.32	29.6 ± 4.13	1.81 ± 0.11	100
Means of main effects§						
	400		32.2 ± 2.05	23.6 ± 2.07	1.61 ± 0.08	100
	500		36.5 ± 2.26	27.8 ± 2.19	1.76 ± 0.06	100
		60	31.2 ± 1.79^{Z}	22.5 ± 1.78^{Z}	1.57 ± 0.07^{Z}	100
		120	37.5 ± 2.00^{Y}	28.8 ± 1.93^{Y}	1.80 ± 0.05^{Y}	100
Two-way ANOVA¶						
Dietary protein			NS	NS	NS	NS
Dietary lipid			*	*	*	NS
Interaction			NS	NS	NS	NS

†, ‡, §, ¶ see footnotes †, ‡, § and ¶ in Table 2.

1701 Table 4. Feed intake and feed efficiency (mean ± standard error mean [SEM]) of the common snook fed diets containing different
1702 protein:lipid ratios.

Diet name‡	CP† (g kg^{-1})	CL (g kg^{-1})	FI (g $fish^{-1}$)	NI (g $fish^{-1}$)	LI (g $fish^{-1}$)	EI (KJ $fish^{-1}$ day^{-1})	PER	FCR
400:60	400	60	33.9 ± 0.3	14.2 ± 0.1	2.04 ± 0.02	5.49 ± 0.05^{c}	1.34 ± 0.02^{b}	1.77 ± 0.03^{a}
400:120	400	120	35.6 ± 0.6	14.9 ± 0.2	4.27 ± 0.08	6.95 ± 0.13^{b}	1.88 ± 0.04^{a}	1.26 ± 0.02^{b}
500:60	500	60	33.8 ± 0.9	17.6 ± 0.4	2.04 ± 0.05	6.69 ± 0.18^{b}	1.47 ± 0.05^{b}	1.31 ± 0.01^{b}
500:120	500	120	36.7 ± 1.3	19.1 ± 0.6	4.41 ± 0.15	12.03 ± 0.43^{a}	1.53 ± 0.06^{b}	1.27 ± 0.14^{b}
Means of main effects§								
	400		34.7 ± 0.50	14.6 ± 0.21^{B}	3.15 ± 0.50	6.22 ± 0.33	1.61 ± 0.12	1.52 ± 0.11
	500		35.3 ± 0.96	18.3 ± 0.50^{A}	3.22 ± 0.53	9.36 ± 1.21	1.51 ± 0.03	1.29 ± 0.06
		60	33.9 ± 0.45^{Z}	15.9 ± 0.78^{Z}	2.03 ± 0.03^{Z}	6.09 ± 0.28	1.41 ± 0.04	1.54 ± 0.11
		120	36.1 ± 0.71^{Y}	17.0 ± 0.98^{Y}	4.34 ± 0.08^{Y}	9.49 ± 1.15	1.71 ± 0.08	1.27 ± 0.00
Two-way ANOVA¶			NS	***	NS	***	*	*
Dietary protein			*	*	***	***	***	**
Dietary lipid			NS	NS	NS	***	***	*
Interaction								

1703 †, ‡, §, ¶ see footnotes †, ‡, § and ¶ in Table 2.

1704

Table 5. Whole body composition (g kg^{-1}) (mean ± standard error of the mean [SEM]) of common snook feed diets containing different protein:lipid ratios.

Diet name[‡]	CP[†] (g kg^{-1})	CL (g kg^{-1})	Moisture	Protein	Lipid	Ash
400:60	400	60	637.5 ± 5.2^{b}	300.1 ± 4.7^{a}	32.5 ± 0.7	71.7 ± 0.7^{a}
400:120	400	120	656.2 ± 2.2^{a}	257.9 ± 5.0^{b}	55.0 ± 0.6	65.1 ± 0.3^{b}
500:60	500	60	657.1 ± 2.3^{a}	269.7 ± 5.1^{b}	39.5 ± 1.2	73.8 ± 0.2^{a}
500:120	500	120	645.5 ± 4.1^{ab}	268.6 ± 7.3^{b}	60.4 ± 1.0	72.9 ± 1.5^{a}
Means of main effects[§]						
	400		646.8 ± 49.1	279.0 ± 99.2	43.8 ± 50.5^{B}	68.4 ± 15.3
	500		651.3 ± 33.4	269.2 ± 39.9	50.0 ± 47.1^{A}	73.4 ± 7.5
		60	647.3 ± 50.9	284.9 ± 74.6	36.1 ± 16.9^{Z}	72.8 ± 58.5
		120	650.9 ± 12.4	263.3 ± 46.4	57.7 ± 13.1^{Y}	69.0 ± 19.0
Two-way ANOVA[¶]						
Dietary protein			NS	NS	***	***
Dietary lipid			NS	**	***	**
Interaction			*	**	NS	*

[†, ‡, §, ¶] see footnotes †, ‡, § and ¶ in Table 2.

Table 6. Condition factor (CF), hepatosomatic index (HSI), glycogen (g kg^{-1}), and triglycerides (mmol/l) in the liver (mean ± standard error of the mean [SEM]) of common snook feed diets containing different protein:lipid ratios.

Diet name‡	CP† (g kg^{-1})	CL (g kg^{-1})	CF	HSI	Liver glycogen	Liver triglycerides
400:60	400	60	0.64 ± 0.0	0.56 ± 0.02	2.02 ± 0.1	5.27 ± 0.4
400:120	400	120	0.66 ± 0.0	0.67 ± 0.02	2.54 ± 0.2	7.01 ± 0.2
500:60	500	60	0.63 ± 0.0	0.61 ± 0.03	2.46 ± 0.2	4.57 ± 0.8
500:120	500	120	0.66 ± 0.0	0.66 ± 0.02	2.37 ± 0.2	7.01 ± 0.6
Means of main effects§						
	400		0.66 ± 0.0	0.64 ± 0.02	2.28 ± 0.1	6.14 ± 0.6
	500		0.64 ± 0.0	0.61 ± 0.03	2.42 ± 0.1	5.66 ± 0.5
		60	0.63 ± 0.0	0.58 ± 0.02^{Z}	2.24 ± 0.1	4.92 ± 0.5^{Z}
		120	0.64 ± 0.0	0.66 ± 0.01^{Y}	2.46 ± 0.1	6.88 ± 0.7^{Y}
Two-way ANOVA¶						
Dietary protein			NS	NS	NS	NS
Dietary lipid			NS	***	NS	**
Interaction			NS	NS	NS	NS

†, ‡, §, ¶ see footnotes †, ‡, § and ¶ in Table 2.

Table 7. Specific activities (U mg protein^{-1}) (mean ± standard error mean [SEM]) of digestive enzymes in the pyloric caeca in common snook fed diets containing different protein:lipid ratios.

Diet name‡	CP† (g kg^{-1})	CL (g kg^{-1})	Alkaline proteases††	Trypsin	Chymotrypsin	Lipase
400:60	400	60	7.91 ± 0.31	4.14 ± 0.27^{b}	7.77 ± 1.29^{a}	3.39 ± 0.49^{b}
400:120	400	120	7.67 ± 0.15	8.52 ± 0.37^{a}	3.23 ± 0.32^{b}	4.93 ± 0.21^{a}
500:60	500	60	7.19 ± 0.04	1.81 ± 0.09^{c}	1.35± 0.29^{b}	0.48 ± 0.01^{c}
500:120	500	120	7.37 ± 0.12	1.74 ± 0.08^{c}	1.20 ± 0.13^{b}	1.57 ± 0.01^{c}
Means of main effects§						
	400		7.79 ± 0.16	6.33 ± 1.00	5.50 ± 1.17	4.16 ± 0.41
	500		7.28 ± 0.19	1.78 ± 0.06	1.27 ± 0.14	1.02 ± 0.24
		60	7.55 ± 0.27	2.98 ± 1.55	4.56 ± 1.55	1.93 ± 0.68
		120	7.52 ± 0.11	5.13 ± 0.48	2.21 ± 0.48	3.25 ± 0.75
Two-way ANOVA¶						
Dietary protein			NS	***	***	***
Dietary lipid			NS	***	**	***
Interaction			NS	***	*	***

†, ‡, §, ¶ see footnotes †, ‡, § and ¶ in Table 2.

††Alkaline protease activities are expressed at 10–2 U mg protein^{-1}. Trypsin, chymotrypsin, and lipase activities are expressed at 10–4 U mg protein^{-1}.

Table 8. Specific activities (U mg protein^{-1}) (mean ± standard error mean [SEM]) of digestive enzymes in intestine in common snook fed diets containing different protein:lipid ratios.

Diet name‡	CP† (g kg^{-1})	CL (g kg^{-1})	Alkaline proteases††	Trypsin	Chymotrypsin	Lipase
400:60	400	60	6.86 ± 0.19	4.83 ± 0.22^{b}	7.10 ± 1.08^{a}	4.03 ± 0.08^{b}
400:120	400	120	5.97 ± 0.03	7.81 ± 0.31^{a}	2.02 ± 0.28^{b}	10.4 ± 0.38^{a}
500:60	500	60	7.91 ± 0.15	1.63 ± 0.07^{c}	1.01 ± 0.17^{b}	0.40 ± 0.07^{c}
500:120	500	120	6.71 ± 0.19	2.04 ± 0.22^{c}	1.32 ± 0.60^{b}	1.81 ± 0.09^{bc}
Means of main effects§						
	400		6.41 ± 0.21^{B}	6.32 ± 0.68	4.56 ± 1.28	6.65 ± 1.77
	500		7.31 ± 0.28^{A}	1.84 ± 0.13	1.20 ± 0.27	1.11 ± 0.31
		60	7.39 ± 0.25^{Y}	3.23 ± 0.72	4.06 ± 1.44	1.54 ± 0.57
		120	6.34 ± 0.18^{Z}	4.93 ± 1.30	1.70 ± 0.46	6.13 ± 1.94
Two-way ANOVA¶						
Dietary protein			***	***	**	***
Dietary lipid			***	***	*	***
Interaction			NS	***	**	***

†, ‡, §, ¶, †† see footnotes †, ‡, §, ¶, †† in Table 2 and Table 6.

Table 9. Specific activities (U/mg of protein) (mean ± standard error mean [SEM]) of hepatic intermediary metabolism enzymes in the common snook fed containing different protein:lipid ratios.

Diet name[‡]	CP[†] (g kg^{-1})	CL (g kg^{-1})	PK	G6PDH	6PGDH	FBPase	ALT[††]
400:60	400	60	14.9 ± 0.51	25.1 ± 0.59	14.3 ± 0.91	7.73 ± 0.87	4.17 ± 0.22
400:120	400	120	17.0 ± 0.17	35.8 ± 0.58	15.9 ± 0.09	7.73 ± 0.02	3.27 ± 0.12
500:60	500	60	17.1 ± 0.39	29.4 ± 0.48	15.3 ± 0.19	7.62 ± 0.06	5.41 ± 0.54
500:120	500	120	19.1 ± 0.31	32.9 ± 0.79	17.7 ± 0.61	7.99 ± 0.09	3.27 ± 0.05
Means of main effects[§]							
	400		15.9 ± 0.52^{B}	29.0 ± 1.80^{B}	15.1 ± 0.54^{B}	7.73 ± 0.08	3.72 ± 0.22
	500		18.1 ± 0.49^{A}	32.6 ± 1.49^{A}	16.6 ± 0.62^{A}	7.80 ± 0.09	4.34 ± 0.53
		60	16.0 ± 0.55^{Z}	27.2 ± 10.3^{Z}	14.8 ± 0.47^{Z}	7.67 ± 0.09	4.79 ± 0.38^{Y}
		120	18.1 ± 0.47^{Y}	34.4 ± 0.78^{Y}	16.9 ± 0.49^{Y}	786 ± 0.07	3.27 ± 0.06^{Z}
Two-way ANOVA[¶]							
Dietary protein			***	***	*	NS	NS
Dietary lipid			***	***	**	NS	***
Interaction			NS	NS	NS	NS	NS

†, ‡, §, ¶, †† see footnotes †, ‡, §, ¶, †† in Table 2 and Table 6.

Figure legends

Figure 1. Growth of common snook fed with two dietary protein levels (400 g kg^{-1} and 500 g kg^{-1}) associated to two dietary lipid levels (60 g kg^{-1} and 120 g kg^{-1}) for 84 days. (**1**) 400:60 (400 g kg^{-1} protein and 60 g kg^{-1} lipid); (**2**) 400:120 (400 g kg^{-1} protein and 120 g kg^{-1} lipid); (**3**) 500:60 (500 g kg^{-1} protein and 60 g kg^{-1} lipid); and (**4**) 500:120 (500 g kg^{-1} protein and 120 g kg^{-1} lipid).

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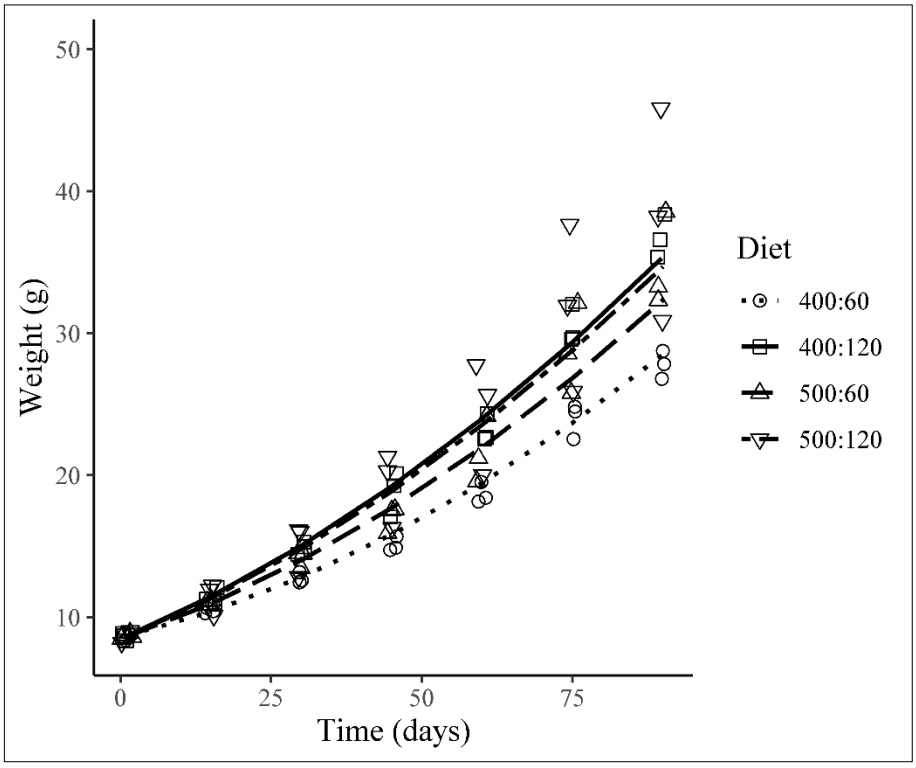

Figure 1. TIF

CAPÍTULO 5

Discusión general

En la acuicultura, una de las etapas más importantes en el establecimiento de una nueva tecnología de cultivo, es la formulación de un alimento balanceado a partir de una selección y proporción apropiada de ingredientes que permitan ser aprovechados eficientemente por el organismo que se desea cultivar. En la presente tesis, se evaluó por primera vez la habilidad de *C. undecimalis* y *O. chrysurus* para metabolizar carbohidratos y lípidos. A pesar de que ambas especies de peces poseen hábitos alimenticios carnívoros con un requerimiento similar de proteína en el alimento (Concha-Frías et al., 2018; Enriquez, 2018; Gracia-López, García-Galano, Gaxiola-Córtes, & Pacheco-Campos, 2003; Tucker, 1987), los resultados obtenidos en el presente estudio revelaron diferentes respuestas fisiológicas a la presencia de estos nutrientes en el alimento entre especies, con potenciales consecuencias en su aprovechamiento.

El efecto de los carbohidratos en el alimento

A pesar de que la habilidad de los peces para metabolizar carbohidratos se encuentra clasificado primariamente por sus hábitos alimenticios (p. ej. herbívoros, omnívoros y carnívoros) (Stone, 2003; Wilson, 1994), la amplia diversidad morfológica y fisiológica existente en los peces, incluso en aquellos categorizados dentro de un grupo alimenticio particular, da lugar a una amplia variedad de respuestas intrínsecas en el metabolismo de este nutriente (Kamalam, Medale, & Panserat, 2017).

En la presente tesis, se evaluó la habilidad de dos especies de peces tropicales para metabolizar carbohidratos, *C. undecimalis* y *O. chrysurus*, que desde una perspectiva bioquímica y ecológica han sido catalogadas como peces carnívoros (Ahumada-Hernández et al., 2014; Concha-Frias et al., 2016; Jimenez-Martinez et al., 2012), los cuales además presentan un tracto digestivo similar (p. ej. estómago, ciegos pilóricos e intestino); sin embargo, poseen diferentes ciclos de vida (De La Morinière et al., 2002; McMichael et al., 1989). Los resultados obtenidos demostraron que ambas especies de peces se adaptaron a la presencia de carbohidratos en el alimento (20% almidón de maíz), esto a través de una reorganización metabólica (p. ej. amilasa, PK y GK). Sin

embargo, a pesar de qué *O. chrysurus* presentó una mayor actividad digestiva de la enzima amilasa respecto a *C. undecimalis*, esta especie exhibió una menor expresión bioquímica y génica de la enzima hepática GK. La baja expresión de la enzima GK observada en *O. chrysurus* podría explicar: 1) la prolongada condición de hiperglucemia observada en esta especie, y 2) la ausencia o falta de estimulación de los mecanismos involucrados en la homeostasis de la glucosa en estado postprandial post-absorción (p. ej. síntesis de glucógeno y la ruta de la pentosa fosfato). Por otra parte, un efecto aditivo de la enzima amilasa a la prolongada hiperglucemia registrada en *O. chrysurus* por efecto de una mejor digestión del almidón de maíz no puede ser descartado.

El catabolismo de aminoácidos no fue afectado por la presencia de carbohidratos en el alimento en ambas especies de peces; sin embargo, *O. chrysurus* presentó un metabolismo mejor adaptado al catabolismo de proteínas respecto a *C. undecimalis*. Interesantemente, a pesar de que el catabolismo de aminoácidos no fue afectado por los carbohidratos, la actividad de la enzima hepática FBPasa disminuyó, indicando que el potencial de gluconeogénesis decreció en ambas especies. Estos resultados indican que la presencia de carbohidratos en el alimento reprimió sustratos gluconeogénicos alternos a los aminoácidos. La FBPasa es clave en la incorporación del glicerol (producto de hidrólisis de triglicéridos) a la ruta de gluconeogénesis (Figura 1), por lo tanto, posiblemente el glicerol fue el sustrato reprimido en esta ruta metabólica.

El crecimiento no fue afectado por los carbohidratos en ambas especies de peces, pero en *C. undecimalis* mejoró la eficiencia alimenticia, indicando que *C. undecimalis* a diferencia de *O. chrysurus* sustituyó parcialmente las proteínas y lípidos por la glucosa de origen exógeno para cubrir sus necesidades energéticas. La pobre habilidad de *O. chrysurus* para utilizar los carbohidratos como sustratos energéticos podría ser atribuido a su baja capacidad para almacenar la glucosa exógena en forma de glucógeno, efecto de una baja expresión bioquímica y génica de la enzima hepática GK (explicación extensa en capítulo 2). El glucógeno funciona como reserva de glucosa-6 fosfato para la producción de ATP vía glucólisis en condiciones de ayuno. La baja disponibilidad de glucógeno hepático en condiciones de ayuno, pudo generar en *O. chrysurus* una mayor demanda de proteína para cubrir sus necesidades energéticas vía gluconeogénesis, lo cual a su vez explicaría el elevado catabolismo de aminoácidos observado en esta especie.

El efecto de los lípidos en el alimento

Un aumento en el contenido de energía digestible por incremento del porcentaje de lípidos en el alimento acompañando con o sin una reducción concomitante en el contenido de energía digestible por proteína, ha demostrado ser extremadamente eficiente para reducir el catabolismo de aminoácidos con propósitos energéticos y mejorar su uso para el crecimiento en algunas especies de peces (NRC, 2011).

En la presente tesis, el incremento del porcentaje de lípidos en el alimento de 6 a 12%, permitió reducir el catabolismo de aminoácidos en *C. undecimalis*; no obstante, el potencial de gluconeogénesis permaneció constante. Por el contrario, en *O. chrysurus*, el catabolismo de aminoácidos no fue afectado por el contenido lípidos en el alimento, pero el potencial de gluconeogénesis incremento cuando se alimentó con 12% de lípidos. Lo anterior indica que *C. undecimalis* reemplazó los aminoácidos por los lípidos en la vía de gluconeogénesis, mientras que *O. chrysurus* los incorporó de forma aditiva. A nivel de proteína, en *O. chrysurus* el catabolismo de aminoácidos decreció cuando el nivel de proteína en el alimento disminuyó de 50 a 40%, mientras que en *C. undecimalis* permaneció constante. Interesantemente, el potencial de gluconeogénesis fue constante a nivel de proteína en ambas especies de peces. En *C. undecimalis*, la respuesta del potencial de gluconeogénesis fue equilibrado respecto al catabolismo de aminoácidos; sin embargo, en *O. chrysurus* la reducción del catabolismo de aminoácidos en 40% proteína sugiere que esta especie incorporó a la vía de gluconeogénesis un sustrato alterno a los aminoácidos, lípidos (glicerol; considerando que la formulación fue a base proteínas y lípidos), lo cual además es consistente con la elevada actividad de la enzima lipasa observada en *O. chrysurus* en los niveles más bajos de proteína (indicador de una mayor digestión de lípidos), particularmente en la combinación 40% proteína y 6% lípidos.

La eficiencia alimenticia mejoró por el incremento del porcentaje de lípidos en el alimento de 6 a 12% en ambas especies de peces, indicando que tanto *C. undecimalis* como *O. chrysurus* fueron capaces de utilizar los lípidos como sustratos energéticos. Respecto al crecimiento, en *C. undecimalis* éste incrementó cuando los niveles de proteína se combinaron con 12% lípidos, mientras que en *O. chrysurus* el mayor crecimiento se registró en el nivel de proteína de 50%, esto independientemente del contenido lípidos en el alimento. Cuando los peces son alimentados

con un exceso de proteína, ésta puede ser utilizada como sustrato energético en el metabolismo intermediario, convertida en glucosa o almacenada como lípidos (depósitos de energía) (Jia et al., 2017; Oliva-Teles et al., 2020; Walton & Cowey, 1982). A nivel de proteína, el potencial de glucólisis presentó un comportamiento similar en ambas especies de peces; sin embargo, el potencial de pentosa fosfato o producción de NADH (poder reductor para la biosíntesis de lípidos) y el contenido de lípidos totales de todo el cuerpo incrementó sólo en *C. undecimalis* cuando se alimentó con 50% proteína. Por otra parte, el catabolismo de aminoácidos fue constante en *C. undecimalis*, pero en *O. chrysurus* decreció en el nivel de proteína de 40%. Estos resultados indican que la ausencia de diferencias en crecimiento a nivel de proteína en *C. undecimalis* se debió a su bajo requerimiento de proteína, mientras que la reducción del crecimiento de *O. chrysurus* en 40% proteína correspondió a un alto requerimiento de este nutriente por la especie.

Conclusión

- La incorporación de un 20% de carbohidratos en el alimento mejora la eficiencia alimenticia (factor de conversión alimenticia y la tasa de eficiencia proteica) en *C. undecimalis*; sin embargo, esto no ocurre en *O. chrysurus*.

- Tanto *C. undecimalis* como *O. chrysurus*, son capaces de adaptarse a la presencia de carbohidratos en el alimento a través de una reorganización metabólica, por estimulación del potencial digestivo y de la glucólisis, e inhibición del potencial de gluconeogénesis; sin embargo, la habilidad de éstas especies para aprovechar los carbohidratos de forma eficiente como sustrato energético depende de su capacidad para incorporarlos a las rutas metabólicas de almacenamiento (p. ej. glucogenosis y pentosa fosfato).

- La inducción nutricional de la enzima hepática GK por los carbohidratos es un factor clave en el almacenamiento y aprovechamiento de este nutriente para *C. undecimalis* y *O. chrysurus*.

- La incorporación de carbohidratos en el alimento suprime el potencial de gluconeogénesis en *C. undecimalis* y *O. chrysurus*, sin embargo, no afecta el catabolismo de aminoácidos, por lo tanto, la presencia de carbohidratos en el alimento suprime la incorporación de los lípidos a la vía de gluconeogénesis.

- El incremento de lípidos en el alimento de 6 a 12% mejora la eficiencia alimenticia (factor de conversión alimenticia y la tasa de eficiencia proteica) tanto en *C. undecimalis* como en *O. chrysurus*.

- En *C. undecimalis* y *O. chrysurus*, el incremento de lípidos en el alimento de 6 a 12% promueve la incorporación de lípidos (glicerol) a la vía de gluconeogénesis; sin emebargo, sólo en *C. undecimalis* se suprime el catabolismo de aminoácidos.

- Para *C. undecimalis*, un contenido de proteína del 40% en el alimento es apropiado para un adecuado crecimiento y eficiente utilización de este nutriente; sin embargo, para *O. chrysurus* es limitante.

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