VICTOR HUGO MARQUES LORENTI

A influência de diferentes razões de ácidos graxos saturados e monoinsaturados no desempenho de crescimento e metabolismo lipídico de juvenis de *Rachycentron canadum*

The influence of different ratios of saturated and monounsaturated fatty acids on growth performance and lipid metabolism of *Rachycentron canadum* juveniles

São Paulo

2021

VICTOR HUGO MARQUES LORENTI

A influência de diferentes razões de ácidos graxos saturados e monoinsaturados no desempenho de crescimento e metabolismo lipídico de juvenis de *Rachycentron canadum*

The influence of different ratios of saturated and monounsaturated fatty acids on growth performance and lipid metabolism of *Rachycentron canadum* juveniles

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em fisiologia geral, na Área de fisiologia.

Orientadora: Renata Guimarães Moreira Whitton

Coorientador: Bruno Cavalheiro Araújo

São Paulo

2021

Ficha Catalográfica

Marques, Victor Hugo
A influência de diferentes razões de ácidos graxos saturados e monoinsaturados no desempenho de crescimento e metabolismo lipídico de juvenis de *Rachycentron canadum*
Número de páginas: 66

Dissertação (Mestrado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Fisiologia geral.

1. Ácidos graxos; 2. Lipídios alternativos; 3. β oxidação; 4. *Sparing effect*
Universidade de São Paulo. Instituto de Biociências. Departamento de fisiologia geral.

Comissão Julgadora:

Prof(a). Dr(a).

Prof(a). Dr(a).

Prof(a). Dr.(a).

Prof(a). Dr.(a).
Orientador(a)

Dedicatória

Dedico este trabalho à minha família, aos meus orientadores e aos meus amigos.

Epígrafe

Éowyn: *Coragem, Merry. Coragem pelos nossos amigos.*

Théoden: *Eomer, leva a sua eored pelo flanco esquerdo. Gamling, siga a bandeira real pelo centro. Gimbold, leve a sua companhia pela direita depois de passar a muralha.*
Para a frente, e não tema as trevas!

Théoden: *Ergam-se, ergam-se cavaleiros de Théonden!*
Lanças devem ser abanadas, escudos devem ser quebrados.... Um dia de espadas, um dia vermelho antes que o sol nasça!
Théoden: *Morte!*
Cavaleiros de Rohan: *Morte!*
Théoden: *Avante Eorlingas!*

O Senhor dos Anéis, O Retorno do Rei – J.R.R, Tolkien

Agradecimentos

Agradeço, primeiramente, a minha família: Noemi, Janete, Guilherme e Milton por cuidarem de mim, me oferecerem apoio e uma educação de qualidade de forma que eu pudesse dar este passo na minha carreira.

A Beatriz Vieira Matos, pelo amor e companheirismo durante todos estes anos.

Aos meus amigos que são essenciais na minha vida e me incentivam sempre. Agradeço também à Universidade de São Paulo (USP), ao Instituto de Biociências (IB) e ao Programa de Pós-Graduação em (Ciências) Fisiologia Geral, por conceder toda estrutura física e profissional para o desenvolvimento de todas as etapas do meu mestrado.

Agradeço à Professora Renata por me orientar durante o curso e fornecer apoio, ensinamentos e oportunidades durante todos estes anos; à Aline Dal Olio Gomes por me orientar durante a IC e me auxiliar durante os passos iniciais do mestrado. Agradeço ao Bruno Araújo por me dar ótimas oportunidades de crescer profissionalmente, me mostrar dinâmicas de trabalho que me identifico muito, e me orientar na carreira que pretendo seguir. A vocês minha gratidão e lealdade são eternas.

Agradeço a todos os colegas do laboratório LAMEROA que me deram suporte e fazem o ambiente de trabalho ser extremamente convidativo. Agradeço à Giovana Branco por me ensinar novas técnicas de análise com muita paciência e qualidade e a todos os outros que não citei os nomes pois são muitos, mas, estiveram comigo em momentos de trabalhar duro e de celebrar. Agradeço a todos vocês.

Agradeço especialmente ao Vagner por se dispor a me ajudar com análises, problemas logísticos e técnicos.

Aos demais parceiros e colaboradores que me ensinaram e facilitaram a condução do projeto: Artur Rombenso, Maria Teresa Viana e Renato Honji.

À FAPESP, por todo auxílio financeiro (2019/09246-9 e 2019/26018-0).

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES).

Agradeço a todas as pessoas que de alguma forma me auxiliaram diretamente ou indiretamente durante o período do meu mestrado. Muito obrigado!

Índice

Lista de abreviações 9

Resumo geral 11

General abstract 12

Introdução Geral 13

Capítulo 1. Different saturated and monounsaturated fatty acids levels in fish oil-free diets to cobia (*Rachycentron canadum*) juveniles: Effects in growth performance and lipid metabolism 20

1. Introdução 23
2. Material e métodos 25
3. Resultados 32
4. Discussão 40
5. Conclusão 45
6. Agradecimentos 46
7. Referências bibliográficas 46

Discussão geral e Conclusão **54**

Referências bibliográficas gerais **58**

Lista de abreviações

ARA: Ácido araquidônico (do inglês, *Arachidonic acid*, 20:4n-6)

BHT: Hidroxitolueno butilado

C18: 18 carbonos (ácidos graxos de cadeia curta)

C18PUFA: Ácido graxo polinsaturados (duas ou mais ligações duplas) com dezoito átomos de carbono

DHA: Ácido docosahexaenoico (do inglês, *docosahexaenoic acid*, 22:6n-3)

EFA: Ácido graxo essencial (do inglês, *Essential fatty acid*)

EPA: Ácido eicosapentaenoico (do inglês, *eicosapentaenoic acid*, 20:5n-3)

FA: Ácidos graxos (do inglês, *fatty acid*)

Fas: Ácido graxo sintase (do inglês, *fatty acid sintase*)

FAME: Éster metílico de ácido graxo (do inglês, *fatty acids methyl-esters*)

FID: Ionizador de chama (do inglês, *Flame Ionization Detector*)

FO: Óleo de peixe (do inglês, *fish oil*)

HSI: Índice hepatossomático (do inglês, *hepatosomatic index*)

LC-PUFA: Ácidos graxos altamente insaturados com mais de 20C e mais de 2 ligações duplas (do inglês, long-chain polyunsaturated fatty acids)

LSI: Índice lipossomático (do inglês, *liposomatic index*)

MUFA: Monoinsaturados com uma ligação dupla (do inglês, monounsaturated fatty acids)

n-3: Ômega-3

n-6: Ômega-6

n-9: Ômega-9

PL: Fosfolipídios (do inglês, phospholipids)

PUFA: Ácidos graxos polinsaturados com duas ou mais ligações duplas (do inglês, polyunsaturated fatty acids)

SFA: Ácido graxo saturado (do inglês, saturated fatty acid)

VO: Óleo vegetal (do inglês, vegetable oil)

VSI: Índice viscerossomático (do inglês, viscerosomatic index)

Resumo

A utilização de óleo de peixe (OP) na aquicultura marinha é importante devido à presença de ácidos graxos (AG) de cadeia longa e altamente insaturados (LC-PUFA) da série n3. No entanto, a produção de OP é limitada, e por esta razão, fontes alternativas de menor custo e produção mais estável como óleos vegetais (OV) vêm sendo investigadas. No entanto esta substituição, na maioria dos casos, impacta negativamente o crescimento e altera o metabolismo lipídico dos animais, principalmente devido à presença nos OV de quantidades elevadas de AG saturados (SFA) e monoinsaturados (MUFA) e ausência de LC-PUFAs. Uma alternativa para produção de dietas com menor custo e que cumpram as necessidades nutricionais dos animais é a utilização de OV suplementado com LC-PUFAs. Contudo, estudos com peixes marinhos mostraram que dietas com altas quantidades de SFA e MUFAs alteram a deposição lipídica no fígado e a expressão de genes relacionados com a síntese e oxidação de AGs. O objetivo do presente estudo foi investigar a influência de fontes lipídicas alternativas, ricas em SFA e MUFA suplementadas com LC-PUFA no crescimento e metabolismo lipídico de juvenis de *Rachycentron canadum*. Foi conduzido um experimento nutricional de oito semanas utilizando quatro dietas isoproteicas e isolipídicas: OP-D (dieta controle com apenas OP), SFA-D (rica em SFA), MIX-D (mesmos níveis de SFA e MUFA) e dieta MUFA-D (rica em MUFA). As dietas, (exceto OP-D), foram suplementadas com ácido araquidônico (ARA), ácido eicosapentaenoico (EPA), e ácido docosaexaenoico (DHA) (3, 5, 10 g kg^{1}, respectivamente). Foram avaliados o perfil de AG muscular e hepático, a expressão de genes de síntese e oxidação de AG e a morfologia do tecido hepático. De forma geral, o desempenho produtivo não foi prejudicado pelas dietas livres de OP, corroborando estudos que demonstram que fontes lipídicas alternativas podem ser usadas em formulações de dietas para juvenis de peixes marinhos quando adequadamente suplementada com LCPUFAs. A deposição de SFA no músculo e no fígado ocorreu de forma desproporcional à inclusão destes AGs nas dietas. De forma inversa os MUFAs foram depositados principalmente no fígado e músculo, refletindo os níveis de inclusão na dieta. Os principais AG que influenciaram esse padrão foram 12:0 e 18:1n-9. A expressão da ácido graxo sintase (*fas*) foi hiper-regulada no grupo OP-D em comparação com os grupos SFA-D e MIX-D. Não houve diferenças nas expressões relativas de carnitina palmitoiltransferase 1 (*cpt-1α*) e lipase lipoproteica (*lpl*). Os resultados da morfologia do fígado indicaram que os peixes alimentados com SFA-D apresentaram uma área de vacúolos lipídicos menor do que aqueles alimentados com outras dietas experimentais. Este estudo mostra que SFA com cadeias de carbono mais curtas, como 12:0 podem ser administrados em dietas para *R. canadum* para estimular o catabolismo dessas moléculas, fornecendo energia para o crescimento e retendo LC-PUFAs nos tecidos, especialmente no músculo, exibindo um filé mais saudável para os consumidores.

Palavras chave: ácidos graxos, lipídios alternativos, SFA, MUFA, β-oxidação

Abstract

The use of fish oil (FO) in marine aquaculture is important because of the presence of long chain and highly unsaturated fatty acids (LC-PUFA) of the n3 series. However, production of FO is limited and for this reason alternative sources of lower cost and more stable production as vegetable oils (VO) have been investigated. However, this substitution in most cases negatively impacts the growth and changes the lipid metabolism of the animals, mainly due to the presence of high amounts of saturated (SFA) and monounsaturated (MUFA) fatty acids (FA) and absence of LC-PUFAs in VO. An alternative to producing low-cost diets that meet the nutritional needs of fish is the use of VO supplemented with LC-PUFA. However, studies with marine fish showed that diets with high amounts of SFA and MUFA alter lipid deposition in the liver and the expression of genes related to the synthesis and oxidation of FA. The objective of the present project is to investigate the influence of alternative lipid sources rich in SFA and MUFA supplemented with LC-PUFA on growth and lipid metabolism of *Rachycentron canadum* juveniles. An 8-week feeding trial was carried out using four isoproteic and isolipidic diets as follows: FO-D (fish oil, as control diet), SFA-D (rich in SFA), MIX-D (same levels of SFA and MUFA), and MUFA-D (rich in MUFA). Experimental diets were supplemented with arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (3, 5, and 10 g kg1, respectively). The growth performance, fatty acid (FA) profile of liver and muscle, hepatocyte morphology, and gene expression related to the FA synthesis and oxidation on the liver were examined. In general, production performance was not impaired in fish-fed FO-free diets, supporting the hypothesis that alternative lipid sources could be used in cobia's aquafeed formulations when the LCPUFA are adequately supplemented. High dietary SFA levels were disproportionally deposited the liver and muscle. Contrariwise MUFA was mainly deposited in the liver and muscle, reflecting the dietary inclusion levels. The main FA influencing this pattern were 12:0 and 18:1n-9. The expression of fatty acid synthase (*fas*) was up-regulated in the FO-D group compared to SFA-D and MIX-D groups. There were no differences in the relative expressions of carnitine palmitoyltransferase 1 (*cpt-1α*) and lipase lipoprotein (*lpl*). The liver morphology results indicated that fish-fed SFA-D presented a smaller lipid vacuoles area than those fed other experimental diets. This study shows that SFA with shorter carbon chains such as 12:0 can be administered in cobia aquafeeds to stimulate these molecules' catabolism, providing energy for growth, and retaining LC-PUFAs in tissues, especially in the muscle, exhibiting a healthier fillet for consumers.

Keywords: Fatty acids, Alternative lipids, SFA, MUFA, β-oxidation

Introdução geral

A produção mundial de pescado apresenta uma alta projeção de aumento para os próximos anos, podendo chegar a 196 milhões de toneladas em 2025, e a maior parcela desse aumento terá a contribuição de países em desenvolvimento. Nesta projeção, a aquicultura será responsável por aproximadamente 52% da produção de pescado, se mantendo como um dos maiores setores em crescimento de produção animal (FAO, 2016). Apesar das boas perspectivas, o setor enfrentará uma queda de crescimento anual de 5.4% para 3% durante este período e esta queda está ligada a vários fatores, sendo o principal a disponibilidade de fontes que atendam às necessidades nutricionais quantitativas e qualitativas das espécies cultivadas (FAO, 2016).

De forma geral, as dietas para peixes de cultivo, principalmente os marinhos, são formuladas utilizando óleo de peixe (OP) como principal fonte lipídica e consequentemente de ácidos graxos essenciais (AGE). A ampla utilização de OP em dietas para a aquicultura marinha está diretamente relacionada com as altas necessidades nutricionais de animais marinhos por AGs de cadeia longa e altamente insaturados da série n-3 (LC-PUFAs n-3) (Teles et al.,2015). Contudo, a captura de peixes em ambiente natural, para a produção de OP vem se mostrando em declínio nas últimas décadas, visto que a disponibilidade destes recursos é limitada e do ponto de vista ambiental pode ser considerada uma atividade altamente impactante (Nordahl, 2011). Outro fator a ser considerado na utilização de OP para a produção de dietas é a alta variabilidade nutricional e alto custo, uma vez que a disponibilidade no mercado é cada vez mais baixa. Portanto, para que a aquicultura atinja um crescimento sustentável, torna-se imprescindível buscar alternativas em detrimento ao

OP (Tocher, 2003; Glencross, 2009; Turchini et al., 2009). Nos últimos anos diversas pesquisas estão sendo desenvolvidas com o intuito de substituir integralmente OP das dietas de peixes marinhos, por fontes alternativas como óleos vegetais (OV). No entanto a composição de AGs destes ingredientes pode impactar negativamente o crescimento e o metabolismo lipídico dos animais (Tocher, 2010).

Os AGs podem ser divididos em três principais grupos: os saturados (SFA, do inglês, *saturated fatty acids*) que não possuem insaturações em suas cadeias carbônicas; os monoinsaturados (MUFAs, do inglês *monounsaturated fatty acids*), que contêm apenas uma insaturação em sua cadeia carbônica; e os polinsaturados (PUFAs, do inglês, *polyunsaturated fatty acids*) que contêm duas ou mais insaturações em sua cadeia carbônica. Os PUFAs podem ainda ser classificados como LC-PUFAs (do inglês, *long chain polyunsaturated fatty acids*), que são AGs que possuem mais de 20 carbonos e mais de duas insaturações (Nelson e Cox, 2018).

De acordo com Tocher (2003), os AGs desempenham inúmeras funções fundamentais para manutenção dos organismos, sendo uma das mais importantes a produção de energia metabólica realizada por processos de β-oxidação. A utilização de AGs no metabolismo energético é dependente da quantidade e razão em que são incluídos nas dietas para peixes (Tocher, 2010). Em uma dieta com excesso de OP os LC-PUFAs são potencialmente desviados para a via de β-oxidação ao invés de serem depositados no tecido muscular (Salini et al., 2015a; Turchini et al., 2013). De acordo com Jump (2005), os AGs advindos da dieta regulam a expressão de genes do metabolismo lipídico, modulando a síntese e oxidação de AGs. De forma geral, quando uma classe específica de AGs é administrada em excesso na dieta para peixes marinhos, estes AGs são oxidados para a produção de energia metabólica (Tocher, 2010), por outro lado quando incluídos em baixas concentrações são preservados e

depositados em tecidos como fígado e músculo (Torstensen et al., 2004; Stubhaug et al., 2007).

Os LC-PUFAs, como C20:4n6 (ácido araquidônico - ARA), C20:5n3 (ácido eicosapentaenoico - EPA) e C22:6n3 (ácido docosahexaenoico - DHA) são considerados imprescindíveis para os vertebrados, incluindo os peixes, pois participam de vários processos fisiológicos importantes, principalmente relacionados ao desenvolvimento estrutural de juvenis, crescimento, reprodução, síntese de hormônios esteroides gonadais além de serem importantes moléculas para a estruturação e adequado funcionamento das membranas celulares de tecidos nervosos e visuais (Arts e Kohler, 2009; Sorbera et al., 2001; Izquierdo et al., 2001). Além disso, EPA e ARA são importantes precursores de eicosanoides, moléculas que atuam como hormônios parácrinos no controle da inflamação e imunidade (Calder, 2006).

Os LC-PUFA são sintetizados a partir de seus precursores, os AGs 18:3n-3 e 18:2n-6, que são considerados AGs essenciais (AGEs) para a maioria dos organismos. De forma geral, peixes dulciaquícolas apresentam um alto potencial de síntese de LC-PUFA, pois possuem alta atividade das enzimas elongases, que adicionam carbonos aos pares na cadeia carbônica de um AG, e dessaturases, que adicionam duplas ligações nas cadeias carbônicas destes AGs (Henderson, 1996; Tocher, 2003). No entanto na maioria das espécies de peixes marinhos a atividade dessas enzimas é bastante reduzida e, devido a esta baixa atividade, torna-se essencial a inclusão de ingredientes ricos em LC-PUFAs como o OP na dieta de peixes marinhos (Turchini et al., 2009).

A dificuldade do processo de substituição de OP nas rações para peixes de cultivo está justamente em encontrar fontes que atendam às necessidades nutricionais em LC-PUFAs. Óleos vegetais são ricos em SFAs, como 16:0 e 18:0, MUFAs, como

18:1n-9 e também PUFAs n6 como o 18:2n-6, mas não possuem quantidades significativas de LC-PUFAs n-3 e n-6 como EPA, ARA e DHA (Glencross, 2009). Como peixes marinhos possuem baixa atividade das enzimas dessaturases e elongases, apenas a inclusão de OV na dieta, não é suficiente para satisfazer as necessidades nutricionais para estes AGs (Tocher, 2003; Glencross, 2009; Turchini et al., 2009). Como o perfil de AGs de alguns tecidos em peixes geralmente reflete a composição da dieta, a substituição de OP por OV pode acarretar na deficiência de LC-PUFA influenciando também na qualidade do pescado a ser consumido (Teles et al., 2015).

Nos últimos anos diversos estudos foram conduzidos objetivando substituir OP por OV. No entanto, a substituição total de OP por OV nas dietas para peixes de cultivo impacta negativamente o metabolismo lipídico dos animais, reduzindo os níveis de LC-PUFA nos tecidos (Bell et al., 2004; Menoyo et al., 2004; Tocher, 2010). Uma forma de evitar tais efeitos é suplementar as dietas com óleos ou extratos produzidos a partir de algas marinhas, que normalmente contêm quantidades elevadas de LC-PUFAs (Trushenski et al., 2011 Tocher, 2015).

Em experimento realizado com *Atractoscion nobilis* no qual avaliou-se a inclusão de DHA, Rombenso e colaboradores (2015) observaram que este AG fica mais disponível para os tecidos como músculo, nos animais alimentados com dietas a base de óleo de soja, enriquecida com SFA e suplementada com DHA. Além disso, quando os animais são alimentados com dietas com substituição total do OP por SFA ou até mesmo MUFAs, a deposição de LC-PUFAs n-3, como DHA, nos tecidos foi semelhante ao padrão observado em animais que foram alimentados apenas com OP. Esta característica demonstra um mecanismo no qual os LC-PUFAs, quando ofertado em menores quantidades, são poupados (*sparing effect*) em detrimento dos SFA e

MUFAs, os quais sugere-se que sejam preferencialmente catabolizados pela via de β-oxidação (Turchini et al., 2011a,b ; Tocher 2003).

Contudo, apesar de SFAs e MUFAs serem substratos comumente catabolizados em processos de β-oxidação, a formulação de dietas contendo altas quantidades desses AGs deve ser cuidadosamente avaliada, pois estes AGs podem influenciar no crescimento dos animais, no metabolismo de AGs e na deposição lipídica nos tecidos (Salini et al., 2015b). Além disso, fatores como digestibilidade e a expressão de genes relacionados à β-oxidação e síntese de AGs também podem ser afetados quando a razão SFA/MUFA é drasticamente alterada (Salini *et al*., 2015a; Mata-Sotres et al., 2021). Juvenis de *Cromileptes altivelis* alimentados com dietas contendo altos níveis de SFAs e MUFAs, sem uma adequada suplementação de LC-PUFA, apresentaram redução no crescimento (Smith et al., 2005). Outros estudos demonstram que juvenis de *Sparus aurata* L., alimentados com dieta rica em SFA, durante 6 meses, tiveram digestibilidade lipídica e crescimento reduzidos (Fountoulaki et al., 2009).

A substituição de OP por fontes alternativas também interfere no perfil de AGs do tecido muscular, reduzindo os níveis de n-3 LC-PUFA com o aumento das quantidades de OV da dieta (Martinez-Lorens et al., 2007). Outros tecidos como fígado também podem ser diretamente afetados com a inclusão de OV nas dietas, como observado por Mozanzadeh e colaboradores (2016) que demonstraram que peixes alimentados com dietas com total substituição de OP por óleo de canola tiveram quantidades inferiores de EPA no fígado. O mesmo estudo mostrou que a substituição total de OP por OV resultou em menores concentrações de DHA, tanto no fígado quanto no músculo, em juvenis de *Sparidentex hasta*. Além da influência no metabolismo lipídico e crescimento, peixes alimentados com dietas à base de OV

podem apresentar alterações histopatológicas, como esteatose hepática e enterites intestinais, como observado em *Sparus aurata* (Caballero et al., 2002, 2004).

A composição da dieta influencia diretamente em processos metabólicos como a transcrição de genes relacionados com a síntese e β-oxidação de AGs (Araújo et al., 2016). Em peixes, os resultados relacionados com a expressão de genes responsáveis pela síntese de AG como ácido graxo desaturase (*fads*), elongase (*elovl*), ácido graxo sintase (*fas*), estearoil *CoA* desaturase (*scd*), ATP citrato liase (*acyl*) e acetil CoA carboxilase (*acc*) e pela β-oxidação de AG como carnitina palmitoiltransferase (*cpt-1*), acil-CoA desidrogenase de cadeia longa (*acadvl*) e acil-CoA oxidase (*acox*), ainda são contraditórios. Alguns estudos encontraram um aumento na expressão da maioria destes genes em salmão do Atlântico (*Salmo salar*) alimentados com dietas ricas em LC-PUFAs (Ostbye et al., 2009; Torstensen et al., 2009), enquanto outros, utilizando a mesma espécie, mostraram um padrão oposto (Jordal et al., 2005; Pratoomyot et al., 2008; Zheng et al., 2004). Essas diferenças podem ser justificadas pela influência de outros fatores como particularidades fisiológicas da espécie, hábito de vida, massa corpórea, estágio de desenvolvimento e condições de cultivo (Stubhaug et al., 2006). Desta forma, a caracterização da expressão destes genes pode ser uma importante ferramenta para compreender os mecanismos fisiológicos relacionados com crescimento e metabolismo lipídico de animais de cultivo, contribuindo para a formulação de dietas específicas de menor custo e no geral, mais sustentáveis.

Popularmente conhecido no Brasil como bijupirá, *Rachycentron canadum* é um teleósteo marinho, pelágico e de hábito alimentar carnívoro com ótimo potencial produtivo devido ao rápido crescimento, podendo alcançar de 4-6 Kg em apenas um ano de cultivo, com conversão alimentar próxima de 1,5:1,0, alta resistência às condições de cultivo e, uma carne branca de alto valor comercial (Benetti et al., 2008;

Chou et al., 2004). Por ser uma espécie marinha e estritamente carnívora, o bijupirá necessita de uma dieta rica em LC-PUFA (Fraser e Davies, 2009). Alguns trabalhos avaliaram aspectos do metabolismo de AGs de bijupirás alimentados com diferentes concentrações de LC-PUFA como EPA, DHA e ARA, visando encontrar níveis adequados desses AGs para cumprir suas necessidades nutricionais (Trushenski et al., 2012; Araújo et al., 2019). Contudo, até o presente momento, não foram realizados estudos que investigaram o metabolismo lipídico desta espécie relacionando o perfil de AGs em resposta a uma dieta utilizando OV ricos em SFA e MUFAs e suplementados com LC-PUFA.

Com isso, o seguinte estudo teve como objetivo investigar alterações no metabolismo lipídico e no desempenho de crescimento de juvenis de *Rachycentron canadum* alimentados com fontes lipídicas ricas em SFA e MUFAs e suplementadas com LC-PUFA, em detrimento ao OP. Os resultados irão contribuir diretamente para a produção de dietas específicas para o bijupirá, utilizando fontes lipídicas de menor custo, de produção mais estável e menos impactante que o OP. Além disso, os resultados irão gerar informações importantes sobre o metabolismo lipídico de *R. canadum* que hoje pode ser considerada a espécie mais promissora para a piscicultura marinha nacional.

Capítulo 1

Different saturated and monounsaturated fatty acids levels in fish oil-free diets to cobia (*Rachycentron canadum*) juveniles: Effects in growth performance and lipid metabolism

Publicado na revista Aquaculture 541, 736843, 2021[1]

Victor H. Marques., Renata G. Moreira., Giovana S. Branco., Renato M. Honji., Artur N. Rombenso., Maria TeresaViana., Paulo H. de Mello., José A. Mata-Sotres., Bruno C. Araújo. 2021. Different saturated and monounsaturated fatty acids levels in fish oil-free diets to cobia (*Rachycentron canadum*) juveniles: Effects in growth performance and lipid metabolism. Aquacutlure, 541, 736843. https://doi.org/10.1016/j.aquaculture.2021.736843

Victor H. Marques[a], Renata G. Moreira[a], Giovana S. Branco[a], Renato M. Honji[b], Artur N. Rombenso[c], Maria Teresa Viana[d], Paulo H. de Mello[e], José A. Mata-Sotres[f], Bruno C. Araújo[b,g*]

[a]*Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Trav. 14, 321, 05508-090, São Paulo, SP, Brazil*

[b]*Centro de Biologia Marinha, Universidade de São Paulo (CEBIMar/USP), Rodovia Manoel Hipólito do Rego, km 131,5, São Sebastião, SP, 11612-109, Brazil*

[c]*CSIRO Agriculture and Food, Livestock & Aquaculture Program, Bribie Island Research Center, Woorim, QLD, 4507, Australia*

[d]*Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California (UABC), km 107 carretera Tij/Eda, 22860 Ensenada, Baja California, Mexico*

[e] *Centro de Aquicultura, Universidade Estadual Paulista "Júlio de Mesquita Filho" (CAUNESP), Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil*

[f] *CONACYT, Instituto de Investigaciones Oceanológicas, UABC, Ensenada, BC, Mexico*

[g] *Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes. Av. Dr. Cândido Xavier de Almeida Souza, 200, 08780-911, Mogi das Cruzes, SP, Brazil*

*Corresponding author: brunoaraujo@umc.br (B.C, Araújo)

Phone number: +55 (11) 94382-9667

Highlights

• First study of dietary SFA:MUFA ratio on performance and FA metabolism of cobia.

• FO-free diets did not hinder the production performance of cobia juveniles.

• High dietary 12:0 stimulate FA catabolism and preserve LC-PUFAs in cobia fillets.

• 12:0 retention in the tissues was not proportional to the dietary levels.

• 18:1n-9 was more retained in the tissues reflecting its dietary levels.

1. Introduction

Fish oil (FO) has traditionally been the primary lipid source in marine commercial aquafeeds, used to provide long-chain polyunsaturated fatty acids (LC-PUFA), which are physiologically required by marine organisms (Bell and Sargent, 2003; Glencross, 2009; Oliva-Teles et al., 2015; Trushenski and Rombenso, 2020). These fatty acids (FA), mainly docosahexaenoic acid (22:6n-3 - DHA), eicosapentaenoic acid (20:5n-3 - EPA), and arachidonic acid (20:4n-6 - ARA), participate in several crucial physiological roles in fish. Among those, growth, reproduction, synthesis of gonadal steroid hormones, function and structure of cellular membranes, and immune system modulation (Izquierdo et al., 2001; Sorbera et al., 2001; Calder, 2006; Arts and Kohler, 2009).

However, the decreasing or static wild stocks fish populations which, apart from being used for human consumption, has led to a shortage of FO availability that hinders its use in aquafeed formulations (Tacon and Metian, 2008; Sprague et al., 2015; Palomares et al., 2020). Although aquaculture continues to grow and solidifies as an important worldwide economic activity, FO must be judiciously used, and alternatives lipid sources, must be promoted to support sustainability (Tocher, 2003; Glencross, 2009; Turchini et al., 2009, 2018). While the vegetable oils (VO) emerge as the main alternative to fully replace FO from aquafeeds (Turchini et al., 2009, 2011a; Tocher, 2015; Oliva- Teles et al., 2015), its low content in LC-PUFA can negatively impact the lipid metabolism and performance of teleost species. Additionally, it can result in histopathological changes, such as hepatic steatosis and intestinal enteritis (Spisni et al., 1998; Caballero et al., 2004; Carvalho et al., 2021). Therefore, high inclusion levels of VO in aquafeeds drastically reduce the tissue LC-PUFA, impairing animal health and human nutrition (Tocher, 2015; Lange, 2020; Schulze et al., 2020).

Current studies have demonstrated that saturated (SFA) and monounsaturated (MUFA) fatty acid incorporation in marine aquafeeds when adequately supplemented with LC-PUFA result in positive production performance with high retention of DHA and EPA, a physiological process named LC-PUFA “sparing effect” (Turchini et al., 2011b). For example, white seabass (*Atractoscion nobilis*) retained high levels of DHA in the muscle tissue when fed diets composed of hydrogenated soybean oil (rich

in SFA) and supplemented with DHA compared to the fish fed with a diet based on FO (Rombenso et al., 2015). Similar results were observed in *R. canadum* (Trushenski et al., 2013a; Woitel et al., 2014a, 2014b; Araújo et al., 2019), and other marine fish species such as *Trachinotus carolinus* (Rombenso et al., 2016a), *Seriola dorsalis* (Rombenso et al., 2016b, 2018), *Totoaba macdonaldi* (Mata-Sotres et al., 2018), *Morone saxatilis* (Araújo et al., 2021), *Dicentrarchus labrax* (Eroldoğan et al., 2013), and *Schophthalmus maximus* (Xu et al., 2021).

Therefore, to reduce the FO content in aquafeeds while maintaining the optimum LC-PUFA levels in the fish tissues, especially in muscle, several research groups are testing cheaper, sustainable and high available lipid sources, such as VO or terrestrial animal by-products. These lipid sources are generally composed by high levels of SFA and MUFA, and properly supplemented with EPA and DHA rich oils, normally produced from marine algae species; providing positive results related to production performance and high retention of DHA and EPA in tissues of marine fish species through the LC-PUFA sparing effect (Woitel et al., 2014a, 2014b; Araújo et al., 2018, 2019; Rombenso et al., 2018).

Rachycentron canadum, also known as cobia, is a carnivorous marine teleost with great productive potential due to high growth rates, reaching from 4 to 6 Kg in a single year. It also presents a feed conversion ratio (FCR) around 1.5, high resistance to handling, and excellent fillet quality (Chou et al., 2004; Benetti et al., 2008). As *R. canadum* is a strict carnivorous species, it is highly dependent on LCPUFA (Fraser and Davies, 2009). Previous studies assessed aspects of the FA metabolism of *R. canadum* fed with different dietary LC-PUFA levels aiming to estimate values of adequate DHA, EPA, and ARA levels to fulfill its nutritional requirements (Trushenski et al., 2012; Araújo et al., 2019). However, studies evaluating cobia lipid metabolism are still unknown, particularly those relating the FA use on FO-free diets rich in SFA or MUFA. Thus, this study aimed to investigate the influence of SFA and MUFA lipid source levels in FO-free diets adequately supplemented with LC-PUFA on the performance and lipid metabolism of *R. canadum* juveniles. This study provides essential information related to the cobia lipid metabolism, contributing towards a more sustainable and specific FO-free diet for this important commercial species.

2. Material and methods

2.1. Experimental diets

The formulation and FA composition of the experimental diets are shown in Tables 1 and 2, respectively. Four isolipidic, isoproteic, and isoenergetic diets were formulated using different lipid sources. The FOD (control diet) was constituted by 88 g Kg−1 of FO as a unique lipid source; the MUFA-D contained 10 g Kg−1 of coconut oil and 60 g Kg−1 of olive oil, the SFA-D contained 60 g Kg−1 of coconut oil and 10 g Kg−1 of olive oil, while the MIX-D consisted of 35 g Kg−1 of each VO (Table 1). The fishmeal used was previously defatted by immersion and washing in hexane – fishmeal 3:1 solution. The diets were manufactured, and supplemented by vacuum coating with the different experimental oils, respecting the basic nutritional FA requirements described for this species (Fraser and Davies, 2009; Trushenski et al., 2011; Araújo et al., 2019). The different LC-PUFA (ARA, EPA, and DHA rich-oils) were added at similar concentrations across the experimental diets (3, 5, and 10 g Kg−1, respectively) meeting the cobia LC-PUFA requirements (Trushenski et al., 2012; Araújo et al., 2019). The experimental diets contained different SFA and MUFA ratios using different proportions of coconut and olive oil. The diets were processed throughout a standard extrusion protocol described by Araújo et al. (2019), dried in a forced air circulation oven, and stored at −20 °C until the beginning of the experiment.

2.2. Fish handling and experimental design

Fish handling followed the Ethics Committee's procedure protocols on the Use of Animals (CEUA n° 356/2019) of the Institute of Biosciences, University of São Paulo (IB/USP). A total of 226 cobia juveniles, were obtained from Redemar Alevinos (Ilha Bela, São Paulo/Brazil) and were transported to the marine experimental facilities at the Marine Biology Center of the University of São Paulo (CEBIMar/ USP, São Sebastião, São Paulo/Brazil). The fish were kept to the new conditions for 15 days in a 10,000 L tank and were hand-fed until apparent satiation twice a day (08:00 and 16:00 h), with a commercial diet (Guabipirá® - Guabi). After that, the animals were selected according to average body mass (25.53 ± 4.53 g) and transferred to 12–1000 L tanks (18 animals per tank, with three repetitions per treatment) in a flow-through seawater system. The temperature was maintained at

24.7 ± 1.29 °C, and seawater was supplied continuously at 10 L min−1, the salinity was 31 PSU, and the dissolved oxygen at 5.9 ± 0.6 mg L−1. The water quality was analyzed every 3 days and contained at lower levels than: total ammonia nitrogen (TAN) 0.05 mg L−1, nitrite 0.05 mg L−1, and the nitrate 1.20 mg L−1. Temperature and dissolved oxygen were measured daily (YSI model 55, YSI Inc., Yellow Springs, OH, USA) (API test kits, Mars Fishcare Inc., Chalfont, PA, USA). At the beginning of the experiment, ten animals from the initial stock were subjected to benzocaine overdose (0.1 g/L) to collect aliquots of liver and muscle tissues sampled, immediately frozen in liquid nitrogen, and then transferred to an ultra-freezer (−80 °C) until processing.

The experimental fish were hand-fed twice a day (08:00 and 16:00 h) until apparent satiation with the respective experimental diet for a total of 8 weeks (56 days). At the end of the nutritional trial, all experimental animals were anesthetized (in benzocaine overdose), and the biometric data were registered. Then, three fish per tank (in total nine per treatment) were euthanized by decapitation at the level of the operculum, and samples of liver and muscle were immediately frozen in liquid nitrogen for posteriorly molecular and metabolic analyzes. Additionally, liver samples were quickly removed, fixed in 4% formalin for 20–24 h, and then washed in running water and preserved in 70° GL ethanol until the routine histological analyses.

Table 1 Formulation and proximate composition of experimental diets (g Kg^{-1}) containing different proportions of SFA and MUFA.

	Dietary SFA/MUFA levels (g kg^{-1})			
Ingredients	*FO-D*	*SFA-D*	*MIX-D*	*MUFA-D*
Defatted Fish meal [a]	550.0	550.0	550.0	550.0
Wheat flour [b]	130.0	130.0	130.0	130.0
Squid meal [c]	78.0	78.0	78.0	78.0
Tapioca [d]	80.0	80.0	80.0	80.0
Hemoglobin [e]	30.0	30.0	30.0	30.0
Taurine [f]	5.0	5.0	5.0	5.0
Premix Min. Vit. [g]	28.0	28.0	28.0	28.0
Stay C [h]	10.0	10.0	10.0	10.0
Sodium Benzoate [i]	0.1	0.1	0.1	0.1
BHT [j]	1.0	1.0	1.0	1.0
Fish Oil [l]	88.0	-	-	-
Coconut oil [m]	-	60.0	35.0	10.0
Olive oil [n]	-	10.0	35.0	60.0
EPA oil 45% [o]	-	5.0	5.0	5.0
DHA oil 50% [p]	-	10.0	10.0	10.0
ARA oil 40% [q]	-	3.0	3.0	3.0
Proximate composition (g kg^{-1})				
Crude Lipid	136.23	137.28	137.85	137.39
Crude Protein	465.70	466.40	465,.5	464.36
Dry Matter	959.09	958.93	959.05	959.06
Ash	97.76	97.02	98.11	97.99
NFE	300.31	299.30	298.19	300.26

NFE (g Kg^{-1}) including fiber = 1000 – (crude protein + crud lipid + ash).
[a] Fish meal (defatted by 3 × hexane extraction) and [l]fish oil of South American origin;
[c] Squid meal South American origin;
[h] Vitamin and mineral premix (IU kg−1 or g/kg of premix): vitamin A, 2.5MIU; vitamin D3, 0.25 MIU; vitamin E, 16.7 g; vitamin K3, 1.7 g; vitamin B1, 2.5 g; vitamin B2, 4.2 g; vitamin B3, 25 g; vitamin B5, 8.3; vitamin B6, 2.0 g; vitamin B9, 0.8; vitamin B12, 0.005 g; biotin, 0.17 g; vitamin C, 75 g; choline, 166.7 g; inositol, 58.3 g; ethoxyquin, 20.8 g; copper, 2.5 g; ferrous iron, 10.0 g; magnesium, 16.6 g; manganese, 15.0 g; zinc, 25.0 g;
[a,b,c,d,e,f,g,h,i,j,l] Nutricon Ltda-Me, São Paulo, Brazil;
[m] Copra Indústria Alimentícia Ltda., Alagoas, Brazil;
[n] Victor Guedes, Ind. Com. S.A., Abrantes, Portugal;
[o] EPA concentrated fish oil (> EPA 45%), Phosphotech Laboratories, ZAC de la Lorie, France.
[p] Incromega™ DHA 500 TG (> 50%DHA), CRODA™, Snaith, East Yorkshire, UK.
[q] ARA concentrated oil (> 40% ARA), Jangsu Tiankai Biotechnology Co., Ltd., Nanjing, China.

Table 2 Fatty acid profile of the experimental diets (% of total fatty acids).

	Diets (%)				Oils (%)		
Fatty acids	*FO-D*	*SFA-D*	*MIX-D*	*MUFA-D*	*ARA*	*EPA*	*DHA*
12:0	-	22.61	12.54	4.17	nd.	nd.	nd.
14:0	8.69	10.24	7.87	4.20	2.29	0.49	0.78
16:0	25.70	15.57	18.17	18.18	8.96	20.44	18.96
16:1n-7	8.79	2.91	3.59	3.57	0.11	0.36	0.28
18:0	6.00	4.55	4.65	4.82	9.70	0.38	0.75
18:1n-9	18.40	16.84	25.99	36.68	28.02	1.35	0.34
18:1n-7	3.62	1.68	2.13	2.55	1.46	0.79	nd
18:2n-6	6.43	5.64	6.22	7.19	n.d.	n.d.	n.d.
18:3n-3	0.90	0.60	0.71	0.75	n.d.	n.d.	0.26
20:1n-9	1.09	0.89	0.90	0.98	0.18	n.d.	0.19
20:4n-6	1.45	2.00	1.88	2.10	44.95	n.d.	n.d.
20:5n-3	5.09	4.70	4.78	4.93	n.d.	53.15	11.53
22:6n-3	6.93	6.87	6.62	6.56	n.d.	12.59	60.27
∑SFA	40.93	56.24	45.56	32.66	21.82	21.31	20.70
∑MUFA	32.67	22.31	32.60	43.78	29.79	2.50	0.81
∑LC-PUFA	13.47	13.57	13.28	13.59	44.95	65.74	72.06
∑PUFA	20.80	19.82	20.21	21.53	44.95	65.74	72.06
∑n-3 PUFA	12.92	12.17	12.11	12.24	n.d.	65.74	72.06
∑n-6 PUFA	7.88	7.64	8.10	9.29	44.95	n.d.	n.d.
SFA/MUFA	1.25	2.53	1.40	0.75	0.73	0.52	25.56

ΣSFA, ΣMUFA, ΣPUFA, Σn-3 PUFA, Σn-6 PUFA and ∑LC-PUFA are the sum of saturated, monounsaturated, polyunsaturated, polyunsaturated n3, polyunsaturated n6 and, long-chain polyunsaturated fatty acids, respectively. SFA/MUFA is the ratio between the sum of total SFA and MUFA.

2.3. Productive performance and biological indexes

At the end of the 8-week feeding trial, the production performance was assessed as the following metrics:

Feed intake (%/d)=100×total amount of the feed consumed (g) / (Wo+Wt)/2]/t.

Weight gained (g)=Wt-Wo.

Specific growth rate (SGR, %d)=(LnWt-LnWo)×100/t.

Feed conversion rate (FCR, g)=feed consumed (g)/weight gain (g).

Hepatosomatic index (HSI, %) =100×(liver weight/Wt).

Viscerosomatic index (VSI, %) =100×(visceral weight/Wt).

Liserosomatic index (LSI, %) =100×(intraperitoneal fat weight/Wt).

Survival rate (SR, %)=Nt×100/No

Wt and Wo corresponds to final and initial fish weight, respectively; Nt and No were final and initial number of fish, respectively; t was the duration of the experiment in days.

2.4. Proximate composition and fatty acid profile

All experimental diets were analyzed according to AOAC (2015). Dry weight and ash content were determined by drying ground samples at 60 °C for 24 h, followed by carbonization in a muffle furnace at 550 °C for 6 h. Crude protein was analyzed by the micro-Kjeldahl method, and the content was calculated by nitrogen conversion (%N × 6.25). The lipid composition of the experimental diets was quantified according to Soxhlet method using petroleum ether as a carrier. Total lipids of the liver and muscle were extracted according to Folch et al. (1957) and quantified, according to Frings et al. (1972). The FA profile of liver and muscle was initiated by lipid extraction using dichloromethane and further methylated following the transmethylation method described by Parrish et al. (2014). The FA composition was analyzed as methyl esters (FAME) using gas chromatograph Scion 436 equipped with a flame ionizer (FID) and CP 8410 auto-sampler. The capillary column used to analyze the FA was CP Wax, 0.25 μm thickness, 0.25 mm inner diameter, and 30 m length. Hydrogen was used as a carrier gas at a linear velocity of 1.4 mL/min cm/s. The column was programmed at 170 °C for 1 min, followed by a 2.5 °C/min ramp to 240 °C and a final hold time of 5 min. The injector and FID temperatures were 250 and 260 °C, respectively. FAME were identified by comparing their retention times to those obtained from commercial standards (Supelco, 37 components; Sigma-Aldrich). Data are presented as a percentage of total FAME based on peak area analyzed.

2.5. RNA extraction and quantitative real-time PCR

Total RNA from liver samples was purified using Trizol Reagent (Sigma®) according to the manufacturer's instructions. To eliminate potential genomic DNA contamination, the samples were treated with DNase using Turbo DNAfree (AM1907 Invitrogen™). The quantity and quality of RNA were measured using gel electrophoresis and spectrophotometer (Nanodrop® One, Thermo Fisher Scientific INC, Wilmington, USA). Only RNA samples with OD 260/280 ratio between 1.90 and 2.10 were used for expression quantification. Total RNA (500 ng) were used as a

template to synthesize cDNA using SuperScript II Reverse Transcriptase (18,064,014 Invitrogen™) according to the manufacturer's instructions. Negative controls with no reverse transcriptase were also performed, subsequently, the cDNA stored at −20 °C.

The primers used in this study (cpt, fas. Lpl and b-actin), were obtained from previous studies performed with this same species (Luo, 2013; Wang et al., 2016) and manufactured by Thermofisher S.A (Invitrogen ™) (Table 3). Conventional PCR amplification was performed to amplify specific products using Top Taq MasterMix (200,403 Qiagen), as follows: initial denaturation at 95 °C for 5 min, 35 denaturation cycles at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, followed by 10 min for the final extension. Amplified PCR products were electrophoresed in 1% agarose gel containing ethidium bromide (Invitrogen™) in Tris-Acetate-EDTA (TAE) buffers and specific bands were visualized by excitation with UV light. To construct standard curves of absolute copy numbers of these genes, the PCR product was purified with 3 M sodium acetate pH 7.0, the DNA was precipitated, and concentration was determined by NanoDrop One Spectrophotometer (ThermoFisher).

The amplification efficiency of primers was evaluated using absolute copy numbers, which the 109–101 DNA copies. All primers used in this study presented efficiency higher to 90%. The relative expression of transcripts in liver (n = 6) was analyzed with the Step One Real-Time PCR System (Applied Biosystems) and calculated using the Fold change method. The reactions were carried out using SYBR-Green Universal Master Mix, 300 nM of each primer (forward and reverse), 5 μL of cDNA (1:100), in a total volume of 20 μL. The cycle thresholds (Cts) were determined, and the reaction included 10 min at 95 °C (maintenance phase), 15 s at 95 °C, followed by 1 min at 60 °C (40 denaturation cycles - cycling phase), and a melting curve of 60–95 °C. The relative mRNA levels of the target genes were normalized using β -actin as an endogenous reference gene, and the relative values were expressed for the expression values of the FO-D group (e.g., fold induction change). The transcript level of each gene was calculated relative to the levels of the other genes using the raw cycle threshold value for each gene and then normalized against.

2.6. Liver histology

The sample tissues fixed with formalin solution (4%) were dehydrated in increasing ethanol concentrations, diaphanized in xylol (dimethybenzene), and embedded in paraplast® according to routine histological procedures. Liver sections (5 µm thick) were obtained using a microtome (Leica RM2255), equipped with disposable blades, and mounted on Poly-l-Lysine solution-coated slides. The slides were stained with Hematoxylin-Eosin (Behmer et al., 1976), analyzed with a light microscope (Leica DM1000) coupled to a camera (Leica DFC295), and documented using an image capture system (Leica Application Suite Professional, LAS V3.6). To evaluate the hepatocyte morphology and measure the average area of these cells, the LAS software was used to obtain the images (1260 pixels by 960 pixels). Fifteen cells per section were quantified, and three sections per samples were used (n = 3 animals per tank; n = 45 cells per animal measured; with 100 µm distance between each section; totalizing 300 µm distance from the first section to the last part; 135 cells per experimental groups). The LAS software was also used to obtain the measurements of the average hepatocyte area (in µm2). Similar analyses have been performed in other teleost species with different tissues (Honji et al., 2015; Araújo et al., 2021). As well as liver of *R. canadum* (Araújo et al., 2019).

2.7. Data analyses

The ratio between tissue FA composition and dietary FA composition (tissue/diet) in liver and muscle was calculated using the mean (%) of a specific FA divided by the mean (%) of the same FA in the tissue. The dotted line in both graphics indicates higher (above 1), lower (below 1), or equal levels (1) of a specific FA present in the tissues relating to the same FA in the diets. The results of productive performance, tissues and proximal dietary composition, fatty acid profile, hepatocyte morphology, the ratio between tissue and dietary FA composition, and the liver gene expression passed by normality and equal variance test and were compared between the experimental groups using one-way ANOVAs tests, followed by Tukey's HSD tests using the SigmaStat software for Windows version 3.5 (SigmaStat Software, CA, USA) with a significance level of 5%. Data were presented as mean ± SEM (standard error of the mean).

Table 3 Primers pairs used for q-PCR analyses.

Gene	Fwd sequece (5′–3′)	Rev sequence (5′–3′)	Size (bp)	Gene bank reference
fas	ACGGTTACGCCAACTCATC	TGCTTCGCTCTTCACCACC	225	FJ842648.1
cpt-1	TGCTGTTGCCACGGGAGATT	CGCTGCTCGGTGTCATCAAG	180	KT075040
lpl	TGAGCACGCAGATGACCAGAG	AGTCCCTTGATCCCTTCCAGTG	170	KT075046
β-act	AGCCATGGAAGATGAAATCG	TCTCTTGCTCTGGGCTTCAT	190	Lou, 2013

fas: Faty acid synthase; *cpt-1*: carnitine palmitoyltransferase I; *lpl*: Lipase lipoprotein; *β-act: β* actine

3. Results

3.1. Production performance and biological indexes

The results related to the overall production performance and biological indexes are presented in Table 4. While no differences were noticed in production performance, significant differences are observed within the indexes evaluated. Fish fed SFA-D and MIX-D presented higher HSI and VSI values than those fed FO-D, while fish fed MUFA-D did not change those indexes compared to the other treatments.

Table 4 Overall performance and biological indexes of *R. canadum* juveniles fed different experimental diets. Formulated to contain different proportions of SFAs and MUFA (means ± standard error).

Indexes	*FO-D* *n=9*	*SFA-D* *n=9*	*MIX-D* *n=9*	*MUFA-D* *n=9*	*P value*
Initial weight (g)	25.50±0.10	25.67±0.06	25.57±0.06	25.53±0.15	0.274
Final weight (g)	100.73±2.86	107.49±4.87	103.09±3.55	98.56±6.42	0.186
Feed intake [1]	1.02±0.06	1.00±0.07	1.03±0.02	0.98±0.07	0.480
Weight Gained [2]	84.32±4.68	94.94±5.96	85.61±3.90	87.08±8.18	0.199
SGR [3]	2.45±0.06	2.56±0.08	2.49±0.06	2.41±0.11	0.233
FCR [4]	1.16±0.08	1.11±0.09	1.17±0.04	1.11±0.10	0.666
HSI [5]	19.23±0.14^{a}	26.01±1.26^{b}	24.08±1.62^{b}	21.02±2.81^{ab}	0.006
VSI [6]	6.73±0.13^{a}	7.89±0.61^{b}	7.70±0.20^{b}	7.44±0.50^{ab}	0.038
LSI [7]	25.39±1.28	28.98±3.12	27.42±1.56	26.77±1.15	0.232
Survival rate [8]	100.00±0.00	100.00±0.00	100.00±0.00	98.15±3.21	0.441

^{a,b} Different letters mean statistical differences of the indexes between animals fed different experimental diets – Tukey test ($P < 0.05$).
[1]Feed intake (%/d)=100×total amount of the feed consumed (g) / [(Wo+Wt)/2]/t.
[2]Weight gained (g)=Wt-Wo.
[3]Specific growth rate (SGR, %d)=(LnWt-LnWo)×100/t.
[4]Feed conversion rate (FCR, g)=feed consumed (g)/weight gain (g).
[5]Hepatosomatic index (HSI, %) =100×(liver weight/Wt).
[6]Viscerosomatic index (VSI, %) =100×(visceral weight/Wt).
[7]Liserosomatic index (LSI, %) =100×(intraperitoneal fat weight/Wt).
[8]Survival rate (SR, %)=Nt×100/No

3.2. Total lipids and fatty acid profile of liver and muscle

Liver and muscle total lipids and FA profiles are presented in Tables 5 and 6, respectively. Despite to not be observed significant differences between the experimental groups in total lipids in both tissues, animals from SFA-D and MIX-D showed a trend to deposit higher lipid levels in the liver compared to FO-D and MUFA-D, while animals from MIX-D and MUFA-D showed higher lipid levels in the muscle compared to those from FO-D and SFA D. In general, most dietary FA were reflected in liver and muscle. Fish fed SFA-D presented the highest 12:0 levels in muscle and liver than those fed the other diets. In the liver of fish fed SFA-D and FO-D diets, it was observed higher percentages of 16:0 than those fed MIX-D and MUFA-D diets. Similarly, a higher 16:0 percentage in muscle of fish fed FO-D was observed compared to the other experimental groups, with no difference among the animals from the other treatments. In both tissues, 18:1n-9 was significantly higher in fish fed MUFA-D followed by MIX-D, SFA D, and FO-D, respectively. As expected, fish fed SFA-D presented higher SFA content, while those fed MUFA-D showed higher MUFA content in both tissues. The SFA/MUFA ratio in liver and muscle augmented as the dietary SFA levels were increased. Thus, higher SFA/MUFA ratio was observed in fish fed SFA-D than those fed the other experimental diets. Fish fed FO-D showed higher EPA levels in muscle and liver than fish fed FO-free diets (SFA D, MIX-D, and MUFA-D). The DHA content in the liver was higher in the FO-D group than in SFA D, while MUFA-D and MIX-D presented similar levels. No statistical difference in DHA content in muscle was observed in animals fed distinct experimental diets. Similarly, no differences in ARA percentage were found among different treatments in both tissues. LC-PUFA and n-3 PUFA were higher in liver of fish fed FO-D, while no significant differences were observed in animals from the other experimental groups.

Table 5 Liver fatty acid composition (% of total fatty acids) and total lipids (mg g^{-1} of wet weight) of *R. canadum* juvenile fed different experimental diets.

Fatty acids	*FO-D*	*SFA-D*	*MIX-D*	*MUFA-D*	*P value*
12:0	0.23 ± 0.16 [a]	5.22 ± 0.41 [b]	3.08 ± 0.25 [c]	0.85 ± 0.15 [a]	<0.001
14:0	5.71 ± 0.60 [a]	8.03 ± 0.60 [b]	5.22 ± 0.38 [a]	3.09 ± 0.42 [c]	<0.001
16:0	24.56 ± 0.94 [a]	23.37 ± 0.84 [a]	21.00 ± 0.26 [b]	19.84 ± 0.39 [b]	<0.001
16:1n-7	12.10 ± 0.88 [a]	8.24 ± 0.42 [c]	7.21 ± 0.34 [bc]	6.27 ± 0.19 [b]	<0.001
18:0	3.30 ± 0.32	3.14 ± 0.19	3.00 ± 0.28	2.89 ± 0.37	0.327
18:1n-9	22.97 ± 1.03 [a]	28.64 ± 1.17 [b]	35.46 ± 1.66 [c]	39.60 ± 0.73 [d]	<0.001
18:1n-7	4.46 ± 0.13 [a]	3.26 ± 0.25 [c]	3.47 ± 0.22 [bc]	3.78 ± 0.13 [b]	<0.001
18:2n-6	7.20 ± 0.51 [ab]	6.19 ± 0.29 [b]	6.40 ± 0.51 [b]	7.75 ± 0.67 [a]	<0.001
18:3n-3	0.70 ± 0.07 [a]	0.35 ± 0.07 [b]	0.51 ± 0.06 [bc]	0.65 ± 0.14 [ac]	<0.001
20:1n-9	0.67 ± 0.08	0.59 ± 0.10	0.68 ± 0.13	0.64 ± 0.05	0.616
20:4n-6	1.90 ± 0.07	2.06 ± 0.18	2.03 ± 0.09	2.21 ± 0.12	0.068
20:5n-3	7.29 ± 0.58 [a]	3.77 ± 0.16 [b]	4.10 ± 0.51 [b]	4.11 ± 0.21 [b]	<0.001
22:6n-3	8.91 ± 0.81 [a]	7.14 ± 0.62 [b]	7.84 ± 0.72 [ab]	8.32 ± 0.66 [ab]	0.010
∑SFA	33.80 ± 0.70 [a]	39.75 ± 0.94 [b]	32.29 ± 0.42 [a]	26.67 ± 0.53 [c]	<0.001
∑MUFA	40.21 ± 0.79 [a]	40.73 ± 1.20 [a]	46.83 ± 1.65 [b]	50.30 ± 0.80 [c]	<0.001
∑LC-PUFA	18.10 ± 0.92 [a]	12.97 ± 0.54 [b]	13.97 ± 1.29 [b]	14.63 ± 0.92 [b]	<0.001
∑PUFA	25.99 ± 1.43 [a]	19.51 ± 0.34 [b]	20.88 ± 1.83 [bc]	23.03 ± 0.92 [ac]	0.001
∑n-3 PUFA	16.90 ± 0.90 [a]	11.26 ± 0.66 [b]	12.45 ± 1.24 [b]	13.07 ± 0.87 [b]	<0.001
∑n-6 PUFA	9.10 ± 0.57 [ab]	8.26 ± 0.46 [a]	8.43 ± 0.59 [a]	9.96 ± 0.56 [b]	0.019
SFA/MUFA	0.84 ± 0.01 [a]	0.98 ± 0.05 [b]	0.69 ± 0.02 [c]	0.53 ± 0.01 [d]	<0.001
Total lipids	202.54 ± 27.71	292.96 ± 22.63	311.52 ± 32.27	229.20 ± 17.01	0.011

Values represent means ± standard deviation (n=3). ΣSFA, ΣMUFA, ΣPUFA, Σn-3 PUFA, Σn-6 PUFA and ∑LC-PUFA are the sum of saturated, monounsaturated, polyunsaturated, polyunsaturated n-3, polyunsaturated n-6 and long-chain polyunsaturated fatty acids, respectively. SFA/MUFA is the ratio between the sum of total SFA and MUFA. [ab] Different letters indicate statistical differences between experimental diets, by Tukey's test ($P < 0.05$). FO-D – Fish Oil; SFA-D – Diet rich in saturated fatty acids; MIX-D – Diet with the same percentages of saturated and monounsaturated fatty acids; MUFA-D – Diet rich in monounsaturated fatty acids.

In general, in the liver (Fig. 1) and muscle (Fig. 2) tissues, the deposition of SFA was not proportional to the inclusion of these FA in the diets. The ratio between tissue deposition and diet levels (tissue/diet) of SFA in fish fed SFA-D showed a decrease in the deposition of this FA class in liver and muscle compared to those fed MUFA-D and FO-D. For all dietary treatments, the tissue/diet ratio of SFA was below 1 both in liver and muscle, and this pattern was influenced mainly by 12:0 levels. Besides, fish fed FO-free diets presented a higher ratio of 12:0 compared to those fed FO-D. The tissue levels of these FA were not proportional to the inclusion in the diets since its ratio is below 1 for all dietary treatments in both tissues.

Table 6 Muscle fatty acid composition (% of total fatty acids) and total lipids (mg g^{-1} of wet weight) of *R. canadum* juvenile fed different experimental diets.

FA	*FO-D*	*SFA-D*	*MIX-D*	*MUFA-D*	*P value*
12:0	0.53 ± 0.26 [a]	15.09 ± 2.04 [b]	8.86 ± 1.88 [c]	3.35 ± 0.88 [d]	<0.001
14:0	8.89 ± 1.02 [a]	9.02 ± 0.49 [a]	6.55 ± 0.21 [b]	4.54 ± 0.75 [c]	<0.001
16:0	24.95 ± 1.37 [a]	19.71 ± 0.75 [b]	19.50 ± 0.77 [b]	19.24 ± 0.74 [b]	<0.001
16:1n-7	10.30 ± 0.76 [a]	4.75 ± 0.48 [b]	4.42 ± 0.19 [b]	4.88 ± 0.44 [b]	<0.001
18:0	5.82 ± 0.84	5.59 ± 0.31	5.60 ± 0.20	4.99 ± 0.78	0.286
18:1n-9	19.37 ± 0.76 [a]	22.39 ± 0.73 [b]	30.02 ± 1.41 [c]	35.76 ± 0.76 [d]	<0.001
18:1n-7	4.37 ± 0.40 [a]	2.56 ± 0.10 [b]	3.03 ± 0.04 [c]	3.43 ± 0.10 [c]	<0.001
18:2n-6	7.10 ± 0.44 [a]	6.30 ± 0.09 [b]	6.72 ± 0.13 [ab]	7.80 ± 0.17 [c]	<0.001
18:3n-3	0.83 ± 0.14 [a]	0.43 ± 0.06 [b]	0.49 ± 0.04 [b]	0.69 ± 0.12 [a]	<0.001
20:1n-9	0.75 ± 0.03	0.61 ± 0.11	0.63 ± 0.07	0.70 ± 0.04	0.261
20:4n-6	1.96 ± 0.31	1.95 ± 0.14	2.13 ± 0.16	2.05 ± 0.34	0.804
20:5n-3	7.22 ± 0.89 [a]	4.18 ± 0.14 [b]	4.35 ± 0.47 [b]	4.32 ± 0.36 [b]	<0.001
22:6n-3	7.91 ± 0.56	7.41 ± 0.48	7.72 ± 1.45	8.25 ± 0.98	0.630
∑SFA	40.20 ± 1.54 [a]	49.42 ± 1.07 [b]	40.49 ± 2.21 [a]	32.11 ± 1.90 [c]	<0.001
∑MUFA	34.78 ± 0.24 [a]	30.32 ± 0.58 [b]	38.10 ± 1.29 [c]	44.77 ± 0.45 [d]	<0.001
∑LC-PUFA	17.09 ± 1.45	13.54 ± 0.48	14.19 ± 2.05	14.62 ± 1.56	0.083
∑PUFA	25.02 ± 1.77 [a]	20.27 ± 0.50 [b]	21.40 ± 2.16 [ab]	23.12 ± 1.78 [ab]	0.038
∑n-3 PUFA	15.96 ± 1.37 [a]	12.02 ± 0.35 [b]	12.56 ± 1.91 [ab]	13.26 ± 1.40 [ab]	0.034
∑n-6 PUFA	9.06 ± 0.42 [ab]	8.25 ± 0.15 [a]	8.85 ± 0.29 [a]	9.86 ± 0.39 [b]	0.002
SFA/MUFA	1.16 ± 0.04 [a]	1.63 ± 0.07 [b]	1.06 ± 0.08 [a]	0.72 ± 0.05 [c]	<0.001
Total lipids	11.22 ± 2.62	7.36 ± 1.63	15.65 ± 4.66	15.07 ± 4.41	0.544

Values represent means ± standard deviation (n=3). ΣSFA, ΣMUFA, ΣPUFA, Σn-3 PUFA, Σn-6 PUFA, and ∑LC-PUFA are the sum of saturated, monounsaturated, polyunsaturated, polyunsaturated n-3, polyunsaturated n-6, and long-chain polyunsaturated fatty acids, respectively. SFA/MUFA is the ratio between the sum of total SFA and MUFA. [ab] Different letters indicate statistical differences between animals fed different experimental diets, by Tukey's test ($P < 0.05$). FO-D – Fish Oil; SFA-D – Diet rich in saturated fatty acids; MIX-D – Diet with the same percentages of saturated and monounsaturated fatty acids; MUFA-D – Diet rich in monounsaturated fatty acids.

The ratio between tissue deposition and diet inclusion of 16:0 were observed above the dotted line also in both tissues of fish fed SFA-D compared to those fed other experimental diets. SFA-D displayed a higher ratio of MUFA in liver and muscle than fish fed MUFA-D and FO-D. For all the dietary treatments, the tissue/diet ratio of MUFA was above the dotted line. This pattern was influenced mainly by the 18:1n-9 levels. The ratio between tissue retention and diet inclusion of this FA was higher in fish fed SFA-D than those fed the other experimental diets. Tissue levels of these FA were not proportional to the diet's inclusion since its ratio was above the dotted line for almost all dietary treatments in both tissues. In muscle and liver, the ratio of tissue deposition and diet inclusion of 18:2n-6 were not

different from all dietary treatments. The ratio was above the dotted line for all dietary treatments. The ratio between tissue and diet of ARA in the liver was higher in fish-fed FO-D than fish fed FO-free diets. The same ARA pattern ratio was observed in muscle excepted by the fish fed MIX-D, which had the same tissue/diet ratio of fish fed FO-D. The tissue/diet ratio of EPA in muscle and liver was higher in fish fed FO-D than those fed FO-free diets. For all fish fed FO-free diets, the tissue/diet ratio of EPA where below 1, while for fish fed FO-D the same ratio was observed above 1. In the liver of fish fed SFA D, the tissue/diet ratio of DHA was lower compared to those fed FO-D, and MUFA-D. However, fish fed SFA-D showed the tissue/diet DHA ratio equal to 1, while the fish fed with the other diets demonstrated this ratio above the dotted line. The ratio between tissue DHA retention and DHA dietary inclusion in muscle was not different from all the dietary treatments. In the same tissue fish fed SFA-D showed the tissue/diet DHA ratio equal to 1, while the fish fed with the other diets showed this ratio above 1.

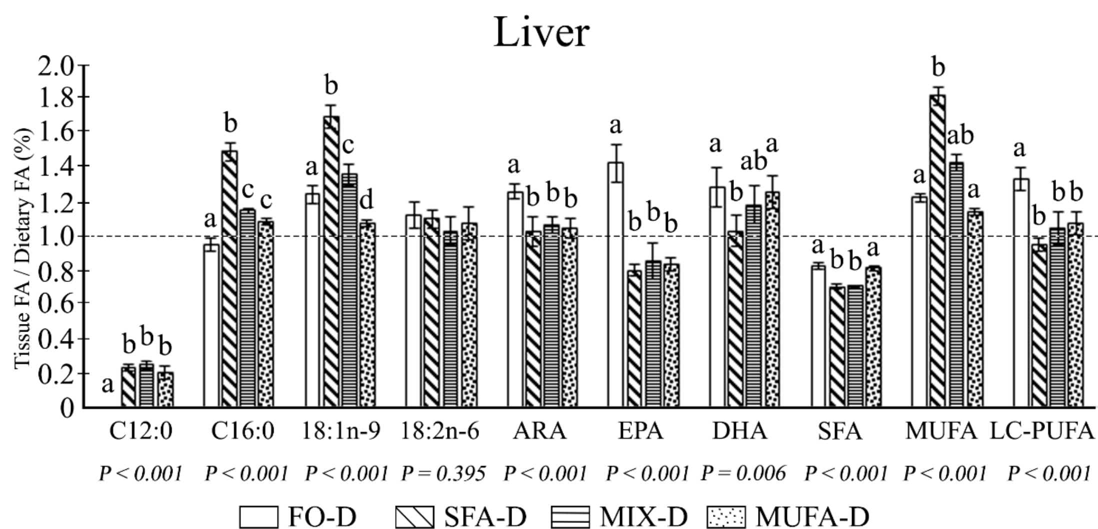

Fig. 1. Ratio between the percentage of total fatty acids in liver tissue of *R. canadum* and the percentage of the same FA in the experimental diet. FO-D – Fish Oil; SFA-D – Diet rich in saturated fatty acids; MIX-D – Diet with the same percentages of saturated and monounsaturated fatty acids; MUFA-D – Diet rich in monounsaturated fatty acids. [ab] Different letters indicate statistical differences among experimental diets, by Tukey's test (*P < 0.05*). The dotted line indicates a ratio of 1 to indicate accumulation (higher than 1) or reduction (lower than 1) of FA in the liver in relation to the same FA in the diets.

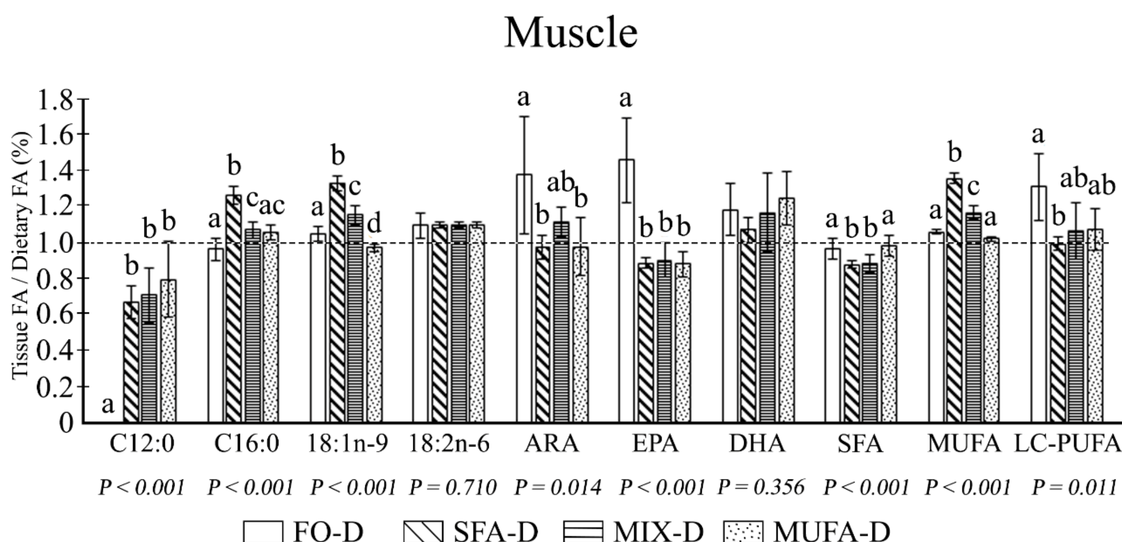

Fig. 2. Ratio between the percentage of total fatty acids in muscle of *R. canadum* and the percentage of the same FA in the experimental diet. FO-D – Fish Oil; SFA-D – Diet rich in saturated fatty acids; MIX-D – Diet with the same percentages of saturated and monounsaturated fatty acids; MUFA-D – Diet rich in monounsaturated fatty acids. [ab] Different letters indicate statistical differences between experimental diets, by Tukey's test ($P < 0.05$). The dotted line indicates a ratio of 1 to indicate accumulation (higher than 1) or reduction (lower than 1) of FA in the liver in relation to the same FA in the diets.

3.3. Gene expression

Data of the relative expression of fas, cpt-1, and lpl are presented in Fig. 5. A single significant difference was observed in the relative expression of fas, which was up-regulated in the liver of FO-D fed fish compared to SFA-D and MIX-D fed animals (P < 0.001). There were no differences in the relative gene expressions of cpt-1 (P = 0.985) and lpl (P = 0.718).

3.4. Liver morphology

The hepatic morphology and lipid vacuole area of the liver of *R. canadum* juveniles fed different experimental diets are presented in Figs. 3 and 4, respectively. The results indicated that the dietary treatments influenced differential hepatocyte morphology (Fig. 3). A gradual decrease in the area of lipid vacuoles was observed in fish fed FO-D, MUFA-D, MIX-D, and SFA D, respectively (P < 0.001) (Fig. 4).

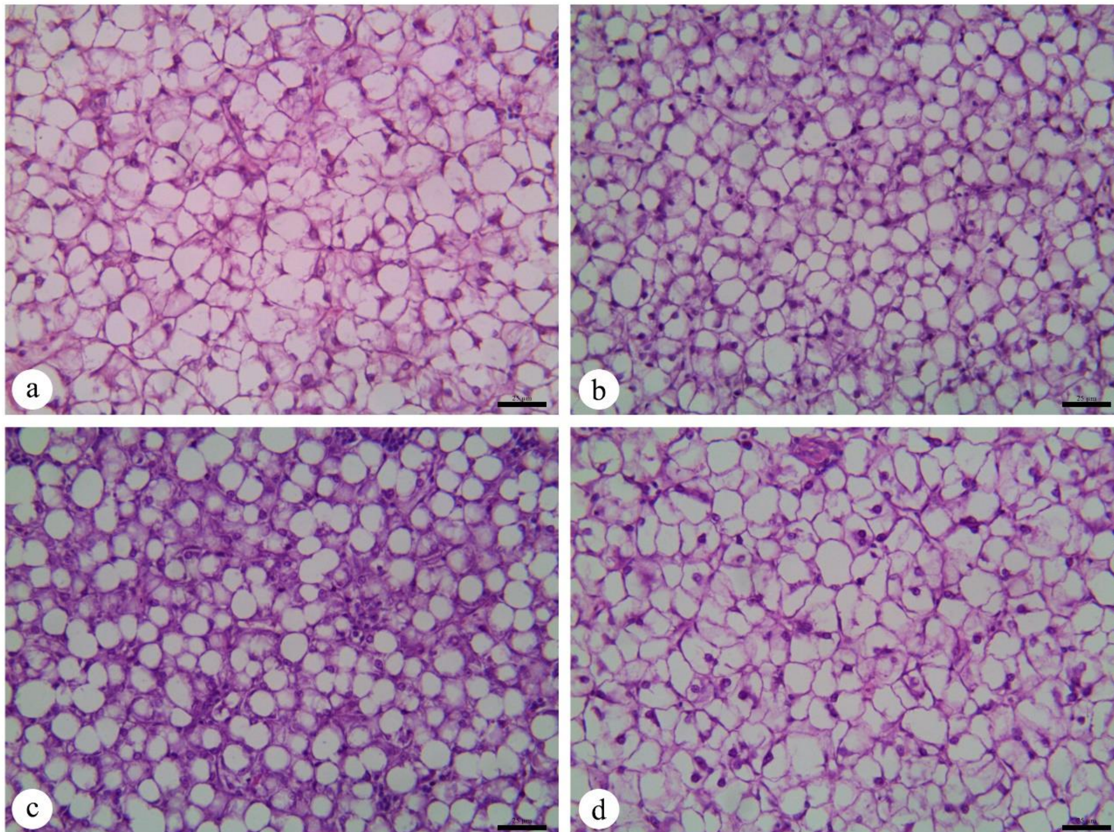

Fig. 3. Hepatocytes morphology of *R. canadum* juvenile fed different experimental diets (n = 3 per tank, 9 per treatment). Hematoxylin-eosin staining. Bars: 25 µm. a: fish fed with FO-D; b: Fish fed with SFA–D; c: Fish fed with MIX-D; d: Fish fed with MUFA-D.

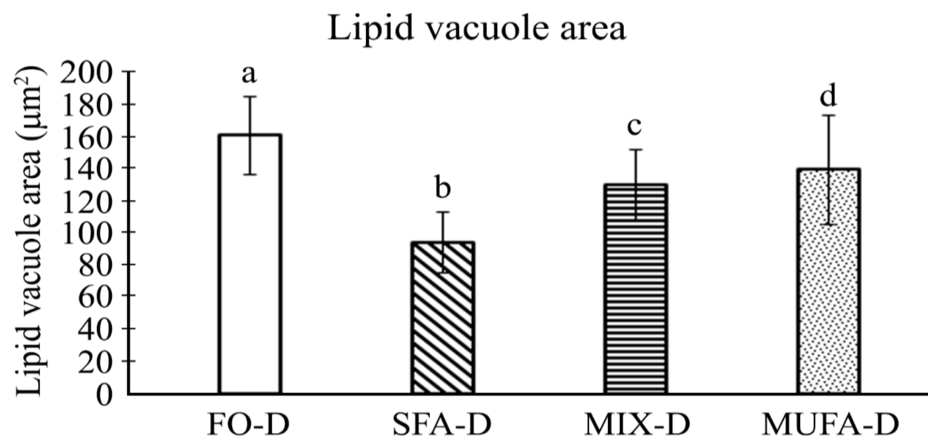

Fig. 4. Lipid vacuoles area (µm2) of *R. canadum* hepatic tissue fed different experimental diets. FO-D – Fish Oil; SFA-D – Diet rich in saturated fatty acids; MIX-D – Diet with the same percentages of saturated and monounsaturated fatty acids. MUFA-D – Diet rich in monounsaturated fatty acids. [ab] Different letters indicate statistical differences among experimental diets, by Tukey's test (*$P < 0.05$*).

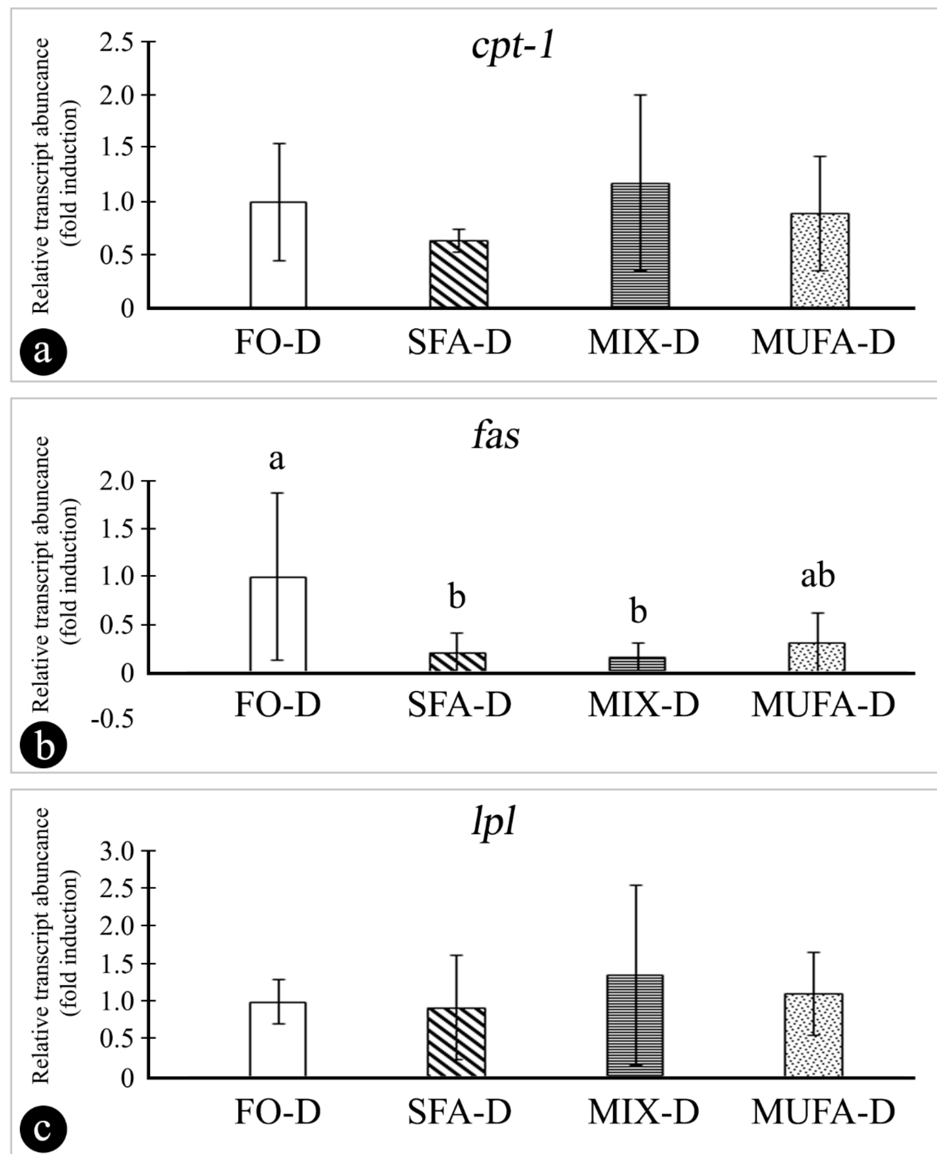

Fig. 5. Relative expression levels of FA synthesis and β-oxidation genes (a) *cpt-1α* (carnitine palmitoyltransferase Iα); (b) *fas* (fatty acid synthase), and (c) *lpl* (lipase lipoprotein). The values represent the mean ± standard errors (n = 6). The transcript level from each gene was calculated relative to the other genes' levels using the raw cycle threshold value for each gene and then normalized against β-actin. The values shown are the fold induction changes relative to the average Ct value for all genes. The different letters [a,b] represent significant ($P < 0.05$) differences among diets. FO-D – Fish Oil; SFA-D – Diet rich in saturated fatty acids; MIX-D – Diet with the same percentages of saturated and monounsaturated fatty acids; MUFA-D – Diet rich in monounsaturated fatty acids.

4. Discussion

The VO can be considered adequate lipid sources to spare or replace FO from marine aquafeeds (Turchini et al., 2011a). However, the inclusion of these alternative lipid sources in FO-free diets can lead to low diet acceptance for some fish species, consequently reducing feed intake and influencing a higher incidence of essential FA deficiencies (Yildirim- Aksoy et al., 2007; Trushenski et al., 2011). Clearly, the experimental diets used in this study were well accepted for *R. canadum* juveniles yielding equivalent production performance to that observed in FO-D fed animals. Due to the adequate supplementation of DHA, EPA, and ARA to the FO-free diets, as recommended to cobia juveniles (Trushenski et al., 2012; Araújo et al., 2019) no negative impacts on the overall performance could be observed. The present results are similar to other FO replacement studies with alternative lipid sources, indicating that VO can be widely used in marine aquafeeds when supplemented with LC-PUFA (Turchini et al., 2011b; Araújo et al., 2019) as reported for marine fish species as *M. saxatilis* (Araújo et al., 2021), S. dorsalis (Rombenso et al., 2018), and *T. carolinus* (Rombenso et al., 2017). Moreover, the trend of weight gain observed with the increase of SFA levels in the experimental diets suggested a beneficial effect of this FA class on the production performance of cobia's juveniles, as previously observed within this same species (Woitel et al., 2014a) and others marine fish species as *A. nobilis* (Rombenso et al., 2015), *T. carolinus* (Rombenso et al., 2016a), *Epinephelus coioides* (Tseng and Lin, 2019) and *Trachinotus ovatus* (Guo et al., 2020).

In general, the SFA and MUFA content of analyzed tissues reflected the dietary FA, while LC-PUFA and PUFA levels were less affected. The muscle FA profile indicated that fish fed SFA-D and MUFA-D did not change the LC-PUFA deposition. Preferential catabolism of SFA and MUFA could contribute to the unbalanced deposition from those FA classes in the muscle, contributing to the LC-PUFA availability. The mechanism modulating this lower deposition of SFA and MUFA in the tissues without altering the LC-PUFA content has been described previously as the LC-PUFA "sparing effect" (Turchini et al., 2011b; Trushenski et al., 2013a, 2015). Our results suggested an LC-PUFA sparing effect due to preferential catabolism of SFA and MUFA in lipolytic tissues as muscle (Glencross, 2009; Turchini et al., 2011b), which are consonant with the literature and also suggested that FA oxidation might be more efficient for SFA than MUFA. According

to Rombenso et al. (2018), LC-PUFA from muscle tissue of S. dorsalis juveniles were less affected by SFA-rich diet than MUFA-rich diet. Moreover, *Lates calcarifer* fed a high level of dietary SFA showed higher or equivalent LC-PUFA levels in muscle tissue than FO-fed animals (Salini et al., 2015a), corroborating the results obtained in this present study.

Our results obtained by comparing the ratio between tissue and dietary FA suggest that higher levels of short-chain SFA resulted in less deposition of these FA in the analyzed tissues. Contrariwise MUFA, was more deposited in the liver and muscle as its dietary inclusion increased. Although the muscle and liver tissues of SFA-D fed fish showed an increased FA content, mainly 12:0, when compared to other dietary treatments, this increase was not proportional to the dietary SFA levels, suggesting that when in surplus, SFA may be preferably oxidized to provide metabolic energy (Tocher, 2003). This result suggested that 12:0 is less deposited/retained in liver and muscle, being highly catabolized by *R. canadum* juveniles. On the other hand, 18:1n-9 levels in liver and muscle increased as the dietary inclusion augmented, suggesting that oxidative metabolism of *R. canadum* have a preference for SFA over MUFA, as reported for other marine carnivores' species as *S. dorsalis* (Rombenso et al., 2018), *Epinephelus marginatus* (Araújo et al., 2018) and also *R. canadum* (Trushenski et al., 2013a).

Several studies have evaluated the effects of high SFA levels on marine fish diets (Trushenski et al., 2013a; Woitel et al., 2014a, 2014b; Mata-Sotres et al., 2018). However, these previous studies used SFA with longer carbon chains compared to 12:0, such as 16:0 and 18:0. The β-oxidation pathway preferentially uses as substrate FA with shorter carbon chains due to the lower energy costs associated with the oxidation of these molecules in energy conversion processes (Schönfeld and Wojtczak, 2016). Therefore, the inclusion of SFA such as 12:0 in cobia aquafeeds, is strategic as the carbon backbone of this FA is smaller and can be easily oxidized, probably without depending on the action of transporters such as acyl-carnitine/carnitine, an alternative pathway that can decrease the energy cost for FA oxidation in the mitochondria (Nelson and Cox, 2018). On the other hand, SFA with larger carbon chains like 18:0 or 16:0 would have a disadvantage compared to 12:0 to enter the mitochondrial β-oxidation pathway. In addition to coconut oil, there are other lauric acid (12:0) rich sources that can be used in aquafeed formulation. Some

insect such as *Hermetia illucens* normally presents in their body fatty acid profile high 12:0 levels (Giannetto et al., 2019). As several insect species have been considered a sustainable alternative source of valuable nutrients in aquafeed formulation as well as a promising strategy in the waste valorization process (Mastoraki et al., 2020) its use as a source of short-chain SFA should also be tested and considered.

It is known that the regulatory mechanisms of carnitine palmitoyltransferase I (CPT I), the main regulatory enzyme of mitochondrial FA oxidation, are affected by the dietary FA composition (Morash et al., 2009). Previous studies showed that the cpt-1 expression was upregulated in response to an increase in SFA or MUFA in aquafeeds (Qiu et al., 2017; Carvalho et al., 2021), contrary to observed in this study. Here, the cpt-1 expression failed to change in response to dietary treatments. However, the same cpt-1 expression pattern observed in fish from different treatments might be related, as mentioned before, to the 12:0 does not need to use the CPT-I transporter to enter in the mitochondria matrix to be metabolized (Nelson and Cox, 2018). Similar results (lower 12:0 tissue deposition and down-regulation of cpt-1) were observed in *E. marginatus* (Araújo et al., 2018), corroborating the assumption that VO rich in short-chain SFA can be considered a viable alternative to stimulate the catabolism and at the same time to preserve LC-PUFA in lipid-relevant tissues.

Differently, 16:0 was retained in tissues of fish-fed diets with lower levels of this FA (FO-free diets). This result suggests a possible 16:0 biosynthesis or retention, probably due to its physiological importance in the phospholipid composition, specifically in phosphatidylcholine, where the levels of this FA are generally high (Tocher et al., 2008). According to Sissener et al. (2020), *Salmo salar* juveniles fed diets with high 16:0 levels inversely resulted in lower levels in the hepatic triglycerides, suggesting a high oxidative potential of 16:0 when in surplus. However, the phospholipid composition showed high 16:0 levels, mainly in the liver, heart, and retina of *S. salar* (Sissener et al., 2020). According to finds and previous results, 16:0 is highly catabolized when offered in excess in the diet, however, this FA is also important in phosphatidylcholines, that act on lipoprotein metabolism (Tocher et al., 2008). Thus 16:0 might have multiple functions in FA metabolism, been partly catabolized. Simultaneously, another fraction might be esterified into

phospholipids. However, it will be modulated by the composition and level of this fatty acid in the diet.

The enzyme fatty acid synthase (FAS) catalyzes the de novo synthesis of fatty acids (Jensen-Urstad and Semenkovich, 2012). The fas expression was up-regulated in liver of fish fed FO-D and MUFA-D, possibly due to the lower SFA levels, especially 12:0, on these diets, while higher levels in SFA-D and MUFA-D. Given the physiological importance of 16:0 in phospholipids composition (Tocher et al., 2008), and the preferential FA oxidation order of SFA (Tocher, 2003), from shorter to longer carbon chains, *fas* expression was possibly up-regulated to compensate the lower SFA levels in FO-D and MUFA-D. Several studies with different fish species have shown more oxidation of SFA and MUFA when offered in excess, and consequently preserving the LCPUFA, especially in the muscle (Turchini et al., 2011b; Salini et al., 2015a; Bowzer et al., 2016; Qiu et al., 2017; Rombenso et al., 2017). Also, high dietary PUFA, as usually occurs in FO-based diets, may not be the most efficient practice, as the excess of these FA are commonly oxidized (Torstensen et al., 2000; Salini et al., 2015b). In *T. carolinus* (Rombenso et al., 2016a), MUFA were accumulated in tissues as the dietary inclusion of this FA increased. In the Atlantic salmon, the 10:0 was preferably oxidized, while 18:1n-9 was retained in the muscle (Denstadli et al., 2011). According to Rombenso et al. (2018), LC-PUFA sparing effect is more efficient in SFA-fed fish than those fed with MUFA-rich diets, which was consonant with our results. This study demonstrated that in *R. canadum* juveniles, the MUFA were less catabolized and preferentially retained in the liver and muscle tissues.

In general, 18:2n-6 showed a similar retention pattern in both liver and muscle tissues. The 18:2n-6 tissue deposition, both in muscle and liver, remained stable, regardless of the dietary treatment. Several studies on marine fish species evidenced a strong trend for 18:2n-6 retention in tissues like muscle and liver (Trushenski et al., 2011; Nayak et al., 2016). However, C18 PUFA usually competes with LC-PUFA for deposition on these same tissues (Lane et al., 2006), resulting in lower LCPUFA retention and availability (Laporte and Trushenski, 2011; Trushenski et al., 2011; Mulligan and Trushenski, 2013; Woitel et al., 2014a, 2014b), commonly influencing in LC-PUFA deficiencies (Trushenski et al., 2013a, 2013b; Rombenso et al., 2015). Marine carnivorous fish such as *R. canadum* have a high LC-PUFA

nutritional requirement (NRC, 2011; Trushenski et al., 2012). Therefore, C18 PUFA/LC-PUFA competition can strongly influence the LC-PUFA content in the tissues negatively affecting the n-3/n-6 ratio in fillet and consequently its nutritional quality. The VO, such as soybean oil, are rich sources of 18:2n-6. Although its frequent application in aquafeed formulations, soybean oil should carefully be evaluated for a possible adverse effect compromising the physiological aspects of the species, such as FA metabolism and fish's nutritional quality (Ogori, 2020). In Atlantic salmon (*S. salar*), high 18:2n-6 content in the diet resulted in a deleterious effect on EPA levels in the liver, heart, and retina, and it modified the eicosanoids synthesized from these precursor FA (Sissener et al., 2020). In the present study, LC-PUFAs, and C18 PUFA, with specific exceptions, remained stable in fish muscles. Our results are in harmony with previous studies, regarding the strong tendency to 18:2n-6 retention in fish tissues (Trushenski et al., 2011; Nayak et al., 2016; Chen et al., 2019).

The VO inclusion in marine aquafeeds can influence an imbalance of FA composition, compromising the integrity of lipolytic and lipogenic tissues (Caballero et al., 2004). The FA synthesis and oxidation in the liver and the enzymes involved in these pathways have a straight relationship with the FA availability on specific tissue (Henderson, 1996). When lipids are in excess in the diet, or when protein synthesis is compromised, there is an increase in the triglyceride's synthesis and deposition in the hepatocytes, generating a morphological pathological pattern known as hepatic steatosis (Caballero et al., 2004). *Sparus aurata* juveniles fed a SFA-rich diet presented lower lipid deposition in the liver (lower lipid vacuoles area) than those fish-fed MUFA-rich diets, which inversely demonstrated larger lipid vacuoles (Riera-Heredia et al., 2020). A gradual decrease in the lipid vacuoles in fish-fed FO-D, MUFAD, MIX-D, SFA D, respectively was observed in the present study. These findings, along with tissue FA profile results, clearly suggested a preferential SFA oxidation than other FAs classes. Apparently, as previously mentioned, the lipid metabolism of *R. canadum* juveniles metabolized the short-chain SFA preferentially. Therefore, its deposition in the liver was differentiated, while MUFA, mainly 18:1n-9, was progressively accumulated in the hepatocytes. Likewise, Fountoulaki et al. (2009) also observed larger lipid deposition in *S. aurata* fed diets rich in 18:1n-9. Besides the livers of fish-fed FO-D and MUFA-D presented

higher lipid vacuole area, these animals showed reduced hepatic lipid content compared to the other experimental groups. This result could be due to lower amount of lipid vacuoles cells in this tissue, which is consonant with HSI values that are lower in fish-fed FO-D and MUFA-D.

The higher lipid vacuole area in the hepatocytes of fish from FO-D and MUFA-D groups might be related to a compromise lipoprotein metabolism (Caballero et al., 2006), or an imbalance between lipid synthesis, oxidation, and transport to other tissues (Postic and Girard, 2008). However, the present study results showed that lpl gene expression, an enzyme that controls the plasma lipoprotein levels and is considered a “gatekeeper” for the uptake of FA into tissues did not differ significantly among different treatments. Similarly, in European seabass (*D. labrax*), there were no differences in the expression of lpl in the liver in response to the different diet compositions (Richard et al., 2006). Nevertheless, the reduction in the lipid's vacuole area in the liver of fish from SFA-D could be related to the increase in lipid oxidation influenced by high SFA levels included in the diet, in particular 12:0.

5. Conclusion

This study demonstrated the feasibility of using alternative FA sources rich in SFA and MUFA when adequately supplemented with LCPUFA (DHA, EPA, and ARA) in FO-free diets for *R. canadum* juveniles. The present study contributes to the generation of knowledge concerning physiological aspects (mainly related to the LC-PUFA sparing effect) of this important commercial species. Our study demonstrates an acceptance of FO-free diets without compromising survival, growth, and FA profile, mainly related to levels of LC-PUFA in muscle, which are essentially important in various physiological processes in the fish and further to human nutrition. SFA and MUFA triggered different metabolic responses in *R. canadum* juveniles, with short-chain SFA being more oxidized than MUFA. Simultaneously these were preferentially deposited as their levels increased in the diet. The 12:0 retention in the tissues was not proportional to dietary inclusion levels, suggesting that SFA of shorter carbon chains can be strategically used in the aquafeeds formulation for marine fish species. Considering the physiological importance of SFAs such as 16:0 and 18:0, SFA of shorter carbon chains can be administered in

larger quantities to stimulate the catabolism of these molecules, providing energy for growth and simultaneously preserving LC-PUFA in tissues, especially in the muscle.

6. Acknowledgements

Authors would like to thanks Vagner Alberto and Valquiria de Oliveira Pereira for all their help with the analysis and purchases. To Prof. Dr. Lucile Maria Floeter-Winter and Dr. Ricardo Andrade Zampieri for the assistance and availability of equipment for the analysis of gene expression. This work was supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) for the master's fellowship of Victor Hugo Marques project number: (2019/09246-9), and for financial and technical assistance (2014/16320-7; 2017/06765-0; 2019/26018-0). We gratefully acknowledge the support of Centro de Biologia Marinha da Universidade de São Paulo (CEBIMar), especially the staff for their support of the feasibility of this trial.

7. References

Araújo, B.C., Honji, R.M., Rombenso, A.N., Brambila-Souza, G., de Mello, P.H., Hilsdorf, A.W.S., Moreira, R.G. 2019. Influences of different arachidonic acid levels and temperature on the growth performance, fatty acid profile, liver morphology and expression of lipid genes in cobia (*Rachycentron canadum*) juveniles. Aquacult. 511, 734245. https://doi.org/10.1016/j.aquaculture.2019.734245

Araújo, B.C., Rodriguez, M., Honji, R.M., Rombenso, A.N., Rio-Zaragoza, O.B., Cano, A., Tinajero, A., Mata-Sotres, J.A., Viana, M.T. 2021. Arachidonic acid modulated lipid metabolism and improved productive performance of striped bass (*Morone saxatilis*) juvenile under sub- to optimal temperatures. Aquacult. 530, 735939. https://doi.org/10.1016/j.aquaculture.2020.735939

Araújo, B.C., Wade, M.W., de Mello, P.H., Rodrigues-Filho, J.A., Garcia, C.E.O., de Campos, M.F., Botwright, N., Hashimoto, D.T., Moreira, R.G., 2018. Characterization of lipid metabolism genes and the influence of fatty acid supplementation in the hepatic lipid metabolism of dusky grouper (*Epinephelus marginatus*). Comp. Biochem. Physiol. A, 219-220, 1–9. https://doi.org/10.1016/j.cbpa.2018.01.018.

Arts, M.T., Kohler, C.C. 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts, M.T., Brett, M.T., Kainz, M.E. (Eds). Lipids in Aquatic Ecosystems. Springer, New York, EUA, p. 237-256.

Association of Official Analytical Chemistis [AOAC]. 2015. Official Methods of Analysis. 14ed. AOAC, Washington, DC, USA.

Behmer, O.A., Tolosa, E.M.C., Neto, A.G.F. 1976. Manual de técnicas para histologia normal e patológica. EDART. São Paulo. Livraria Editora Ltda. 239p.

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquacult. 218, 491–499. https://doi.org/10.1016/S0044-8486(02)00370-8

Benetti, D.D., Orhun, R., Sardenberg, B., O'Hanlon, B., Welch, A., Hoenig, R., Zink, I., Rivera, J.A., Denlinger, B., Bacoat, D., Palmer, K., Cavalin, F. 2008. Advances in hatchery and grow-out technology of cobia *Rachycentron canadum* (Linnaeus). Aquac. Res. 39, 701-711. https://doi.org/10.1111/j.1365-2109.2008.01922.x

Bowzer, J., Jackson, C., Trushenski, J. 2016. Hybrid striped bass feeds based on fish oil, beef tallow, and EPA/DHA supplements: insight regarding fish oil sparing and demand for n-3 long-chain polyunsaturated fatty acids. J. Anim. Sci. 94, 978–988. https://doi.org/10.2527/jas.2015-9199

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernandez, A.J., Rosenlund, G. 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. J. Fish Dis. 27, 531–541. 10.1111/j.1365- 2761.2004.00572.x

Caballero, M.J., Gallardo, G., Robaina, L., Montero, D., Fernandez, A., Izquierdo, M. 2006. Vegetable lipid sources affect in vitro biosynthesis of triacylglycerols and phospholipids in the intestine of sea bream (*Sparus aurata*). Br. J. Nutr. 95, 448–454. https://doi.org/10.1079/BJN20051529

Calder, P.C. 2006. Polyunsaturated fatty acids and inflammation. Prostag. Leukotr. Ess. Fat. Acids. 197-202. https://doi.org/10.1016/j.plefa.2006.05.012

Carvalho, M., Montero, D., Torrecillas, S., Castro, P., Zamorano, M.J., Izquierdo, M. 2021. Hepatic biochemical, morphological and molecular effects of feeding microalgae and poultry oils to gilthead sea bream (*Sparus aurata*). Aquacult. 532, 736073. https://doi.org/10.1016/j.aquaculture.2020.736073

Chen, Y., Sun, Z., Liang, Z., Xi, Y., Su, J., Luo, Q., Zhu, J., Liu, Q., Han, T., Wang, A. 2019. Effects of dietary fish oil replacement by soybean oil and L-carnitine supplementation on growth performance, fatty acid composition, lipid metabolism and liver health of juvenile largemouth bass, *Micropterus salmoides*. Aquacult. 516, 734596. https://doi.org/10.1016/j.aquaculture.2019.734596

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H., Chen, H.Y. 2004. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquacult. 299, 325–333. https://doi.org/10.1016/S0044-8486(03)00395-8

Denstadli, V., Bakke, A.M., Berge, G.M., Krogdahl, A., Hillestad, M., Holm, H., Ruyter, B. 2011. Medium-Chain and Long Chain Fatty Acids Have Different Postabsorptive Fates in Atlantic Salmon. J. Nutr. 141. 10.3945/jn.111.141820

Eroldoğan, T.O., Yılmaz, A.H., Turchini, G.M., Arslan, M., Sirkecioğlu, N.A., Engin, K., Özşahinoğlu, I., Mumoğullarında, P. 2013. Fatty acid metabolism in European sea bass (*Dicentrarchus labrax*): effects of n-6 PUFA and MUFA in fish oil replaced diets. Fish Physiol. Biochem. 39, 941–955. https://doi.org/10.1007/s10695-012-9753-7

Folch, J., Less, M., Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-503. PMID: 13428781.

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., Alexis, M.N. 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata L.*), effects on growth performance, flesh quality and fillet fatty acid profile: recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquacult. 289, 317– 326. https://doi.org/10.1016/j.aquaculture.2009.01.023

Fraser, T.W.K., Davies, J.D. 2009. Nutritional requirements of cobia, *Rachycentron canadum* (Linnaeus): a review. Aquac. Res. 40, 1219-1234. https://doi.org/10.1111/j.1365-2109.2009.02215.x

Frings, C.S., Fendly, T.W., Dunn, R.T., Quenn, C.A. 1972. Improved determination of total lipids by the sulpho-phospho-vanilin reaction. Clin. Chem. 18, 673-674.

Giannetto, A., Oliva, S., Lanes, C.F.C., Pedron, F.A., Savastano, D., Baviera, C., Parrino, V., Lo Paro, G., Spanò, N.Z., Cappello, T., Maisano, M., Mauceri, A., Fasulo, S. 2019. *Hermetia illucens* (Diptera: Stratiomydae) larvae and prepupae: biomass production, fatty acid profile and expression of key genes involved in lipid metabolism. J. Biotchnol. 307, 44-54. https://doi.org/10.1016/j.jbiotec.2019.10.015

Glencross, B.D. 2009. Exploring the nutritional demand for essential fatty acid by aquaculture species. Rev. Aquacult. 1, 71-124. https://doi.org/10.1111/j.1753-5131.2009.01006.x

Guo, H., Chen, C., Yan, X., Li, Y., Wen, X., You,C., Monroig, O., Tocher, D.R., Wang, S. 2020. Effects of different dietary oil sources on growth performance, antioxidant capacity and lipid deposition of juvenile golden pompano *Trachinotus ovatus.* Aquacult. 530, 735923. https://doi.org/10.1016/j.aquaculture.2020.735923

Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5-22. http://dx.doi.org/10.1080/17450399609381859

Honji, R.M., Caneppele, D., Pandolfi, M., Lo Nostro, F.L., Moreira, R.G., 2015. Gonadotropins and growth hormone family characterization in na endangered Siluriform species, *Steindachneridion parahybae* (Pimelodidae): relationship with annual reproductive cycle and induced spawning in captivity. Anat. Rec. 298, 1644–1658. https://doi.org/10.1002/ar.23174

Izquierdo, M.S., Fernández-Palacios, H., Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquacult. 197, 25 – 42. https://doi.org/10.1016/S0044-8486(01)00581-6

Jensen-Urstad, A.P.L., Semenkovich, C.F. 2012. Fatty acid synthase and liver triglyceride metabolism: Housekeeper or messenger? Biochim. Biophys. Acta. 747–753. https://doi.org/10.1016/j.bbalip.2011.09.017

Lane, R. L., Trushenski, J.T., Kohler, C.C. 2006. Modification of fillet composition and evidence of differential fatty acid turnover in sunshine bass *Morone chrysops* × *M. saxatilis* following change in dietary lipid source. Lipids. 41, https://doi.org/10.1007/s11745-006-5053-2

Lange, K.W. Omega-3 fatty acids and mental health. 2020. Glob. Health J. 4, 18-30. http://dx.doi.org/10.1016/j.glohj.2020.01.004

Laporte, J., Trushenski, J.T. 2011. Growth performance and tissue fatty acid composition of largemouth bass fed diets containing fish oil or blends of fish oil and soy-derived lipids. N. Am. J. Aquac. 73, 435–444. https://doi.org/10.1080/15222055.2011.623947

Luo, Y., 2013. Nutrition Requirement of Dietary Protein and Optimal Replacement of Fish Meal Protein by Rapeseed Meal and Corn Gluten Meal in Cobia, *Rachycentron canadum* L. Ocean University of China, China ((MA. Sc.dissertation), in Chinese with English abstract).

Mastoraki, M., Ferrándiz, P.M.,Vardali, S.C., Kontodimas, D.C., Kotzamanis, Y.P., Gasco, L., Chatzifotis, S., Antonopoulou, E. 2020. A comparative study on the effect of fish meal substitution with three different insect meals on growth, body composition and metabolism of European sea bass (*Dicentrarchus labrax* L.). Aquacult. 528, 735511. https://doi.org/10.1016/j.aquaculture.2020.735511

Mata-Sotres, J.A., Tinajero-Chavez, A., Barreto-Curiel, F., Pares-Sierra, G., Del Rio-Zaragoza, O.B., Viana, M.T., Rombenso, A.N. 2018. DHA (22:6n-3) supplementation is valuable in *Totoaba macdonaldi* fish oil-free feeds containing poultry by-product meal and beef tallow. Aquacult. 497, 440–451. https://doi.org/10.1016/j.aquaculture.2018.08.015

Morash, A.J., Bureau, D.P., McClelland, G.B. 2009. Effects of dietary fatty acid composition on the regulation of carnitine palmitoyltransferase (CPT) I in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. B. 152, 85–93. 10.1016/j.cbpb.2008.10.005

Mulligan, B.L., Trushenski, J.T., 2013. Use of standard or modified plant-derived lipids as alternatives to fish oil in feeds for juvenile Nile Tilapia. J. Aquat. Food Prod. Technol. 22, 47–57. https://doi.org/10.1080/10498850.2011.623336

National Research Council (NRC) 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington, DC.

Nayak, M., Saha, A., Pradhan, A., Samanta, M., Giri, S.S. 2016. Dietary fish oil replacement by linseed oil: Effect on growth, nutrient utilization, tissue fatty acid composition and desaturase gene expression in silver barb (*Puntius gonionotus*) fingerlings. Comp. Biochem. Physiol. B. 205, 1-12. https://doi.org/10.1016/j.cbpb.2016.11.009

Nelson, D.L., Cox, M.M. 2018. Lehninger: Principles of biochemistry. Freeman, New York, EUA. pag 341-362.

Ogori, A.F. 2020. Source, Extraction and Constituents of Fats and Oils. J. Food Sci. Nutr. 6, 2 – 8. 10.24966/FSN-1076/100060

Oliva-Teles, A., Enes, P., Peres. H. 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Davis, D.A. Feed and Feeding Practices in Aquaculture. Elsevier P. 203-233. https://doi.org/10.1016/b978-0-08-100506-4.00008-8

Palomares, M.L.D., Froese, R., Derrick, B., Meeuwig, J.J., Nöel, S.-L., Tsui, G., Woroniak, J., Zeller, D., Pauly, D. 2020. Fishery biomass trends of exploited fish populations in marine ecoregions, climatic zones and ocean basins. Estuar. Coast. Shelf Sci. 243, 106896. https://doi.org/10.1016/j.ecss.2020.106896

Parrish, C.C., Nichols, P.D., Pethybridge, H., Young, J.W. 2014. Direct determination of fatty acids in fish tissues: quantifying top predator trophic connections. Methods. 177, 85–95. https://doi.org/10.1016/0952-3278(90)90017-f.

Postic, C., Girard, J. 2008. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab. J. 34 (6 part 2), 643–648. https://doi.org/10.1016/S1262-3636(08)74599-3

Qiu, H., Jin, M., Li, Y., You, L., Hou, Y.M., Zhou, Q.C. 2017. Dietary lipid sources influence fatty acid composition in tissue of large yellow croaker (*Larmichthys crocea*) by regulating triacylglycerol synthesis and catabolism at the transcriptional level. PLoS One. 12 (1), e0169985. https://doi.org/10.1371/journal.pone.0169985

Richard, N., Mourente, G., Kaushik, S., Corraze, G. 2006. Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). Aquacult. 261, 1077–1087. https://doi.org/10.1016/j.aquaculture.2006.07.021

Riera-Heredia, N.R., Moya, A.S., Pecino, S.A., Fontanillas, R., Gutiérrez, J., Capilla, E., Navarro, I. 2020. The combination of palm and rapeseed oils emerges as a good dietary alternative for optimal growth and balanced lipid accumulation in juvenile gilthead sea bream reared at an elevated temperature. Aquacult. 526, 735396. https://doi.org/10.1016/j.aquaculture.2020.735396

Rombenso, A.N., Trushenski, J.T., Jirsa, D., Drawbridge, M. 2015. Successful Fish Oil Sparing in white seabass Feeds Using Saturated Fatty Acid-Rich Soybean Oil and 22:6n-3 (DHA) Supplementation. Aquacult. 448, 176–185. https://doi.org/10.1016/j.aquaculture.2015.05.041

Rombenso, A.N., Trushenski, J.T., Drawbridge, M. 2018. Saturated lipids are more effective than others in juvenile California yellowtail feeds—Understanding and harnessing LC-PUFA sparing for fish oil replacement. Aquacult. 493, 192–203. https://doi.org/10.1016/j.aquaculture.2018.04.040

Rombenso, A.N., Trushenski, J.T., Jirsa, D., Drawbridge, M. 2016b. Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are essential to meet LC-PUFA requirements of juvenile California Yellowtail (*Seriola dorsalis*). Aquacult. 463, 123-134. http://dx.doi.org/10.1016/j.aquaculture.2016.05.004

Rombenso, A.N., Trushenski, J.T., Schwarz, M.H. 2016a. Fish Oil Replacement in Feeds for Juvenile Florida Pompano: Composition of Alternative Lipid Influences Degree of Tissue Fatty Acid Profile Distortion. Aquacult. 458, 177–186. https://doi.org/10.1016/j.aquaculture.2016.03.009

Rombenso, A.N., Trushenski, J.T., Schwarz, M.H. 2017. Beef tallow is suitable as a primary lipid source in juvenile Florida Pompano feeds. Aquac. Nutr. 23, 1274 -1286. https://doi.org/10.1111/anu.12502

Salini, M.J., Turchini, G.M., Glencross, B.D. 2015a. Effect of dietary saturated and monounsaturated fatty acids in juvenile barramundi *Lates calcarifer*. Aquac. Nutr. 1-12. https://doi.org/10.1111/anu.12389

Salini, M.J., Irvin, S.J., Bourne, N., Blyth, D., Cheers, S., Habilay, N., Glencross, B.D. 2015b. Marginal efficiencies of long chain-polyunsaturated fatty acid use by barramundi (*Lates calcarifer*) when fed diets with varying blends of fish oil and poultry fat. Aquacult. 449, 48–57. https://doi.org/10.1016/j.aquaculture.2015.02.027

Schönfeld, P., Wojtczak, L. 2016. Short and medium chain fatty acids in energy metabolism: the cellular perspective. J. Lipid Res. 57, 943–954. https://doi.org/10.1194/jlr.R067629

Schulze, M.B., Minihane, A.M., Saleh, R.N.M., Risérus, U. 2020. Intake and metabolism of omega-3 and omega-6 polyunsaturated fatty acids: nutritional implications for cardiometabolic diseases. Lancet Diabetes Endocrinol. 8 (11), 915-930. https://doi.org/10.1016/S2213-8587(20)30148-0

Sissener, N.H., Araujo, P., Sælea, O., Rosenlund, G., Stubhaug, I., Sanden, M. 2020. Dietary 18:2n-6 affects EPA (20:5n-3) and ARA (20:4n-6) content in cell membranes and eicosanoid production in Atlantic salmon (*Salmo salar* L.). Aquacult. 522, 735 –798. https://doi.org/10.1016/j.aquaculture.2020.735098

Sorbera, L. A., Astuarino, J. F., Carrillo, M., Zanuy, S. 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocytes maturation in a marine teleost, the european sea bass (*Dicentrarchus labrax*). Biol. Reprod. 64, 382-389. https://doi.org/10.1095/biolreprod64.1.382

Sprague, M., Dick, J.R., Tocher, D.R. 2015. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. Sci. Rep. 6, 21892. https://doi.org/10.1038/srep21892

Spisni, E., Tugnoli, M., Ponticelli, A., Mordenti, T., Tomasi, V. 1998. Hepatic steatosis in artificially fed marine teleosts. J. Fish Dis. 21, 177–184. https://doi.org/10.1046/j.1365-2761.1998.00089.x

Tacon, A.G.J., Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded: aquafeeds: Trends and future prospects. Aquacult. 285, 146–158. https://doi.org/10.1016/j.aquaculture.2008.08.015

Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11 (2), 107–184. https://doi.org/10.1080/713610925

Tocher, D.R. 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquacult. 449, 94–107. https://doi.org/10.1016/j.aquaculture.2015.01.010

Tocher, D.R., Bendiksen, E.A., Campbell, P.J., Bell, J.G. 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquacult. 280, 21–34. https://doi.org/10.1016/j.aquaculture.2008.04.034

Torstensen, B.E., Lie, O., Froyland, L. 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) – Effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids. 35, 653–664. https://doi.org/10.1007/s11745-000-0570-6

Trushenski, J.T., Rombenso, A.N. 2020. Trophic Levels Predict the Nutritional Essentiality of Polyunsaturated Fatty Acids in Fish—Introduction to a Special Section and a Brief Synthesis. N. Am. J. Aquac. 82, 241–250. https://doi.org/10.1002/naaq.10137

Trushenski, J.T., Crouse, C.C., Rombenso, A.N., 2015. Effects of fish oil sparing on fillet fatty acid composition in hybrid striped bass are influenced by dietary levels of saturated and unsaturated fatty acids. N. Am. J. Aquac. 77, 160–169. https://doi.org/10.1080/15222055.2014.963769

Trushenski, J.T, Mulligan, B., Jirsa, D., Drawbridge, M. 2013b. Sparing fish oil with soybean oil in feeds for White Seabass: effect of inclusion rate and soybean oil composition. N. Am. J. Aquac. 75, 305–315. https://doi.org/10.1080/15222055.2012.720650

Trushenski, J., Schwarz, M., Lewis, H., Laporte, J., Delbos, B., Takeuchi, R., Sampaio, L.A. 2011. Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia *Rachycentron canadum*. Aquac. Nutr. 17, 437- 447. https://doi.org/10.1111/j.1365-2095.2010.00779.x

Trushenski, J., Woitel, F., Schwarz, M., Yamamoto, F. 2013a. Saturated fatty acids limit the effects of replacing fish oil with soybean oil with or without phospholipid supplementation in feeds for juvenile cobia. N. Am. J. Aquac. 75, 316–328. https://doi.org/10.1080/15222055.2012.713897

Trushenski, J., Schwarz, M., Bergman, A., Rombenso, A., Delbos, B. 2012. DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. Aquacult. 326–329, 81–89. https://doi.org/10.1016/j.aquaculture.2011.11.033

Tseng, Y., Lin, Y-H. 2019. Effects of dietary supplementation with coconut oil on the growth, fatty acid profiles and some lipid metabolism relative gene expressions

of orange-spotted grouper *Epinephelus coioides*. Aquac. Nutr. 26 (1), 1-10. https://doi.org/10.1111/anu.12981

Turchini, G.M., Trushenski, J., Glencross, B.D. 2018. Toughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds. N. Am. J. Aquac. 81 (1), 13-39. https://doi.org/10.1002/naaq.10067

Turchini, G.M., Torstensen, B.E., Ng, W.K. 2009. Fish oil replacement in finfish nutrition. Rev. Aquacult. 1, 10–57. https://doi.org/10.1111/j.1753-5131.2008.01001.x

Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), 2011a. Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton.

Turchini, G.M., Francis, D.S., Senadheera, S.P.S.D., Thanuthong, T., De Silva, S.S. 2011b. Fish oil replacement with different vegetable oils in Murray cod: evidence of an "omega-3 sparing effect" by other dietary fatty acids. Aquacult. 315, 250–259. https://doi.org/10.1016/j.aquaculture.2011.02.016

Wang, Z., Mai, K., Xu, W., Zhang, Y., Liu, Y., Ai, Q. 2016. Dietary methionine level influences growth and lipid metabolism via GCN2 pathway in cobia (Rachycentron canadum). Aquacult. 454, 148 – 156.

Woitel, F.R., Trushenski, J.T., Schwarz, M.H., Jahncke, M.L. 2014a. More judicious use of fish oil in cobia feeds: I. Assessing the relative merits of alternative lipids. N. Am. J. Aquac. 76, 222–231. https://doi.org/10.1080/15222055.2014.893469

Woitel, F.R., Trushenski, J.T., Schwarz, M.H., Jahncke, M.L. 2014b. More judicious use of fish oil in cobia feeds: II. Effects of graded fish oil sparing and finishing. N. Am. J. Aquac. 76, 232–241 https://doi.org/10.1080/15222055.2014.893470

Xu, H., Bi, Q., Liao, Z., Sun, B., Jia, L., Wei, Y., Liang, M. 2021. Long-term alternate feeding between fish oil- and terrestrially sourced oil-based diets mitigated the adverse effects of terrestrially sourced oils on turbot fillet quality. Aquacult. 531, 735974. https://doi.org/10.1016/j.aquaculture.2020.735974

Yildirim-Aksoy, M., Lim, C., Davis, D.A., Shelby, R., Klesius, P.H. 2007. Influence of dietary lipid sources on the growth performance, immune response, and

resistance to Nile Tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* challenge. J. Appl. Aquac. 19, 29–49. https://doi.org/10.1300/J028v19n02_02

Discussão Geral e Conclusões

Atualmente a aquicultura presencia um paradoxo em suas atividades: cultivar os organismos, diminuindo os níveis de inclusão de OP nas dietas, a fim de conduzir uma atividade mais sustentável. Porém, a substituição do OP por fontes lipídicas alternativas, geralmente pobres em LC-PUFA, se depara com limitações de aspectos fisiológicos das espécies como digestibilidade, suscetibilidade às patologias, redução de desempenho produtivo e qualidade nutricional do filé, principalmente relacionada aos níveis de LC-PUFA n-3 (DHA e EPA), característica que está sendo cada vez mais procurada pelos consumidores (Tacon e Metian, 2008; Trushenski e Bowzer, 2013). Por outro lado, a continuação da dependência em dietas a base de OP aumenta os preços do processo de cultivo e consequentemente, do produto final, além de não diminuir a pressão antrópica nos oceanos, causadas pelo aumento na demanda de OP, devido a intensificação da aquicultura (Tocher et al., 2015; Alhazzaa et al., 2018).

A viabilidade econômica de suplementar as dietas com óleos ou extratos produzidos a partir de algas marinhas, que normalmente contêm quantidades elevadas de LC-PUFA (Trushenski et al., 2011; Tocher, 2015) ainda não foi atingida. Existem também estratégias nutricionais durante o ciclo de cultivo como, alimentar os animais com dietas livres de OP em períodos anteriores a engorda e, nas fases de engorda, fornecer dietas a base de OP; uma técnica chamada de *finishing*. Apesar de promissora, os gastos economizados na pré-engorda são perdidos durante o período de engorda (Turchini et al., 2011b).

Os transgênicos são uma das alternativas mais recentes ponderadas sobre a questão da substituição de OP nas dietas para aquicultura (Tocher et al., 2015). *Yarrowia lipolytica* e *Camelina sativa* são plantas terrestres que possuem potencial na

área de biotecnologia aplicada a formulação de dietas, utilizando organismos geneticamente modificados que sintetizam maiores quantidades de LC-PUFA n3 (Xue et al., 2013; Ruiz-Lopez et al., 2014). Contudo, a utilização de organismos geneticamente modificados apresenta impedimentos em políticas e legislações de alguns países, além da aceitação pelos consumidores (Tocher et al., 2015).

Através de anos de pesquisa na substituição de OP por fontes lipídicas alternativas que forneçam AG que atendam as necessidades nutricionais dos organismos cultivados, muitas informações foram geradas sobre as necessidades de AG de diversas espécies (Tocher, 2010). A partir disso, atualmente a necessidade em LC-PUFA não é mais considerada única e sim, dividida em três tipos: necessidade suficiente para a prevenção de patologias nutricionais (necessidade fisiológica), necessidade para sustentar crescimento e desempenho produtivo ótimos e necessidade para manter níveis desejáveis de EPA e DHA no filé (Tocher et al., 2015; Rombenso et al., 2021). Este último vem sendo cada vez mais exigido pelos consumidores, principalmente na comparação entre peixes cultivados e selvagens (Rombenso et al., 2021). Apesar das alternativas anteriores produzirem modestos aumentos nos níveis de LC-PUFA n-3 no filé, nenhuma delas se mostra economicamente viável até o momento. Contudo, a informação sobre as necessidades nutricionais de LC-PUFA de diversas espécies de importância comercial foi necessária para o surgimento de uma nova linha de pesquisa.

Conhecendo a necessidade fisiológica de LC-PUFA para uma dada espécie, a manipulação dos grupos de AG presentes na dieta pode ser feita para que a transferência de LC-PUFA da dieta para os tecidos seja otimizada de forma a preservar os LC-PUFA nos tecidos e induzir o catabolismo de outros AG como SFA e MUFA (Turchini et al., 2011; Rombenso et al., 2021). Os SFA mostram-se

particularmente mais interessantes para este contexto pois, são oxidados mais rapidamente e exercem menor influência no perfil de AG do tecido muscular em comparação com os MUFA, otimizando o processo de LC-PUFA *sparing effect* (Rombenso et al., 2018). Dessa forma, os níveis limitados de LC-PUFA presentes nas dietas para aquicultura podem ser utilizados com maior eficiência tendo melhores níveis de retenção no tecido muscular (Rombenso et al., 2021).

O presente estudo demonstrou a viabilidade na utilização de óleos vegetais ricos em SFA e MUFA, aliada a suplementação com LC-PUFA (DHA, EPA e ARA) em dietas livres de OP para juvenis de bijupirá. Dessa forma, fontes de óleos vegetais, especialmente aquelas ricas em SFA, surgem como alternativas na substituição de subprodutos de peixes em dietas para aquicultura. Tais resultados são importantes para que futuramente possa ser reduzido o custo da nutrição, que representa até 70% dos custos totais do cultivo (Henry et al., 2015), para peixes marinhos e carnívoros como o bijupirá. Espécies marinhas e carnívoras como o bijupirá são as que mais dependem do OP e outros insumos de origem marinha como a farinha de peixe (Alhazzaa et al., 2018), por várias particularidades fisiológicas como a baixa ou até mesmo nula atividade enzimática relacionada a biossíntese de LC-PUFA a partir de precursores PUFA C18 (Teles et al., 2015).

Alternativas ao OP que forneçam níveis adequados de LC-PUFA são extremamente necessárias para a aquicultura. Neste estudo a fonte de SFA utilizada foi o óleo de coco, rico em 12:0. Contudo, existem outros ingredientes que são potenciais fontes de lipídios para peixes de cultivo e são ricos em SFA de cadeia curta, como o óleo de inseto (*Hermetia illucens*) que é um subproduto da farinha de inseto e possuem composição similar ao óleo de coco, com 21-49% de 12:0 (Li et al., 2016; Ogori et al., 2020). É importante salientar que não foi objetivo deste trabalho

avaliar o custo de produção associado a esta dieta, e sim avaliar inicialmente as bases fisiológicas deste incremento de SFA e MUFA aliada a suplementação com LC-PUFA nesta espécie.

Apesar da manipulação da dieta para induzir o LC-PUFA *sparing effect* ser uma alternativa promissora, é improvável que apenas este fator seja suficiente para que a aquicultura atinja um patamar de sustentabilidade e reduza totalmente o OP das dietas. Deve-se buscar abordagens que cubram múltiplos fatores, tanto nutricionais, como no caso dos LC-PUFA e digestibilidade de ingredientes alternativos, quanto em fatores essenciais como sanidade e resistência a patologias, qualidade de água, particularidades fisiológicas das espécies em cada estágio de cultivo, entre outros. Neste contexto o conhecimento de aspectos fisiológicos das espécies cultivadas é extremamente importante e dessa forma, a fisiologia pode ser utilizada como ferramenta para melhorar aspectos produtivos, nutricionais e sanitários durante o cultivo.

Como ações futuras, sugere-se que mais estudos sejam realizados avaliando diferentes níveis de inclusão de óleos ricos em SFA e MUFA no metabolismo lipídico de espécies comerciais, principalmente, marinhas. Além disso, estudos que avaliem aspectos fisiológicos de espécies comerciais em condições mais próximas do cultivo real da espécie podem gerar grandes avanços neste contexto. Parcerias com empresas, universidades e pisciculturas devem ser firmadas para a realização de experimentos em maior escala, cobrindo mais estágios de vida dos organismos e em condições de cultivo, e chegando mais próximo de transcrever conhecimentos gerados nos laboratórios para o empreendimento aquícola (Rombenso et al., 2021).

Referências bibliográficas gerais

Alhazzaa, R., Nichols, P.D., Carter, C.G. 2018. Sustainable alternatives to dietary fish oil in tropical fish aquaculture. Reviews in Aquaculture, 11: 1195-1218.

Araújo, B.C., Honji, M.R., Rombenso, A.N., Brambila-Souza, G., Mello, P.H., Hilsdorf, A.W.S., Moreira, R.G. 2019. Influences of different arachidonic acid levels and temperature on the growth performance, fatty acid profile, liver morphology and expression of lipid genes in cobia (*Rachycentron canadum*) juveniles. Aquaculture, 511, 734245.

Araújo, B.C., Wade, N.M., Salini, M., Glencross, B.D. 2016. Influence of variation in dietary fatty acid and fasting on the hepatic lipid composition and gene expression by barramundi (*Lates calcarifer*). Aquaculture Research, 48: 3879–3893.

Arts, M.T., Kohler, C.C. 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts, M.T., Brett, M.T., Kainz, M.E. (Eds). Lipids in Aquatic Ecosystems. Springer (Publ.), New York, EUA, p. 237-255.

Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R. 2004. Replacement of dietary fish oil with increasing levels of linseed oil: Modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. Lipids, 39: 223–232.

Benetti, D.D., Orhun, R., Sardenberg, B., O'Hanlon, B., Welch, A., Hoenig, R., Zink, I., Rivera, J.A., Denlinger, B., Bacoat, D., Palmer, K., Cavalin, F. 2008. Advances in hatchery and grow-out technology of cobia *Rachycentron canadum* (Linnaeus). Aquaculture Research, 39:701-711.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M.S. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 214, 253–271.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernandez, A.J., Rosenlund, G. 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. Journal of Fish Diseases, 27, 531–541.

Calder, P.C. 2006. Polyunsaturated fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids, 75: 197-202.

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H., Chen, H.Y. 2004. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquaculture, 299, 325–333.

FAO, 2016. The State of World Fisheries and Aquaculture 2016. Rome. P. 170-190.

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., Alexis, M.N. 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata L.*); effects on growth performance, flesh quality and fillet fatty acid profile: recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquaculture, 289, 317– 326.

Fraser, T.W.K., Davies, J.S. 2009. Nutritional requirements of cobia, *Rachycentron canadum* (Linnaeus): a review. Aquaculture Research, 40:1219-1234.

Glencross, B.D. 2009. Exploring the nutritional demand for essential fatty acid by aquaculture species. Reviews in Aquaculture, 1, 71-124.

Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Archive of Animal Nutrition, 49:5-22.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E. 2015. Review on the use of insects in the diet of farmed fish: Past and future. Animal Feed Science and Technology, 203, 1–22.

Izquierdo, M.S., Fernández-Palacios, H., Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197, 1 – 4.

Jordal, A.E.O., Torstensen, B.E., Tsoi, S., Tocher, D.R., Lall, S.P., Douglas, S .2005. Dietary rapessed oil affects the expression of genes involved in hepatic lipid metabolism in Atlantic salmon (*Salmo salar*). Journal of Nutrition, 135, 2355–2361.

Jump, D.B., Botolin, D., Wang, Y., Xu, J., Christian, B., Demeure, O. Fatty Acid Regulation of Hepatic Gene Transcription. Recent Advances in Nutritional Sciences, 135: 2503-2506.

Li, S., Ji, H., Zhang, B., Tian, J., Zhou, J., Yu, H. 2016. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue

fatty acid composition and lipid deposition in juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture, 465, 43-52.

Mata-Sotres, J.A., Marques, V.H., Barba, D., Braga, A, Araújo, B.C., Viana, M.T., Rombenso, A.N. 2021. Increasing dietary SFA:MUFA ratio with low levels of LC-PUFA affected lipid metabolism, tissue fatty acid profile and growth of juvenile California Yellowtail (*Seriola dorsalis*). Aquaculture, 543,737011.

Menoyo D., Izquierdo M.S., Robaina, L., Gines, R., Lopez-Bote, C.J., Bautista, J.M. 2004. Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soybean oils. The British Journal of Nutrition, 92: 41–52.

Mozanzadeh, M.T., Agh, N., Yavari, V., Marammazi, J.G., Mohammadian, T., Gisbert, E. 2016. Partial or total replacement of dietary fish oil with alternative lipid sources in silvery-black porgy (*Sparidentex hasta*). Aquaculture, 451, 232–240.

Nelson, D.L., Cox, M.M. 2018. Lehninger: Principles of Biochemistry. Freeman, New York, EUA, 341-362.

Nordahl, G. 2011. Is the Aquaculture Industry Caught in a Fishmeal Trap? Master Thesis in Economic Analysis. Norwegian School of Economics and Business Administration, p. 109.

Ostbye, T.K., Kjaer, M.A., Rora, A.M.B., Torstensen, B., Ruyter, B. 2009. High n3 EFA levels in the diet of Atlantic salmon affect muscle and mitochondrial membrane lipids and their susceptibility to oxidative stress. Aquaculture Nutrition, 17:177-190.

Ogori, A.F. 2020. Source, Extraction and Constituents of Fats and Oils. Journal of Food Science and Nutrition, 6, 2 – 8.

Pratoomyot, J., Bendiksen, E.A., Bell, J.G., Tocher, D.R. 2008. Comparison of effects of blended vegetable oils and contaminant-stripped fish oil on growth performance, composition and gene expression in Atlantic salmon (*Salmo salar* L.). Aquaculture, 280,170–178.

Rombenso, A.N., Turchini, G.M., Trushenski, J.T. 2021. The omega-3 sparing effect of saturated fatty acids: A reason to reconsider common knowledge of fish oil replacement. Reviews in Aquaculture, 1-5.

Rombenso, A.N., Trushenski, J.T., Jirsa, D., Drawbridge, M. 2015. Successful Fish Oil Sparing in white seabass Feeds Using Saturated Fatty Acid-Rich Soybean Oil and 22:6n-3 (DHA) Supplementation. Aquaculture, 448, 176–185.

Rombenso, A.N., Trushenski, J.T., Drawbridge, M. 2018. Saturated lipids are more effective than others in juvenile California yellowtail feeds—Understanding and harnessing LC-PUFA sparing for fish oil replacement. Aquaculture, 493, 192–203.

Ruiz-Lopez, N., Haslam, R.P., Napier, J.A., Sayanova, O. 2014. Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. The Plant Journal. 77, 198–208.

Salini, M.J., Irvin, S.J., Bourne, N., Blyth, D., Cheers, S., Habilay, N., Glencross, B.D. 2015a. Marginal efficiencies of long chain-polyunsaturated fatty acid use by barramundi (*Lates calcarifer*) when fed diets with varying blends of fish oil and poultry fat. Aquaculture, 449, 48–57.

Salini, M.J., Turchini, G.M., Glencross, B.D. 2015b. Effect of dietary saturated and monounsaturated fatty acids in juvenile barramundi *Lates calcarifer*. Aquaculture Nutrition, 23, 1-12.

Smith, D.M., Williams, I.H., Williams, K.C., Barclay, M.C., Venables, W.N. 2005. Oxidation of medium-chain and long-chain fatty acids by polka dot grouper *Cromileptes altivelis*. Aquaculture Nutrition, 11, 41–48.

Sorbera, L. A., Astuarino, J. F., Carrillo, M., Zanuy, S. 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocytes maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biology of Reproduction, 64, 382-389.

Stubhaug, I., Lie, O., Torstensen, B.E. 2006. β-oxidation capacity in liver increases during parr–smolt transformation of Atlantic salmon (*Salmo salar* L.) fed vegetable and fish oil. Journal of Fish Biology, 69, 504–517.

Stubhaug, I., Lie, O., Torstensen, B.E. 2007. Fatty acid productive value and b-oxidation capacity in Atlantic salmon (*Salmo salar L.*) fed on different lipid sources along the whole growth period. Aquaculture Nutrition, 13, 145–155.

Tacon, A.G.J., Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded: aquafeeds: Trends and future prospects. Aquaculture, 285, 146–158.

Teles, A.O., Enes, P., Peres. H. 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Davis, D.A. Feed and Feeding Practices in Aquaculture. Elsevier P. 203-233

Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science, 11(2), 107–184.

Tocher, D.R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research, 41, 717 -732.

Tocher, D.R. 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture, 449, 94–107.

Torstensen, B.E., Froyland, L., Lie, O. 2004. Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. Aquaculture Nutrition, 10, 175–192.

Torstensen, B.E., Nanton, D., Olsvik, P.A., Sundvold, H., Stubhaug, I. 2009. Gene expression of fatty acid binding proteins (FABPs), fatty acid transport proteins (cd36 and FATP) and β-oxidation related genes in Atlantic salmon (*Salmo salar* L.) fed fish oil or vegetable oil. Aquaculture Nutrition, 15:440-445.

Trushenski J.T., Bowzer J.C. 2013. Having your omega 3 fatty acids and eating them, too: Strategies to ensure and improve the long-chain polyunsaturated fatty acid content of farm-raised fish. In: De Meester F, Watson RR, Zibadi S, eds. Sustainable Long Chain Omega-3 Fatty Acids in Cardiovascular and Mental Health. New York, NY: Springer. p 319-340.

Trushenski, J., Schwarz, M., Lewis, H., Laporte, J., Delbos, B., Takeuchi, R., Sampaio, L.A. 2011. Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia *Rachycentron canadum*. Aquaculture Nutrition, 17, 437- 447.

Trushenski, J., Schwarz, M., Bergman, A., Rombenso, A., Delbos, B. 2012. DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. Aquaculture, 326–329, 81–89.

Turchini, G.M., Torstensen, B.E., Ng, W.K. 2009. Fish oil replacement in finfish nutrition. Reviews in Aquaculture, 1, 10–57.

Turchini, G.M., Francis, D.S., Senadheera, S.P.S.D., Thanuthong, T., De Silva, S.S. 2011a. Fish oil replacement with different vegetable oils in Murray cod: evidence of an “omega-3 sparing effect” by other dietary fatty acids. Aquaculture, 315, 250–259.

Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), 2011b. Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton.

Turchini, G.M., Hermon, K., Cleveland, B.J., Emery, J.A., Ran- kin, T. & Francis, D.S. 2013. Seven fish oil substitutes over a rainbow trout grow-out cycle: I) Effects on performance and fatty acid metabolism. Aquaculture Nutrition, 19, 82–94.

Xue, Z., Sharpe, P.L., Hong, S.P., Yadav, N.S., Xie, D., Short, D.R., Damude, H.G., Rupert, R.A., Seip, J.E., Wang, J., Pollak, D.W., Bostick, M.W., Bosak, M.D., Macool, D.J., Hollerbach, D.H., Zhang, H., Arcilla, D.M., Bledsoe, S.A., Croker, K., McCord, E.F., Tyreus, B.D., Jackson, E.N., Zhu, Q. 2013. Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. Nature Biotechnology, 31, 734–740

Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G., Teale, A.J. 2004. Effects of diets containing vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in liver of Atlantic salmon (*Salmo salar*). Aquaculture, 236: 467–483.