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FARELO DE SOJA COMO ALTERNATIVA PROTEICA NA DIETA DE MATRINXÃ (*Brycon amazonicus*)

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FARELO DE SOJA COMO ALTERNATIVA PROTEICA NA DIETA DE MATRINXÃ (*Brycon amazonicus*)[1]

Autora: Ana Amélia Nunes Fossati

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RESUMO

O Brasil é um dos países que mais cresce em produção de pescado cultivado no mundo, destacando-se pelo aumento na produção de peixes nativos, como o tambaqui, pacu, matrinxã, entre outros. Embora esta produção esteja crescendo, a formulação de dietas ainda está baseada na utilização de farinha de pescado como principal fonte proteica, o que é muito negativo do ponto de vista ecológico. Por este motivo, fontes proteicas alternativas de origem vegetal vem sendo estudas para tentar suprir essa necessidade na alimentação de peixes. O objetivo deste trabalho foi avaliar os efeitos de três dietas: SBM (100% de farelo de soja como proteína na dieta), SBM/FM (50% de farelo de soja e 50% de farinha de peixe como proteína) e FM (100% de farinha de peixe como proteína) no crescimento muscular, nos parâmetros de crescimento, composição dos filés e histopatologia do fígado e intestino em matrinxãs juvenis. O tratamento FM apresentou os maiores valores de peso corporal e de filés nos animais. Enquanto o tratamento SBM apresentou maior comprimento de sarcômero e maior número de fibras por área, os demais parâmetros musculares não apresentaram diferenças estatísticas entre os tratamentos. O peso final, ganho de peso e ganho em peso diário diminuíram conforme a substituição proteica aumentava. Já para crescimento específico, conversão alimentar aparente e eficiência proteica, os tratamentos SBM/FM e FM obtiveram resultados similares. O tratamento SBM apresentou maiores valores de ácidos graxos poli-insaturados e ômega-6 nos filés enquanto o tratamento FM apresentou maiores valores de DHA e ômega-3. A maioria das análises histológicas de fígado e intestino apresentaram estruturas morfológicas normais, apenas com o aparecimento de algumas alterações como aumento de vacúolos e aumentos dos sinusóides entre os hepatócitos no tratamento SBM nos fígados analisados, e encurtamento de vilosidades no tratamento SBM em comparação com os outros tratamentos nos intestinos analisados. Em conclusão, observou-se que a substituição parcial SBM/FM pode obter valores similares ao tratamento FM quanto à vários dos parâmetros analisados, sendo uma alternativa promissora após maiores estudos.

Palavras-chave: performance; histologia; intestino; fígado; nutrição; proteína vegetal.

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SOYBEAN MEAL AS A ALTERNATIVE PROTEIN IN MATRINXÃ DIET (*Brycon amazonicus*)[1]

Autora: Ana Amélia Nunes Fossati

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ABSTRACT

Brazil is one of the fastest growing countries in fish production in the world, especially due to the increase in the production of native fish, such as tambaqui, pacu, matrinxã, among others. Although this production is growing, the formulation of diets is still based on the use of fish as the main source of protein, which is very negative from the ecological point of view. For this reason, alternative protein sources of plant origin have been studied to try to meet this need in fish feed. The objective of this work was to evaluate the effects of three diets: SBM (100% soybean meal as protein in the diet), SBM / FM (50% soybean meal as protein) and FM (100%) on muscle growth, growth parameters, fillet composition, and histopathology of the liver and intestine in juvenile matrinxans. The FM treatment presented the highest body weight and fillet values in the animals. While the SBM treatment presented higher sarcomere length and greater number of fibers per area, the other muscle parameters did not present statistical differences between the treatments. The final weight, weight gain and daily weight gain decreased as protein substitution increased. As for specific growth, apparent feed conversion and protein efficiency, SBM / FM and FM treatments obtained similar results. The SBM treatment presented higher values of polyunsaturated and omega-6 fatty acids in fillets while FM treatment had higher values of DHA and omega-3. Most histological analyzes of the liver and intestine showed normal morphological structures, only with the appearance of some alterations such as increase of vacuoles and increases of sinusoids between hepatocytes in SBM treatment in the analyzed livers, and shortening of villi in the SBM treatment in comparison to the other treatments in the intestines analyzed. In conclusion, it was observed that the partial substitution SBM / FM can obtain values similar to FM treatment for several of the parameters analyzed, being a promising alternative after further studies.

Keywords: performance; histology; intestine; liver; nutrition; plant protein.

[1] Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (99p.). March, 2019.

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LISTA DE ABREVIATURAS E SÍMBOLOS

am: *ante meridiem*
ANOVA: analysis of variance
CAPES: Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior
CF: fator de condição
CP: crude protein
DHA: ácido docosahexaenóico
FM: fish meal
FCR: feed conversion rate
GE: gross protein
GP: ganho de peso
GPD: ganho de peso diário
HPLTC: cromatografia líquida de alta eficiência
INPA: Instituto Nacional de Pesquisas da Amazônia
Lp: lâmina própria
MUFA: ácidos graxos monoinsaturados
n-3: ômega 3
n-6: ômega 6
OsO_4: tetróxido de ósmio
pH: potencial hidrogeniônico
pm: *post meridiem*
PP: plant proteins
PUFA: ácidos graxos poliinsaturados
SAS: Statistical Analyses System
SBM: soybean meal
S.E.M: standard error of the mean
SFA: ácidos graxos saturados
SGR: specific growth rate
SPC: soy protein concentrate
TEM: transmission electron microscopy
v: vacúolos absortivos supranucleares

CAPÍTULO I

1. INTRODUÇÃO

A produção de pescado brasileira cresceu 8% em 2017, alcançando valores de produção de 691.700 toneladas de peixes cultivados, em comparação as 640.410 toneladas produzidas em 2016 (PeixeBR, 2018). A matrinxã está entre a espécies de pescado de importância comercial na região norte do Brasil, contribuindo com 5.702,1 toneladas no ano de 2011 (Brasil, 2011). O estado do Amazonas é um dos maiores produtores de peixes nativos do país, concentrando sua produção em espécies como: Tambaqui, Pirarucu e Matrinxã (PeixeBr, 2019).

Matrinxã é o nome popular dado a espécie *Brycon amazonicus*, originária da bacia Amazônica. Esse peixe tem despertado o interesse de aquicultores devido a uma série de aspectos: carne amplamente apreciada, hábito alimentar onívoro, apresentar fácil adaptação ao cultivo e reprodução artificial dominadas, características que aumentam o potencial para a piscicultura. Além disto, apresenta alta resistência e adaptabilidade às rações comerciais, o que facilita o manejo alimentar e proporciona uma acelerada taxa de crescimento em cativeiro (Gomiero et al., 2003).

O pescado é uma fonte de proteína animal mais saudável e nutritiva do que a carne de outras espécies. A carne do pescado apresenta alto valor biológico, possuindo características interessantes: proteína altamente digestível, baixo valor de ácidos graxos saturados, alta concentração de ômega 3 e ácidos poli-insaturados, rica em minerais como o cálcio, fósforo, magnésio e ferro, entre outros, além de conter uma ampla variedade de vitaminas hidro e lipossolúveis (Tacon e Metian, 2013).

O conhecimento da composição centesimal da carne dos peixes é essencial para que se possa conhecer a qualidade do produto e viabilizá-la para o consumo humano. Além disto, permite avaliar a eficiência da transferência dos nutrientes do alimento oferecido ao peixe para a carne do pescado, assim como sua forma de processamento (Arbeláez-Rojas, Fracalossi e Fim, 2002). A qualidade é extremamente importante, pois além do valor nutritivo, os preços do pescado também dependem da qualidade, a qual se evindecia através de fatores como: textura da carne, composição química, rendimento e processo de captura e beneficiamento do produto (Simões et al., 2007).

As fases de crescimento dos peixes exige percentuais elevados de proteína na composição das dietas. Com a depleção crescente dos estoques pesqueiros, surge a necessidade de substituição da proteína de origem animal pela proteína de origem vegetal nas dietas para peixes (Collins et al., 2013). O desenvolvimento de uma aquicultura sustentável é parcialmente dependente de uma dieta para peixes também sustentável, ou seja, substituição de parte ou totalidade da proteína e óleo derivados de fonte marinha. Estratégias estão sendo pensadas no intuito de substituir as fontes de proteína e óleo originária de peixes por outras fontes provenientes da produção agrícola (Carter et al., 2012).

Neste contexto, a utilização de fontes proteicas sustentáveis para a produção aquícola tem levado os especialistas em nutrição aquícola a explorar novas fontes proteicas para substituir a farinha de peixe nas últimas décadas (Cowey et al., 1971; Dabrowski et al., 1979; Dabrowski et al., 1989; Shiau et al., 1989). Diversos estudos atuais continuam a utilizar o farelo de soja como fonte de proteína principal, devido ao alto valor proteico, balanço adequado de aminoácidos e baixo custo (Barnes et al., 2014; Kokou et al., 2015; Gu et al., 2016; Liu et al., 2017; Kotzamanis et al., 2018).

Embora as fontes proteicas vegetais possuam diversos benefícios em sua utilização na dieta, existem alguns limitantes no seu uso, como: baixa digestabilidade e palatabilidade, perfil aminoacídico diferente das proteínas de origem animal, além de possuir em sua constituição antinutrientes que podem afetar o desenvolvimento e fisiologia do animal (Glencross et al., 2004).

Como as exigências nutricionais variam para cada espécie, existem relatos de trabalhos demonstrando benefícios e malefícios na substituição da farinha de peixe pelo farelo de soja. O farelo de soja pode ser utilizado como substituto à farinha de peixe em até 69% da proteína total, sem causar prejuízos ao crescimento ou conversão alimentar no bacalhau, já o salmão do atlântico apresentou menor crescimento quando a dieta teve uma redução de 25% para 5% de farinha de peixe em substituição por proteína vegetal que consistia em uma mistura de farinha de girassol, farinha de glúten de milho, farelo de soja e glúten de trigo (Pratoomyot et al., 2010).

O objetivo deste estudo foi avaliar o desempenho zootécnico dos peixes, a composição centesimal e de ácidos graxos dos filés, assim como avaliar as características histomorfológicas da musculatura, fígado e intestino de Matrinxãs (*B. amazonicus*), alimentados com dietas contendo substituições substituição parcial e total da farinha de peixe pelo farelo de soja.

2. REVISÃO BIBLIOGRÁFICA

2.1 A Matrinxã (*Brycon amazonicus*)

A Matrinxã (*B. amazonicus*) é uma espécie originária da Bacia Amazônica, que já vem sendo cultivada na região sudeste do Brasil, atendendo principalmente ao mercado de pesque-pague (Figura 1). Pertencente à família *Characidae*, considerada a mais ampla dos peixes de água doce neotropicais. A aceitabilidade ampla pelos consumidores e considerado na mais alta categoria no mercado norte do Brasil (Almeida e Franco, 2007), especialmente na região Amazônica (Zanuzzo et al., 2018).

FIGURA 1. Matrinxã (*Brycon amazonicus*). Fonte: Acervo pessoal.

A espécie apresenta grande potencial de crescimento (800 a 1000 gramas no primeiro ano) e carne nobre. A matrinxã em fase adulta pode chegar a um comprimento total de 80 cm e peso ao redor de 5 kg, na fase juvenil pode alcançar 22,8 cm de comprimento e 0,148 kg de peso (Gadelha & Araújo, 2013). Apresenta alta relevância comercial em países da América do Sul, sendo uma das principais espécies produzidas (Venturini et al., 2019). No Brasil, os maiores produtores de espécies nativas são Rondônia, Amazonas e Pará (região Norte), Mato Grosso e Goiás (região Centro-Oeste) e Maranhão (região Nordeste). O estado de Amazonas possui a terceira maior produção, com 100% de produção de peixes nativos (28 mil toneladas), liderada pelo Tambaqui e com boa presença de Matrinxã e Pirarucu, entre outras espécies (PeixeBR, 2018).

Em seu ambiente natural alimentam-se de frutos, sementes e insetos, sendo seu hábito alimentar onívoro, o que consequentemente facilita o consumo

e aceitabilidade de rações e favorece seu cultivo (Macedo-Viegas et al., 2000). A espécie também tem boa conversão alimentar quando alimentada com dieta artificial (Ribeiro et al., 2016). Dentro da aquicultura é altamente valorizada para pesca esportiva (Saccol et al., 2017). Devido a aceitação de ração, é possível formular e fornecer diversas dietas adequadas a seu crescimento. Izel et al. (2004) constataram que entre os níveis proteicos testados em dieta de matrinxã juvenil, a que continha 28% de proteína bruta (PB) proporcionou o maior crescimento em peso diário dos peixes (4,0 g/dia), e as melhores taxas de conversão alimentar foram obtidas com rações contendo entre 25 e 28% de PB, sugerindo que a exigência por PB da matrinxã pode ser atendida entre estes valores.

Quanto á análise bromatológica dos filés, não foram observadas diferenças significativas entre os diferentes valores de proteína fornecidos na ração (Izel et al., 2004). O aumento da massa corporal e melhor conversão alimentar da matrinxã está relacionado com a quantidade proteica da dieta (Ferreira et al., 2013). O índice de conversão alimentar da espécie é de 1 grama para cada 3,83 gramas de proteína bruta em dietas contendo 32% PB. O ganho de peso médio é de 0,65 g/dia submetidos à alimentação com mesma quantidade de proteína bruta (Canevesi et al., 2014). O teor de proteína bruta aumentou e o extrato etéreo diminiu, conforme o nível proteico das dietas de matrinxã aumentavam, afirmando que o teor de proteína foi inversamente proporcional ao teor de extrato etéreo na carcaça de peixes (Mattos et al., 2018).

Quanto as características de qualidade do filé, Almeida et al. (2009) verificaram que o matrinxã é uma espécie com carne considerada gorda, possuindo 9,4% de lipídios totais, sendo o ácido araquidônico o metil-éster mais encontrado no tecido muscular. Os ácidos graxos predominantes na fração lipídica encontrados nas cabeças de matrinxã foram os ácidos palmítico, esteárico, oleico e linoleico. Porém, variaram em quantidades de 20,30 à 22% de lipídios totais, enquanto a razão entre ômega 6 e ômega 3 encontrada para a espécie foi de 8,05 (Moreira et al., 2003). Cabe ressaltar que a carne da matrinxã é uma excelente fonte calórica para alimentação humana, devido ao seu alto teor de ácidos graxos benéficos a saúde. De acordo com Batista et al. (2004), matrinxãs de 11 meses de idade apresentavam peso médio de 1,23 kg (±0,0894) e comprimento padrão de 34,7 cm (±1,23). Apresentavam carne semi-gorda (7,5g/100g ± 0,1 extrato etéreo), além disso a composição centesimal do músculo continha 72,3% de umidade, 18,4% de proteína, 0,9% de cinzas. Pizango-Paima et al. (2001) verificaram que o teor de proteína bruta encontrado nos filés de matrinxã variava de 53-73% da matéria seca. Relataram também a ocorrência de relação inversa entre os teores de proteína e de extrato etéreo no músculo dos peixes. Os autores sugerem que a composição centesimal do filé de matrinxã está diretamente relacionada com a composição centesimal da

dieta. No estudo de Arbeláez-Rojas et al. (2002) encontrou-se teor de gordura corporal dos juvenis de matrinxã entre 13,5 e 14,95%. Observaram também que o maior crescimento dos juvenis de matrinxã, no sistema intensivo com alto fluxo de água ocorreu provavelmente devido ao maior desenvolvimento das fibras musculares, refletido pelo maior peso seco, quando comparado ao grupo de peixes que não foi submetido a nado contínuo. Entretanto, os peixes cultivados no sistema semi-intensivo de água parada, o peso corporal esteve representado por maior acúmulo de gordura. Franco et al. (2010) verificaram que o peso dos filés de matrinxã foram de 391–398g. Os valores de umidade, proteína bruta, cinzas e lipídios foram de 72,9, 20,0, 1,25 e 3,37%, respectivamente. Os autores destacam que a composição do filé pode influenciar no rendimento, sabor, textura e estabilidade oxidativa da gordura. Macedo-Viegas et al. (2000) encontraram rendimento médio de filé de 38,6-40,0% em matrinxãs de pesos médio de 400 a 700 gramas.

Sabe-se que o colágeno é o principal componente do tecido conectivo muscular dos peixes, e está estritamente relacionado à textura, sendo mais encontrado os colágenos do tipo I e V. Suárez-Mahecha et al. (2007) observaram em seus estudos de textura post-mortem em matrinxã que as fibras de colágeno no tecido conectivo pericelular indicaram estreita relação com o amolecimento post-mortem. Imediatamente após a morte, as fibras de colágeno foram observadas claramente, mantendo uma arquitetura ordenada junto à fibra muscular. Após armazenamento de 12 horas a –30°C, as fibras do tecido conectivo pericelular se desintegraram, ocasionando perda do arranjo arquitetônico em relação à fibra muscular.

2.2 Ingredientes protéicos da dieta: Farelo de soja e farinha de peixe

Sabe-se que somente uma pequena quantidade do pescado capturado é consumido pelo homem. De 25 a 75% (dependendo da espécie) da matéria-prima remanescente é utilizada pela alimentação animal ou está sendo desperdiçada no processamento industrial, fato este que demonstra a urgência em aprimorar o gerenciamento dos recursos naturais, modernização e planificação logística. Quando os peixes começaram a desaparecer da costa devido à pesca excessiva e a fatores biológicos, percebeu-se a necessidade de administração imediata, uma vez que os recursos marinhos logo esgotariam diane da pressão de pesca exercida (Ogawa e Maia, 1999). Há mais de 20 anos, Ogawa e Maia (1999) observaram no litoral sul que os peixes pelágicos como a sardinha, os demersais como a pescada, corvina, castanha, linguado e cação, e os camarões rosa e sete-barbas, estão se tornando mais escassos, devido principalmente a sobrepesca. A busca por fontes nutritivas semelhantes as do pescado para permitir a criação de peixes em cativeiro é um desafio. Entregar

todos os nutrientes de forma balanceada envolve o conhecimento das necessidades nutricionais da referida espécie.

As proteínas e os aminoácidos são indispensáveis na nutrição animal, pois possuem importante papel estrutural e metabólico (NRC, 2011), sendo constituintes fisiológicos em todas as etapas de desenvolvimento, além de serem responsáveis pela formação de enzimas e hormônios (Pezzato et al., 2004). Contudo, nenhuma espécie possui exigência nutricional específica por proteína, mas sim, por um adequado balanceamento de aminoácidos (Bicudo e Cyrino, 2009). Para peixes, 10 aminoácidos tem sido descrito como essenciais: arginina, fenilalanina, histidina, isoleucina, leucina, lisina, metionina, treonina, triptofano e valina (NRC, 2011), os mesmo para a maior parte das espécies. A composição aminoacídica da farinha de peixe e o farelo de soja e a exigência aminoacídica para tilápias encontram-se nas Tabelas 1 e 2.

Tabela 1. Composição de aminoácidos essenciais digestíveis (incluindo cistina e tirosina) da farinha de peixe e farelo de soja (base na matéria úmida).

Aminoácido (%)	**Alimento**	
	Farinha de Peixe	**Farelo de soja**
Arginina	3,42	3,36
Histidina	1,15	1,17
Isoleucina	2,24	2,18
Leucina	3,79	3,67
Lisina	4,04	3,10
Metionina	1,40	0,50
Metionina + cistina	2,00	1,06
Fenilalanina	2,20	2,23
Fenilalanina + tirosina	3,65	3,44
Treonina	2,17	1,66
Triptofano	0,27	0,53
Valina	2,87	2,24

Fonte: Furuya et al., 2001; Guimarães et al., 2008.

Tabela 2. Exigências de aminoácidos essenciais (incluindo cistina e tirosina) para tilápias (base na matéria úmida).

Aminoácido (%)	**>100 g**
Lisina	1,38
Metionina	0,47
Metionina + cistina	0,83
Treonina	1,07
Arginina	1,14
Fenilalanina + tirosina	1,50
Histidina	0,47

Isoleucina	0,84
Leucina	0,92
Triptofano	0,27
Valina	0,75

Fonte: Furuya, 2010.

A deficiência ou excesso de um dos aminoácidos na dieta, ou seja, uma dieta desbalanceada, acarreta em redução da mobilização dos aminoácidos para a formação dos tecidos musculares, resultando na diminuição do crescimento, ganho em peso, eficiência alimentar e resistência a doenças (Rodrigues et al., 2013). A alimentação é o maior custo na produção intensiva de peixes, sendo geralmente os ingredientes proteicos os mais onerosos e de maior importância para o crescimento (Ahmed e Khan, 2006). O custo da ração representa cerca de 60% do custo de produção (Teixeira et al., 2008). Entre os ingredientes, a soja faz o preço por kg de ração ser menor em comparação à utilização de farinha de peixe, consequentemente o custo final da produção do pescado pode diminuir (Hernández et al., 2007). Desta maneira, ao se encontrar um nível de inclusão do farelo de soja como substituto da farinha de peixe que não prejudique o desempenho dos peixes, pode ser uma ótima alternativa para diminuição do custo de produção, e consequentemente aumentar a lucratividade do produtor.

Entre os ingredientes proteicos utilizados em dietas para peixes, a farinha de peixe é considerada uma das mais onerosas, e embora esta seja rica em aminoácidos essenciais, a necessidade diária proteica dos peixes se situa entre os 30 a 50%, na espécie matrinxã entre 36-45% (Ferreira et al., 2013). Aumentando a inclusão de farinha de peixes à dieta de tilápia, de acordo com Boscolo et al. (2010) repercute em maior crescimento dos animais. Esses resultados podem estar associados a maior quantidade de metionina na dieta devido a àdição da farinha, gerando maior disponilidade deste aminoácido na alimentação. As proteínas animais possuem maior valor biológico em comparação as de origem vegetal, além de serem mais palatáveis, promovendo maior consumo pelos peixes (Rodrigues et al., 2013). Contudo, na última década, o aumento do preço da farinha de peixe, a regulação intensa de efluentes de incubação e o debate sobre a sustentabilidade têm intensificados os estudos em alternativas à farinha de peixe (Jirsa et al., 2015).

A farinha de peixe inteiro e a de resíduo de peixe apresentam bom equilíbrio de aminoácidos essenciais e ácidos graxos, teores variáveis de gordura (4-20%) e de matéria mineral (11-23%). O alto custo e a excelente qualidade nutricional da farinha de peixe levam a priorização desta para dietas de reprodutores e animais jovens, fases de maior exigência nutricional (Rodrigues et al., 2013). Além disso, grande parte da farinha de peixe produzida

tem sido destinada para indústria de rações para animais de companhia, como gatos e cães, por serem produtos de maior valor agregado.

Os produtos a base de soja como farelo de soja e proteína concentrada de soja estõ entre as proteínas de origem vegetal mais promissoras e utilizadas em diversos estudos de substituição proteica à farinha de peixe (Bonaldo et al., 2006). A soja também é uma excelente alternativa do ponto de vista de produção, pois o Brasil produziu 119.996 milhões de toneladas na safra de 2017/2018, sendo o segundo maior produtor da semente do mundo (EMBRAPA, 2018). Proteínas de fonte vegetal, que são abundantes e de menor custo, vêm sendo utilizadas na alimentação de peixes como substitutos da farinha de peixe em diversas espécies, embora altos níveis de substituição possam acarretar em uma menor ingestão de alimentos e, consequentemente, menor crescimento (Liu et al., 2016). O farelo de soja é atualmente o principal ingrediente de origem vegetal utilizado em rações para peixes, devido ao seu alto percentual proteico (44 a 50% PB) e balanço aminoácidico razoável, com exceção da metionina que necessita de complementação. Tem se mostrado eficiente na substituição da farinha de peixe, podendo compor até 50% da base proteica da dieta, sendo que para espécies onívoras, por exemplo, essa substituição pode ser de até 100% (Rodrigues et al., 2013).

Existem estudos em que a substituição parcial da farinha de peixe pelo farelo de soja concentrado permite os mesmos índices produtivos (Zhao et al., 2010; Azarm & Lee, 2014; Bansemer et al., 2015; Chu et al., 2016), enquanto outros autores sugerem que ingredientes a base de soja reduzem ganho de peso e eficiência alimentar (Silva-Carrillo et al. 2012; Yu et al. 2013). Embora a soja esteja se consolidando como alternativa proteica nas rações, alguns fatores ainda devem ser considerados. De acordo com Wang et al. (2015), amostras de fígado de carpas pretas alimentadas com farelo de soja comparadas com a de animais alimentados com farinha de peixe mostraram polarização do núcleo dos hepatócitos, presença de vacúolos lipídicos e pontos isolados de necrose, embora tenha apresentado histologia normal nas carpas capim e carpa “Gibel”. De acordo com Honorato et al. (2014), dietas com 24 e 28% de PB com inclusão de silagem biológica de pescado afetaram a estrutura hepática, ocasionando nos hepatócitos, deslocamento nuclear para periferia. Essa alteração é indicativa de alteração metabólica do órgão. As fontes protéicas de origem vegetal são ingredientes eficientes pois apresentam disponibilidade ao logo de todo o ano, composição homogênea e custo inferior a proteína animal (Rodrigues et al., 2013). Chou et al. (2004) observaram que a concentração de lipídios no músculo de Cobia (*Rachycentron canadum*) aumentou conforme os níveis de substituição de farinha de peixe por farelo de soja aumentavam.

Em peixes que consomem grandes quantidades de ingredientes a base de plantas cruas, um ponto negativo pode estar associado à presença dos

fatores anti-nutricionais n o trato gastrintestinal. O grão de soja cru, por exemplo, contém a presença de lecitina e glicina, que são capazes de se ligar a superfície das células intestinais e podem ocasionar reações patológicas ao tecido. Porém, em estudo com trutas arco-íris em que 35% de proteína bruta a base de proteína de soja foi incluída na dieta, nenhuma inflamação foi detectada na parte distal do intestino, sugerindo que rações que continham soja tratada e purificada foram capazes de inativar fatores anti-nutricionais evitando os danos teciduais (Brinker e Reiter, 2011).

Os antinutrientes são substâncias que afetam negativamente o desempenho dos peixes e apresentam-se normalmente em ingredientes de origem vegetal. A soja possui antinutrientes como os inibidores da tripsina, agentes hemaglutinantes, saponinas, taninos e outros compostos fenólicos e polissacarídeos não amiláceos. Geralmente estas substâncias podem ser inativadas ou ter sua ação reduzida através do emprego de métodos como o processamento a quente ou tratamentos químicos (Rodrigues et al., 2013). Embora a soja e seus subprodutos possam conter antinutrientes, sabe-se que a sensibilidade a estes é variável entre as espécies de peixes, sendo que várias conseguem crescer com dietas que possuem quantidades praticamente iguais de farinha de peixe e farelo de soja sem efeitos adversos (Chou et al., 2004). Dentre os efeitos dos antinutrientes destacam-se os efeitos sobre as modificações na permeabilidade da mucosa intestinal, redução na absorção de nutrientes no intestino, redução da ação de enzimas digestivas, necrose hepática, gastroenterites, redução da digestibilidade do amido, proteína e lipídeos, redução na taxa de passagem no trato gastrointestinal com consequente redução do consumo, retardo no crescimento, entre outros efeitos negativos (Rodrigues et al., 2013). Outro estudo relata aumento no acúmulo de gordura no fígado do salmão do Atlântico alimentado com dieta contendo proteína de soja concentrada, embora não tenha afetado o peso do estômago e do fígado.

As condições de processamento sobre os ingredientes podem alterar o consumo e a qualidade das dietas. O processo de extrusão, por exemplo, pode levar a degradação de vitaminas termolábeis e aminoácidos, como a lisina e cisteína pela temperatura elevada empregada. O uso de alta temperatura também pode desativar fatores antinutricionais que afetam a qualidade da dieta (Francis, Makkar & Becker, 2001). De acordo com Collins et al. (2013) o crescimento reduzido de animais alimentados com dietas a base de proteínas vegetais pode ser explicado pela redução proteica, aminoacídica, lipídica e energia digestível, se não forem compensados pelo aumento da ingestão. Em salmonídeos se observam os efeitos negativos do uso de proteínas vegetais no desempenho que geralmente estão associados aos antinutrientes. Proteínas vegetais tendem a ter menor digestabilidade do que a farinha de peixe. Este problema poderia ser resolvido diminuindo a passagem da dieta pelo trato gastrointestinal. Um tempo maior de retenção do alimento pode favorecer a ação de enzimas gástricas e microrganismos fermentativos, melhorando a utilização de nutrientes antes indisponíveis (Collins et al., 2013).

2.3 Conformação muscular em peixes

A fibra muscular esquelética é uma célula alongada, que possui entre 1 e 40 mm de comprimento e entre 10 a 100 µm de diâmetro (Figura 2). São células multinucleadas, os núcleos são ovais e se localizam na periferia da célula (Banks, 1991). O tecido muscular estriado esquelético é formado pelas fibras musculares, que são células especializadas localizadas abaixo da membrana plasmática (Dal Pai-Silva et al., 2005). Na maioria das espécies de peixes, a musculatura estriada esquelética está organizada em unidades morfofuncionais, os miômeros e os miosseptos (Alexander, 1969).

Nos peixes, a taxa de crescimento está diretamente relacionada com o crescimento da musculatura estriada esquelética (Almeida, 2011). Desta forma, a avaliação da morfologia do tecido muscular estriado esquelético é uma maneira importante de se avaliar o crescimento do animal.

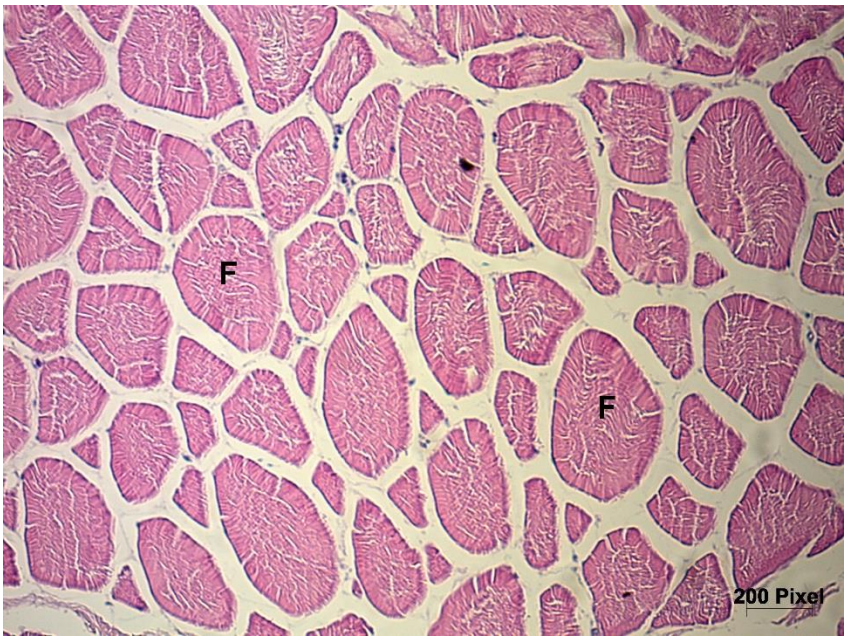

FIGURA 2. Corte transversal de músculo esquelético de *Brycon amazonicus*. Legenda: As fibras (F) estão agrupadas em feixes ou fascículos. Fonte: Acervo pessoal.

Finas membranas de tecido conectivo, chamadas iocomatas, dividem o músculo esquelético em segmentos denominados miômeros, estes, são compostos de fibras musculares paralelas umas as outras ao longo do eixo longitudinal do peixe. Cada miótomo é constituído por inúmeras fibras musculares, com diâmetro entre 50 e 300 µm e comprimento entre 5 a 20 nm (Ogawa e Maia, 1999). As variações no diâmetro das fibras musculares esqueléticas irão depender do músculo, idade, sexo, nutrição e espécie. Este aumento de massa muscular pode ocorrer de duas formas, através do aumento de volume da célula, processo denominado hipertrofia, e através da proliferação

de células totipotentes (células satélites), chamado hiperplasia (Junqueira & Carneiro, 2013).

Estriações transversais estão orientadas perpendicularmente em relação ao maior eixo da fibra muscular, estas são compostas por bandas, onde *bandas* I são mais claras e *bandas* A mais escuras. No interior das *bandas* I existe uma faixa mais densa denominada *linha* Z, esta em distância a *linha* Z adjacente recebe a denominação de sarcômero, ou seja, a unidade de contração muscular que contém miofilamentos contidos entre duas *linhas* Z (Banks, 1992). Cada fibra muscular contém muitos feixes cilíndricos de filamentos, as miofibrilas, estruturas que medem de 1 a 2 µm de diâmetro, são paralelas ao maior eixo da fibra muscular e são estruturadas em um arranjo repetitivo de sarcômeros (Junqueira & Carneiro, 2013). Os miofilamentos contidos na *banda* I são os filamentos finos de actina, já a *banda* A é formada pelos filamentos finos de actina e filamentos grossos de miosina. A miosina é um filamento de aproximadamente 10 nm de diâmetro e 1,5 µm de comprimento, enquanto a actina possui cerca de 5 nm de diâmetro e 1 µm de comprimento (Figura 3). Outras miofibrilas presentes no sarcômero são a troponina e a tropomiosina, ambas são proteínas reguladores do mecanismo de contração muscular (Banks, 1991).

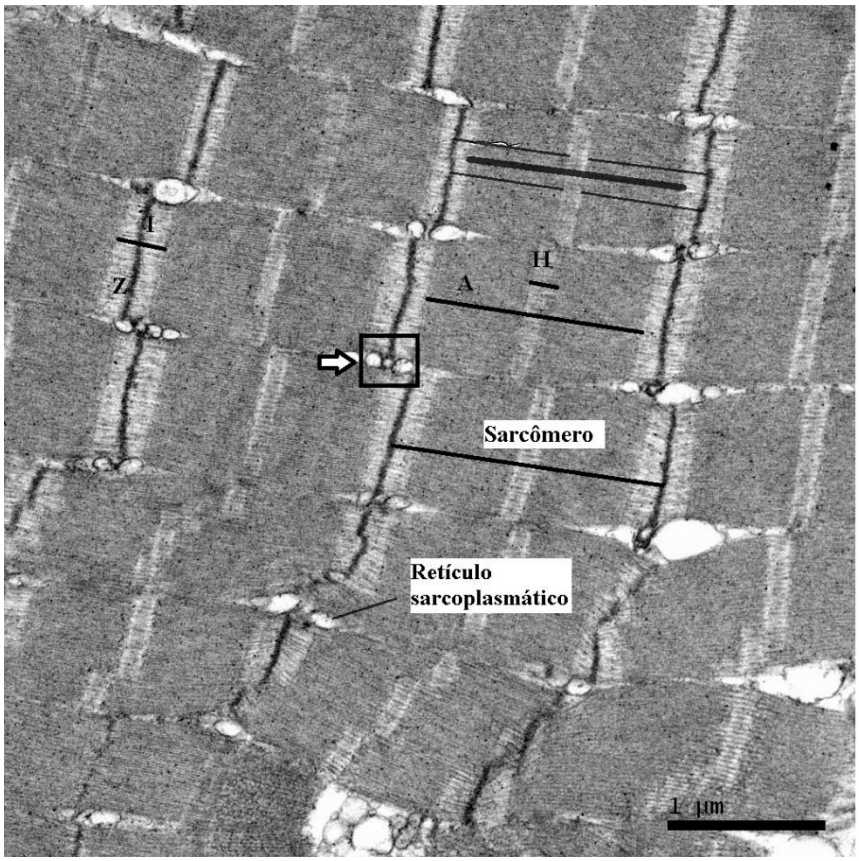

FIGURA 3. Elétron micrografia de corte de músculo estriado de *Brycon amazonicus*. Legenda: Sarcômero com as regiões A, I, Z e H. Na parte superior do desenho está ilustrada a posição dos filamentos finos e grossos no sarcômero. Em peixes, as tríades estão localizadas na altura das linhas Z de cada sarcômero (seta). (25.000X). Fonte: Acervo pessoal.

Existem três tipos de fibras musculares identificadas conforme suas características metabólicas: tipo I – contração lenta, oxidativa; tipo IIa – contração rápida, oxidativa – glicolítica; tipo IIb – contração rápida, glicolítica (Banks, 1991). A miofibrila é uma estrutura cilíndrica de diâmetro de 1 a 2 µm e comprimento de 10 a 100 µm, estas são divididas em bandas. A unidade contrátil da fibrila é o sarcômero, localizado entre duas linhas Z adjacentes (Ogawa e Maia, 1999).

As fibras musculares esqueléticas se adaptam morfologicamente conforme as demandas funcionais a elas requeridas. O uso elevado resulta no aumento do tamanho anatômico do músculo. Este aumento ocorre devido a hipertrofia das fibras musculares individuais. A hipertrofia resulta da elevação do número de miofibrilas e não do aumento de espessura miofibrilar. As miofibrilas se dividem durante o processo de crescimento do animal (Banks, 1991).

A degradação do tecido conectivo acontece mesmo em armazenamento à frio, sendo um processo natural, demonstrando perda do tecido interfibrilar, aumento dos espaços na miocomata e na separação entre miótomos. Estas modificações na conformação muscular ocorrem devido a degradação do colágeno (León et al., 2018). Em estudo com matrinxãs observou-se que a proteína bruta no músculo branco e vermelho do filé não diferiu nos diferentes meses do ano (Carvalho & Urbinati, 2005), demonstrando que a mobilização de proteínas em períodos de restrição alimentar não é proveniente da musculatura. Asaduzzaman et al. (2017) descrevem que o músculo estriado de tilápia apresenta fibras musculares em padrão de mosaico com diferentes diâmetros após 60 dias de arraçoamento. As amostras apresentavam maiores frequências de fibras de diâmetro de classe 40, 50 e 60 µm. A densidade de fibras musculares é um ótimo indicador de crescimento, qualidade e textura do músculo, além disso, se observa que a densidade de fibras musculares decresce com o avançar do tempo, fenômeno comum do crescimento muscular. Michelato et al., (2016) encontraram frequências do diâmetro das fibras de tilápia valores que variavam de 8,93-15,50% da classe <20 µm, 78,25-83,31% da classe 20-50 µm, 5,69-9,81% da classe >50 µm. A frequência de fibras de <20 µm de diâmetro encontrada confirma que o processo de crescimento por hiperplasia em músculo branco também ocorre nas tilápias adultas. Enquanto a altra frequência de fibras de diâmetros de 20-50 µm e >50 µm caracterizam crescimento por hipertrofia.

2.4 Histologia e histopatologia do fígado

O fígado é uma glândula tubular que possui diversas funções, que são realizadas por dois tipos celulares: o hepatócito e a célula de von Kupffer (Figura 4). As funções hepáticas consistem em: síntese (açúcares, proteínas plasmáticas, fatores de coagulação, lipídios, uréia, corpos cetônicos), secreção (sais biliares, ácidos biliares), excreção (pigmentos biliares), armazenamento (lipídios, vitaminas, glicogênio), biotransformação (substâncias tóxicas, drogas, hormônios) e metabolismo (lipídios, proteínas, carboidratos) (Banks, 1991).

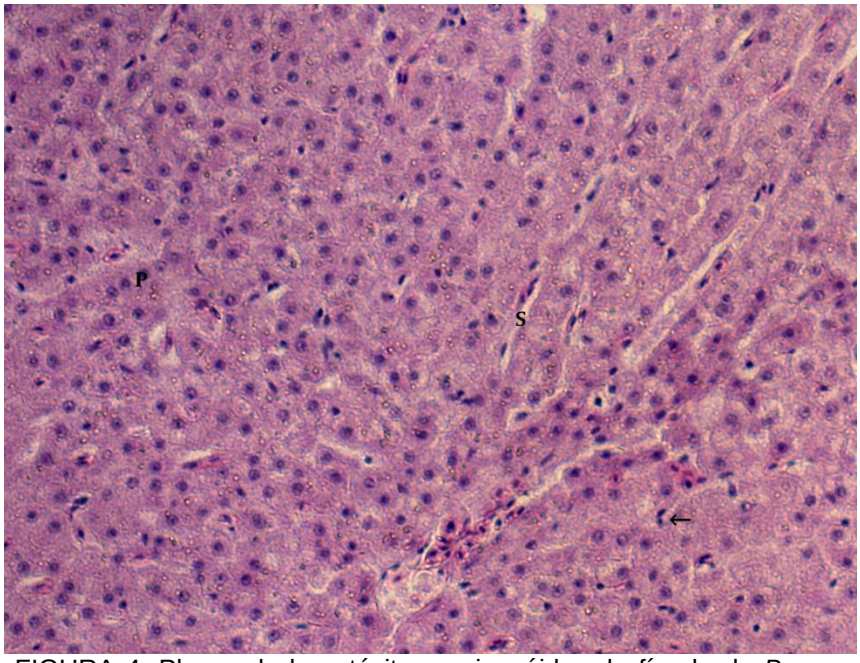

FIGURA 4. Placas de hepatócitos e sinusóides do fígado de *Brycon amazonicus*. Legenda: As placas de hepatócitos (P) estão separadas por sinusóides (S), estes são revestidos por células de Kupffer (←) e células endoteliais. Fonte: Acervo pessoal.

A histologia do fígado de peixes difere dos mamíferos, pois os hepatócitos não possuem a tendência de se organizar em cordões ou lóbulos. Os canalículos biliares se juntam aleatoariamente até formar os condutos biliares, estes fusionam-se formando a vesícula biliar que armazena a bile. A bile é direcionada ao intestino pelo colédoco, onde exerce função de emulsificação de gorduras e neutralização da acidez do quimo, auxiliando a digestão e absorção de lipídios e vitaminas lipossolúveis no intestino (Rotta, 2003).

O tecido conjuntivo intralobular é escasso em todas as espécies. Os lóbulos hepáticos são a unidade morfológica do fígado, essas massas poligonais e prismáticas de tecido são organizadas em placas de hepatócitos interdigitadas entre os capilares sinusóides anastomosados. As placas de hepatócitos e sinusóides partem de um vaso centralmente localizado, a veia centrolobular. Os hepatócitos apresentam núcleo vesicular com nucléolos proeminentes, este encontra-se centralizado e é circundado por citoplasma acidófilo que contém material basófilo. O sangue dos vasos interlobulares é transportado pelos sinusóides para a veia centrolobular. O sinusóides são desprovidos de lâmina basal e permitem movimentação de materiais entre o plasma e os hepatócitos. O espaço perisinusoidal (espaço de Disse) fica entre os hepatócitos e os sinusóides. Algumas células, fibras reticulares e microvilos dos hepatócitos podem estar presentes nesse espaço (Banks, 1991).

A célula de von Kupffer, membro do sistema macrofágico, reveste regiões dos sinusóides hepáticos. As placas de hepatócitos que se interdigitam com os sinusóides são contínuas com os ductos biliares extra-hepáticos, os quais se abrem no duodeno (Banks, 1991). A aparência histológica do hepatócito

pode variar conforme as condições fisiológicas do animal. Animais em jejum apresentam hepatócitos pequenos, turvos e mal delineados. Após alimentação, os hepatócitos se apresentam grandes, bem delineados e contendo inclusões lipídicas e de glicogênio, gerando uma aparência vesiculada (Banks, 1991).

As células secretoras e os túbulos condutores de bile formam os componentes glandulares exócrinos do fígado. Os canalículos biliares, pequenos ductos revestidos por epitélio cúbico, confluem para os ductos biliares interlobulares na periferia dos lóbulos. Os ductos biliares interlobulares se anastomosam com os ramos da artéria hepática e a veia porta, formando a tríade portal. O espaço porta contém nervos, pequenos vasos linfáticos, além de tecido conjuntivo intra-lobular. Este espaço, com tríade portal evidente, é ponto de referência para estudos histológicos de patologias (Banks, 1991).

A artéria hepática carrega metabólitos e é rica em oxigênio, atendendo os requerimentos metabólicos do fígado. Já a veia porta, carregada de substância provenientes da absorção intestinal, é pobre em oxigênio. As substâncias tóxicas e metabólitos são direcionadas para as células de von Kuppfer e hepatócitos para o processamento metabólico (Banks, 1991).

Os ductos biliares interlobulares confluem e formam os ductos intra-hepáticos. Estes, tornam-se ductos extra-hepáticos, formando os ductos hepáticos, o ducto cístico da vesícula biliar e o ducto colédoco (biliar comum), que conduz a bile ao duodeno (Banks, 1991).

Os hepatócitos sintetizam substâncias como albumina, fibrinogênio, α e β-globulinas, lipoproteínas e colesterol. O glicogênio é sintetizado através da glicogênese, sendo armazenado nos hepatócitos. Responsável também pela destoxicação, o fígado altera e eliminina diversos compostos químicos nocivos ao organismo. Embora as reações de conjugação sejam um meio de destoxificar as substâncias, nem todas resultam na destoxificação (Banks, 1991).

O potencial mitótico dos hepatócitos persiste por toda a vida do animal. Embora lesões agudas por substâncias tóxicas possam ser seguidas pela recuperação do órgão, a exposição crônica pode levar a alterações na função orgânica, diminuição do tamanho do órgão e no aumento do tecido conjuntivo fibroso intra-hepático (cirrose) (Banks, 1991).

O fígado de tilápia alimentada com silagem de pescado apresentou-se em distribuição cordonal de hepatócitos e sinusóides revestidos de células endoteliais. Os hepatócitos apresentavam citoplasma claro com núcleo central, somente as vezes sendo periférico. Em dietas com percentagem maior de inclusão de silagem de peixe pode-se observar congestão multifocal dos sinusóides e baixa concentração de glicogênio intracitoplasmático, até desarranjo da estrutura cordonal dos hepatócitos, pontos de necrose e deslocamento de núcleo para a periferia em casos mais graves (Honorato et al., 2014).

Peixes alimentados com dietas com concentrado protéico de soja apresentaram variados níveis de vacuolização citoplasmática, deslocamento de núcleo para a perfiferia do hepatócito, com modificação da forma normal circular,

apresentavam também congestão vascular e agregados mononucleares infiltrados multifocais aleatórios (Figuras 5, 6 e 7). Os resultados deste estudo com substituição de farinha de peixe por concentrado protéico de soja, sugerem que juvenis de *Totoaba macdonaldi* alimentados com essas dietas alternativas podem apresentar um processo inflamatório no intestino devido aos efeitos dos antinutrientes e da interação entre estes (López et al., 2015).

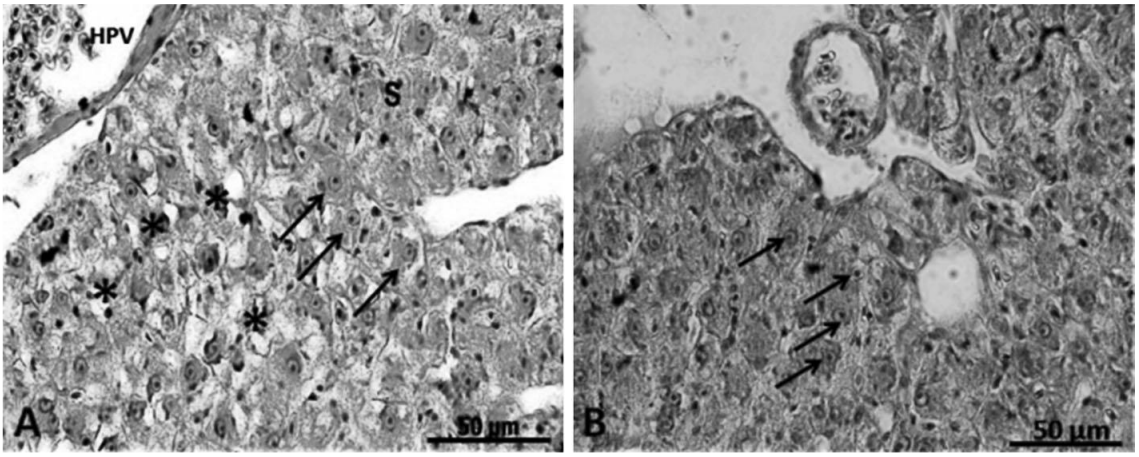

FIGURA 5. Fotomicrografia de secções histológicas do fígado de juvenis *Totoaba macdonaldi* alimentados com dieta a base de farinha de peixe (dieta de referência). Legenda: *HPV* Veia portal hepática, *S* sinusoides. *Flechas* indicam hepatócitos com núcleo normal, e *asteriscos* indicam hepatócitos com vacúolos lipídicos. Coloração Hematoxilina e eosina, 40X (Pax-it software 6, MIS, Inc., USA). Fígados de espécimes do grupo controle mostram histologia normal uniforme e consistente com ocasional, moderados vacúolos no citoplasma. Fonte: López et al., 2015.

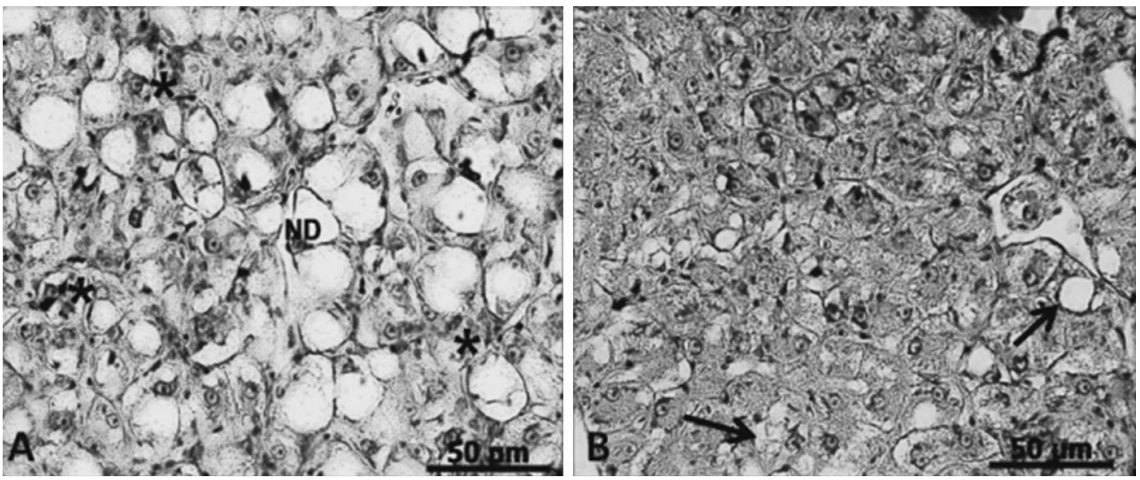

FIGURA 6. Fotomicrografia de secções histológicas do fígado de juvenis *Totoaba macdonaldi* alimentados com dieta contendo 30% SPC suplementada com taurina (S30T) (**a**) e sem taurina (S30) Legenda: (**b**). *ND* núcleo deslocado. *Flechas* indicam hepatócitos vacuolizados com núcleo deslocado, e *asteriscos* indicam linfócitos. Coloração Hematoxilina e eosina, 40X (Pax-it software 6, MIS, Inc., USA). Fonte: López et al., 2015.

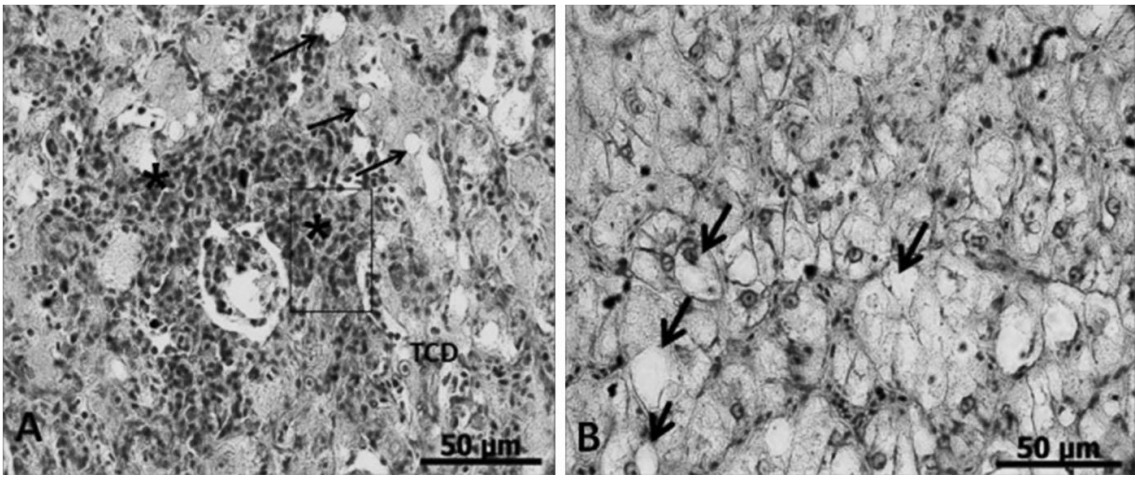

FIGURA 7. Fotomicrografia de secções histológicas do fígado de juvenis *Totoaba macdonaldi* alimentados com dieta contendo 60% SPC suplementada com taurina (S60T) (**a**) e sem taurina (S60) (**b**). Legenda: *TCD* tecido com congestão vascular. *Flechas* indicam hepatócitos vacuolizados com núcleo deslocado, e *asteriscos* indicam agregados multinucleares multifocais. Coloração Hematoxilina e eosina, 40X (Pax-it software 6, MIS, Inc., USA). Fonte: López et al., 2015.

Espirito Santo et al., (2015) não encontraram lesões hepáticas em tilapias alimentadas com diferentes níveis de proteína de soja concentrada, observaram apenas o arranjo típico de hepatócitos em cordão, com sinusóides, veias centrais e ductos normais. Em estudo com *Lates calcarifer* alimentados com dietas a base de farelo de soja observou-se que o número de sinusóides hepáticos foi afetado, aumentando nos animais que se alimentaram com dietas contendo de 50-100% de substituição na dieta, ouseja, maiores quantidades de farelo nas dietas (Ma et al., 2018). Depleção de vacúolos nos hepatócitos de Salmão Atlântico foram observados por Gu et al., (2015) em animais alimentados com dietas contendo isoflavonas, além destes hepatócitos apresentarem os menores diâmetros, comparado com os outros tratamentos. A depleção de glicogênio pode ser causada por redução da função digestiva e/ou ingestão da dieta.

2.5 Histologia e histopatologia do intestino

O intestino de peixes é um tubo simples, não sendo separado em delgado e grosso como ocorre em mamíferos. Em teleósteos se verificam, no mínimo, dois segmentos intestinais, mesmo sem a separação entre delgado e grosso. Na primeira porção ocorre a absorção de monossacarídeos, aminoácidos e ácidos graxos, já a segunda parte é reponsável pela entrada de moléculas por pinocitose (Rotta, 2003).

O comprimento do intestino varia com o hábito alimentar e características dos alimentos ingeridos, sendo que os onívoros possuem intestino em formato de “N”. Peixes herbívoros e onívoros possuem a capacidade de alterar a estrutura e propriedades absortivas do sistema digestivo conforme a

sua dieta (grandes variações na composição bromatológica), sendo considerados substrato dependentes (Rotta, 2003).

Na junção gastroduodenal, a membrana mucosa se apresenta em projeções digitiformes (vilos ou vilosidades), estas estão contidas em pregas onde também se encontra parte da túnica submucosa (Figura 8). O epitélio da mucosa é formado por três tipos celulares: as células de revestimento ou absortivas, as células caliciformes e as células argentafins (Banks, 1991).

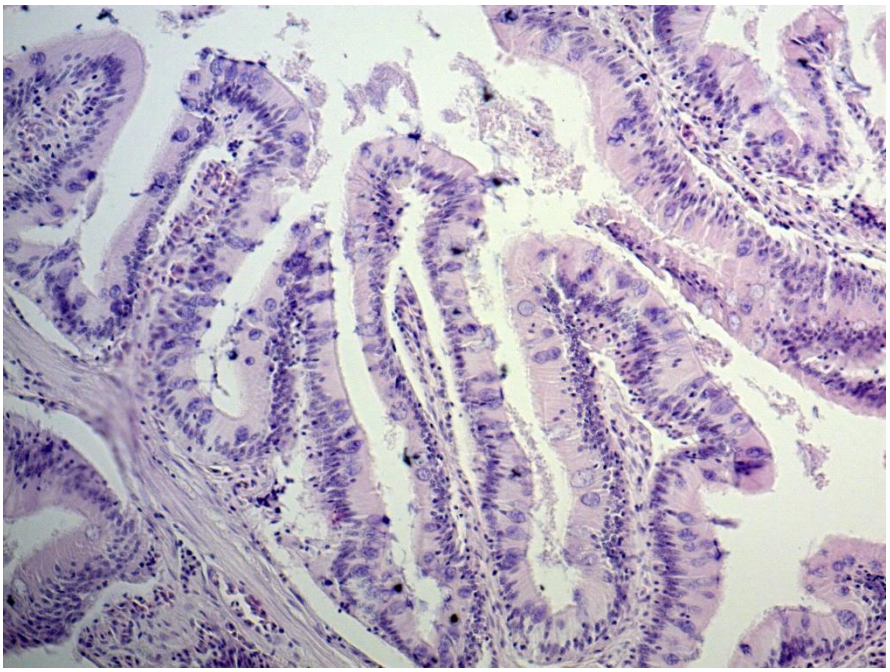

FIGURA 8. Corte histológico de intestino de *Brycon amazonicus* evidenciando as vilosidades intestinais.

As células absortivas são células prismáticas epiteliais típicas. O citoplasma acidófilo, finamente granulado, possui núcleo alongado e situa-se na região basal. As células caliciformes, tedem em aumentar sua quantidade conforme a aproximação do reto. Sua função é secretar muco, facilitando o deslocamento do conteúdo luminal (Banks, 1991). As glândulas intestinais estão localizadas na base dos vilos. A lâmina própria é formada por tecido conjuntivo frouxo, onde é ocupado na maior parte por glândulas. Vasos linfáticos estão presentes e penetram no tecido conjuntivo. A muscular da mucose consiste em feixes de músculo liso e se interdigitam com as glândulas dentro dos vilos (Branks, 1991). A submucosa contém glândulas tubuloacinosas simples e ramificadas, abrindo-se em glândulas intestinais. A túnica muscular é formada por músculo liso, sendo responsável pela peristalse. A túnica serosa é típica (Banks, 1991). O duodeno apresenta mucosa altamente pregueada com glândulas intestinais proeminentes, os vilos tendem a ser largos, com formato regular e ponta arredondada. O jejuno é semelhante ao duodeno, porém os vilos são estreitos, pequenos e menos numerosos. O íleo é semelhante ao jejuno,

sendo as células caliciformes uma característica importante. Os vilos são claviformes e as pregas não estão presentes (Banks, 1991). As células caliciformes, as células das glândulas submucosas duodenais e as células que revestem as glândulas intestinais do intestino delgado são responsáveis pela secreção de suco intestinal. A digestão completa dos alimentos e absorção são funções essenciais do intestino delgado. A diminuição no tempo de deslocamento do quimo no intestino pode provocar diarreia, absorção deficiente ou má-digestão (Banks, 1991).

O intestino proximal apresenta epitélio de aspecto normal, enterócitos diferenciados, núcleo basal e boa vacuolização no citoplasma ao longo das dobras. Na base das dobras se encontram enterócitos indiferenciados, pouca vacuolização no citoplasma e pouca mitose é encontrada. Células caliciformes estão distribuídas ao longo do epitélio, células inflamatórias são encontradas na parte basal do epitélio e poucas na lâmina própria (Van den Ingh et al., 1991). No intestino distal observa-se muitas pregas da mucosa de menor comprimento, estroma delicado e enterócitos altamente vacuolizados. Na base das pregas encontram-se células indiferenciadas sem vacúolos e com pouca mitose. As células caliciformes encontram-se distribuídas ao longo de todo o epitélio. As pregas apresentam tecido fibroso, centro muscular, e pregas secundárias irregulares cobertos por células indiferenciadas na base e enterócitos vacuolizados no topo. Em direção ao topo observa-se a maior quantidade de células caliciformes substituindo enterócitos absortivos (Van den Ingh et al., 1991).

O uso de farelo de soja nas dietas ocasiona mudanças histopatológicas no intestino de peixes, conhecida como “soybean meal-induced enteritites” (SBMIE) (Figura 9). É caracterizada por encurtamento das pregas da mucosa intestinal, aumento da lâmina própria, infiltração de células inflamatórias e diminuição de vacúolos nos enterócitos (Gu et al., 2016).

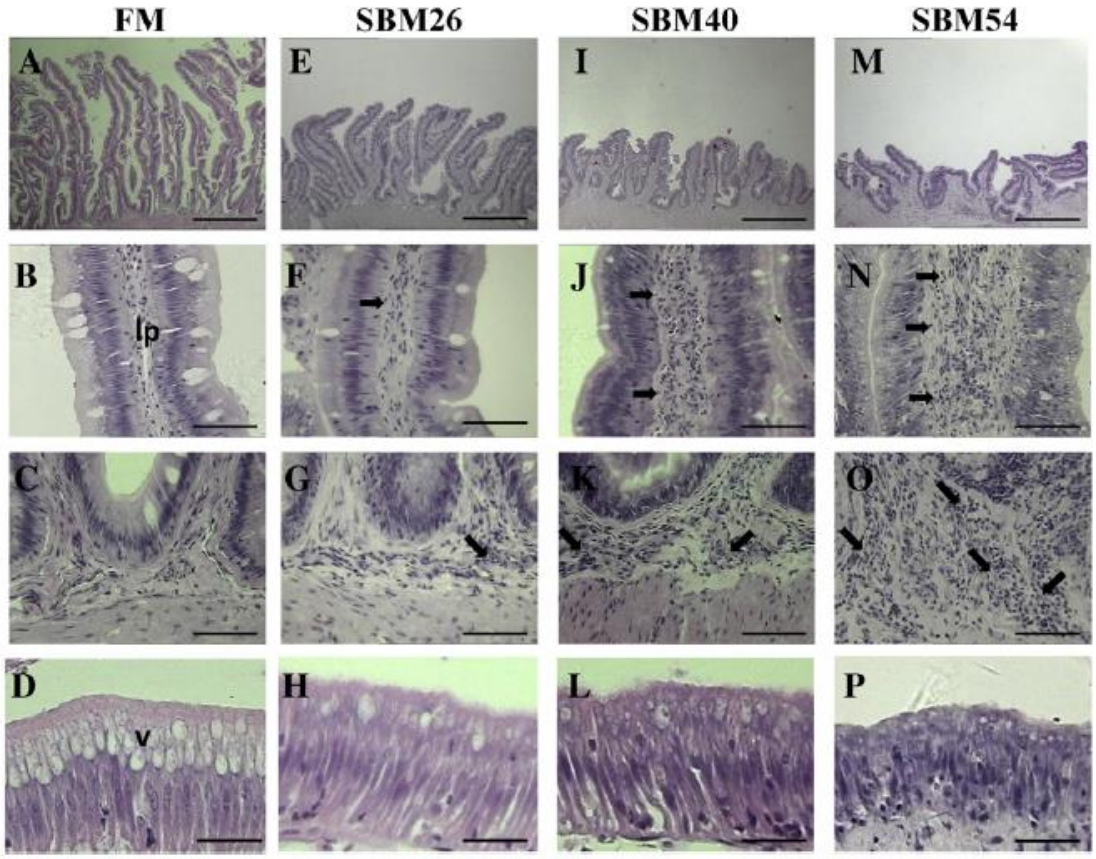

FIGURA 9. Fotomicrografias de modificações histopatológicas de secções de intestino de "Turbot" alimentadas com dietas a base de farelo de soja. Legenda: Imagens histomorfológicas representativas de secções do intestino distal de "Turbot", coradas com Hematoxilina e Eosina, retratando o aumento dose-dependente da severidade das mudanças inflamatórias com o aumento da suplementação de farelo de soja em "Turbot" alimentados com dietas FM (a-d), SBM26 (e-h), SBM40 (i-l) e SBM54 (m-p). FM – fishmeal; SBM – Soybean meal. Imagens representativas da diminuição da altura e aumento da fusão das dobras da mucosa com o aumento do nível de farinha de peixe na dieta (bar = 50 µm). (b, f, j, n) Imagens representativas do aumento de largura e infiltração celular (leucócito) (flechas) na lâmina própria com o aumento do nível de inclusão de farelo de soja (bar = 50 µm). (c, g, k, o) Imagens representativas do aumento de altura e infiltração celular (leucócito) (flechas) da submucosa com o aumento do nível de inclusão de farelo de soja (bar = 50 µm). (d, h, l, p) Imagens representativas da redução do número de vacúolos absortivos supranucleares nos enterócitos, núcleo deslocado em direção ao ápice das células com o aumento do nível de inclusão de farelo de soja (bar = 20 µm). Os dados são expressos como médias ± SD (n = 12). lp: lâmina própria; v: vacúolos absortivos supranucleares. Fonte: Gu et al., (2016).

Krogdahl et al., (2015) observaram que 2-4 g/kg de saponinas de soja na dieta ocasiona sinais de inflamação no intestino distal de salmões, mesmo sem a presença de outros componentes de leguminosas, confirmando que a severidade das mudanças inflamatórias no intestino distal é dose-dependente e que a saponina é o agente causador envolvido nessas alterações. Liu et al., (2017) encontraram em seus estudos, modificações intestinais nas dietas a partir de 20% de substituição de FM por SBM. Os linfócitos aumentaram em densidade

e migraram para a lâmina própria e epitélio. Muitos destes linfócitos se encontravam abaixo do núcleo dos enterócitos, o núcleo encontrava-se deslocado para as regiões mais altas das células, as células caliciformes apresentavam modificação em sua forma, de fusiformes para redonda e os enterócitos se apresentavam malformados e irregulares, confirmando assim que que quanto maior a porcentagem de inclusão de SBM nas dietas maiores os sinais e gravidade das enterites.

3. HIPÓTESES E OBJETIVOS

A hipótese geral do trabalho propõe que as dietas a base de farelo de soja em substituição a farinha de peixe na dieta de matrinxãs, resulta em desempenho semelhante sem prejuízos à qualidade do filé.

O objetivo geral do estudo foi: analisar o desempenho zootécnico, qualidade do produto final (filé) e características histológicas das células musculares, hepáticas e intestinais.

Os objetivos específicos foram:

1. Avaliar o desempenho zootécnico de matrinxãs alimentadas com dietas contendo farelo de soja como substituto da farinha de peixe;

2. Verificar a composição centesimal do filé de matrinxãs alimentadas com dietas contendo farelo de soja como substituto da farinha de peixe;

3. Determinar o perfil de ácidos graxos dos filés de matrinxãs alimentadas com dietas contendo farelo de soja como substituto da farinha de peixe;

4. Observar o comprimento de sarcômero das fibras musculares de filés de matrinxãs alimentadas com dietas contendo farelo de soja como substituto da farinha de peixe;

5. Analisar as características morfológicas das fibras musculares de matrinxãs alimentadas com dietas contendo farelo de soja como substituto da farinha de peixe, através de análise histológica;

6. Observar a composição estrutural e alterações no tecido hepático e intestinal através de análises histológicas de amostras coletadas das matrinxãs alimentadas com dietas contendo farelo de soja como substituto da farinha de peixe.

CAPÍTULO II*

*Artigo apresentado de acordo com a normas da revista North American Journal of Aquaculture.

Responses of dietary soybean meal levels on white skeletal muscle histology of Matrinxã (*Brycon amazonicus*) juveniles

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Abstract

Partial or total replacement of fishmeal by soybean meal was examined in matrinxã (*Brycon amazonicus*) for ninety two days. A diet with fishmeal (FM) as the sole protein source was compared to diets with 50% and 100% of replacement (SBM / FM and SBM). A significant difference was observed in the weight of the animals and of the fillets between treatments, with FM treatment being the highest values. No significant differences were found between treatments in the fiber distribution, diameter, radius, area and circumference parameters. While in sarcomere length and fiber number per area it was observed that the SBM treatment presented significantly higher values. The difference found between the length of the sarcomere and the number of fibers per area between the different treatments is much more related to the age of the animals and consumption of the feed than necessarily the protein source used in the diet. Based on the results, it was observed that the lower muscle deposition occurred due to the lower consumption of SBM-containing diets, so future studies could use palatabilizers to try to reduce this difference.

The Matrinxã (*Brycon amazonicus*) is a freshwater native fish species from the Amazon Basin. The specie is promising for Brazilian aquaculture because the omnivorin habit, rapid growth rate, great acceptance of his meat in the Market and established reproduction techniques (Almeida and Franco 2007; Canevesi et al. 2014; Ferreira et al. 2013; Vargas et al. 2013). The composition and characteristics of muscular tissue in

farmed matrinxãs demonstrated that quality of muscle could be improved by diet formulation improvements (Almeida et al. 2009).

Feeding is the highest cost in intensive fish production, with protein being the most expensive and most important ingredient for the growth of organisms (Ahmed and Khan 2006). Currently, fish meal is the main protein ingredient of animal origin used in the formulation of fish feed (Liu et al. 2012), because it presents high levels of protein, fat, minerals and energy (Pastore et al. 2013). However, this is a limited and finite resource, and its overuse is criticized by environmental organizations (Hardy 2010). In addition, its low availability and high cost make it necessary to search for alternative ingredients with lower cost and constant availability (Naylor et al. 2000). For these reasons, it has been studied the use of protein sources of vegetable origin, which present lower cost, good balance of amino acids and supply throughout the year with constant and regular nutritional composition (Coutinho et al. 2017).

In this context, soybean meal has been suggested to replace fishmeal in diets (Hardy 2010). Soybean meal is a more economical source than protein sources of animal origin and is available worldwide with a consistent pattern of composition and quality (NRC 2011). However, the use of soybean meal may be limited due to the presence of antinutritional factors, which can result in a lower nutrient utilization leading to lower animal growth and consequently lower muscle deposition.

Protein is the fundamental component for the growth animal, and consequently, body muscle deposition. In fish, much of the body mass is composed of muscle tissue, the proportion being much higher when compared to other vertebrates (Santos 2006).

The characterization of white muscle density and muscle collagen are important for obtaining a quality fish fillet (Videler 2011). Muscular growth in the larval and

juvenile stages of commercial species is of great importance for the final size of the fish (Aguiar et al. 2005), can be influenced by several factors, including nutrition (Koumans and Akster 1995). In fish, there are two forms of muscle growth, hyperplasia, which is the generation of new muscle fibers and hypertrophy, expansion of existing muscle fibers (Asaduzzaman et al. 2017). Unlike mammals, fish have the ability to produce muscles through hyperplasia throughout their life (Rowlerson and Van Veggetti 2001; Vélez et al. 2017). The number and size of muscle fibers can vary according to several factors, such as: different species, exercise, temperature and especially diet (Johnston 1999). Sarcomeres are a pattern of repeated bands in the striated muscle, where the sequence and stacking of several sarcomeres forms myofibril (Van der Meulen et al. 2005).

It is known that the protein gain is linked to the muscular growth of the fish, however, the regulation of muscle growth by nutrients remains poorly documented. Based on all these facts, the aim of this study was to evaluate the muscle growth, observed different sizes of fibers and sarcomeres in white skeletal muscle of Matrinxã that received different diets with fishmeal substitution for soybean meal.

METHODS

Diets. - Three isonitrogenous and isoenergeticas diets (Crude protein (CP): 34%, gross energy (GE): 390 Kcal/100g) using common feed industry ingredients were manufactured at the Instituto Nacional de Pesquisas da Amazônia (INPA), using extrusion technology. The ingredients were ground in hammer mill with a 0.3 mm sieve, homogenized, humidified and the feed was processed by extrusion with a 2 mm sieve. Soya bean meal (SBM) was used to replace 100 or 50% of fishmeal protein (diets SBM and SBM/FM, respectively) (Table 1).

TABLE 1. Ingredients (g/kg) and proximal composition analyzed (%) of the experimental diets for *Brycon amazonicus*.

Ingredients (g/kg)	Experimental diets		
	SBM	SBM/FM	FM
Soybean meal 46	645.00	220.00	0.00
Fish meal 61	0.00	280.00	426.00
Corn grain 7,92	115.00	200.00	220.00
Whear bran	60.00	145.00	220.00
Rice	60.00	100.00	100.00
Soybean oil	52.00	30.00	20.00
Dicalcium phosphate	50.00	15.00	4.00
Vitamin and mineral premix[a]	10.00	10.00	10.00
Calcareous	8.00	0.00	0.00
Proximal composition analyzed (%)			
Dry matter	93.93	94.47	93.80
Crude protein	33.37	34.07	34.30
Ether Extract	5.00	5.40	6.43
Ash	10.07	9.00	9.40

Note. SBM = Soybean meal; SBM/FM = Soybean meal/Fish meal; FM = Fish meal. [a] Vitamin and mineral premix meeting NRC (2011) recommendations.

Fish and experimental design. - The experiment was conducted at INPA, Manaus, Brazil. Two hundred and seventy matrinxã (*Brycon amazonicus*) of initial mean weight 141.59 ± 0.5 g were randomly distributed among six excavated tanks (45 fish per tank). Duplicate groups of matrinxã were fed one of the three diets, twice a day (09:00 and 16:00 h) and seven days for week, until apparent satiation for ninety two days. Experiment was conducted under natural photoperiod. The water quality parameters: temperature (30-33ºC), pH (8.35-8.50), transparency (25-55 cm) and oxygen dissolved (7.60-13.60 mg L^{-1}) were measured and recorded every day. At the end of the trial, 26 fish per treatment were euthanized by hypothermia and decapitation, weighed, medidos. Twenty one fish were sampled, 18 fish were used for light microscopy and three fish for electron

microscopy. Fish management was according to the ethics of animal use by the Brazilian Society of Laboratory Animal Science (COBEA).

Histological analysis. - Samples of white skeletal muscle from epaxial region were fixed in 10% buffered formalin for 24 hours, and later preserved in 70% alcohol for analysis. The samples were passed through a process of paraffin embedment, and the paraffin blocks then cut using a microtome (MICROM International GmbH, Walldorf, Germany). The cross sections (5 μm) were submitted to haematoxylin-eosin staining, and analyzed by light microscopy (Zeiss). Pictures were taken using a microscope camera (Carl Zeiss MicroImaging) for posterior analysis.

An image analysis system Image-Pro Plus 4.5.0.29 (Media Cybernetics, USA) was used for the morphometric analysis, and the smallest diameter in 200 muscle fibres was determined per animal, which were grouped into diameter class (<20 μm, 20-50 μm and >50 μm) to evaluate the contribution of hyperplasia and hypertrophy to muscle growth (Almeida et al. 2008). The fiber counts by area were according to the methodology of Gundersen et al. (1988), while the morphometric measurements (diameter, area, radius and circumference) were performed in 200 muscle fibers per repetition, through software Image Pro Plus 4.5.0.29 analysis (Media Cybernetics, USA).

Transmission electron microscopy (TEM). - The methodology used for TEM was according to Wang et al. (2015). Samples of white skeletal muscle (2 x 2 x 2 mm) were fixed in 2.5% glutaraldehyde, dehydrated in ethanol immersion at increasing concentrations, post-fixed in OsO4 for 1 hour and imbibed in resin. The ultrafines of each sample were placed in grids, stained with uranyl acetate and citrate solution. The images were taken in TEM (JEOL JEM 1200 Exll). In each micrograph (10 per sample), 10 sarcomeres were measured by Image-Pro Plus® software 4.5.0.29

Statistical analysis. - Data are presented as the mean ± S.E.M. (standard error of the mean). All data were submitted to Kolmogorov-Smirnov normality test and Levene's homogeneity, before were subjected to one-way ANOVA. Differences between means at P<0.05 were analyzed using the 5% Tukey test. Statistical Analysis System (SAS 9.4) and GraphPad Prism 7.0 were used to perform statistical analysis and graphing.

RESULTS

Growth parameters

The FM-fed fish had the highest weights of the whole fish and fillet (Table 2).

TABLE 2. Effect of partial and total substituition of fish meal by soybean meal protein in pratical diets for *Brycon amazonicus* after 92 days of experiment.

Parameters	Diets			*P* value
	SBM	SBM/FM	FM	
Total average weight (g)	301.54±8.38c	370.46±10.50b	410.54±9.17a	<0.0001
Average fillet weight (g)	100.38±2.90c	124.23±3.13b	136.08±2.83a	<0.0001
Feed intake (g)	354,30±9,39c	414,52±7,08b	477,88±9,66a	<0.0001
Apparent feed conversion (FI/WG)	2.07±0,10	1.96±0,09	1.93±0,07	0.4839

Note. Mean values and standard error (±SE) are presented for each parameter. FI: food intake; WG: weight gain; *Means in the same row bearing diferente letters differ significantly (P <0.05 level).

Muscle fibers histomorphometry and MET analysis

There was no significant difference between treatments on the frequency of distribution of muscle fibers for the different size classes, where similar values were observed for the muscle fiber class <20 μm (P = 0.2833), 20 - 50 μm (P = 0.4640) and for fibers> 50 μm (P = 0.9668), (Figure 1). However, there was a predominance of fibers of

classes 20-50 μm and> 50 μm in all treatments, indicating a greater participation of hypertrophy on muscle fibers, regardless of the diet consumed.

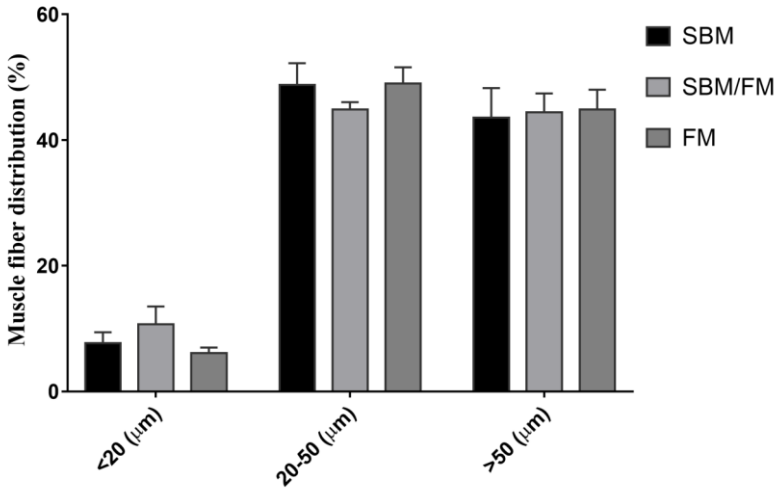

FIGURE 1. Muscle fiber distribution into three diameter classes (<20 μm, 20-50 μm and >50 μm) in matrinxãs fed with different % of fishmeal replacement per soybean meal. Not significant (P>0.05).

In the analysis of muscle fibers histomorphometry (Figure 2), there was no difference between treatments on the parameters of diameter μm (P = 0.4370), radius μm (P = 0.4369), area μm2 (P = 0.4407) and circumference μm (P = 0.4376). Fish fed the SBM diet had the highest number of muscle fibers per area (P <0.0001) in relation to fish fed SBM / FM and FM diets (Figure 2). There was a difference between the treatments (P = 0.0083) on the sarcomere length, where the SBM diet presented the highest sarcomere length in relation to the SBM / FM and FM diets, as was also observed in the number of fibers by area. In the histological analysis of muscle fibers, a mosaic pattern characterized by fibers of different diameters was observed (Figure 3).

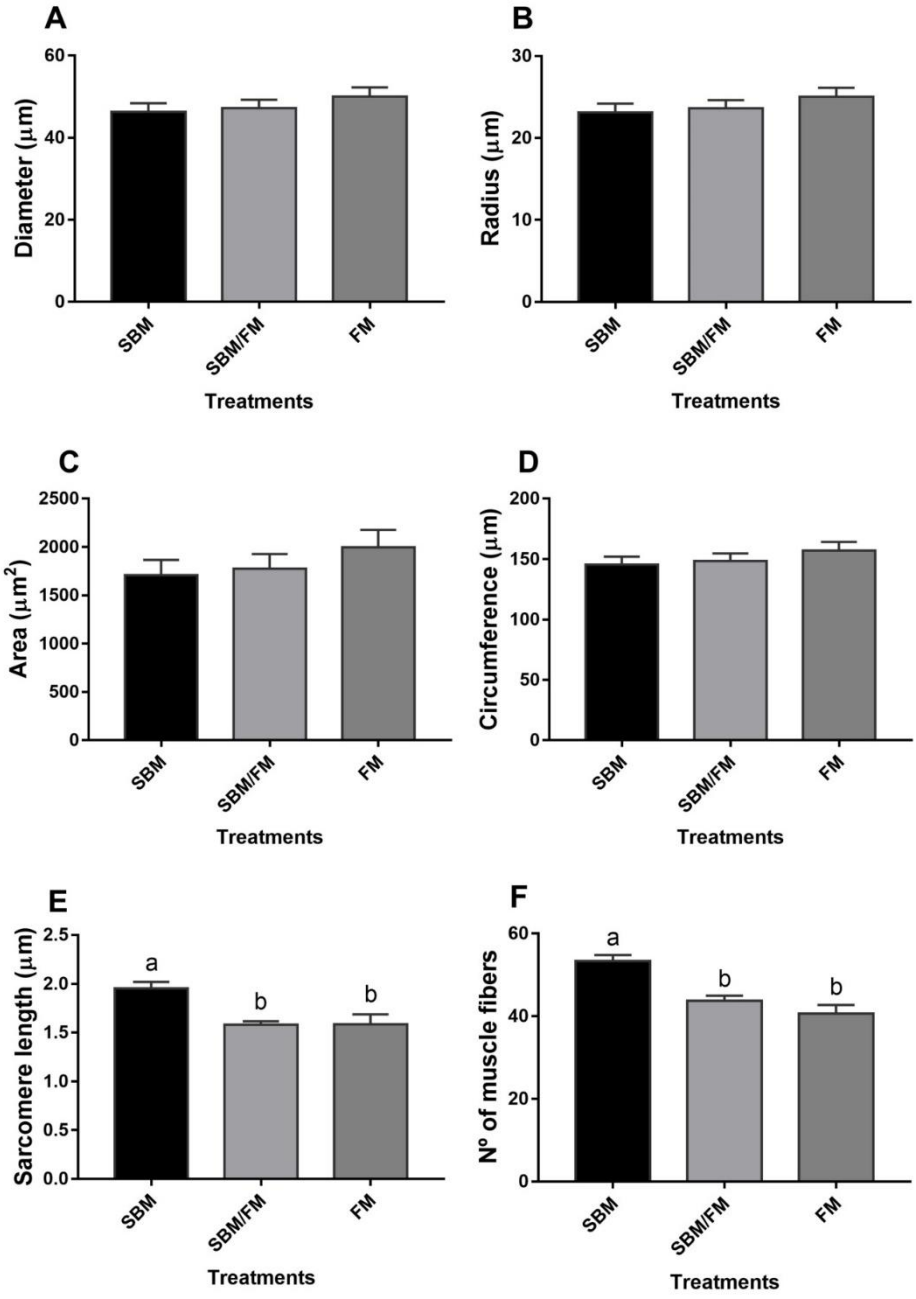

FIGURE 2. Muscle fibers histomorphometry of matrinxans fed with different% of fish meal substitution by soybean meal. A: Diameter; B: Ray; C: Area; D: Circumference. Not significant (P>0.05). E: Sarcomere length; F: Number of muscle fibers. Different letters in the columns indicate significant differences (P = 0.0083) among treatments. SBM: 100% of protein is soybean meal; SBM/FM: 50% of fish meal replaced by soybean meal; FM: 100% of protein is fish meal.

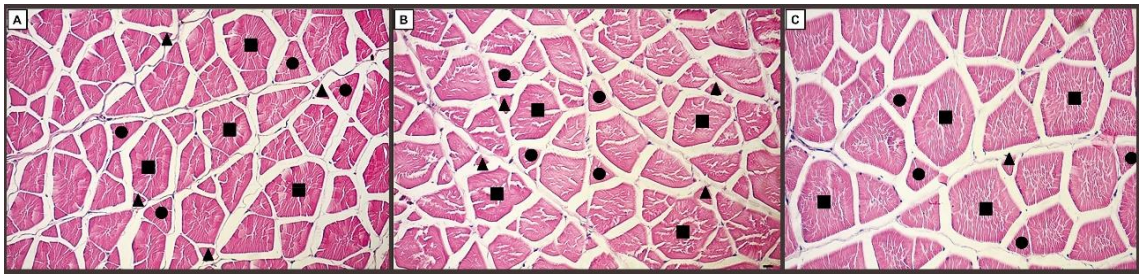

FIGURE 3. Transversal sections of white skeletal muscle of matrinxã (*Brycon amazonicus*) fed diets with diferent sources of protein at 92th day of the experiment. A: transverse section muscle of fish fed with soybean meal protein. B: Transverse section muscle of fish fed with soybean meal and fishmeal protein. C: Transverse section muscle of fish fed with fishmeal protein. Square marks indicated above 50 µm diameter fibers, circle marks indicated between 20 – 50 µm diameter fibers and triangle marks indicated below 20 µm diameter fibers. Object: 40x.

In the MET analysis, the SBM treatment presented a higher sarcomere length (1.95 µm) when compared to SBM / FM (1.58 µm) and FM (1.59 µm) treatments. The structure of the muscle fiber is shown in Figure 4. The collagen fibers of the pericellular connective tissue maintain an orderly architecture next to the muscle fiber. The muscle presents the Z line, located at the center of the actin filaments. No changes were observed in the tissue conformation in relation to the different treatments.

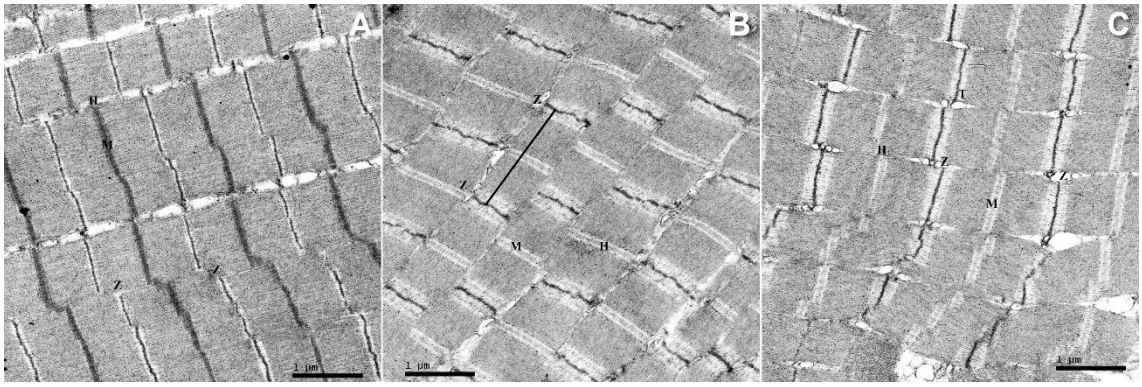

FIGURE 4. Electron micrographs showing longitudinal sections of white muscle. A: transverse section muscle of fish fed with SBM. B: Transverse section muscle of fish fed with SBM/FM. C: Transverse section muscle of fish fed with FM. Bar = 1 µm.

DISCUSSION

Growth parameters

The present study showed a significant difference between the protein sources in the fish weight gain. It is probable that the lower weight of the animals fed with soybean meal diet may be the result of a lower intake of this diet, in which the main protein source of this diet was soybean. It is known that soy protein has low palatability, anti-nutritional factors and amino acid imbalance (Refstie et al. 2001; Cheng et al. 2018). Thus, both the palatability and the difference in the biological value of the protein sources may have influenced the gain in weight, since sources of protein of plant origin may present lower digestibility due to the antinutritional factors, leading to a lower utilization of the nutrients and consequently damaging the growth of fish.

The nutritive value of the protein varies among the ingredients, either by quantitative composition, amino acid balancing and availability (Pezzato et al. 2004). For an adequate use of dietary protein, amino acids must be present in amounts and proportions suitable to maximize fish growth (Wilson 1985), this may suggest that the lower growth of the animals fed with total replacement of fishmeal by bran soybean may be related to a possible imbalance/utilization of essential amino acids for protein deposition in the organism (Gatlin et al. 2007).

Soybean anti-nutrients, such as trypsin inhibitors, saponins and lectins, reduce the growth and digestive function of animals (Krogdahl et al. 2003). Several studies have demonstrated the decline in the zootechnical performance of animals fed high-soybean diets as the main protein (Lee et al. 2016; Liang et al. 2017; Zhang et al. 2018). However, although generalizations can be used as references, the effects of soybean meal on the performance and condition of the fish should be evaluated on a case-by-case basis due to

the difference in utilization and sensitivity to soybean meal inclusion by each species (Sales 2009).

The variation in growth provided by the different diets, in addition to influencing the productive efficiency of the fish farmer, can also influence fish quality and thus affect the fish processing companies, as different growth rates can induce the appearance of muscular and adipose tissues differentiated, and may influence the quality of the carcass (Sänger and Stoiber 2001).

Muscle fibers histomorphometry and MET analysis

Our results showed that the protein sources did not differ in relation to the frequency of distribution of muscle fibers, and a higher frequency of mixed-growth (20-50 μm) and hypertrophic (> 50 μm) muscle fibers was observed in all treatments, demonstrating that fish were already at an advanced stage of growth. The higher frequency of fibers in hyperplasia is characterized by juvenile fish, whereas the higher frequency of mixed growth and hypertrophy is characterized by adult fish (Almeida et al. 2010), since in general the efficiency of protein synthesis decreases with age (Lobley 2003). The progressive increase in the frequency of fibers of larger diameters (class 30-40 μm) coincides with the period of weight gain and length of the animal (Leitão et al. 2011). Muscular growth in fish is different from mammals, as it occurs throughout the entire growth period of the animal, which can be influenced by several factors, such as: temperature, exercise, photoperiod, diet and diet composition (Johnston 1999).

In a study carried out on gilthead sea bream (*Sparus aurata*), the authors reported positive correlation ($r^2 = 0.60$) between fiber area and weight in animals treated with diets containing different amounts of protein and lipids (La Serrana et al. 2013). In agreement,

Silva et al. (2011) found that hypertrophic white muscle fibers were the major constituents of *Pagellus bogaraveo* muscle growth, correlating well with the weight gain of the animals. Melo et al. (2016) observed in their studies with tilapia that the larger the fiber size, the lower the number of fiber per area, agreeing with the results found in the present study, where the fish fed the SBM diet presented the highest number of muscle fibers per area and although there was no statistical difference, there was also a tendency to a smaller size of muscle fibers in this treatment.

According to Valente et al. (2016), the dorsal muscle cross sectional area was significantly affected by conditional dietary, and the diameter of white muscle fibers did not differ statistically in treatments of up to 75% inclusion of plant proteins (PP), although animals fed with 100% PP obtained the lowest diameter values in Senegales sole. The total number of fibers and the percentage of smaller fibers (<30 μm) were similar in all treatments.

In fish, the muscular arrangement is formed by different types of muscular fibers, being the growth through the association of the processes of hypertrophy and hyperplasia. However, new cells originate from satellite cells or through the division of adult fibers (Devincenti et al. 2015), which could explain the difference found in the sarcomere length of the fibers between the treatments SBM, SBM / FM and FM . The growth of muscle fibers occurs through the activation of satellite cells, these proliferate and fuse in a preexisting fiber, the nuclei begin to synthesize myofibrillar proteins that cause the increase of muscle fiber volume through the formation of new sarcomeres (Goldspink 1972). This phenomenon could explain why the FM and SBM/FM treatments had smaller sarcomeres, since they were in full development, consequently, there was a greater deposition of muscle in the animal. The number of muscle fibers per area and sarcomere

length showed similar results, indicating that the sarcomere length is not directly related to the diameter of the muscle fiber. In the present study, the effect of the sarcomere length of the sarcomere was similar to that of the sarcomere (Table 1) than species, since the variation between species is small.

CONCLUSIONS

The results suggest a decrease in zootechnical performance and muscle deposition the higher the percentage of soybean meal inclusion in the diet. However, the parameters: fiber distribution, diameter, radius, area and circumference had no difference between treatments, demonstrating that factors were more related to the age of the animal than to the diet. The length of the sarcomere can be determinant in the texture and shelf-life of fillet. Later studies could use palatabilizing ingredients in order to increase the consumption of the soy-based diet for the animals and thus evaluate the growth values.

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REFERENCES

Aguiar, D. H., Barros, M. M., Padovani, C. R., Pezzato, L. E., Pai-Silva, M.D. 2005. Growth characteristics of skeletal muscle tissue in *Oreochromis niloticus* larvae fed on a lysine supplemented diet. Journal of Fish Biology 67: 1287-1298.

Ahmed, I., Khan, M. A. 2006. Dietary branched-chain amino acid valine, isoleucine and leucine requirements of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). British Journal of Nutrition 96: 450-460.

Alami-Durante, H., Wrutniak-Cabello, C., Kaushik, S. J., Médale, F. 2010. Skeletal muscle cellularity and expression of myogenic regulatory factors and myosin heavy chains in rainbow

trout (*Oncorhynchus mykiss*): Effects of changes in dietary plant protein sources and amino acid profiles. Comparative Biochemistry and Physiology, Part A 156: 561-568.

Almeida, F. L. A., Carvalho, R. F., Pinhal, D., Padovani, C.R., Martins, C., Dal Pai-Silva, M. 2008. Differential expression of myogenic regulatory factor MyoD in pacu skeletal muscle (*Piaractus mesopotamicus* Holmberg 1887: Serrasalminae, Characidae, Teleostei) during juvenile and adult growth phases. Micron 39: 1306-1311.

Almeida, N. M., Franco, M. R. B. 2007. Fatty acid composition of total lipids, neutral lipids and phospholipids in wild and farmed matrinxã (*Brycon cephalus*) in the Brazilian Amazon area. Journal of the Science of Food and Agriculture 87: 2596-2603.

Almeida, N. M., Janzantti, N. S., Franco, M. R. B. 2009. Determination of essential fatty acids in captured and farmed Matrinxã (*Brycon cephalus*) from the Brazilian Amazonian Area. Journal of the American Oil Chemists' Society 86: 717-722.

Almeida, F. L. A., Pessoti, N. S., Pinhal, D., Padovani, C. R., Leitão, N. J., Carvalho, R. F., Martins, C., Portella, M. C., Pai-Silva, M. D. 2010. Quantitative expression of myogenic regulatory factors MyoD and myogenin in pacu (*Piaractus mesopotamicus*) skeletal muscle during growth. Micron 41: 997–1004.

Asaduzzaman, M. D., Ikeda, D., Abol-Munafi, A. B., Bulbul, M., Ali, MD. E., Kinoshita, S., Watabe, S., Kader, M. D. A. 2017. Dietary supplementation of inosine monophosphate promotes cellular growth of muscle and upregulates growth-related gene expression in Nile tilapia *Oreochromis niloticus*. Aquaculture 468: 297-306.

Canevesi, F. C., Muniz, C. C., Aiub, J. A., Oliveira Júnior, E. S. 2014. Desenvolvimento da matrinxã *Brycon amazonicus* (Spix & Agassiz, 1829) em fase de alevino com diferentes níveis de proteína bruta na ração. Revista de Ciências Agroambientais 12: 157-160.

Cheng, Z., Chen, S., An, M., Wang, Q., Sun, J., Fang, Z., Xing, K. 2018. Effects of replacing fish meal with soybean meal, with or without dietary arginine, on growth performance, immune indices and intestinal morphology of grouper, *Epinephelus malabaricus*. Aquaculture Research 49: 2954-2964.

Coutinho, F., Simões, R., Monge-Ortiz, R., Furuya, W. M., Pousão-Ferreira, P., Kaushik, S., Oliva-Teles, A., Peres, H. 2017. Effects of dietary methionine and taurine supplementation to low-fish meal diets on growth performance and oxidative status of European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 479: 447-454.

D'aout, K., Curtin, N. A., Williams, T. L., Aerts, P. 2001. Mechanical properties of red and White swimming muscles as a function of the position along the body of the Eel *Anguilla anguilla*. The Journal of Experimental Biology 204: 2221-2230.

Devincenti, C. V., Longo, M. V., Castro, M. G., Díaz, A. O. 2015. Morphological and histochemical characterization of the pectoral fin muscle of the stripped weakfish, *Cynoscion guatucupa*. Acta Zoologica (Stockholm) 96: 199–208.

Espírito Santo, N. G., Fernandes, V. A. G., Pierri, B. S., Fracalossi, D. M. 2015. Replacement of animal protein sources by soy protein concentrate for juvenile Nile tilápia. Boletim Instituto da Pesca 41: 707-717.

Ferreira, M. S., Aride, P. H. R., Silva, M.N.P., Val, A. L. 2013. Efeito da quantidade de proteína na dieta e treinamento físico sobre parâmetros fisiológicos e zootécnicos de Matrinchã (*Brycon amazonicus*, Gunther 1869). Acta Amazônica 43: 439-446.

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, A., Nelson, R. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38: 551-579.

Goldspink, G. 1972. Postembryonic growth and differentiation of striated muscle. In The Structure and Function of Muscle, vol. 1, (second edition) (ed. G. H. Bourne), 179–236. New York: Academic Press.

Ma, Z., Hassan, M.M., Allais, L., He, T., Leterme, S., Ellis, A. V., McGraw, B., Qin, J. G. 2018. Replacement of fishmeal with commercial soybean meal and EnzoMeal in juvenile barramundi *Lates calcarifer*. Aquaculture Research 49: 3258–3269.

Gundersen, H. J. G., Bendtsen, T. F., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., Pakkeneberg, B., Sorensen, F. B., Vesterby, A., West, M. J. 1988. Some new, simple and eficient stereological methods and their use in pathological research and diagnosis. Journal of pathology, microbiology and immunology 96: 379-394.

Hardy, R.W. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research 41: 770 776.

Johnston, I. A. 1999. Muscle development and growth: potential implications for flesh quality in fish. Aquaculture 177: 99-115.

Koumans, J. T. M., Akster, H. A. 1995. Myogenic cells in development and growth of fish. Comparative Biochemistry and Physiology 10: 3-20.

Krogdahl, A., Bakke-Mckellep, A. M., Baeverfjord, G. 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar L.*). Aquaculture Nutrition 9: 361-371.

La Serrana, D. G., Fontanillas, R., Koppe, W., Fernández-Borràs, J., Blasco J., Martín-Pérez, M., Navarro, I., Gutiérrez, J. 2013. Effects of variable protein and lipid proportion in gilthead sea bream (*Sparus aurata*) diets on fillet structure and quality. Aquaculture nutrition 19: 368 – 381.

Lee, S. M., Azarm, H. M., Chang, K. H. 2016. Effects of dietary inclusion of fermented soybean meal on growth, body composition, antioxidant enzyme activity and disease resistance of rockfish (*Sebastes schlegeli*). Aquaculture 459: 110-116.

Leitão, N. J., Pai-Silva, M. D., Almeida, F. L. A., Portella, M. C. 2011. The influence of initial feeding on muscle development and growth in pacu *Piaractus mesopotamicus* larvae. Aquaculture 315: 78-85.

Lobley, G.E. 2003. Protein turnover—what does it mean for animal production? *Canadian* Journal of Animal Science 83: 327–340.

Liang, X. F., Hu, L., Dong, Y. C., Wu, X. F., Qin, Y. C., Zheng, Y. H., Shi, D. D., Xue, M., Liang, X. F. 2017. Substitution of fish meal by fermented soybean meal affects the growth performance and flesh quality of Japanese seabass (*Lateolabrax japonicus*). Animal Feed Science and Technology 229: 1-12.

Liu, X., Ye, J., Wang, K., Kong, J., Yang, W., Zhou, L. 2012. Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Research 43: 745–755.

Melo, C. C. V., Costa, D. V. da., Gonçalves, A. C. S., Leira, M. H., Botelho, H. A., Oliveira, K. K. C., Freitas, R. T. F. 2016. Desenvolvimento dos tecidos muscular e adiposo em linhagens de tilápia do Nilo *Oreochromis niloticus*. Caderno de Ciências Agrárias 8: 72-82.

Michelato, M. A., Zaminhan, M., Boscolo W. R., Nogaroto, V., Vicari, M., Artoni, R. F., Furuya, V. R. B., Furuya, W. M. 2017. Dietary histidine requirement of Nile tilapia juveniles based on growth performance, expression of muscle-growth-related genes and haematological responses. Aquaculture 467: 63-70.

Monzer, S., Nasser, N., Babikian, J., Saoud, I. P. 2017. Substitution of fish meal by soybean meal in diets for juvenile marbled spinefoot, *Siganus rivulatus*. Journal of Applied Aquaculture 29: 102-216.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kaustsky, N., Beveridge, M. C. 2000. Effect of aquaculture on world fish supplies. Nature 405: 1017–24.

NRC. 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

Pastore, S. C. G., Gaiotto, J. R., Ribeiro, F. De A. S., Nunes, A. J. P. 2013. Formulação de rações e boas práticas de fabricação. In: D. M. Fracalossi; J. E. P. Cyrino (Eds.); In: NUTRIAQUA: Nutrição e alimentação de espécies de interesse para a aquicultura brasileira. (1st ed.), Florianópolis: Sociedade Brasileira de Aquicultura e Biologia Aquática. 375.

Pezzato, L.E., Barros, M.M., Fracalossi, D.M., Cyrino, J.E.P. 2004. Nutrição de peixes. In: Cyrino, J.E.P., Urbinati, E.C., Fracalossi, D.M., Castagnolli, N. Tópicos especiais em piscicultura de água doce tropical intensiva. São Paulo: Tecart, 75-169.

Refstie, S., Storebakken, T., Baeverfjord, G., Roem, A. J. 2001. Longterm protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. Aquaculture 193: 91–106.

Sales, J. 2009. The effect of fish meal replacement by soyabean products on fish growth: a meta-analysis. British Journal of Nutrition 102: 1709-1722.

Santos, V. B. 2007. Morphological aspects of fish lateral muscles. Boletim do Instituto de Pesca, 33: 127-135.

Sigurgisladottir, S., Sigurdardottir, M. S., Ingvarsdottir, Torrissen, O. J., Hafsteinsson, H. 2001. Microstructure and texture of fresh and smoked Atlantic salmon, *Salmo salar* L., fillets from fish reared and slaughtered under different conditions. Aquaculture Research 31: 1-10.

Silva, P., Valente, L. M. P., Olmedo, M., Álvarez-Blázquez, B., Galante, M. H., Monteiro, R. A. F., Rocha, E. 2011. Influence of temperature on muscle fibre hyperplasia and hypertrophy in larvae of blackspot seabream, *Pagellus bogaraveo*. Aquaculture Research 42: 331-340.

Spierts, I. L. Y., Van Leeuwen, J. L. 1999. Kinematics and muscle dynamics of C- and S-Starts of Carp (*Cyprinus carpio* L.). Journal of Experimental Biology 201: 393-406.

Sanger, A. M., Stoiber, W. 2001. Muscle fiber diversity and plasticity. In: Johnston, I. A. Muscle development and growth. London: Academic, 18.

Valente, L. M. P., Cabral, E. M., Sousa, V., Cunha, L. M., Fernandes, J. M. O. 2015. Plant protein blends in diets for Senegalese sole affect skeletal muscle growth, flesh texture and the expression of related genes. Aquaculture 453: 77-85.

Van der Meulen, T., Schipper, H., Van Leeuwen, J. L., Kranenbarg, S. 2005. Effects of decreased muscle activity on developing axial musculature in nicb107 mutant zebrafish (*Danio rerio*). The Journal of Experimental Biology 208: 3675-3687.

Vargas, S. C., Oliveira Filho, P. R. C., Natori, M. M., Lima, C. G., Macedo Viegas, E. M. 2013. Evaluation of different stunning methods on aspects of animal welfare and meat quality of matrinxã (*Brycon cephalus*). Italian Journal of Food Science 25: 255-262.

Vélez, E.J., Lutfi, E., Azizi, S., Perelló, M., Salmerón, C., Riera-Codina, M., Ibarz, A., Fernández-Borràs, J., Blasco, J., Capilla, E., Navarro, I., Gutiérrez, J. 2017. Understanding fish muscle growth regulation to optimize aquaculture production. Aquaculture 467: 28-40.

Videler, J. J. 2011. An opinion paper: emphasis on white muscle development and growth to improve farmed fish flesh quality. Fish Physiology and Biochemistry 37: 337-343.

Wang, C., Zhu, X., Han, D., Jin, J., Yang, Y., Xie, S. 2015. Responses to fishmeal and soybean meal-based diets by three kinds of larval carps of different food habits. Aquaculture Nutrition 21: 552-568.

Wilson, R.P. 1985. Amino acid and protein requirement of fish. In: Cowey, C.B., Mackie, A.M., Bell, J.G. Nutrition and feeding in fish. London: Academic Press. 1-17.

Zhang, C., Rahimnejad, S., Wang, Y., Lu, K., Song, K., Wang, L., Mai, K. 2018. Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): Effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. Aquaculture 483: 173-182.

CAPÍTULO III*

* Artigo apresentado nas normas da revista Aquaculture nutrition.

Soybean meal as an alternative to fish meal in diets for matrinxã (*Brycon amazonicus*): growth performance, body composition and histopathology of liver and intestine

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Abstract
Matrinxãs (Brycon amazonicus) were fed with diets on total replacement (100% protein) of fishmeal by soybean meal (SBM), 50% replacement (SBM / FM) and no substitution (0% SBM (FM), to evaluate the morphometric characteristics, zootechnical performance, centesimal composition and lipid profile of fillets and histopathological characteristics of the liver and intestine. The morphometric characteristics were similar in FM and SBM/FM treatments, fillet yield was similar among the three treatments. Final weight gain, weight gain and daily weight gain decreased with increasing substitution of fishmeal by soybean meal. For the specific growth rate, apparent feed conversion and protein efficiency ratio, SBM/FM and FM treatments were similar. The SBM diet presented lower humidity and lower ethereal extract than the others. The lipid profile showed higher amounts of DHA and n-3 in FM treatment and higher amounts of PUFA and n-6 in SBM treatment. Most of the livers analyzed histologically had normal structure, some animals had focal necrosis, SBM treatment had fewer vacuoles and larger sinusoids than the others, while SBM/FM and FM presented larger hepatocyte size. Most of the intestines evaluated had normal structure, although the SBM treatment showed shorter villi compared to the others, a greater amount of discontinuity of the epithelium and more centralized nuclei of the mucosal cells. Goblet cells were present in normal amounts and mostly in rounded form. In conclusion, the substitution of fish meal by soybean meal as a protein source in diets for matrinxã can be performed partially (50% FM per SBM) without leading to a large fish injury.

KEY WORDS: *performance; histology; intestine; liver; nutrition; plant protein;*

Introduction

Fish meal is the main protein source used in aquaculture diets (Espirito Santo et al., 2015). However, because it is a limited and finite resource, and therefore one of the most costly ingredients in the formulation of fish diets, there is a need to find alternatives to this in diets, especially vegetable proteins (Bonaldo et al., 2006). In this way, the substitution of fish meal by other protein

sources in the formulation of fish diets is an alternative to reduce the pressure on fish stocks, in order to reduce this depletion, besides the reduction of production costs, in view of the increasing cost of fish meal on the market.

In this context, soybean meal has been shown to be the most promising vegetable protein, as it presents characteristics such as: lower price, constant supply and adequate amino acid balance (Kader et al., 2010; Tomas-Vidal et al., 2011; Bowyer et al., 2012; Yu et al., 2013; Zhang et al., 2014; Booman et al., 2018). Although soybean meal presents a lower cost and greater availability of the ingredient throughout the year, with a constant quality standard in relation to fish meal, the use of soy protein in diets should consider factors that may be limiting for its inclusion such as the presence of antinutritional factors, low palatability and lower digestibility, which can cause negative effects on animal performance and health (Brinker & Reiter, 2011).

Soybean meal replacement was showing positive results in literature such as: similar growth to FM diet (Peng et al., 2014), lipid reduction in fillet (Masagounder, Hayward & Firman, 2014) and normal gut morfology (Barnes et al., 2014) or negatives such as: lower performance (Hartviksen et al., 2014), lower protein retention in fillet (Jeon et al., 2014) and enteritite (Gu et al., 2016).

Matrinxã (*Brycon amazonicus*) is a neotropical freshwater species native to the Amazon basin. It is important for commercial and sport fishing, especially in the northern region of Brazil (Canevesi et al., 2014), its omnivorous food habit and good acceptance of commercial feed, may allow the use of vegetable sources of protein in the diet, sources of animal protein.

Considering the above, the use of soy products in fish feed has shown different results regarding zootechnical performance and level of incorporation among the species. The effects of this substitution on the matrinxã diet are not yet known. The objective of this study was to analyze the substitution of soybean meal as a source of protein in the diet of matrinxã (*Brycon amazonicus),* evaluating the effect on the zootechnical performance, centesimal composition and fillet lipid profile, and histopathology of the liver and intestine.

Materials and methods

Fish, experimental set-up and sampling

The experiment was carried out at the Amazonian Research Institute, Graduate Program in Aquaculture, Manaus, Brazil. The matrinxã (*Brycon amazonicus)* had an initial mean weight of 141.59 ± 0.5g, obtained from the institution itself. Prior to the experiment, the fish were fed commercial FM-base diets (Nutripiscis® Amazonia Juvenil, Brazil). Seventy-eight animals were randomly distributed in six tanks excavated and fed the experimental diet for 92 days. The tanks had natural water in a closed system. Water quality follow-up was carried out by temperature monitoring (30-33ºC), diluted oxygen (7.60-13.60 mg L-1), transparency (25-55 cm) and pH (8.35-8.50), with the help of the YSI Professional Plus Multiparameter Water Quality Meter (YSI, Pro Plus, Yellow

Springs-Ohio, USA). The animals were fed twice a day (9.00 am and 4.00 pm) to apparent satiety during the 92 day experimental period.

At the end of the experiment, the animals were desensitized by hypothermia and subsequent decapitation. All animals were measured, weighed and filleted. Eighteen samples of fillets were lyophilized and sent for lipid and fatty acid profile. Twelve samples per treatment were used for centesimal analysis, six liver samples and twelve intestinal samples were used for histological analysis.

Experimental Diets

The experimental diets were prepared to be isoproteic (34% crude protein) and isolipidic (390 kcal / 100g). The diets were made at the Institute of Amazonian Research (INPA). The ingredients were ground in hammer mills with 0.3 millimeters sieve and the final feed was extruded. Experimental diets were formulated to contain different percentages of fishmeal replacement by soybean meal. Experimental diets were composed of: 100% soybean meal (diet SBM), 50% soybean meal / 50% fish meal (SBM / FM diet) and 100% fish meal (diet FM). The ingredients used to make the experimental diets and proximal composition of the diets are shown in Table 1.

Table 1 Ingredients (g/kg) and proximal composition analyzed (%) of the experimental diets for *Brycon amazonicus*.

Ingredients (g/kg)	Experimental diets		
	SBM	**SBM/FM**	**FM**
Soybean meal 46	645.00	220.00	0.00
Fish meal 61	0.00	280.00	426.00
Corn grain 7,92	115.00	200.00	220.00
Whear bran	60.00	145.00	220.00
Rice	60.00	100.00	100.00
Soybean oil	52.00	30.00	20.00
Dicalcium phosphate	50.00	15.00	4.00
Vitamin and mineral premix	10.00	10.00	10.00
Calcareous	8.00	0.00	0.00
Proximal composition analyzed (%)			
Dry matter	93.93	94.47	93.80
Crude protein	33.37	34.07	34.30
Ether Extract	5.00	5.40	6.43
Ash	10.07	9.00	9.40
Fatty acid profile (Percentage of total fatty acids)			
14:00	1.03	6.98	9.69
16:00	104.55	104.90	105.87
16:1n-9	0.35	1.26	1.58
16:1n-7	1.13	9.03	12.45
16:1n-5	0.23	0.41	0.47
17:00	0.79	1.02	1.07
17:1n-9	0.36	0.66	0.97
18:00	26.79	26.71	26.79

18:1n-9	174.23	201.36	212.49
18:1n-7	13.07	15.12	16.08
18:1n-5	0.50	0.45	0.34
18:2n-6	402.12	282.33	231.01
18:3n-6	1.54	1.12	0.83
18:3n-3	41.51	32.60	29.36
20:00	2.28	0.68	0.85
20:1n-9	1.74	5.06	6.78
22:00	2.84	8.56	12.79
24:00:00	1.22	0.72	1.18
ΣSFA	139.49	149.86	158.24
ΣMUFA	191.61	236.46	255.83
ΣPUFA	445.17	328.30	279.47
Σn-6	403.66	283.45	231.84
Σn-3	41.51	44.85	47.63

1 Guarantee levels per kg of product - Premix (DSM-Roche®): Vit. A, 24,000 IU; Vit. D3, 6.000 IU; Vit. E, 300 mg; Vit. K3, 30 mg; Vit. B1, 40 mg; Vit. B2, 40 mg; Vit. B6, 35 mg; Vit. B12, 80 mg; B.C. folic acid, 12 mg; Pantothenate Ca, 100 mg; Vit. C, 600 mg; Biotin, 2 mg; Choline, 1.000 mg; Niacin; Iron, 200 mg; Copper, 35 mg; Manganese, 100 mg; Zinc, 240 mg; Iodine, 1.6 mg; Cobalt, 0.8 mg. SBM = Soybean meal; SBM / FM = Soybean meal / Fish meal; FM = Fish meal. Premix.

Evaluation of performance parameters

After 92 experimental days, the fish were fasted for 24 hours for gastrointestinal tract emptying. Consequently, animals were anesthetized to measure individual weight (g) and total length (cm) and to evaluate production performance.

The following zootechnical parameters were evaluated: Weight gain (g) = (final body weight - initial body weight); Daily weight gain (g) = (weight gain) / (experimental days); Apparent feed conversion = (consumed diet) / (weight gain); Condition factor (%) = (final weight / total final leigh³) *100; Specific growth rate (%.day-1) = ((ln (final weight) – ln (initial leight)) / experiment days) *100; Protein efficiency index = ((weight gain) / (crude protein consumption in dry matter)).

Centesimal composition and fatty acids analyses

Samples of the experimental rations and fish fillets were analyzed according to the protocol recommended by AOAC (2005). Dry matter was obtained by the difference between the initial weight and the final sample submitted to a muffle at 105 ° C for 8 hours and carcasses were submitted to pre-drying at 55 ° C for 72 h before muffle (Method number 950.46). Protein was obtained by digestion with sulfuric acid, distillation and titration by the Kjeldhal method (Method number 981.10) (Model MA-036, Piracicaba - São Paulo, Brazil), while the ethereal extract was conducted through an extractor with solvent (petroleum ether) (Soxhlet extractor; Model TE-0.44, Piracicaba - São Paulo, Brazil). Mineral matter was obtained from a 550 ° C muffle for 6 hours (Method n. 920,153) (Modle 2000B, Belo Horizonte - Minas Gerais, Brazil).

The total lipids of the diets and files were determined by the method proposed by Bligh & Dyer (1959). Lipid class composition of diet and fillet was

determined by high-performance thin-layer chromatography (HPTLC) using Thermo Scientific gas chromatograph, trace ultra 3300 model with CP-7420 (Select FAME) fused silica capillary column. Correction factors were used to obtain the values of fatty acid concentrations according to Visentainer (2012). Absolute quantification of EMAGs (methyl esters of fatty acids) was performed using the methyl ester of trichosanoic acid (23:0me), branded SIGMA (USA). Percentages were determined using ChromQuest Software version 5.0.

Liver and intestine histopathology

Samples for histological evaluation of liver and intestine were immersed in buffered formalin (10%) and after 24 hours were stored in alcohol 70% for further analysis. After, the samples were dehydrated and embedded in paraffin. Sections (4-6 µm) were made and stained with hematoxylin and eosin, and analyzed by light microscopy (Zeiss microscope). The images were taken using a microscopic camera (Carl Zeiss MicroImaging). Histological changes of the liver were categorized according to pathological findings: hepatocyte vacuolation associated with peripheral nucleus, vascular congestion, mononuclear aggregate infiltration, lymphocytes presence (López et al., 2015), focal necrosis (Evans et al. 2005), decreased hepatocyte diameter and nucleus, increased sinusoidal volume (Monfared and Salati, 2013), vascular congestion, clusters of lymphocytes and fibroblasts around necrotic biliary duct, biliary duct cholangiofibrosis, thin wall of central veins (Agamy, 2012). The histological changes of the intestine were categorized according to the criteria: presence of goblet cells, appearance of the epithelium, nucleus location, cytoplasmic vacuolization, presence of inflammatory cells in the lamina propria (Van den Ingh et al., 1991), the presence of basophilic granulocytes in the lamina propria and mucosa (Urán et al., 2008) and presence of and the presence of clusters of bacteria in the lumen (Hansen et al., 2006).

Statistical analyses

All data are presented as mean ± standard error. The normality of the data was verified by the Kolmogorov-Smirnov test and the homogeneity by the Levene test. Subsequently, the data were submitted to one-way ANOVA, followed by Tukey's test, at a 5% significance level. Data that did not present normal distribution were analyzed by Kruskal-Wallis analysis, followed by Dunn's test, at 5% significance level. The analyzes were performed using statistical software Statistical Analysis System 9.4 and GraphPad Prism 7.0.

Results

The results of the morphometric characteristics can be observed in Table 2. At the end of the experimental period a significant difference between the treatments was observed in all the variables, although these differences were observed, it is important to point out that the SBM/FM treatment did not have

significantly different results of FM treatment. No difference was observed between treatments for morphometric relationships.

Table 2 Body morphometric and fillet characteristics of matrinxã fed with different protein sources in the diet.

Variables (cm)	Treatments			*P*-value*
	SBM	**SBM/FM**	**FM**	
Total length	27.23±0.26b	28.57±0.23a	29.35±0.22a	<0.0001
Standard Length	23.93±0.20b	24.92±0.21a	25.61±0.20a	<0.0001
Head length	5.37±0.06b	5.58±0.04[a]	5.68±0.08a	0.0023
Fish height	7.77±0.08b	8.30±0,11[a]	8.57±0,10a	<0.0001
Width of trunk	3.19±0.06b	3.33±0.05ab	3.45±0.04a	0.0015
Fillet height	5.61±0.11b	5.86±0.08ab	6.03±0.11a	0.0177
Fillet lenght	15.27±0.20b	16.43±0.18a	16.35±0.18a	<0.0001
Fillet width	0.77±0.03b	0.82±0.02ab	0.90±0.03a	0.0050
Morphometric relationships				
HL/SL	0.22±0.01	0.22±0.01	0.22±0.01	0.6454
SL/TL	0.88±0.01	0.87±0.01	0.87±0.01	0.4238
WT/SL	0.13±0.01	0.13±0.01	0.13±0.01	0.9819

Data represented by mean ± standard error. * Values followed by different letters on the same line differ from each other by the Tukey test. HL / SL (Head length / standard length); SL / TL (Standard Length / Total Length); WT / SL (Width of trunk / standard length).

The yield characteristics of the fish can be observed in Figure 1. We observed a significant difference between the treatments for the variables of fish weight and fillet weight, however, fillet yield was not different between the treatments. The mean body weight and fillet weight were higher in fish fed the FM diet, while fish fed the SBM diet presented lower values of body weight and fillet.

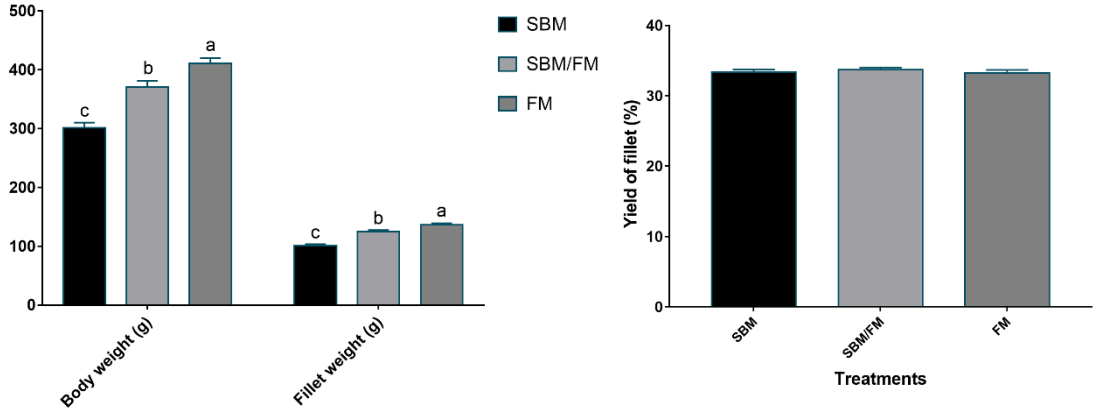

Figure 1 Performance characteristics of matrinxã fed with different protein sources in the diet. Different letters indicate a significant difference between the treatments by the Tukey test.

The data of the zootechnical performance of matrinxans fed with different protein sources in the diet can be observed in Table 3. We observed difference between the treatments for the variables of final weight, weight gain, daily weight gain and condition factor. The variables of specific growth rate, apparent feed conversion and protein efficiency rate did not differ between treatments. Fish fed the FM diet had the highest values of final weight, gain in weight and daily gain in weight, differing from other treatments, while fish fed the SBM diet presented the lowest values of weight. For the condition factor variable, fish fed the FM diet and the SBM / FM diet had the highest values, differing statistically from the fish fed with the SBM diet.

Table 3 Zootechnical performance of matrinxã feed with different protein sources in the diet.

Variables	Treatments			*P*-value*
	SBM	**SBM/FM**	**FM**	
Final weight (g)	301.54±8.38^{c}	370.46±10.50^{b}	410.54±9.17^{a}	<0.0001
Weight Gain (g)	180.61±8.38^{c}	222.90±10.50^{b}	255.10±9.17^{a}	<0.0001
Daily weight gain (g/day)	2.01±0.09^{c}	2.48±0.12^{b}	2.83±0.10^{a}	<0.0001
Condition factor (%)	1.49±0.02^{b}	1.58±0.20^{a}	1.62±0.02^{a}	0.0003
Specific growth rate (% day^{-1})	1.00±0.03	1.01±0.03	1.07±0.02	0.1819
Apparent feed conversion	2.07±0.10	1.96±0.09	1.93±0.07	0.4839
Protein efficiency rate	1.37±0.06	1.42±0.07	1.40±0.05	0.8678

Data represented by mean ± standard error. * Values followed by different letters on the same line differ from each other by the Tukey test.

The centesimal composition of the matrinxã fillets can be observed in Figure 2. There was a difference between the treatments for moisture content and ethereal extract. It was observed a higher moisture content in fish fed with a diet of 100% replacement of fish meal with soybean meal. For the ethereal extract content, a higher value was observed for the mixed treatment, followed by the FM treatment, while the fish fillets fed with the 100% soybean meal diet had the lowest ethereal extract value.

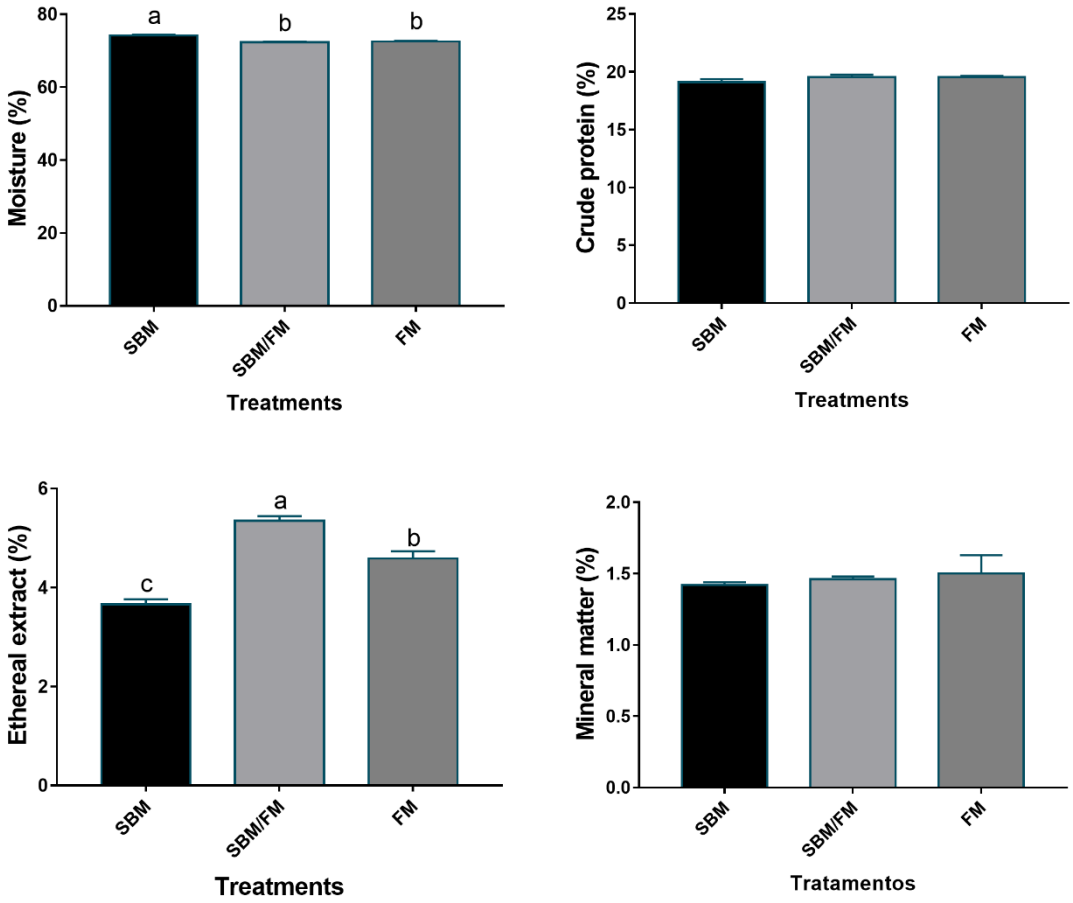

Figure 2 Fillet centesimal composition of matrinxã fed with different protein sources in the diet. Different letters indicate a significant difference between the treatments by the Tukey test.

The results of fillet fatty acid composition analyzes of matrinxã fed with different protein sources in the diet can be observed in Table 4. A significant difference was observed between treatments for fatty acids 14:00; 16:1n-9; 16: 1n-7; 18: 1n-9; 18: 1n-7; 18: 2n-6; 18: 3n-3; 20: 3n-6; 20: 3n-3; 24:00:00 and DHA, in addition to significant difference between the sum of saturated, monosaturated, polyunsaturated, omega 3 and 6 fatty acids.

Table 4 Fatty acid composition (percentage of total fatty acids) of matrinxã fillets fed with different protein sources in the diet.

Fatty acids (%)	Diets			*P*- value*
	SBM	**SBM/FM**	**FM**	
14:00	8.57±0.47^{b}	10.91±0.25^{a}	10.59±0.31^{a}	0.0070
16:00	196.07±10.59	201.05±2.10	186.54±5.70	0.3930
16:1n-9	2.75±0.13^{b}	3.19±0.03^{ab}	3.43±0.11^{a}	0.0100
16:1n-7	7.69±0.43^{b}	12.95±0.15^{a}	13.67±0.42^{a}	<0.0001
18:00	82.99±4.66	81.13±0.99	74.92±2.85	0.2534
18:1n-9	251.01±14.29^{b}	300.25±3.32^{a}	287.20±9.70^{ab}	0.0335

18:1n-7	8.95±0.47[b]	11.14±0.20[a]	11.21±0.34[a]	0.0063
18:2n-6	164.09±9.21[a]	124.82±1.96[b]	99.22±3.46[c]	0.0006
18:3n-3	14.07±0.66[a]	13.14±0.26[a]	10.70±0.34[b]	0.0049
20:3n-6	8.36±0.42[a]	3.81±0.08[b]	3.93±0.17[b]	<0.0001
20:3n-3	8.59±0.38[a]	4.15±0.03[ab]	3.71±0.04[b]	0.0429**
24:00	4.41±0.13[a]	1.37±0.03[ab]	1.16±0.04[b]	0.0111**
DHA	5.81±0.40[c]	11.49±0.17[b]	14.90±0.09[a]	<0.0001
ΣSFA	292.03±15.84	295.19±3.32	273.87±8.23	0.3730
ΣMUFA	270.40±15.32[b]	329.01±3.73[a]	317.75±10.63[ab]	0.0202
ΣPUFA	200.93±11.02[a]	168.08±2.36[b]	144.83±4.15[b]	0.0036
Σn-6	172.46±9.6[a]	137.14±1.96[b]	112.45±3.72[b]	0.0013
Σn-3	28.47±1.43[b]	30.93±0.42[ab]	32.38±0.44[a]	0.0458

Data represented by mean ± standard error. * Values followed by different letters on the same line differ from one another by analysis of variance, followed by Tukey's test. ** Values followed by different letters on the same line differ from each other by Kruskal-Wallis analysis, followed by Dunn's test.

The percentage observed for each fatty acid and the sum of the major groups of fatty acids, for each treatment, can be observed in Figure 3.

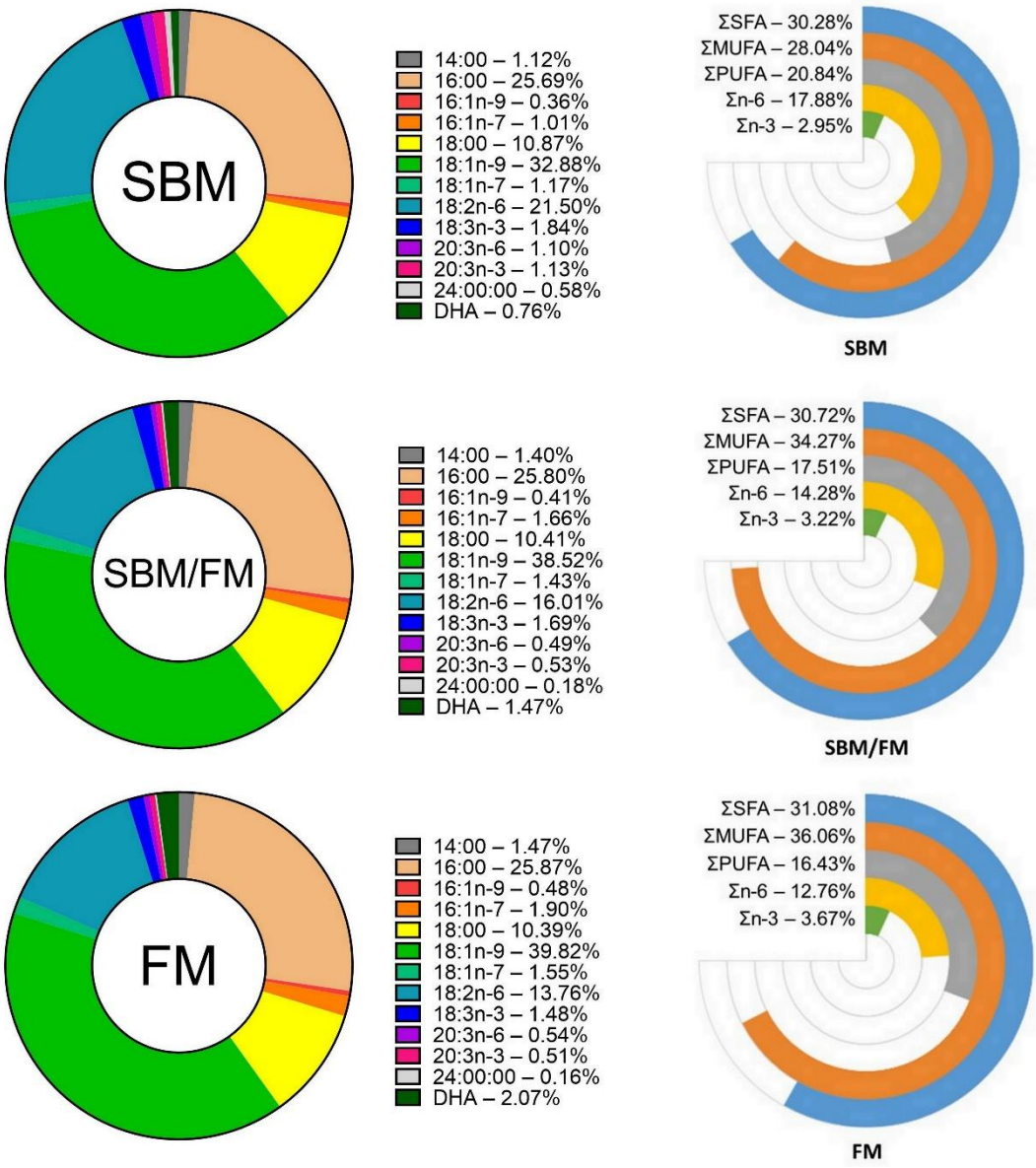

Figure 3 Percentage of the fillet fatty acids composition of matrinxã fed with different protein sources in the diet.

Most treatments had normal liver structure, although SBM/FM and FM treatments had higher numbers of vacuoles and increased hepatocytes. The SBM treatment presented a greater sinusoid increase than the other treatments. In a few animals, focal necrosis was observed in the liver, but there was no difference in the amount found between the groups (Figure 4). In the intestine, there was a greater amount of shortened villi and some irregularities in the mucosa, although most of the samples observed showed villi of normal size and epithelial integrity. SBM treatment also had more centralized nuclei of enterocytes, although most had basal nuclei. The amount of goblet cells was normal and rounded in all treatments (Figure 5). There was no increase in

cytoplasmic vacuolization or thickening of the lamina propria, frequent characteristics of intestinal inflammation.

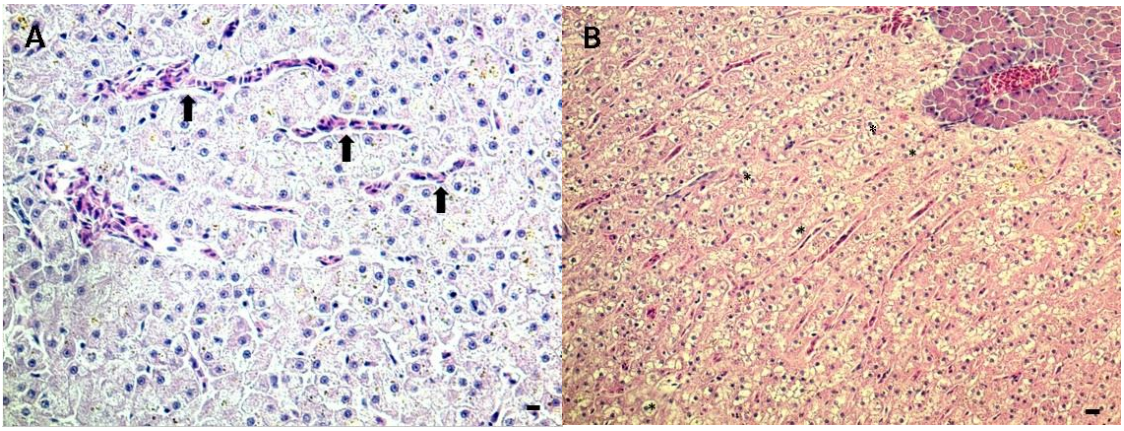

Figure 4 Photomicrographs of histological sections *Brycon amazonicus* liver. Arrows indicate increased sinusoids, asterisks indicate hepatocytes with lipid vacuoles, HE, 20X.

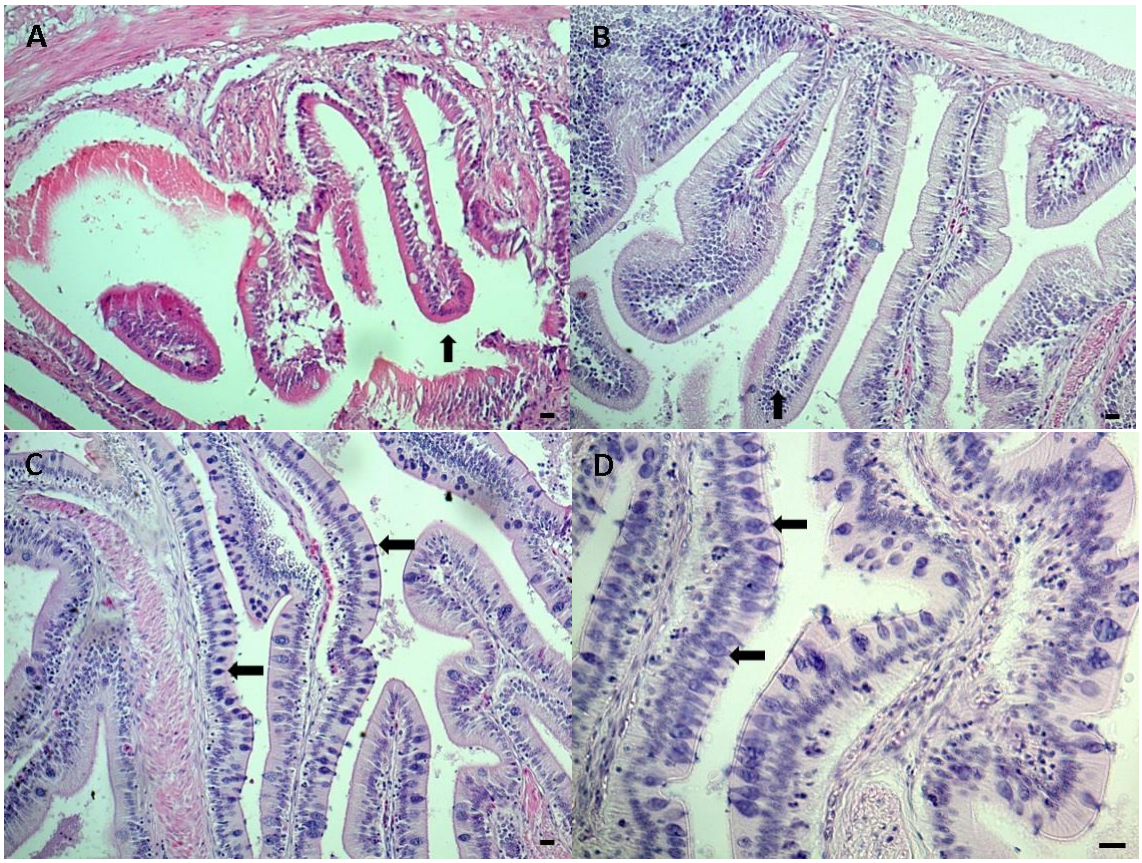

Figure 5 Representative histopathological images of *Brycon amazonicus* intestine. Arrows indicate intestinal villi of different sizes. Asterisks indicate goblet cells. Hematoxylin and eosin staining. A, B and C, 20X. D, 40X.

Discussion

We know the need to find alternatives to fish meal in fish diets, considering the decrease in fish stocks, the cost of the diets and also the uniformity of the product and the constant availability of the product. In recent years, soybean has been studied as one of the main ingredients used as a vegetable protein alternative used in fish diets, however, the use of this ingredient in diets, as it contains antinutritional factors, can lead the animals to low performance and pathological alterations in some organs (Yuan et al., 2017, Yaghoubi et al., 2016, Maans et al., 2018).

The present study showed that the morphometric characteristics were lower in fish fed with the SBM diet. Although fillet and body weight decreased as the replacement of fishmeal by soybean meal increased, fillet yield was not different between treatments.

Regarding the zootechnical performance, it was observed that the final weight gain, weight gain and daily weight gain decreased with the increase of soybean meal inclusion in substitution of fish meal. In contrast, in the other analyzed parameters, the SBM/FM treatment was not significantly different from the FM treatment, demonstrating that the partial replacement of the animal protein source by the plant source can be performed without harming all parameters of productive performance.

García et al. (2015) observed that the growth of juveniles *Tinca Tinca* L. was impaired by the use of 45% substitution of fishmeal by soybean meal, while the substitution of 25-45% impaired total length, weight, specific growth, condition factor and feed conversion. Bañuelos-Vargas et al. (2014) found that the daily growth rate and diet intake significantly reduced with the replacement of fishmeal by soybean protein concentrate in a study with juveniles *Totoaba macdonaldi*.

According to McGoogan and Gatlin (1997), the palatability of diets containing soybean meal as the main source of protein may be responsible for the limitation of feed intake and the drop in fish growth, and it is recommended the use of attractants to stimulate the consumption of diets . The fact that fish fed diets based on soybean meal grow less suggests that this response may be related to problems with palatability and attractiveness induced by the inclusion of soybean meal in the diet. However, the low food efficiency may be due to the decrease in assimilation of soy protein offered in the diet, as observed in a study by Monzer et al. (2017), where even for the species *Siganus rivulatus* that has herbivorous alimentary habit, the assimilation or digestion of the soybean meal is not efficient. In addition, the presence of antinutrients present in soybean, associated with high soybean inclusions in diets, may impair the health and performance of the animals (Ribeiro et al., 2015).

Omnes et al. (2017) observed that the addition of tannins, responsible for the reduction in the palatability of soybean, in the diets for *Dicentrarchus labrax* led to the decrease of the consumption of these, causing a significantly lower growth in the animals compared to the control group. Tannin can form complexes with some amino acids and inhibit digestive enzymes, reducing digestibility by decreasing the bioavailability of the protein. In studies with different amounts of crude protein in the diet of matrinxã, values of 16.5-21.4% of crude protein, 11.7-

14.6% of ethereal extract and 2.3-3.7% of ashes in fillets were found. The protein content behaves inversely proportional to the content of ethereal extract in the carcass of the animal (Mattos et al., 2018). The present study had higher values of crude protein and lower values of ethereal extract, these are probably related to the diets used in the experiment.

Several studies have demonstrated success in the zootechnical performance of low-weight diets (Bowyer et al., 2012; Rossi Jr., Siddiqui, Khan & Siddiqui, 2014; Tomasso & Gatlin III, Tomás-Vidal et al. 2011) as with high soybean meal inclusions (Chu et al., 2016, Hossain et al., 2018, Sarker et al., 2012, Song et al., 2014). Diets with a 25% substitution of fish meal for non-genetically modified soybean meal provided improvements in the final weight of the fish, weight gain, daily gain and protein efficiency of *Sciaenops ocellatus*, demonstrating that the species shows lower sensitivity to the antinutrients present in the SBM, demonstrating that the tolerance to these compounds depends on several factors such as the species (Minjarez-Osorio et al., 2016). Liao et al., (2015) found higher values of gain in weight and protein efficiency of *Apostichopus japonicus* fed diets with 40% and 60% of fishmeal replacement per soybean meal compared to control diet. The success of partial or total replacement of fishmeal with soybean meal is linked to several factors, including fish species, culture system and diet regime, diet formulation and soy product composition and processment. In the present study, the specific growth rate, apparent feed conversion and protein efficiency ratio were not influenced by the inclusion of soybean meal as a substitute for fishmeal.

Studies show that the replacement of the protein source in the diet has little influence on the centesimal composition of the fish. In our study, we observed the influence of the dietary protein source on the moisture and ethereal extract variables of the matrinxã fillets, noting that the body protein content did not affect the different protein sources in the diet. In contrast, the level of FM replacement in the diet influenced only the body protein content of *Argyrosomus regius* (Kotzamanis et al., 2018). In a study evaluating the substitution of animal protein for plant protein in diets for *Myxocycyprinus asiaticus*, it was observed that fish fed the control diet had higher body lipid content than animals fed diets with 20-100% substitution (Yu et al., 2013), and in the present study, we observed that the partial substitution of the protein source led to a higher ethereal extract content in the fillet.

Pizando-Paima, Pereira Filho and Oliveira-Pereira (2001) found values of 63,0% protein content in the dry matter 21.6% of ethereal extract in matrinxã extracted from the Negro River and tributaries, affirming that the body composition of matrinxã indicates a relation between the centesimal composition of diet and fillet.

In *Senegale sole* steaks fed diets with up to 100% substitution of fish meal for proteins of plant origin, polyunsaturated fatty acids were found as the predominant class (39-43%), followed by saturated fatty acids (26- 28%) and monounsaturated fatty acids (22-27%). DHA was more abundant in animals fed FM (20.93%) than in other treatments. The content of Σ n-3 PUFA was higher in

the control treatment (33.35%) based on fish meal (Cabral et al., 2013). The results were similar to those observed in the present study.

Evans et al. (2005) also observed focal necrosis in the liver of some *Ictalurus punctatus* fed diets containing treated or untreated soybean, but found no significant difference between treatments. Novriadi et al. (2017) verified that the liver condition of Trachinotus carolinus showed low numerical value of glycogen granulation, inflammation and altered nuclear position as the amount of fermented soybean meal increased in the diets. Treatment with 75% substitution had less morphological changes in the liver than treatment with 50% substitution. The increased glycogen deposition in the liver, a sign of hepatic dysfunction, tends to increase as the inclusion of plant protein in the diet increases. However, this was not found in the present study, where most of the hepatocytes had the normal histological appearance. Espirito Santo et al., (2015) observed the structural aspect of the liver of tilapia fed with concentrated soy protein, finding liver with normal cellular architecture, with cord hepatocytes arrangement, with normal sinusoids and presence of central veins and bile duct. Matrinxã, like tilapia, is a species of omnivorous food habit, which may facilitate physiological adaptations to high-protein diets.

The use of soybean meal in high amounts in fish diets is associated with signs of inflammation of the intestine (Ferrara et al., 2015, Gued et al., 2016, Hedrera et al., 2013, Venold et al., 2012). Wang et al. (2017) observed a significant decrease in bowel villi height (117.97 mm) of *Epinephelus coioides* fed a 100% replacement diet of fishmeal per soybean meal compared to a control diet (321.84 mm). Significant decrease in villi size was found in *Lateolabrax japonicus* fed diets containing 75% substitution (287.83 μm) compared to basal diet (443.22 μm), this decrease of villi may lead to problems for the animal, since the integrity of the mucosal epithelium acts as a barrier against pathogens while absorbing nutrients (Zhang et al., 2018). Sahlmann et al., (2015) found that the inclusion of SBM (167 g / kg) in the diet did not induce intestinal inflammation in *Salmo salar* juveniles. Ferrara et al. (2015) observed that the inflammatory reactions in the gut begin soon after the administration of the SBM-containing diets in their composition, and progressively decrease with the adaptation of the fish to the vegetal component of the diet. A similar response may have occurred in the present study, since some signs of inflammation were found in the intestines of the animals, but the majority had normal histological structure.

The development of efficient and economically viable ways to eliminate alcohol-soluble components of soybean meal can totally modify the use of this ingredient in feed formulations, leading to an increase in their addition in diets (Nguyen et al., 2015). As well, future studies investigating the adequate balance of essential amino acids in diets containing high amounts of soybean meal may clarify the optimum growth of the fish (Peng et al., 2013). The results of this study indicate that the partial replacement of fishmeal with soybean meal is a viable alternative for diets of juveniles *Brycon amazonicus*, with few adverse effects on their growth. Subsequently, the use of smaller percentages of substitution could be tested to know the better zootechnical performance and less pathological

changes in the animals fed diets containing soybean meal as a component of the protein source in its composition. As well as, to use palatabilizantes in the diets based on soybean meal in order to improve the consumption of the rations that use this ingredient.

Acknowledgment

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References

Agamy, E. 2012. Histopathological Changes in the Livers of Rabbit Fish (Siganus canaliculatus) Following Exposure to Crude Oil and Dispersed Oil. Toxicologic Pathology, 40: 1128-1140.

Bañuelos-Vargas, I., López, L. M., Jiménez, A P., Pérez-Jiménez, A., Peres, H. (2014) Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on hepatic intermediary metabolism and antioxidante status of totoaba juveniles (*Totoaba macdonaldi*). *Comparative Biochemistry and Physiology, Part B*, **170**, 18-25.

Bansemer, M. S., Forder, R. E. A., Howarth, G. S., Suitor, G. M., Bowyer, J., Stone, D. A. J. (2014) The effect of dietary soybean meal and soy protein concentrate on the intestinal mucus layer and development of subacute enteritites in Yellowtail Kingfish (*Seriola lalandi*) at suboptimal water temperatura. *Aquaculture Nutrition*, **21**, 300-310.

Barnes, M. E., Brown, M. L., Bruce, T., Sindeler, S., Neiger, R. (2014) Rainbow Trout Rearing Performance, Intestinal Morphology, and Immune Response after Long-term Feeding of High Levels of Fermented Soybean Meal. North American Journal of *Aquaculture*, **76**, 333-345.

Bligh, E. C. & Dyer, W. J. 1959. A rapid method of total lipid. Extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Bonaldo, A., Roem, A. J., Pecchini, A., Grilli, E., Gatta, P. P. (2006) Influence of dietary soybean meal levels on growth, feed utilization and gut histology of Egyptian sole (*Solea aegyptiaca*) juveniles. *Aquaculture*, **261**, 580-586.

Booman, M., Forster, I., Vederas, J. C., Groman, D. B., Jones, S. R. M. (2018) Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). *Aquaculture*, **483**, 238-243.

Bowyer, J. N., Qin, J. G., Smullen, R. P., Adams, L. R., Thomson, M. J. S., Stone, D. A. J. (2012) The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds at different water temperatures: 1. Solvent extracted soybean meal. *Aquaculture*, **384-387**, 35-45.

Cabral, E. M., Fernandes, T. J. R., Campos, S. D., Castro-Cunha, M., Oliveira, M. B. P. P., Cunha, L. M., Valente, L. M. P. (2013) Replacement of fish meal by plant protein sources up to 75% induces good growth performance without affecting flesh quality in ongrowing Senegalese sole. *Aquaculture*, **380-383**, 130-138.

Canevesi, F. C., Muniz, C. C., Aiub, J. A., Oliveira Jr., E. S. (2014) Desenvolvimento da Matrinxã *Brycon amazonicus* (Spix & Agassiz, 1829) em fase de alevino com diferentes níveis de proteína bruta na ração. *Revista de Ciências Agroambientais*, **12**, 157-160.

Chu, Z. J., Yu, D. H., Dong, G. F., Gong, S. Y. (2016) Partial replacement of fish meal by soybean meal with or without methionine and phytase supplement in diets for juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture Nutrition*, **22**, 989-996.

Empresa Brasileira de Pesquisa Agropecuária – Embrapa. 2018. Soja em números (safra 2017/2018). Available at: < https://www.embrapa.br/soja/cultivos/soja1/dados-economicos> Acces on: 02 Nov. 2018.

Espirito Santo, N. G., Fernandes, V. A. G., Pierri, B. S., Fracalossi, D. M. (2015) Replacement of animal protein sources by soy protein concentrate for juvenile Nile Tilapia. *Boletim Instituto da Pesca*, **41**, 707-717.

Evans, J. J., Pasnik, D. J., Peres, H., Lim, C., Klesius, P. H. (2005) No apparent diferences in intestinal histology of channel catfish (*Ictalurus punctatus*) fed heat-treated and non-heat-treated raw soybean meal. *Aquaculture nutrition*, **11**, 123-129.

Ferrara, E., Gustinelli, A., Fioravanti, M. L., Restucci, B., Quaglio, F., Marono, S., Piccolo, G. (2015) Histological and micro-/macro-morphological evaluation of intestine in sharpsnout seabream (*Diplodus puntazzo*) fed soybean meal-based diets added with MOS and inulin as prebiotics. *Aquaculture International*, **23**, 1525-1537.

García, V., Celada, J. D., González, R., Carral, J. M., Sáez-Royuela, M., González, A. (2015) Response of juvenile tench (*Tinca tinca* L.) fed practical diets with different protein contents and substitution levels of fish meal by soybean meal. A*quaculture Research*, **46**, 28-38.

Gu, M., Bai, N., Zhang, Y., Krogdahl, A. (2016) Soybean meal induces enteritis in turbot *Scophthalmus maximus* at high supplementation levels. *Aquaculture*, **464**, 286-295.

Hansen, A. C., Rosenlund, G., Karlsen, O., Olsvik, P. A., Hemre, G. I. (2006) The inclusion of plant protein in cod diets, its effects on macronutrient digestibility, gut and liver histology and heat shock protein transcription. *Aquaculture Research*, **37**, 773-784.

Hartman, L., Lago, B.C. A rapid preparation of fatty methyl esters from lipids. Lab. Pract. V. 22, p. 475-477, 1973.

Hartviksen, M., Vecino, J. L. G., Ringo, E., Bakke, A. M., Wadsworth, S., Krogdahl, A., Ruohonen, K., Kettunen, A. (2014) Alternative dietary protein sources for Atlantic salmon (*Salmo salar* L.) effect on intestinal microbiota, intestinal and liver histology and growth. *Aquaculture Nutrition*, **20**, 381-398.

Hedrera, M. I., Galdames, J. A., Jimenez-Reyes, M. F., Reyes, A. E., Avendaño-Herrera, R., Romero, J., Feijóo, C. G. (2013) Soybean Meal Induces Intestinal Inflammation in Zebrafish Larvae. *Plos One*, **8**, 1-10.

Hossain, S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N. M., Islam, J., Maekawa, M., Fujieda, T. (2018) Substitution of dietary fishmeal by soybean meal with inosine administration influences growth, digestibility, immunity, stress resistance and gut morphology of juvenile amberjack *Seriola dumerili*. *Aquaculture*, **488**, 174-188.

Jeon, G. H., Kim, H. S., Myung, S. H., Cho, S. H. (2014) The effect of the dietary substituition of fishmeal with tuna by-product meal on growth, body composition, plasma chemistry and amino acid profiles of juvenile Korean rockfish (*Sebastes schlegeli*). *Aquaculture Nutrition*, **20**, 753-761.

Jiang, Y., Zhao, P. F., Lin, S. M., Tang, R. J., Chen, Y. J., Luo, L. (2018) Partial substitution of soybean meal with fermented soybean residue in diets for juvenile largemouth bass, *Micropterus salmoides*. *Aquaculture Nutrition*, **24**, 1213-122.

Kader, M. A., Koshio, S., Ishikawa, M., Yokoyama, S., Bulbul, M. (2010) Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. *Aquaculture*, **308**, 136-144.

Kotzamanis, Y., Kouroupakis, E., Illia, V., Haralabous, J., Papaioannou, N., Papanna, K., Richards, R., Gisbert, E. (2018) Effects of high-level fishmeal replacement by plant proteins supplemented with different levels of lysine on growth performance and incidence of systemic noninfectious granulomatosis in meagre (*Argyrosomus regius*). *Aquaculture Nutrtion*, **24**, 1738-1751.

Liao, M., Ren, T., He, L., Han, Y., Jiang, Z. (2015) Optimum dietary proportion of soybean meal with fish meal, and its effects on growth, digestibility, and digestive enzyme activity of juvenile sea cucumber *Apostichopus japonicas*. *Fisheries Science*, **81**, 915-922.

López, L. M., Flores-Ibarra, M., Bañuelos-Vargas, I., Galaviz, M. A., True, C. D. 2015. Effect of fishmeal replacement by soy protein concentrate with taurine supplementation

on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (Totoaba macdonaldi). Fish Physiology and Biochemistry. 41: 921-936.
Ma, Z., Hassan, M. M., Allais, L., He, T., Leterme, S., Ellis, A. V., McGraw, B., Qin, J. G. (2018) Replacement of fishmeal with commercial soybean meal and EnzoMeal in juvenile barramundi *Lates calcarifer*. *Aquaculture Research*, **49**, 3258-3269.
Masagounder, K., Hayward, R. S., Firman, J. D. (2014) Replacing fish meal with increasing levels of meat and bone meal, soybean meal and corn gluten meal, in diets of juvenile bluegill, *Lepomis macrochirus*. *Aquaculture Research*, **45**, 1202-1211.
Mattos, B. O., Bueno, G. W., Honczaryk, A., Pereira-Filho, M., Roubach, R. (2018) Crude protein levels in the diet of juvenile *Brycon amazonicus*. *Amazonian Journal of Agricultural and Environmental Sciences*, **61**, 1-6.
McGoogan, B. B., Gatlin, D. M. (1997) Effects of Replacing Fish Meal with Soybean Meal in Diets for Red Drum *Sciuenops ocellatus* and Potential for Palatability Enhancement. *Journal of the World Aquaculture Society*, **28**, 374-385.
Minjarez-Osorio, C., Castillo-Alvarado, S., Gatlin III, D. M., González-Félix, M. L., Perez-Velazquez, M., Rossi, W. R. (2016) Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study. *Aquaculture*, **453**, 122-129.
Monfared, A. L., Salati, A. P. 2013. Histomorphometric and biochemical studies on the liver of rainbow trout (Oncorhynchus mykiss) after exposure to sublethal concentrations of phenol. Toxicology and Industrial Health, 29 (9): 856-861.
Monzer, S., Nasser, N., Babikian, J., Saoud, P. (2017) Substitution of fish meal by soybean meal in diets for juvenile marbled spinefoot, *Siganus rivulatus*. *Journal of Applied Aquaculture*, **29**, 1-16.
Ngandzali, B. O., Zhou, F., Xiong, W., Shao, Q. J., Xu, J. Z. (2011) Effect of dietary replacement of fish meal by soy bean protein concentrate on growth performance and phosphorus discharging of juvenile black sea bream, *Acanthopagrus schlegelii*. *Aquaculture nutrition*, **17**, 526-535.
Nguyen, H. P., Khaoian, P., Fukada, H., Suzuki, N., Masumoto, T. (2015) Feeding fermented soybean meal diet supplemented with taurine to yellowtail *Seriola quinqueradiata* affects growth performance and lipid digestion. *Aquaculture Research*, **46**, 1101-1110.
Novriadi, R., Rhodes, M., Powell, M., Hanson, T., Davis, D. A. (2017) Effects of soybean meal replacement with fermented soybean meal on growth, serum biochemistry and morphological condition of liver and distal intestine of Florida pompano *Trachinotus carolinus*. *Aquaculture Nutrition*, **24**, 1066-1075.
Omnes, M. H., Goasduff, J. L., Delliou, H. L., Bayon, N. L., Quazuguel, P., Robin, J. H. (2017) Effects of dietary tannin on growth, feed utilization and digestibility,and carcass composition in juvenile European seabass (*Dicentrarchus labrax* L.). *Aquaculture Reports*, **6**, 21-27.
Peng, M., Xu, W., Ai, Q., Mai, K., Liufu, Z., Zhang, K. (2013) Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). *Aquaculture*, **392-395**, 51-58.
Peng, M., Xu, W., Mai, K., Zhou, H., Zhang, Y., Liufu, Z., Zhang, K., Ai, Q. (2014) Growth performance, lipid deposition and hepatic lipid metabolismo related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil. *Aquaculture*, **433**, 442-449.
Pizango-Paima, E. G., Pereira-Filho, M., Oliveira-Pereira, M. I. (2001) Composição corporal e alimentar do Matrinxã, *Brycon cephalus* (Günther, 1869), na Amazônia Central. *Acta Amazônica*, **31**, 509-520.
Ribeiro, L., Moura, J., Santos, M., Colen, R., Rodrigues, V., Bandarra, N., Soares, F., Ramalho, P., Barata, M., Moura, P., Pousão-Ferreira, P., Dias, J. (2015) Effect of

vegetable based diets on growth, intestinal morphology, activity of intestinal enzymes and haematological stress indicators in meagre (*Argyrosomus regius*). *Aquaculture*, **447**, 116-128.

Rossi Jr., W., Tomasso, J. R., Gatlin III, D. M. (2015) Performance of Cage-Raised, Overwintered Hybrid Striped Bass Fed Fish Meal- or Soybean-Based Diets. *North American Journal of Aquaculture*, **77**, 178-185.

Sahlmann, C., Gu, J., Kortner, T. M., Lein, I., Krogdahl, A., Bakke, A. M. (2015) Ontogeny of the Digestive System of Atlantic Salmon (*Salmo salar* L.) and Effects of Soybean Meal from Start-Feeding. *Plos One*, **10**, 1-23.

Sarker, M. S. A., Satoh, S., Kamata, K., Haga, Y., Yamamoto, Y. (2012) Partial replacement of fish meal with plant protein sources using organic acids to practical diets for juvenil yellowtail, *Seriola quinqueradiata*. *Aquaculture Nutrition*, **18**, 81-89.

Siddiqui, M. I., Khan, M. A., Siddiqui, M. I. (2014) Effect of soybean diet: Growth and conversion efficiencies of fingerling of stinging cat fish, *Heteropneustes fossilis* (Bloch). *Journal of King Saud University Science*, **26**, 83-87.

Song, Z., Li, H., Wang, J., Li, P., Sun, Y., Zhang, L. (2014) Effects of fishmeal replacement with soy protein hydrolysates on growth performance, blood biochemistry, gastrointestinal digestion and muscle composition of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture*, **426-427**, 96-102.

Torrecillas, S., Mompel, D., Caballero, M. J., Montero, D., Merrifield, D., Rodiles, A., Robaina, L., Zamorano, M. J., Karalazos, V., Kaushik, S., Izquierdo, M. (2017). Effect of fishmeal and fish oil replacement by vegetablemeals and oils on gut health of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **468**, 386-398.

Urán, P. A., Gonçalves, A. A., Taverne-Thiele, J. J., Schrama, J. W., Verreth, J. A. J., Rombout, J. H. W. M. (2008) Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish & Shellfish Immunology*, **25**, 751-760.

Venold, F. F., Penn, M. H., Krogdahl, A., Overturf, K. (2012) Severity of soybean meal induced distal intestinal inflammation, enterocyte proliferation rate, and fatty acid binding protein (Fabp2) level differ between strains of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **364-365**, 281-292.

Visentainer, J. V. (2012) Aspectos analíticos da resposta do detector de ionização em chama para ésteres de ácidos graxos em biodiesel e alimentos. *Química Nova*, **35**, 274-279.

Yaghoubi, M., Mozanzadeh, M. T., Marammazi, J. G., Safari, O., Gisbert, E. (2016) Dietary replacement of fish meal by soy products (soybean meal and isolated soy protein) in silvery-black porgy juveniles (*Sparidentex hasta*). *Aquaculture*, **464**, 50-59.

Yu, D. H., Gong, S. Y., Yuan, Y. C., Lin, Y. C. (2013) Effects of replacing fish meal with soybean meal on growth, body composition and digestive enzimes activities of juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture Nutrition*, **19**, 84-90.

Yuan, Y. C., Lin, Y. C., Yang, H. J., Gong, Y., Gong, S. Y., Yu, D. H. (2013) Evaluation of fermented soybean meal in the practical diets for juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture Nutrition*, **19**, 74-83.

Wang, Y., Wang, L., Zhang, C., Song, K. (2017) Effects of substituting fishmeal with soybean meal on growthperformance and intestinal morphology in orange-spotted grouper (*Epinephelus coioides*). *Aquaculture Reports*, **5**, 52-57.

Zhang, C., Rahimnejad, S., Wang, Y., Lu, K., Song, K., Wang, L., Mai, K. (2018) Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): Effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. *Aquaculture*, **483**, 173-182.

1. CONSIDERAÇÕES FINAIS

A matrinxã começa a ter destaque na produção nacional devido ao fato de ser uma espécie nativa, onívora, com carne apreciada pelos consumidores, com protocolo de reprodução estabelecido e produção eficiente, facilitada por sua fácil adaptação sà dietas comerciais. Porém, as dietas comerciais são compostas por proteínas de origem animal, principalmente a farinha de peixe. O custo oneroso, a falta de padronização, produção contína e a preocupação ambiental, fazem com que novas alternativas sejam estudadas para substituição das fontes proteicas originaria de peixes, tradicionamente utilizadas.

Em diversos aspectos do desempenho animal e qualidade do produto final pode-se observar que não houve diferença estatística entre parâmetros, como: rendimento de filé, taxa de crescimento específico, conversão alimentar aparente e taxa de eficiência proteica dos tratamentos com 50% de substituição de farinha de peixe por farelo de soja e o tratamento controle de farinha de peixe. Esses resultados demonstram que esta substituição parcial da proteína animal por proteína vegetal é possível para matrinxãs. Em estudos futuros poderia ser realizada a adição de palatabilizantes e atrativos, afim de tentar aumentar o consumo das dietas a base de farelo de soja, assim os tratamentos poderiam ter valores de ingestão mais parecidos.

Como ainda não está totalmente estabelecida a exigência proteica desta espécie, assim como a composição aminoacídica ideal para sua dieta é desconhecida. Logo, a composição aminoacídica das rações e filés poderiam ser analisadas, para se conhecer o quanto a dieta pode influenciar na composição e qualidade do produto final nesta espécie.

REFERÊNCIAS

ALEXANDER, R. The orientation of muscle in the myomers of fishes. **Journal of the Marine Biological Association of the United Kingdom**, Cambridge, v. 49, n. 2, p. 263-290, 1969.

ALMEIDA, N. M.; FRANCO, M. R. B. Fatty acid composition of total lipids, neutral lipids and phospholipids in wild and farmed matrinxã (*Brycon cephalus*) in the Brazilian Amazon área. **Journal of the Science of Food and Agriculture**, London, v. 87, p. 2596-2603, 2007.

ALMEIDA, N. M.; JANZANTTI, N. S.; FRANCO, M. R. B. Determination of essential fatty acids in captured and farmed matrinxã (*Brycon cephalus*) from the Brazilian Amazon Area. **Journal of the American Oil Chemists' Society**, Champaign, v. 86, p. 717-722, 2009.

ALMEIDA, F.L.A. **Expressão gênica de fatores que controlam o crescimento muscular do pacu (Piaractus mesopotamicus).** 2011. 127 f. Tese de doutorado (Biologia celular e estrutural) - Instituto de Biologia, Universidade Estadual de Campinas, Campinas, 2011.

ARBELÁEZ-ROJAS, G. A.; FRACALOSSI, D. M.; FIM, J. D. Composição corporal de Tambaqui, *Colossoma macropomum*, e Matrinxã, *Brycon cephalus*, em sistemas de cultivo intensivo, em igarapé, e semi-intensivo, em viveiros. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 31, n. 3, p. 1059-1069, 2002.

ASADUZZAMAN, M. *et al*. Dietary supplementation of inosine monophosphate promotes cellular growth of muscle and upregulates growth-related gene expression in Nile tilapia *Oreochromis niloticus*. **Aquaculture**, Amsterdam, v. 468, p. 297-306, 2017.

AZARM, H. M.; LEE, S. M. Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. **Aquaculture Research**, Oxford, v. 45, n. 6, p. 994-1003, 2014.

BANSEMER, M. S. *et al*. J. The effect of dietary soybean meal and soy protein concentrate on the intestinal mucus layer and development of subacute enteritites in Yellowtail Kingfish (*Seriola lalandi*) at suboptimal water temperature. **Aquaculture Nutrition**, Oxford, v. 21, p. 300-310, 2015.

BANKS, W. J. (ed.). **Histologia veterinária aplicada**. São Paulo: Manole, 1991. 592 p.

BARNES, M. E. *et al*. Rainbow trout rearing performance, intestinal morphology, and immune response after long-term feeding of high levels of

fermented soybean meal. **North American Journal of Aquaculture**, Bethesda, v. 76, n. 4, p. 333-345, 2014.

BATISTA, G. M. *et al*. Alterações bioquímicas post-mortem de Matrinxã *Brycon cephalus* (GÜNTHER, 1869) procedente da piscicultura, mantido em gelo. **Food Science and Technology**. Campinas, v. 24, n. 4, p. 573-581, 2004.

BOSCOLO, W. R. *et al*. Rações orgânicas suplementadas com farinha de resíduos de peixe para juvenis da tilápia do Nilo (*Oreochromis niloticus*). **Revista Ciência Agronômica**, Fortaleza, v. 41, n. 4, p. 686-692, 2010.

BRASIL. Ministério da Pesca e Aquicultura. **Boletim estatístico da pesca e aquicultura:** 2011. Brasília: Ministério da Pesca e Aquicultura, 2011. 60 p.

BRINKER, A.; REITER, R. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality. **Aquaculture**, Amsterdam, v. 310, p. 350–360, 2011.

CANEVESI, F. C. *et al*. Desenvolvimento da matrinxã *Brycon amazonicus* (Spix & Agassiz, 1829) em fase de alevino com diferentes níveis de proteína bruta na ração. **Revista de Ciências Agroambientais**, Alta Floresta, v. 12, n. 2, p. 157-160, 2014.

CARTER, C. G. *et al*. Protein synthesis in gilthead sea bream: response to partial fishmeal replacement. **British Journal of Nutrition**, Wallingford, v. 108, n. 12, p. 2190–2197, 2012.

CARVALHO, E. G.; URBINATI, E. C. Crescimento, desenvolvimento gonadal e composição muscular de matrinxãs (*Brycon cephalus*) submetidos à restrição alimentar e realimentação durante um ano. **Ciência Rural**, Santa Maria, v. 35, v. 4, p. 897-902, 2005.

CHU, Z. J. *et al*. Partial replacement of fish meal by soybean meal with or without methionine and phytase supplement in diets for juvenile Chinese sucker, *Myxocyprinus asiaticus*. **Aquaculture Nutrition**, Oxford, v. 22, n. 5, p. 989-996, 2016.

COLLINS, S. A. *et al*. Effect of plant protein sources on growth rate in salmonids: meta-analysis of dietary inclusion of soybean, pea and canola/rapeseed meals and protein concentrates. **Aquaculture**, Amsterdam, v. 400/401, p. 85-100, 2013.

COWEY, C. B. *et al*. Studies on the nutrition of marine flatfish. Growth of the plaice *(Pleuronectes platessa)* ondiets containing proteins derived from plants and other sources. **Marine Biology**, Berlin, v. 10, n. 2, p. 145-153, 1971.

DABROWSKI, K.; KOZAK, B. The use of fishmeal and soybean meal as a protein in the diet of grass carp. **Aquaculture**, Amsterdam, v. 18, p. 107-114, 1979.

DABROWSKI, K. *et al*. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, feed utilization and proteolytic enzyme activities in rainbow trout (*Salmo gardneri).* **Aquaculture**, Amsterdam, v. 77, p. 29–49, 1989.

DAL PAI-SILVA, M.; DAL PAI, V.; CARVALHO, R.F. Célula muscular estriada esquelética. *In*: CARVALHO, H.F.; COLLARES-BUZATO, C.B (ed.). **Células:** uma abordagem multidisciplinar. São Paulo: Manole. 2005, p. 83-94.

EMBRAPA - EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. **Soja em números (safra 2017/2018)**. Londrina: Embrapa Soja, 2018. Disponível em: https://www.embrapa.br/en/soja/cultivos/soja1/dados-economicos. Acesso em: 9 Jan. 2018.

ESPIRITO SANTO, N. G. *et al*. Replacement of animal protein sources by soy protein concentrate for juvenile Nile tilapia. **Boletim Instituto de Pesca**, São Paulo, v. 41, p. 707-717, 2015.

FERREIRA, M. S. *et al*. Efeito da quantidade de proteína na dieta e treinamento físico sobre parâmetros fisiológicos e zootécnicos de matrinchã (*Brycon amazonicus*, Günther 1869). **Acta Amazonica**, Manaus, v. 43, n. 4, p. 439-446, 2013.

FRANCIS, G.; MAKKAR, H P. S.; BECKER, K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. **Aquaculture**, Amsterdam, n. 199, n. 3/4, p. 197-227, 2001.

FRANCO, M. L. R. S. *et al*. Effects of hot and cold smoking processes on organoleptic properties, yield and composition of matrinxa fillet. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 39, n. 4, p. 695-700, 2010.

FURUYA, W. M. (ed.). **Tabelas brasileiras para a nutrição de tilápias**. Toledo, PR: GFM, 2010. 100 p.

FURUYA, W. M. *et al*. Coeficientes de digestibilidade aparente e valores de aminoácidos digestíveis de alguns ingredientes para tilápia do Nilo (*Oreochromis niloticus*). **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 30, p. 1143-1149, 2001.

GU, M.; BAI, N.; ZHANG, Y.; KROGDAHL, A. Soybean meal induces enteritis in turbot Scophthalmus maximus at high supplementation levels. **Aquaculture**, Amsterdam, v. 464, p. 286-295, 2016.

GU, M. *et al*. Effects of diet supplementation of soya-saponins, isoflavones and phytosterols on Atlantic salmon (*Salmo salar*, L) fry fed from start-feeding. **Aquaculture Nutrition**, Oxford, v. 21, n. 5, p. 604-613, 2015.

GUIMARÃES, I. G. *et al*. Nutrient digestibility of cereal grain products and by-products in extruded diets for Nile tilapia. **Journal of the World Aquaculture Society**, Baton Rouge, v. 39, n. 6, p. 781-789, 2008.

HARTVIKSEN, M. *et al*. Alternative dietary protein sources for Atlantic salmon (*Salmo salar*) effect on intestinal microbiota, intestinal and liver histology and growth. **Aquaculture Nutrition**, Oxford, v. 20, n. 4, p. 381-398, 2014.

HONORATO, C A. *et al*. Histologia do fígado de tilápia do Nilo (*Oreochromis niloticus*) alimentados com dietas contendo silagem biológica de pescado. **Pesquisa Veterinária Brasileira**, Rio de Janeiro, v. 34, n. 1, p. 64-68, 2014.

IZEL, A. C. U. *et al*. Avaliação de níveis protéicos para a nutrição de juvenis de matrinxã (*Brycon cephalus*). **Acta Amazonica**, Manaus, v. 34, n. 2, p. 179-184, 2004.

JIRSA, D. *et al*. Alternative protein blends as a replacement for fish meal in diets for white seabass, *Atractoscion nobilis*. **Aquaculture Nutrition**, Oxford, v. 21, n. 6, p. 861-867, 2015.

JUNQUEIRA, L. C. U.; CARNEIRO, J. **Histologia básica**. 12. ed. Rio de Janeiro: Guanabara Koogan, 2013. 538 p.

KOKOU, F. *et al*. Effects of Fish Meal Replacement by a Soybean Protein on Growth, Histology, Selected Immune and Oxidative Status Markers of Gilthead Sea Bream, *Sparus aurata*. **Journal of the World Aquaculture Society**, Baton Rouge, v. 46, n. 2, p. 115-128, 2015.

KOTZAMANIS, Y. *et al*. Effects of high-level fishmeal replacement by plant proteins supplemented with different levels of lysine on growth performance and incidence of systemic noninfectious granulomatosis in meagre (*Argyrosomus regius*). **Aquaculture Nutrition**, Oxford, v. 24, n. 6, p. 1738-1751, 2018.

KROGDAHL, A. *et al*. Soya saponins induce enteritis in Atlantic salmon (*Salmo salar L*.). **Journal of Agricultural and Food Chemistry**, Washington, DC, v. 63, n. 15, p. 887-902, 2015.

GADELHA, E. S.; ARAÚJO, J. C. Criação de Matrinxã em cativeiro. **Publicações em Medicina Veterinária e Zootecnia (PUBVET)**, Londrina, v. 7, n. 5, p. 312-419, 2013.

GLENCROSS, B. D. *et al*. A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*)

or rainbow trout (*Oncorhynchus mykiss*). **Aquaculture**, Amsterdam, v. 237, n. 1/4, p. 333-346, 2004.

LEÓN, J. G. *et al*. Efecto crioprotector del sorbitol en la microestructura del músculo del yamú (*Brycon amazonicus)* durante su almacenamiento a 2 y -18 °C. **Revista Facultad Nacional de Agronomía Medellín**, [Bogotá], v. 72, n. 1, p. 8763-8774, 2018.

LIU, H. *et al*. Effect of subtituition of dietary fishmeal by soya bean meal on diferente sizes of gibel carp (*Carassius auratus gibelio*): nutriente digestibility, growth performance, body composition and morphometry. **Aquaculture Nutrition**, Oxford, v. 22, p. 142-157, 2016.

LIU, H. *et al*. Effect of substituition of dietary fish meal by soybean meal on diferente sizes of gibel carp (*Carassius auratus gibelio):* digestive enzyme gene expressions and activities, and intestinal and hepatic histology. **Aquaculture Nutrition**, Oxford, v. 23, p. 129-147, 2017.

LÓPEZ, L. M. *et al*. Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (*Totoaba macdonaldi*). **Fish Physiology and Biochemistry**, Amsterdam, v. 41, n. 4, p. 921-936, 2015.

MA, Z. *et al*. Replacement of fishmeal with commercial soybean meal and EnzoMeal in juvenile barramundi *Lates calcarifer*. **Aquaculture Research,** Oxford, v. 49, n. 10, p. 3258-3269, 2018.

MACEDO-VIEGAS, E. M. *et al*. Efeito das classes de peso sobre a composição corporal e o rendimento de processamento de matrinxã (*Brycon cephalus*). **Acta Scientiarum. Animal Sciences**, Maringá, v. 22, n. 3, p. 725-728, 2000.

MATTOS, B. O. *et al*. Níveis de proteína bruta na dieta de juvenis de matrinxã (*Brycon amazonicus*). **Amazonian Journal of Agricultural and Environmental Sciences**, Belém, v. 61, p. 1-6, 2018.

MICHELATO, A. *et al*. Dietary lysine requirement to enhance muscle development and fillet yield of finishing Nile tilapia. **Aquaculture**, Amsterdam, v. 457, p. 124-30, 2016.

MOREIRA, A. B. *et al*. Composição de ácidos graxos e teor de lipídeos em cabeças de peixes: Matrinxã (*B. cephalus*), Piraputanga (*B. microlepsis*) e Piracanjuba (*B. orbygnyanus*), criados em diferentes ambientes. **Ciência e Tecnologia de Alimentos**, Campinas, v. 23, n. 2, p. 179-183, 2003.

NRC - NATIONAL RESEARCH COUNCIL. **Nutrient requirements of fish and shrimp**. Washington DC: The National Academies Press, 2011. 376 p.

OGAWA, M.; MAIA, E. L. **Manual de pesca**. São Paulo: Livraria Varela, 1999. v. 1 - Ciência e Tecnologia do Pescado.

PEIXE BR. **Anuário PEIXEBR da piscicultura 2018**. São Paulo: Associação Brasileira da Piscicultura, [2018]. 71 p. Disponível em: https://www.peixebr.com.br/anuario2018/. Acesso em: 8 jan. 2018.

PEIXE BR. **Anuário PEIXEBR da piscicultura 2019**. São Paulo: Associação Brasileira da Piscicultura, [2019]. 148 p. Disponível em: https://www.peixebr.com.br/anuario-peixe-br-da-piscicultura-2019/. Acesso em: 28 fev. 2019.

PEZZATO, L. E. *et al*. Nutrição de peixes. *In*: CYRINO, J. E. P. *et al*. **Tópicos especiais em piscicultura de água doce tropical intensiva**. São Paulo: Tecart, 2004. p. 75-169.

PIZANGO-PAIMA, E. G.; PEREIRA-FILHO, M.; OLIVEIRA-PEREIRA, M. I. Composição corporal e alimentar do matrinxã, *Brycon cephalus* (*Gunther, 1869*), na Amazônia central. **Acta Amazonica**, Manaus, v. 31, n. 3, p. 509-520, 2001.

RIBEIRO, A. S. *et al*. Anesthetic properties of Ocimum gratissimum essential oil for juvenile matrinxã. **Acta Scientiarum. Animal Sciences**, Maringá, v. 38, n. 1, p. 1-7, 2016.

RODRIGUES, A. P. O. *et al*. **Piscicultura de água doce:** multiplicando conhecimentos. Brasília, DF: Embrapa, 2013. 440 p.

ROTTA, M. A. **Aspectos gerais da fisiologia e estrutura do sistema digestivo dos peixes relacionados à piscicultura**. Corumbá: Embrapa Pantanal, 2003. 48 p. (Documentos, 53).

SACCOL, E. M. H. *et al*. Oxidative and biochemical responses in *Brycon amazonicus* anesthetized and sedated with Myrcia sylvatica (G. Mey.) DC. and Curcuma longa L. essential oils. **Veterinary Anaesthesia and Analgesia**, Oxford, v. 44, n. 3, p. 555-566, 2017.

SANTIAGO, C. B.; LOVELL, R. T. Aminoacid requeriments for growth of Nile tilápia. **The Journal of Nutrition**, Bethesda, v. 118, n. 12, p. 1540-1546, 1988.

SHIAU, S. Y. *et al*. Replacement of fishmeal with soybean meal in male tilapia (*Oreochromis niloticus* X *O. aureus*) fingerling diets at a suboptimal protein level. **Journal of the World Aquaculture Society**, Baton Rouge, v. 20, n. 4, p. 230– 235, 1989.

SILVA-CARRILLO, Y. *et al*. The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in

juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). **Aquaculture**, Amsterdam, v. 364–365, p. 180–185, 2012.

SIMÕES, M.R. *et al*. Composição físico-química, microbiológica e rendimento do filé de tilápia tailandesa (Oreochromis niloticus). **Ciência e Tecnologia de Alimentos**, Campinas, v. 27, n. 3, p. 608-613, 2007.

SUÁREZ-MARECHA, H. *et al*. Efeito do resfriamento sobre a textura post-mortem da carne do peixe matrinxã *Brycon cephalus*. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 59, p. 1067-1073, 2007.

TACON, A. G. J.; METIAN, M. Fish matters: importance of aquatic foods in human nutrition and global food supply. **Reviews in Fisheries Science**, Boca Raton, v. 21, n. 1, p. 22-38, 2013.

TEIXEIRA, E. A. *et al*. Composição corporal e exigências nutricionais de aminoácidos para alevinos de tilápia (*Oreochromis sp*.). **Revista Brasileira de Saúde e Produção Animal**, Salvador, v. 9, p. 239-246, 2008.

VAN DEN INGH, T. S. G. A. M. *et al*. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. **Aquaculture**, Amsterdam, v. 94, n. 4, p. 297-305, 1991.

VARGAS, S. C. *et al*. Evaluation of diferente stunning methods on aspects of animal welfare and meat quality of matrinxã (*Brycon cephalus*). **Italian Journal of Food Science**, Pinerolo, v. 25, n. 3, p. 255-262, 2013.

VENTURINI, F. P. *et al*. A multi-biomarker approach to lambda-cyhalothrin effects on the freshwater teleost matrinxa Brycon amazonicus: single-pulse exposure and recovery. **Fish Physiology and Biochemistry**, Amsterdam, v. 45, n. 1, p. 341-353, 2019.

WANG, C. *et al*. Responses to fishmeal and soybean meal-based diets by three kinds of larval carps of different food habits. **Aquaculture Nutrition**, Oxford, v. 21, n. 5, p. 552-568, 2015.

YU, D. *et al*. Effects of replacing fish meal with soybean meal on growth, body composition and digestive enzyme activities of juvenile Chinese sucker, *Myxocyprinus asiaticus*. **Aquaculture Nutrition**, Oxford, v. 19, n. 1, p. 84–90, 2013.

ZANUZZO, F. S. *et al*. A description of liver and blood changes in matrinxã (*Brycon amazonicus)* during induced spawning. **Aquaculture**, Amsterdam, v. 495, p. 345-350, 2018.

ZHAO, H. *et al*. Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis*

niloticus GIFT strain) less than 2 g. **Aquaculture Nutrition**, Oxford, v. 16, n. 6, p. 648–653, 2010.

APÊNDICES

APÊNDICE 1 – Normas utilizadas para a preparação do Capítulo 2.

Author Guidelines: North American Journal of Aquaculture.

Editorial Policy

We encourage the submission of original papers on all aspects of aquaculture, including broodstock selection and spawning, nutrition and feeding, health and water quality, facilities and production technology, and the management of ponds, pens, and raceways. We will consider papers dealing with ways to improve the husbandry of any aquatic species—marine or freshwater, vertebrate or invertebrate—raised for commercial, scientific, recreational, enhancement, or restoration purposes that may be of interest to practitioners in North America.

Papers concerning fisheries science per se should be submitted to the American Fisheries Society's (AFS) sister publication Transactions of the American Fisheries Society; those dealing with management should be submitted to the North American Journal of Fisheries Management; those dealing with the health of fish and other aquatic organisms should be submitted to the Journal of Aquatic Animal Health; and those with a focus on marine and estuarine species and habitats should be submitted to Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science.

Authors are also cautioned not to republish original data without full attribution and explicit permission; see "Dual Publication of Scientific Information" in Transactions of the American Fisheries Society 110:573–574, 1981.

Authors must also confirm that all of their research meets the ethical guidelines and legal requirements of the country in which it was performed. For investigators in the United States, AFS has developed the document "Guidelines for the Use of Fishes in Research," which addresses both field and laboratory research with fish. A free version of this document is available for viewing and/or downloading at http://fisheries.org/policy-media/science-guidelines/guidelines-for-the-use-of-fishes-in-research/.

Please note that this journal uses CrossRef Similarity Check (Powered by iThenticate) software to screen papers for unoriginal material. By submitting your paper, you are agreeing to any necessary originality checks your paper may have to undergo during the peer review and production processes.

Manuscript Submission and Review

Manuscript Categories

Manuscripts may be submitted in any of the following categories: (1) Articles are full reports of substantial, controlled research and critical reviews of such research; they will be judged on their scientific merit and relevance to practical aquaculture. Critical reviews of timely topics will also be considered in this category. (2) Communications are shorter papers reporting nonreplicated experiments, the exploratory culture of new species or life stages, the testing of new techniques, and so forth; they will be judged primarily on the biological insights that they provide as well as their practical contribution to aquaculture. (3)Technical Notes are short papers that deal with operational improvements, describe new techniques or equipment, or present unusual observations; they will judged on their practical contribution and general interest. (4)Comments are critiques of papers published by this journal (responses to which will be invited from the original authors), brief presentations of additional observations or data related to previously published papers, or short discussions of technical issues of interest to the aquacultural community.

Papers that are judged to be especially topical, important, and/or likely to be of wide interest may be "featured," that is, given special treatment that includes accelerated production; waiver of page charges and publication fees; waiver of the fee for printing figures in color (if applicable); free online access for two months; and special promotion. Authors who wish to have their papers featured should indicate that at the time of

submission, briefly explaining why their papers merit such treatment. Decisions about featuring papers rest with the editors.

Submission Procedures

Manuscripts and associated correspondence should be submitted at the journal's online submission and tracking site, http://mc.manuscriptcentral.com/naja (this site may also be accessed through the Publications section at the American Fisheries Society's Web site, www.fisheries.org). Detailed instructions, including acceptable file formats, are available at the site.

Although the submission site permits authors to include a cover letter, such letters are generally not necessary; they should be included only when they contain information that cannot easily be incorporated into the standard submission form.

Review Process

Submitted papers will be critically reviewed by at least two experts in the relevant discipline(s) and evaluated by one of the journal's editors. A manuscript may be returned to its author without review if it is judged to be of poor quality or inappropriate for this journal.

All submissions are electronically screened for the inappropriate use of material from previously published sources. In submitting a paper, you are stipulating that, except where explicitly indicated otherwise, all of the statements, data, and other elements reflect your own work and not that of others. All allusions to the work of others should be properly cited; exact quotations from other sources should be in quotation marks. Authors are also cautioned not to repeat long passages from their own publications. Failure to follow these requirements may result in rejection of the paper and, in extreme cases, restrictions on publishing in this journal.

Authors have the option of not having their names revealed to the reviewers (to facilitate the selection of reviewers, however, the editor and associate editor will always be aware of the authors' identities). If authors wish to exercise this option, they should (1) check the appropriate block on the submission page, (2) put the title page in a separate file that can be excluded from the manuscript file that the reviewers receive, and (3) remove from their manuscript any other information that may reveal their identities.

Review of manuscripts relies on volunteers. We strive to get decisions to authors in 9–12 weeks. If revisions are requested, authors should make them promptly, normally within 30 days of receiving the editor's decision (short extensions will be allowed if there are justifiable delays). If a revision is not received within the allowed time, the paper will be considered withdrawn; late revisions will be treated as new submissions and may have to go through the review process again.

Revisions should be accompanied by detailed, point-by-point responses to the reviewers' and editors' comments. These responses should be put in a separate file designated "Response to Decision Letter" rather than in the cover letter. This file will automatically be included in a PDF containing the revised text, tables, figures, and supplementary material (if any) that is available to the reviewers and associate editor as well as the editor; otherwise, only the editor will have access to the responses.

Rejected papers.—If a paper is rejected, authors should not submit a revised version unless the editor has specifically invited them to do so. However, authors may request reconsideration of rejected papers when they believe that the review process was flawed in some way (e.g., suspicion of bias or inadequate understanding of the paper on the part of the reviewers and/or the associate editor). Such requests should be directed to the editor or AFS staff and should include a detailed statement as to why the paper should be reconsidered along with supporting material as necessary. They should also indicate the author's preferred remedy, which may range from a simple reexamination of the reviews and recommendation by the original editor to a completely fresh review by a new editor, associate editor, and reviewers. Decisions about requests for reconsideration

will be made by the director of publications in consultation with the original editor and/or the editor-in-chief. Every effort will be made to give authors a fair hearing.

Publication Charges

Traditional publication.—Publication charges are US$100 per printed page (or $150 per published page for special section articles) and will be billed when the paper is in proof. Full and partial subsidies are available to voting members of the American Fisheries Society who certify that grant or agency funds are not available. Manuscript reviews are not affected by requests for subsidies; however, at least one author must be an AFS member by the time that the paper is published. Every paper published in the journal is subject to a $30 fee to offset processing costs. If you choose to include color figures in the print edition, please note the fee is $500 per figure.

Open access.—Authors may make their papers open access by paying a fee of $3,000 (+ valued-added tax [VAT]). Waivers will not be granted for open access papers. Page charge and color figure fees still apply.

Reprints.—Authors will get free access to their article on Wiley's Author Services. They will be able to share their article for free with up to 10 other people. Authors may also purchase reprints of their articles from the printer when they receive their proofs.

Manuscript Preparation

Components

A typical manuscript will have the following components:

Title page.—The title page should give the title of the paper and the name(s) and complete mailing address(es) of the author(s). In addition to accurately reflecting the content of the paper, the title should be short (preferably no more than 12 words) and to the point. A suggested running head (shortened version of the title) should also be included on the title page. Keywords are not used in this journal, however, and so should not be included.

Abstract.—Articles and communications require abstracts; comments do not. The abstract should consist of one paragraph (up to 300 words for an article and up to 200 words for a communication) that concisely states why and (generally) how the study was done as well as what the results were and what they mean. Because abstracts tend to be more widely read than complete papers, authors should take care to make them comprehensive, clear, and interesting. It should not simply outline the contents (e.g., avoid statements to the effect that such-and-such is presented) or present the methods in detail. Citations and footnotes are not allowed in abstracts, and abbreviations should be used sparingly. Detailed statistical results (e.g., P-values) should be reserved for the main text.

Introduction.—The introduction should provide a context for the work to be reported, particularly its purpose and importance. In doing so, it should present at least a summary review of previous literature on the subject.

Methods.—Descriptions of the methods employed in the study should be detailed enough to enable readers to repeat it. Previously published descriptions may be cited in lieu of presenting complete new ones provided that the sources are readily available (in general, avoid citations to theses, dissertations, agency reports, and similar sources in this instance). If more than one method was used or a particular method entails a series of major steps, present each method or step in a separate subsection. Appropriate tables and figures can reduce the need for detailed verbal descriptions of methods. Papers focusing entirely on techniques or models do not require a separate section on methods.

Results.—As a rule, it is preferable to present detailed results in tables and/or figures and to devote the text to summary statements and analyses. Display data in tables if numerical precision is important, in figures if trends are paramount. Although the presentation of a large amount of raw data is generally not meaningful, data should not be refined to the point that the reader cannot verify the analyses or use the information

for other purposes. In presenting the results of statistical tests, report the type of test, the test statistic, the degrees of freedom, and the significance level (P-value). Although the value 0.05 is commonly used as the threshold in hypothesis testing, we have no specific requirements in this area; in the interest of providing useful information, authors should report all P-values. It is very important that statistical designs and models be appropriate for the studies in which they are used; we encourage authors to have a statistician review their work before submitting a paper for publication. Lastly, statistical results should be presented in biologically meaningful terms rather than in purely statistical jargon.

Discussion.—The merits of a paper can be greatly enhanced by a good discussion. In it authors should indicate the significance of their research, how it relates to current knowledge, and any avenues that it suggests for further research. Informed speculation is acceptable as long as it is clearly identified as such. Authors should avoid merely restating their results and/or (re)summarizing the literature.

Acknowledgments.—In this section authors may acknowledge the sources of their funding and thank those who contributed directly to the project or the preparation of the manuscript. Dedications and acknowledgment of emotional support from family and friends are not appropriate. If all authors are employees of the U.S. Government, this section should state that the mention of specific products does not constitute endorsement by their agency.

References.—References should be selected with a view to relevance and availability, with preference given to peer-reviewed publications that are widely available. Internal reports, papers presented at conferences, articles in preparation, and so forth should be treated as unpublished and cited like personal communications (i.e., parenthetically in the text alone). Authors should obtain written permission to cite such material. Common reference formats are given below; a more complete list is given in chapter 8 of the AFS style guide, which is available at the AFS Web site (https://fisheries.org/books-journals/writing-tools/style-guide/) as well as the manuscript submission site.

Footnotes.—Footnotes should be kept to a minimum. Typically, they are used to report changes of address for authors, identify additional sources of data, or explain technical nomenclature (e.g., ages of anadromous fish and structures of fatty acids).

Tables.—In general, tables should be designed to present related information as simply and directly as possible. A good rule of thumb is to establish the point(s) that the table is intended to make, then to select the information required to do that and determine the most logical order in which to present it. Detailed guidelines for the preparation of tables can be found in chapter 12 of the AFS style guide, but a few of the more important ones may be mentioned here:

1. We prefer to print tables in “portrait” orientation but will allow ones in “landscape” orientation as long as they take up no more than two pages.
2. Tables that are too long or too wide to fit on one page can be carried over to a facing page, but authors should try to avoid creating tables that span more than two pages. In general, very large tables should appear as supplements in the online version of the article only.
3. Tables should contain only three horizontal rules (lines)—one before the column headings, one after those headings, and one at the bottom of the table—and no vertical rules.
4. Captions no longer need to be detailed enough that tables can be understood apart from the text, but they should provide enough information that readers can easily perceive the tables’ purpose and structure (if there is more than one table with the same general structure, the captions to the latter ones can be shortened by referring the reader to the first such table for details). Captions should not merely list the contents of tables in a mechanical way.

5. There should be only one set of column headings. If the information to be presented seems to require more than that, the table should be redesigned (e.g., by switching the rows and columns) or split into two or more tables.
6. Bold, centered headings may be used within the body of the table to distinguish different types of data as long as they do not conflict with the column headings.
7. Only the first letter of a row or column heading should be capitalized (along with words or symbols that would be capitalized in ordinary text).
8. The data within the body of the table should not be crowded; if need be, blank rows can be inserted to separate data into logical groups or provide guides for the eye.
9. Significant differences should be indicated by lowercase letters, beginning with the letter “z” (“z” may mark either the highest or the lowest value[s], but subsequent letters have to follow suit); in most cases, there should be no omissions in the sequence of the letters. The letters should be set on the same lines as the values to which they pertain (not as superscripts) and be separated from those values by single spaces.
10. Values less than 1.00 should be preceded by zeroes (e.g., 0.78).
11. Values need not be reported to all significant digits if a lesser number of digits conveys the information in a meaningful way.
12. Footnotes should be indicated by superscripted lowercase letters, beginning with the letter “a”; the letters may appear in the row and column headings as well as the body of the table but not in the caption. The footnotes per se should be listed on separate lines at the bottom of the table.

Figure captions.—Figure captions should be given in a separate list; they may also be given with the figures themselves, but this is not required. Figure captions should follow the same general rules as those for tables. To the extent possible, however, panel descriptions, (full) variable names, units of measure, legends, and so forth should be included in the figure itself rather than in the caption; in no case should they be given in both places. Different panels may be designated “A,” “B,” and so forth, but it is preferable to give them substantive labels (e.g., “Treatment” and “Control”).
Figures.—Figures include visual materials such as graphs, maps, diagrams, and photographs. Figures have proved to be one of the most troublesome aspects of the publishing process. As the compositor has only limited ability to modify figures, they frequently have to be sent back to the authors for correction.
At the most fundamental level, figure design should follow certain commonsense principles: figures should be as simple and straightforward as possible; have a high enough resolution to be easily readable (300 dpi or more); and be consistent in the use of lettering, line widths, and other graphic elements. In addition, they need to conform to AFS style. It is particularly important to remember that most figures will be reduced by up to 50% when printed and thus need to be designed with this in mind. We recommend that authors use a copier to reduce each figure to the width of one or two printed columns (3.50 and 7.25 inches, respectively), depending on the dimensions of the particular figure, and verify that all elements are still legible. The following are particularly problematical: bold type (which tends to blur), italic type (which tends to become less visible), dashed lines (which tend to appear continuous) and dotted lines (which tend to disappear entirely). Additional guidelines for the preparation of figures may be found in the AFS style guide.
In the print version of the journal, all figures will be reproduced in black and white unless authors have made specific arrangements with the publisher to cover the extra costs of color printing. In the online version, however, color figures will be reproduced in color at no additional charge. Note that the availability of an acceptable version in color does not obviate the need for a legible version in black and white and that in some cases there may be no alternative to using color. Because color printing is expensive, authors are advised not to use color to distinguish phenomena when other means (different shading,

symbols, and so forth) are adequate. If color has to be used, avoid using similar colors or shades that may be difficult for readers to distinguish. Also, in deference to readers with color blindness, avoid using red and green in the same figure.
Digital files in EPS and TIFF formats are preferred; figures should be submitted as separate files rather than being imbedded in text files.
Call-outs.—Call-outs are in-text references to tables, figures, and supplementary material (appendices and supplements [discussed below]), e.g., "(Table 1)." All tables and figures in the main article should be called out at appropriate places; appendices and supplements may be called out either individually or generally, depending on their purpose and how closely related to the article they are. Call-outs to tables and figures should be in strict numerical order. Call-outs need not be repeated each time a result shown in a table or figure is mentioned as long as it is clear which table or figure contains it.
Mathematical and statistical expressions.—Chapter 4 of the AFS style guide covers the treatment of these expressions in detail, but a few general points may be mentioned here:

1. Symbols representing variables and parameters should be italicized if they consist of single letters in the Latin alphabet (e.g., K and F). All other symbols except Greek letters may be italicized or not, provided that the treatment is consistent (e.g., CPUE or CPUE); Greek letters should never be italicized.
2. Natural logarithms may be expressed as loge or ln; logarithms with other bases should identify the base (e.g., log10).
3. Long equations should be "broken" at logical points, normally after an operator such as a plus or minus sign.
4. Definitions of variables and parameters may be run into the text if only a few such terms are involved. If there are a number of them or they are used in more than one equation, a list is preferable (see section 4.8 of the AFS style guide).
5. Avoid the expressions "the mean length was 45.2 ± 3.84 mm" and "the mean (±SD) length was 45.2 ± 3.84 mm" because they are at best awkward and at worst inaccurate. Use the expressions "the mean ± SD length was 45.2 ± 3.84 mm" or "the mean length was 45.2 mm (SD, 3.84)" instead.

Appendices and supplements.—In addition to the standard elements of a paper, authors may submit certain supplementary material, such as additional data or results, the derivations of equations, computer code, and so forth. For publication purposes, such material will be treated either as an appendix (which will appear with the article in both the print and online versions) or as a supplement (which will appear only in the online version). Of course, all material that is essential to understanding an article should be included in the article itself. Closely related material that will be of interest to a large number of readers may be placed in an appendix. Other material may be made available through a supplement if the editors deem it important enough for readers to have ready access to. In terms of format, appendices should be regarded as extensions of articles and thus follow AFS style strictly. Supplements, by contrast, may be in any format that is suitable for their contents; however, (1) there should be consistency between the symbols, abbreviations, and so forth used in the supplement and those used in the article and (2) either the title of the supplement or the first paragraph should make clear how it relates to the article.
Style and Format
Published articles represent the culmination of research efforts, often lengthy and highly sophisticated ones. To do those efforts justice, however, the articles must be well written; poorly written articles not only place an unnecessary burden on readers, they also cast doubt on the quality of the research itself. The introduction to the AFS style guide should

be a particularly valuable resource in this regard; in a few pages, it identifies the errors in composition mostly commonly encountered in the papers submitted to AFS journals and shows how to correct them. We also encourage authors to have other fisheries professionals critique their initial drafts with respect to presentation as well as substance. Authors whose native language is not English should make a point of having English speakers review their manuscripts before submission; free assistance is available from the International Fisheries Section of AFS (http://bit.ly/1UhIdQz).

In writing for AFS journals, authors are also expected to follow certain style conventions pertaining to capitalization, spelling, punctuation, mathematical expressions, technical terms, and so forth. For instance, we require that the letter P (indicating the degree of statistical significance) be capitalized as well as italicized, whereas some journals require that it be lowercased. Although some of the more important style conventions are noted below, all of them are discussed in detail in the AFS style guide. Authors would be well advised to become familiar with the main elements of AFS style and to consult the guide frequently in preparing their manuscripts.

Resources for authors.—As suggested above, the principal resource on matters of style is the AFS style guide. Authors may also find it helpful to consult the Chicago Manual of Style (University of Chicago Press, Chicago) and Scientific Style and Format (Council of Science Editors, Chicago), though the AFS style guide always takes precedence.

The standard resource for word usage and spelling is Webster's Third New International Dictionary, as updated by the latest edition of Merriam-Webster's Collegiate Dictionary. Appendix A of the AFS style guide shows the proper way to spell many of the terms used in fisheries writing (some of which are not in the dictionary), including terms for which our preferred spelling differs from that in the dictionary.

The standard resource for the common and scientific names of North American fish species is the current edition of Common and Scientific Names of Fishes from the United States, Canada, and Mexico (American Fisheries Society, Bethesda, Maryland). For other aquatic species, authors should follow the companion publications World Fishes Important to North Americans and Common and Scientific Names of Aquatic Invertebrates from the United States and Canada (the volumes Mollusks, Decapod Crustaceans, and Cnidaria and Ctenophora are currently available in the latter series).

In most cases, scientific names should be included only at first mention in the abstract and text; full common names (e.g., "Coho Salmon" rather than simply "Coho") should be used elsewhere. The format for the first mention is

Coho Salmon Oncorhynchus kisutch,

in which all parts of the common name are capitalized and the scientific name follows the common name but is not given in parentheses. See chapter 9 of the AFS style guide for additional information on the treatment of species' names; the accepted plurals of fish names are given in Appendix C of the guide.

In papers about population dynamics, we prefer the notation used by W. E. Ricker in Computation and Interpretation of Biological Statistics of Fish Populations (Fisheries Research Board of Canada Bulletin 191, 1975). However, all symbols should be defined anew in every paper. Our standard sources for chemical and enzyme names are the current editions of the Merck Index (Merck & Co., Rahway, New Jersey) and Enzyme Nomenclature (Academic Press, San Diego, California), respectively. The preferred treatment of allozymes is noted in the article "Gene Nomenclature for Protein-Coding Loci in Fish" by J. B. Shaklee et al. (Transactions of the American Fisheries Society 119:2–15, 1990). Additional information on the treatment of these and other technical matters may be found in chapter 11 of the AFS style guide.

Manuscript format.—As an aid to reviewers and editors, authors should

1. use double spacing for all components of the paper, including the title page, footnotes, and tables;

2. number all pages sequentially and provide continuous line numbering beginning with the title page;
3. use a 12-point font throughout;
4. use three levels of headings, as follows: for the major sections of the paper (Methods, Results, Discussion, Acknowledgments, and References), type them flush left with initial letters capitalized (except for prepositions and conjunctions) in ordinary type, preceded by "[A]" (e.g., [A]Methods); for subsections in Results and Discussion, type them flush left with initial letters capitalized in ordinary type preceded by "[B]" (e.g., [B]Treatment 1); and for subsections in Methods and sub-subsections in Results and Discussion, run them into the text with only the initial letter of the first word capitalized, all words italicized, preceded by "[C]," and followed by a period and a long dash (e.g., [C] Sampling design.—); and
5. turn off automatic hyphenation and justification.

General style conventions.—A detailed presentation of AFS style is beyond the scope of these guidelines. The following conventions, however, are so general as to apply to virtually every paper:

1. Only symbols and abbreviations included in Webster's dictionaries or listed at the end of these guidelines (as well as at the back of each printed issue of the journal) may be used without definition. All others should be defined at first use (e.g., index of biotic integrity [IBI]). Abbreviations should not be introduced unless they are used at least two more times.
2. All measurements should be given in metric units. The only exceptions are a few quantities that are typically expressed only one way (e.g., g [of medication]/lb [of feed]).
3. Single-digit numbers should be spelled out unless they are used with units of measure or in conjunction with larger values (e.g., 2 mg/L; 8 Walleyes and 16 Saugers). Numbers with four or more digits should contain commas; those less than 1.00 should be preceded by zeroes.
4. Ratios involving two values or units of measure should be indicated by forward slashes (e.g., 0.30 g/d); ratios involving three such terms should be indicated by negative exponents (e.g., 0.01 g · g–1 · d–1).
5. Ages of fish should be expressed by Arabic numerals and not contain plus signs (e.g., a fish is age 1 [not age 1+] from the January 1 after it hatches to the following December 31).
6. Dates should be expressed as month–day–year (e.g., January 11, 2011). Note that the term "Julian day" does not mean day of the year and should not be used in that context.
7. Time should be expressed in terms of the 24-hour clock followed by the word "hours" (e.g., 1435 hours rather than 2:35 p.m.).

Reference formats.—Text citations should conform to the author–year system. Examples of common types are as follows:
(Johnson 1995)
(Johnson and Smith 1996)
(Johnson et al. 1997, 1998) [three or more authors]
(Johnson et al. 1999, 2001; Smith 2000)
(Johnson 2000a, 2000b)
(Johnson, in press)
(E. M. Johnson, National Marine Fisheries Service, personal communication)
Note that with one exception citations should be listed in chronological order; the exception is that all citations to the same author(s) should be grouped together (see the fourth example above).

In reference lists, references should be in strict alphabetical order by authors' last names; if there are two or more references with the same authors, those references should then be listed chronologically. All authors must be named in references.

Detailed information on reference formats may be found in chapter 8 of the AFS style guide. The more common types are as follows:

Articles in journals

Pace, M. L., and J. D. Orcutt. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography 26:822–830.

Note that (1) except for the first author, authors' initials come before their last names; (2) only the first word of the title of the article is capitalized (along with any other words that would be capitalized in ordinary text); and (3) the name of the journal is given in full.

Books

Krebs, C. J. 1989. Ecological methodology. Harper and Row, New York.

Chapters in books

Omernik, J. M. 1995. Ecoregions: a spatial framework for environmental management. Pages 49–62 in W. S. Davis and T. P. Simon, editors. Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Government reports

Reports that are issued on a regular basis are treated much like articles in journals (the principal difference being that page numbers should not be given); other reports are treated like books:

Everest, F. H., C. E. McLemore, and J. F. Ward. 1980. An improved tri-tube cryogenic gravel sampler. U.S. Forest Service Research Note PNW-350. [journal format]

USEPA (U.S. Environmental Protection Agency). 1998. Water quality criteria and standards plan: priorities for the future. USEPA, 822-R-98-003, Washington, D.C. [book format]

Electronic publications

References to books and reports should be formatted in the usual way even if they are only available online (or are available in print form but were accessed online):

Baldwin, N. A., R. W. Saalfield, M. R. Dochoda, H. J. Buettner, and R. L. Eshenroder. 2000. Commercial fish production in the Great Lakes, 1867–1996. Great Lakes Fishery Commission, Ann Arbor, Michigan.

Uniform resource locator (URL) addresses may be given for sources that are difficult to locate, but they should be omitted otherwise.

If a journal is available in print form, authors should use the standard reference format even if they accessed the article online. If a journal is only available electronically, the format depends on the way(s) in which articles are designated. Two possible formats are as follows:

Gallagher, M. B., and S. S. Heppell. 2010. Essential habitat information for age-0 rockfish along the central Oregon coast. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science [online serial] 2:60–72.

Kimmerer, W. J. 2004. Open-water processes of the San Francisco Estuary: from physical forcing to biological responses. San Francisco Estuary and Watershed Science [online serial] 2(1):article 1.

Note that digital object identifiers (DOIs) should only be included for articles still in press.

Databases should be cited as follows:

Pacific States Marine Fisheries Commission. 2015. PTAGIS (Columbia Basin PIT Tag Information System) [online database]. Pacific States Marine Fisheries Commission, Portland, Oregon. Available: www.ptagis.org.

The "author" should be the organization(s) that maintain(s) the database; if there are more than five such organizations, use the name of the database as the author. The year

should be the year in which the database was accessed. If additional information is necessary to enable readers to locate the exact source, it may be given in the text citation.

Software packages should be cited only in the text (see section 13.3 of the AFS style guide).

APÊNDICE 2 - Normas utilizadas para a preparação do Capítulo 3.

Author guideline: Aquaculture Nutrition

1. SUBMISSION

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at http://mc.manuscriptcentral.com/anu

Click here for more details on how to use ScholarOne.

For help with submissions, please contact Inghild Øye at the Editorial Office: an@hi.no

2. AIMS AND SCOPE

Aquaculture Nutrition provides a global perspective on the nutrition of all cultivated aquatic animals. Topics range from extensive aquaculture to laboratory studies of nutritional biochemistry and physiology.

Aquaculture Nutrition publishes papers which strive to:

● increase basic knowledge of the nutrition of aquacultured species and elevate the standards of published aquaculture nutrition research

● improve understanding of the relationships between nutrition and the environmental impact of aquaculture

● increase understanding of the relationships between nutrition and processing, product quality, and the consumer.

● help aquaculturalists improve their management and understanding of the complex discipline of nutrition

● help the aquaculture feed industry by providing a focus for relevant information, techniques, tools and concepts.

3. MANUSCRIPT CATEGORIES

● Original Articles

● Letter to the Editor

● Review

4. PREPARING THE SUBMISSION

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A covering letter must be included, signed by the corresponding author (i.e., the author to whom correspondence should be addressed), and stating on behalf of all the authors that the work has not been published and is not being considered for publication elsewhere. Authors are encouraged to suggest four potential referees for their manuscripts.

The manuscript should be submitted in separate files: main text file; figures.

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The text file should be presented in the following order:

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ii. A short running title of less than 40 characters;

iii. The full names of the authors with corresponding author marked with *;

iv. The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;

v. Acknowledgments;

vi. Abstract and keywords;

vii. Main text;

viii. References;

ix. Tables (each table complete with title and footnotes);

x. Figure legends;

xi. Appendices (if relevant).

Figures and supporting information should be supplied as separate files.

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Please refer to the journal's Authorship policy in the Editorial Policies and Ethical Considerations section for details on author listing eligibility.

Acknowledgments

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

Conflict of Interest Statement

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the Editorial Policies and Ethical Considerations section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Abstract

Please provide an abstract of no more than 200 words containing the major keywords.

Keywords

Please provide six keywords.

Main Text

● The journal uses British spelling; however, authors may submit using either option, as spelling of accepted papers is converted during the production process.

● Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

References

List all sources in the reference list alphabetically by name. In text citations should follow the author-date method. This means that the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998), and a complete reference should appear in the reference list at the end of the paper.

References are styled according to the sixth edition of the Publication Manual of the American Psychological Association. A sample of the most common entries in reference lists appears below. Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

Journal article:

Phelps, L. (1996). Discriminative validity of the WRAML with ADHD and LD children. Psychology in the Schools, 33, 5-12.

Book edition:

Bradley-Johnson, S. (1994). Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school (2nd ed.). Austin, TX: Pro-ed.

References should refer only to material listed within the text.

Footnotes

Footnotes should be placed as a list at the end of the paper only, not at the foot of each page. They should be numbered in the list and referred to in the text with consecutive, superscript Arabic numerals. Keep footnotes brief; they should contain only short comments tangential to the main argument of the paper and should not include references.

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure Legends

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

Figures

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted.

Click here for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

Color figures: Figures submitted in colour may be reproduced in color online free of charge. Please note, however, that it is preferable that line figures (e.g. graphs and charts) are supplied in black and white so that they are legible if printed by a reader in black and white.

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Appendices will be published after the references. For submission they should be supplied as separate files but referred to in the text.

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The following points provide general advice on formatting and style.

● Abbreviations: In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use theabbreviation only.

● Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website for more information about SI units. The salinity of sea water should be given as g L-1. Use the form g mL-1 not g/mL. Avoid the use of g per 100g, for example in food composition, use g/kg composition. If other units are used, these should be defined on first appearance in terms of SI units, e.g. mmHg.

● Numbers: numbers under 10 are spelt out, except for: measurements with a unit (8mmol/l); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).

● Trade Names: Chemical substances should be referred to by the generic name only. Trade names should not be used.Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.

Resource Identification Initiative

The journal supports the Resource Identification Initiative, which aims to promote research resource identification, discovery, and reuse. This initiative, led by the Neuroscience Information Framework and the Oregon Health & Science University Library, provides unique identifiers for antibodies, model organisms, cell lines, and tools including software and databases. These IDs, called Research Resource Identifiers (RRIDs), are machine-readable and can be used to search for all papers where a particular resource was used and to increase access to critical data to help researchers identify suitable reagents and tools.

Authors are asked to use RRIDs to cite the resources used in their research where applicable in the text, similar to a regular citation or Genbank Accession number. For antibodies, authors should include in the citation the vendor, catalogue number, and RRID both in the text upon first mention in the Methods section. For software tools and databases, please provide the name of the resource followed by the resource website, if available, and the RRID. For model organisms, the RRID alone is sufficient.

Additionally, authors must include the RIIDs in the list of keywords associated with the manuscript.

To Obtain Research Resource Identifiers (RRIDs):

1) Use the Resource Identification Portal, created by the Resource Identification Initiative Working Group.

2) Search for the research resource (please see the section titled “Search Features and Tips” for more information).

3) Click on the “Cite This” button to obtain the citation and insert the citation into the manuscript text.

If there is a resource that is not found within the Portal, authors are asked to register the resource with the appropriate resource authority. Information on how to do this is provided in the “Resource Citation Guidelines” section of the Portal.

If any difficulties in obtaining identifiers arise, please contact rii-help@scicrunch.org for assistance.

Example Citations:

Antibodies: "Wnt3 was localized using a rabbit polyclonal antibody C64F2 against Wnt3 (Cell Signaling Technology, Cat# 2721S, RRID: AB_2215411)"

Model Organisms: "Experiments were conducted in c. elegans strain SP304 (RRID:CGC_SP304)"

Cell lines: "Experiments were conducted in PC12 CLS cells (CLS Cat# 500311/p701_PC-12, RRID:CVCL_0481)"

Tools, Software, and Databases: "Image analysis was conducted with CellProfiler Image Analysis Software, V2.0 (http://www.cellprofiler.org, RRID:nif-0000-00280)"

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· Observational studies: STROBE

· Systematic reviews: PRISMA

· Case reports: CARE

· Qualitative research: SRQR

· Diagnostic / prognostic studies: STARD

· Quality improvement studies: SQUIRE

· Economic evaluations: CHEERS

· Study protocols: SPIRIT

· Clinical practice guidelines: AGREE

We also encourage authors to refer to and follow guidelines from:

· Future of Research Communications and e-Scholarship (FORCE11)

· The Gold Standard Publication Checklist from Hooijmans and colleagues

· Minimum Information Guidelines from Diverse Bioscience Communities (MIBBI) website

· FAIRsharing website

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Author Guidelines updated 18th July 2018

7. VITA

Ana Amélia Nunes Fossati, filha de Mario Luiz Fossati e Débora Nunes Fossati, nasceu no dia 15 de janeiro de 1987 no município de Telêmaco Borba, Paraná.

Cursou o ensino fundamental na Escola Estadual de Ensino Médio e Fudamental Santo Augusto e ensino médio na Escola Cenescista Padre Anchieta, ambas no município de Santo Augusto, Rio Grande do Sul. Em 2005 ingressou no curso de Medicina Veterinária na Universidade Federal do Rio Grande do Sul (UFSM). Em 2010 e 2011 cursou a especialização em Produção, tecnologia e higiene de alimentos de origem animal na Universidade Federal do Rio Grande do Sul (UFRGS). Em 2012 cursou o mestrado em Ciências Veterinárias na Universidade Federal do Rio Grande do Sul (UFRGS) na área de Concentração: Medicina Veterinária Preventiva e Patologia/Inspeção de Produtos de Origem Animal e Tecnologia. Em 2015, iniciou o curso de Doutorado junto ao Programa de Pós-graduação em Zootecnia, na Universidade Federal do Rio Grande do Sul (UFRGS) na área de concentração: Produção Animal, como bolsista CAPES.