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Bruno Bianchi Loureiro

OTIMIZAÇÃO NA PRODUÇÃO DO CONCENTRADO PROTEICO DE FARELO DE ARROZ E UTILIZAÇÃO NA ALIMENTAÇÃO DO JUNDIÁ (*Rhamdia quelen*)

Santa Maria, RS
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Tese apresentada ao Curso de Pós-Graduação em Zootecnia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Zootecnia**

Orientadora: Profª. Drª. Leila Picolli da Silva

Santa Maria, RS
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Aprovado em 27 de março de 2020:

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Santa Maria, RS
2020

DEDICATÓRIA

A minha família, minha esposa **Naglezi** inspiração de vida e dedicação e ao meu amado filho **Gonçalo**, meu maior e melhor presente.
Ao meus pais (**Luiz e Jucélia**), minha irmã e cunhado (**Cristiane e Junior**).
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Dedico este trabalho.

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RESUMO

OTIMIZAÇÃO NA PRODUÇÃO DO CONCENTRADO PROTEICO DE FARELO DE ARROZ E UTILIZAÇÃO NA ALIMENTAÇÃO DO JUNDIÁ (*Rhamdia quelen*)

AUTOR: Bruno Bianchi Loureiro
ORIENTADORA: Leila Picolli da Silva

O presente estudo foi realizado com o objetivo de avaliar os efeitos da inclusão do concentrado proteico de farelo de arroz (CPFA) sobre os parâmetros de crescimento, respostas metabólicas (plasmáticas e hepáticas), deposição de nutrientes e enzimas digestivas de jundiás (*Rhamdia quelen*): em substituição a farinha de peixe. Para o desenvolvimento e escolha do melhor processo para obtenção do CPFA a partir do farelo de arroz desengordurado e desfitinizado (FADD), foi testada a influência do processo de extrusão do FADD sobre a extração e concentração da proteína do FADD *in natura* e extrusado. Os resultados obtidos indicaram que o processo de extrusão *per se* ou combinado com a metodologia de concentração, não foi eficiente para elevar o teor de proteína bruta (PB) do farelo *in natura*, apresentando valores inferiores ($P<0,05$) em comparação ao concentrado proteico obtido a partir da FADD *in natura.* Após a obtenção e escolha do concentrado com maior teor de PB (42,39%), a proteína do CPFA foi inclusa em diferentes níveis (25, 35, 45 e 55%) em substiuição a proteína da farinha de peixe, nas dietas para jundiás. Um ensaio biológico foi conduzido durante 45 dias experimentais, onde 300 jundiás, com peso médio inicial 10,28 ± 0,19 g foram distribuídos aleatoriamente e alocados em 15 tanques de polipropileno (20 peixes por tanque) com volume de 100 L conectados a um sistema de recirculação de água termorregulado. Os peixes foram alimentados com as dietas experimentais, três vezes ao dia (9h, 13h30min e 17h), até a saciedade aparente. O delineamento experimental foi inteiramente casualizado com cinco tratamentos e três repetições. Os dados foram submetidos à análise de variância (ANOVA) e as médias comparadas ao teste de Tukey ($P<0,05$). Ao final do período experimental, foram observadas queda no desempenho ($P<0,05$) sobre os parâmetros de ganho de peso, taxa de crescimento específico e ganho de peso relativo nos peixes alimentados com as dietas contendo 45 e 55% de inclusão do CPFA. Para proteína total depositada, foi verificada a redução ($P<0,05$) nos peixes que receberam as dietas contendo 35% ou mais da inclusão do CPFA. A maior atividade da enzima digestiva tripsina foi observada nos peixes alimentados com as dietas 35% de CPFA. Não foram observadas diferenças ($P<0,05$) para as respostas metabólicas plasmáticas e hepáticas. A concentração proteica realizada a partir do FADD *in natura,* permitiu a obtenção de um concentrado com maior teor de proteína bruta (42,39%). Quanto aos resultados do ensaio biológico, foi observado que a inclusão de até 35% do CPFA em substituição a proteína advinda da farinha de peixe não afeta negativamente os parâmetros de crescimento, metabólicos, deposição de nutrientes e enzimas digestivas de jundiás (*Rhamdia quelen*)

Palavras-chave: Extrusão, proteína, farelo de arroz desengordurado e desfitinizado, enzimas digestivas.

ABSTRACT

RICE MEAL RESIDUE AS A PROTEIN INGREDIENT IN THE FEEDING OF SILVER CATFISH (*Rhamdia quelen*)

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ADVISER: Leila Picolli da Silva

The present study was carried out with the objective of evaluating the effects of the inclusion of de-oiled and de-phytinized rice bran protein concentrate (DRBPC) on the parameters of growth and performance, metabolic responses (serum and liver), nutrient deposition and digestive enzymes of silver catfish (*Rhamdia quelen*), fed with diets containing different levels of replacement of protein from fishmeal by DRBPC. For the development and choice of the best DRBPC, obtained from de-oiled and dephytinized rice bran (DDRB), the influence of the DDRB extrusion process on the improvement of the extraction and concentration of the fresh and extruded DDRB protein was tested, obtaining two protein concentrates: DRBPC and DRBPCext. The results obtained indicated that the extrusion process *per se* or combined with the concentration methodology was not efficient to increase the crude protein (CP) content of the DDRB, presenting lower values ($P < 0.05$) compared to protein concentrate from DRBPC. After obtaining and choosing the concentrate with the highest CP content (42.39%), DRBPC protein was included in different levels (25, 35, 45 and 55%) of fishmeal replacement in silver catfish diets. A biological assay was conducted during 45 experimental days, where 300 silver catfish, with initial body weight 10.28 ± 0.19 g were randomly distributed and allocated in 15 polypropylene tanks (20 fish per tank) with a volume of 100 L connected to a thermo regulated water recirculation system. The fish were fed with experimental diets, three times a day (9 am, 1:30 pm and 5 pm), until apparent satiety. The experimental design was completely randomized with five treatments and three replications. The data were submitted to analysis of variance (ANOVA) and the means compared to the Tukey test ($p < 0.05$). At the end of the experimental period, a decrease in performance was observed ($P < 0.05$) on weight gain, specific growth rate and relative weight gain in fish fed diets containing 45 and 55% of DRBPC inclusion. For total protein deposited, a reduction ($P < 0.05$) was verified in fish that received diets containing 35% or more of the inclusion of DRBPC. The highest activity of the digestive enzyme trypsin was observed in fish fed diet 35% of DRBPC. Based on the results obtained, it was found that the DDRB extrusion process was not efficient to facilitate the extraction of the bran protein by the protein concentration process. The protein concentration carried out from the DDRB *in natura*, allowed to obtain a concentrate with a higher crude protein content (42.39%). As for the results of the biological test, it was observed that the inclusion of 25% of the DRBPC in replacement of the protein coming from fishmeal did not negatively affect the parameters of growth and metabolic, deposition of nutrients and digestive enzymes of silver catfish (*Rhamdia quelen*).

Keywords: Extrusion, protein, de-oiled and de-phytinized rice bran, digestive enzymes.

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1 INTRODUÇÃO

Segundo a Organização das Nações Unidas para a Alimentação e a Agricultura (FAO, 2014a; 2014b), mais de 16% da proteína de origem animal consumida pela população mundial é advinda do pescado e considerando a soma das fontes de origem vegetal e animal, esse valor chega a quase 7% do total de fontes proteicas consumida. Verdadeiramente, a aquicultura desponta como uma atividade agropecuária de elevado crescimento nas últimas décadas. De acordo com o relatório SOFIA (2018), entre 1961 e 2016 houve aumento de 3,2% no consumo médio anual de pescado no mundo, o que veio acompanhado por todas as demandas associadas ao seu cultivo, principalmente aquelas relacionadas à nutrição e eficiência nutricional no uso combinado de ingredientes para compor as rações balanceadas.

A nutrição de peixes é considerada um dos maiores obstáculos a serem enfrentados nas próximas décadas, principalmente no que se refere ao grande número de espécies com potencial para cultivo, com diferentes hábitos alimentares e exigências nutricionais (BITTENCOURT et al., 2010). Dentre as fontes proteicas utilizadas na aquicultura, a farinha de peixe é um dos principais ingredientes, pois apresenta alta palatabilidade, digestibilidade (GIRIJA; MURTHY, 2019) e proteína de elevado valor biológico, resultante do perfil de aminoácidos adequadamente equilibrado (DANIEL, 2018). No entanto, é um ingrediente escasso e com grande variação na sua composição nutricional (KOKOU; FOUNTOULAKI, 2018). Várias são as tentativas de sua substituição parcial ou total por fontes de origem vegetal que sejam economicamente viáveis, produzidas de acordo com a demanda e que apresentem qualidade nutricional compatível com fontes proteicas animais.

Grande parte dos ingredientes vegetais apresentam restrições quanto ao seu uso na nutrição de peixes, como menor digestibilidade e palatabilidade devido a presença de grande quantidade de carboidratos não solúveis, como fibras e amido resistente e outros compostos presentes nesses ingredientes (DANIEL, 2018; SÁNCHEZ-MUROS et al., 2018), presença de fatores antinutricionais (WELKER et al., 2016; NGUGI et al., 2017; CHAKRABORTY et.al, 2019) e desequilíbrio no perfil de aminoácidos (DANIEL, 2018). Melhoria na qualidade nutricional e digestibilidade de fontes proteicas vegetais podem ser obtidas através da aplicação de processos químicos e/ou físicos, tendo em vista que as proteínas podem ser modificadas buscando-se aumento do valor biológico, possibilitando a aplicação de coprodutos e resíduos vegetais das agroindústrias na alimentação animal.

Entre os coprodutos e resíduos agroindustriais com potencial para produção de ingredientes sustentáveis cita-se o resíduo de farelo de arroz desengordurado e desfitinizado

(FADD), o qual possui maior teor de proteína bruta (20%) e menor quantidade de fósforo (0,09%) na sua composição em comparação ao grão de arroz.

Diversos fatores podem interferir na determinação da exigência de proteína desde a qualidade da fonte proteica até a participação de fontes energéticas não proteicas (FRACALOSSI; CYRINO, 2013). Segundo Luchesi et al (2014), para o bom desempenho dos peixes é importante conhecer, além das exigências das espécies, o valor biológico dos ingredientes que serão utilizados na formulação das dietas.

Diante do exposto, há necessidade de estudos voltados à busca de soluções sustentáveis para essa problemática. O desenvolvimento e aprimoramento de técnicas para obtenção de concentrados proteicos possibilitam a utilização de fontes vegetais, melhorando o valor biológico da proteína, através da redução de nutrientes pouco digestíveis, como as fibras. A utilização de concentrados proteicos permite agregar valor comercial e interesses tecnológicos de resíduos e subprodutos vegetais para obtenção de produtos diferenciados.

1.1 OBJETIVOS

1.1.1 Objetivo Geral

Desenvolver processo para a obtenção do concentrado proteico de farelo de arroz a partir do Farelo de arroz desengordurado e desfitinizado (FADD) testando a eficiência de uso no desempenho e respostas metabólicas do jundiá (*Rhamdia quelen*).

1.1.2 Objetivos Específicos

Desenvolver processo de extração e concentração proteica do FADD;

Avaliar características nutricionais e tecnológicas do concentrado proteico obtido a partir do FADD;

Avaliar o melhor nível de substituição da proteína advinda da farinha de peixe pela proteína do concentrado proteico de FADD na dieta de jundiá (*Rhamdia quelen*), avaliando parâmetros de crescimento, metabólicos, deposição de nutrientes e enzimas digestivas.

1.2 REVISÃO BIBLIOGRÁFICA

1.2.1 Proteína na nutrição de peixes

As proteínas são consideradas os principais nutrientes orgânicos dos tecidos dos peixes (LIMA et al., 2015). Quando digeridas são hidrolisadas em aminoácidos livres que serão disponibilizados pela corrente sanguínea para os órgãos e tecidos, sintetizando novas proteínas, que serão destinadas a crescimento, reprodução e manutenção corporal (NRC, 2011). Deste modo, são caracterizadas como o nutriente mais importante para o crescimento animal em todas as fases de desenvolvimento.

Considera-se imprescindível a ingestão regular de proteína, devido à exigência continua de aminoácidos tanto para síntese de novas proteínas (crescimento e reprodução), quanto para reposição das proteínas degradadas pelo organismo do peixe (SAKOMURA, et al., 2014). O fornecimento de dietas com proteínas de baixo valor biológico ou desequilíbrio em aminoácidos podem causar a redução na eficiência alimentar, perda de peso e baixo desempenho dos animais, devido à mobilização proteica tecidual para manutenção das funções vitais (WILSON, 2002).

Esse desequilíbrio de aminoácidos no que se refere à falta ou excesso de proteína na dieta é indesejável, devido à proteína ser o macronutriente mais oneroso da dieta (LIMA et al., 2015; CRAIG et al., 2017), podendo representar até 70% dos custos totais de produção (FERREIRA et al., 2013). Portanto, fica evidente a importância do fornecimento de dietas com níveis de proteína adequados para garantir a quantidade de aminoácidos para atender as exigências de uma espécie em particular.

Peixes exigem níveis elevados teores de proteína quando comparados aos demais animais de produção. O bagre americano (*Ictalurus punctatus)* apresenta exigência proteica de 28-32%, 35-40% para tilápia, 38-42% para robalo híbrido e 40-45% para truta (CRAIG et al., 2017), enquanto aves, suínos e ruminantes apenas de 18%, 16% e 11%, respectivamente (TAKAHASHI, 2005). A proteína na nutrição de peixes desperta grande interesse nutricional e de pesquisa pela elevada inclusão nas rações, com variação em torno de 30 a 60% da matéria seca da dieta. Para melhor utilização da proteína e desempenho dos animais é importante conhecer as exigências nutricionais em proteínas e aminoácidos de cada espécie de peixe (CRAIG et al., 2017), para que se possa obter o melhor aproveitamento da dieta para o crescimento e saúde dos animais.

A farinha de peixe continua sendo a fonte de proteína mais utilizada na elaboração de dietas para aquicultura mundial (BANDARA, 2018), pois apresenta elevado teor de proteína aliado a perfil de aminoácidos adequado e equilibrado (DANIEL, 2018). Ainda apresenta elevada palatabilidade e digestibilidade, ácidos graxos essenciais, vitaminas e minerais (GIRIJA; MURTHY, 2019)

A farinha de peixe apresenta-se como um produto com menor disponibilidade no mercado consumidor, resultante da alta demanda pela indústria de rações (LI et al. 2015). De acordo com esse cenário, a viabilidade econômica e o aumento do crescimento da aquicultura são afetados pela demanda de proteína para fabricação de rações. Dessa maneira, buscam-se alternativas para redução ou substituição da farinha de peixe por fontes proteicas de menor custo, boa qualidade nutricional que resultem em bom desempenho zootécnico. Aspectos que podem ser encontrados com a utilização de fontes proteicas alternativas.

1.2.2 Proteínas vegetais como fontes alternativas na alimentação de peixes

Os ingredientes de origem vegetal são cada vez mais utilizados na substituição da farinha de peixe como fontes de proteína na elaboração de dietas aquícolas. Esse fato ocorre devido a ampla disponibilidade, além da existência de uma grande variedade de espécies vegetais em todo o mundo, com potencial para serem inseridas na nutrição de peixes.

Diversas são as pesquisas direcionadas a utilização de fontes vegetais como ingredientes proteicos alternativos e viáveis à substituição das fontes de origem animal na alimentação de peixes (AL-THOBAITI et al., 2018; DANIEL, 2018; CHAKRABORTY et al., 2019; AZIZA E EL-WAHAB, 2019). Apesar da grande quantidade de espécies vegetais com amplo potencial para uso como fonte proteica, apenas uma pequena parcela é atualmente utilizada na alimentação animal.

Fontes vegetais como farinha de colza (DOSSOU et al., 2018), farinha de microalgas (RADHAKRISHNAN et l., 2016; LEITE et al., 2019), farinhas de glúten de trigo, glúten de milho e de bagaço de pitaya (AL-THOBAITI et al., 2017), concentrado proteico de farelo de arroz (LOUREIRO et al., 2019), concentrado proteico de arroz (CAI et al., 2018a) concentrado proteico de soja (MOHD-FAUDZI et al., 2018; BISWAS et al., 2019) e concentrado proteico de milho (KHALIFA et al., 2018) são amplamente pesquisadas como ingredientes promissores para alimentação de peixes.

Existe ainda, a possibilidade da utilização de fontes proteicas de animais terrestres na nutrição aquícola, porém essa hipótese causa preocupação e receio público em muitos países

devido aos riscos associados a zoonoses e sua transmissão (FUERTES et al., 2013). Com isso, a transição de uso das fontes proteicas de origem animal por ingredientes proteicos vegetais é uma importante demanda para garantir sustentabilidade dos sistemas produtivos e estabilidade de mercado aquícola (NGUGI et al., 2017).

Normalmente a substituição da farinha de peixe tem sido alcançada em diferentes níveis que variam, por exemplo, entre 25-50% (LOVATTO et al., 2016, 2017) ou 50-100% (ANDERSON et al., 2016), sendo dependente da fonte proteica vegetal utilizada. Porém, o uso de fontes vegetais em rações aquícolas ainda é um desafio, pois as informações sobre a disponibilidade de nutrientes são questionáveis (CABRAL et al., 2011), além disso possuem baixa disponibilidade nutricional (CHENG et al., 2016), como por exemplo, o farelo de arroz, farelo de girassol, farelo de trigo e farelo de soja. Geralmente, o uso de alimentos com elevados teores de proteína advinda de fontes vegetais, pode causar redução do desempenho de crescimento dos peixes e afetar a integridade intestinal (KOKOU et al., 2017).

Para que uma fonte proteica de origem vegetal seja considerada uma alternativa viável à farinha de peixe ou outra fonte de origem animal, deve possuir alguns pré-requisitos como: alta disponibilidade, produção constante ao longo do ano em grande escala, facilidade de manuseio, transporte e armazenagem. Além de apresentar determinados aspectos nutricionais como: elevado teor de proteína, perfil de aminoácidos favorável, baixo teor de fibra e carboidratos insolúveis, ser livre ou possuir reduzida presença de antinutrientes, boa palatabilidade e digestibilidade (GATLIN et al., 2007).

Para viabilizar a utilização das fontes vegetais, métodos como cozimento, torrefação e extrusão são utilizados, pois reduzem a presença de fatores antinutricionais e melhoram a digestibilidade da proteína (NYINA-WAMWIZA et al., 2010). Ainda, métodos de concentração proteica podem ser usados na obtenção de fontes proteicas minimizadas de fatores antinutricionais, melhorando a digestibilidade, reduzindo o teor de fibras e aumentando o conteúdo de aminoácidos.

1.2.3 Farelos de arroz como fonte proteica

O arroz (*Oryza sativa*) é um dos cereais mais produzidos e consumidos no mundo, sendo o principal componente na dieta da população mundial, com cerca de 161 milhões de hectares cultivados e produção total de 756,5 milhões de toneladas (SOSBAI, 2018). A produção anual de arroz no Brasil, na safra 2018/2019 foi em torno de 10,6 milhões de toneladas, sendo o estado do Rio Grande do Sul o maior produtor do País com quase oito

milhões de toneladas produzidas (CONAB, 2020).

Para produzir o arroz branco, após o descasque é feito o polimento. Esse último processo de beneficiamento do grão gera o subproduto farelo de arroz integral (FAI). Aproximadamente 9% desse farelo é obtido pelo beneficiamento do cereal para o consumo humano (SOUSA, 2019; CORDEIRO, 2015), o qual é usado para nutrição animal, incluindo peixes. O FAI apresenta elevado teor de amido que pode variar conforme o grau de extração e mostra-se uma excelente fonte de energia, além de ter bons níveis de fósforo, proteína e gordura (ROSTAGNO et al., 2011) é rico em vitaminas (vitamina E e do complexo B), aminoácidos e ácidos graxos insaturados (ácido oléico, linoléico e linolênico) (ARAÚJO, 2019). A composição química do FAI é variável tendo cerca de 13% de proteína bruta (PB), 21% de fibra em detergente neutro (FDN), 14% de extrato etéreo (EE) e 1,6% de fósforo total (P) (ROSTAGNO et al., 2005).

Entretanto, a utilização do FAI na alimentação animal é limitada, principalmente pela presença de teores significativos de fibras insolúveis e fatores antinutricionais como as lípases, substâncias antiproteolíticas e ácido fítico (CORDEIRO, 2015; SUPRIYATI et al., 2015), que é um poderoso quelante com atividade negativa sobre o aproveitamento e disponibilidade dos nutrientes para animais monogástricos (SANCHEZ et al., 2019), interferindo no desempenho e metabolismo (IMOROU TOKO et al., 2008), limitando sua utilização intensiva na elaboração de rações. Devido a elevada percentagem de óleo e enzimas lipolíticas, o FAI possui maior tendência a rancificação, tornando-se um coproduto instável com reduzido tempo de armazenamento (KAWSKI, 2015), não devendo ser armazenado por mais de duas semanas (LUCHESE e JUSTINO, 2003).

Deste modo, uma alternativa utilizada para evitar a rancificação do FAI é a de extração do óleo, através de solventes químicos ou por prensagem, resultando no farelo de arroz desengordurado (FAD) com teores mais elevados de proteína e fósforo total, maior teor de vitaminas e minerais, além de melhor estabilidade, quando comparado ao FAI (ROSTAGNO et al., 2005). O FAD pode ser armazenado por maior período, viabilizando sua utilização. Entretanto, este coproduto ainda possui elevado teor de fibras insolúveis e ácido fítico em sua composição, que é uma forma de fósforo indisponível para animais monogástricos e que forma quelatos com metais di- e tri-valentes como cálcio, magnésio, manganês, ferro e zinco, diminuindo a disponibilidade destes compostos orgânicos no trato gastrointestinal (KUMAR et al., 2011).

Tecnologias de extração dos lipídios e extração do ácido fítico já são consolidadas na cadeia produtiva arrozeira. A partir dessas extrações tem-se o subproduto Farelo de arroz

Desengordurado e Desfitinizado (FADD), que apresenta reduzida concentração de ácido fítico, proteína bruta acima de 18% (LOUREIRO et al., 2019) e maior percentual de lisina (0,82%), quando comparado à maioria dos cereais.

De acordo com Ferreira (2011), os valores obtidos nas análises de proteína bruta e fósforo total do FAD e FADD foram 17,29% e 2,9%; 20,74% e 0,33%, respectivamente. Embora ocorra melhora nos índices, o conteúdo de proteína ainda é pouco satisfatório e o teor de fibras ainda é elevado para o FADD ser potencializado como ingrediente proteico na dieta de peixes.

Diante deste cenário, buscam-se estratégias para agregar maior valor nutricional e econômico ao farelo de arroz, bem como ampliar e potencializar seu uso como ingrediente na nutrição. Além disso, como desafio no processamento proteico devemos considerar o tipo de concentração ou modificação proteica realizada.

1.2.4 Estratégias tecnológicas para utilização de proteínas vegetais na nutrição de peixes

Devido ao aumento da demanda mundial de proteína, há grande interesse por proteínas vegetais na nutrição animal. Comparadas à outros constituintes orgânicos, as proteínas são muito complexas devido à suas estruturas, heterogeneidade e participação em associações muito estáveis o que limita os rendimentos de extração (LINDEN; LORIENT, 1996).

Portanto, como desafios no processamento proteico, devemos considerar o tipo de concentração ou modificação proteica realizada, pois através do uso de tecnologias em alimentos é possível utilizar proteínas presentes nos vegetais de forma mais eficiente, com o desenvolvimento de fontes proteicas de melhor qualidade, tornando seu uso cada vez mais comum no desenvolvimento de novos alimentos. Dentre as diversas alternativas para melhorar a disponibilidade proteica em produtos de origem vegetal, tem-se os processos de extrusão e de concentração proteica.

1.2.4.1 Processo de extrusão

A extrusão pode ser definida como um processo de combinação de várias operações de modificações físico-químicas de um ingrediente ou alimento em apenas um equipamento. Possui capacidade de realizar mistura, hidratação, aquecimento, cozimento, cisalhamento, gelatinização do amido, desnaturação do amido e materiais proteicos, caramelização,

destruição de microrganismos, redução ou inativação de substâncias antinutricionais, moldagem, texturização e secagem, formando novas estruturas (FELLOWS, 2006; VERNAZA et al., 2009). A extrusão *per se* não é capaz de aumentar o conteúdo proteico dos ingredientes, contudo é capaz de melhorar a disponibilidade proteica devido à modificação estrutural de fibras e também pelo processo de desnaturação proteica.

A utilização do processo de extrusão é considerada tecnologia com diversas vantagens, pois apresenta baixo custo (ERIKSSON, 2019), alta versatilidade, eficiência e produtividade, curto tempo de reação, baixa ou nenhuma geração de resíduos, reduzida perda de nutrientes, produção de produtos com diferentes formatos e, principalmente, não produz efluentes (AKHTAR; MALIK; ALAM, 2015). Apesar de a extrusão possuir inúmeras vantagens, o controle do processo é complicado, pois muitos alimentos são de natureza complexa e ainda envolvem muitas variáveis físicas (LOPES, 2010).

Existem dois tipos de variáveis, as independentes ou fatores que podem interferir diretamente na qualidade do produto e as dependentes ou respostas, que são alteradas em consequência das independentes (HUBER, 1991). As variáveis independentes compreendem: o ingrediente utilizado, umidade da matéria-prima, configuração da matriz, geometria e velocidade do parafuso, temperatura das jaquetas, pré-condicionamento do canhão (aquecimento) e a taxa de alimentação. Já as variáveis dependentes incluem a viscosidade, taxa de cisalhamento e fluxo, pressão, energia, tempo de residência, temperatura e características do produto (HARPER, 1989).

De acordo com Emin e Schuchmann (2017), as modificações na estrutura do produto durante a extrusão apresenta relação com as condições do processamento, as quais possuem ligação direta com a escolha consciente e controlada dos parâmetros de extrusão à serem utilizados. No entanto, existem poucas informações relacionadas aos efeitos sobre os fatores temperatura, pressão e tempo de permanência do alimento dentro do canhão para extrusados voltados a alimentação de monogástricos (BERTIPAGLIA et al., 2008).

O uso da extrusão como processo físico no desenvolvimento de uma metodologia de concentração proteica para FADD, vem como auxílio para ser utilizada em consórcio com outros processos, pois somente o processo de extrusão não é capaz de aumentar significativamente o teor de proteína de um farelo vegetal. Esta afirmação vai de acordo aos resultados encontrados por Bertipaglia et al. (2008) ao trabalhar com soja e milho, Abd El-Hady e Habiba (2003) trabalhando com sementes de leguminosas e Becker et al. (2013) com farelos de arroz.

Durante o processo de extrusão as proteínas sofrem mudanças estruturais que ocorrem

na seguinte ordem: desnaturação, combinação, rompimento de algumas ou todas as combinações através do aquecimento e cisalhamento para formar uma fase concentrada, possível formação de ligações covalentes em elevadas temperaturas, formação de ligações não covalentes e pontes dissulfeto via refriamento e, transferência de regiões amorfas para o estado vítreo se o teor de umidade for baixo (MITCHELL; AREAS, 1992).

A passagem do FADD pelo processo de extrusão visa a melhora na disponibilidade proteica devido a gelatinização do amido (OMOSEBI, OSUNDAHUNSI, FAGBEMI, 2018) e desnaturação das proteínas, pela exposição de locais para ação de enzimas (MIRANDA, 2006) a serem utilizadas posteriormente no processo de concentração proteica.

De acordo com Glencross (2011), a própria gelatinização e expansão do amido elevam seu valor nutricional através do aumento da digestibilidade do amido para a maioria das espécies de peixes.

1.2.4.2 Concentração proteica

A concentração proteica geralmente é realizada para fins de necessidade nutricional, funcional, sensorial e econômica do ingrediente, através da extração ou inativação de antinutrientes e substâncias tóxicas que estão fortemente associados às proteínas (LINDEN; LORIENT, 1996). Neste contexto, a concentração proteica de fontes vegetais apresenta-se como uma promissora linha de pesquisa na obtenção de produtos proteicos alternativos a farinha de peixes (LOVATTO et al., 2017).

Após a concentração proteica é muito difícil manter a estrutura nativa da proteína, pois a maioria dos agentes modificantes permanece ligada a proteínas, podendo diminuir a biodisponibilidade de alguns aminoácidos (PACHECO, 1996) acarretada pelas mudanças nas estruturas terciária e quaternária das proteínas.

As técnicas para concentração das proteínas variam conforme os grupos proteicos dos ingredientes em questão, que são classificados de acordo com o perfil de aminoácidos (LEHNINGER et al., 2004). No caso de proteínas vegetais, que estão fortemente ligadas a compostos indigestíveis, têm-se como objetivo separar as proteínas destes compostos (celulose, lignina, polifenóis, polissacarídeos não amiláceos entre outros), bem como a diminuição de riscos à poluição ambiental (LINDEN; LORIENT, 1996).

Apesar de a obtenção de concentrados proteicos vegetais ser uma alternativa promissora na nutrição de peixes, tecnologicamente há obstáculos para produção e utilização desses produtos. Inicialmente, em diversas situações os métodos de obtenção de concentrados

proteicos são onerosos devido à utilização de equipamentos ou solventes de custo elevado. Outro ponto é o baixo rendimento do produto final, com valores variando de 10 à 30% em peso de produto, dependendo da fonte proteica utilizada. O baixo rendimento deve-se ao fato das proteínas serem estruturas muito complexas e heterogêneas, além de associadas a outros compostos (LINDEN; LORIENT, 1996). Devido a esta heterogeneidade, várias técnicas físicas e químicas podem ser utilizadas para extração e concentração de proteínas.

Nos últimos anos, muitas pesquisas já foram desenvolvidas com o uso de diferentes concentrados proteicos vegetais, como substitutos parciais a farinha de peixe na nutrição aquícola, sendo possível destacar o uso de concentrado proteico de arroz (CPA) como fonte comparável a farinha de peixe em proteína e gordura (OUJIFARD et al., 2012). O CPA já foi utilizado em dietas para *Megalobrama amblycephala* (ABASUBONG et al., 2018; CAI et al., 2018a, 2018b), *Rhamdia quelen* (LOUREIRO et al., 2019), *Acipenser baerii* (SICURO et al., 2015), *Litopenaeus vannamei* (OUJIFARD et al., 2015), *Pelodiscus sinensis* (SUN et al., 2018) e *Sparus aurata L.* (BAEZA-ARIÑO et al., 2016).

Com isso, podemos inferir que a busca por fontes proteicas livres de fatores indesejáveis como excesso de fibras se dará pelo desenvolvimento de concentrados e isolados proteicos vegetais. Além disso, ressalta-se a importância no equilíbrio de aminoácidos que deve ser presente nesses novos ingredientes, a fim de que possibilitem a metabolização proteica e digestibilidade desse ingrediente.

1.2.5 Caracterização da espécie de peixe estudada: jundiá (*Rhamdia quelen*)

Dentre as principais espécies de peixes cultivadas, as pertencentes a família dos Siluriformes apresentam destaque pela sua qualidade de carne e rendimento de carcaça. O jundiá, *Rhamdia quelen* (Siluriformes, *Pimelodidae*) é um peixe de couro, podendo ser encontrado desde a região sudeste do México até a região central da Argentina (BALDISSEROTTO et al., 2010), que tem se destacado pelo fácil manejo e reprodução (GARCIA et al., 2017), crescimento satisfatório até mesmo nas épocas mais frias do ano (RODRIGUES et al., 2012), podendo atingir em torno de 600-800 g em oito meses de cultivo em densidade de 2-4 peixes/m^2 (BARCELLOS et al., 2004).

A produção brasileira de jundiá advinda do cultivo em cativeiro apresentou crescimento superior a 60% no período compreendido entre 2007 – 2011, com produção de 667 para 1.747,30 toneladas, respectivamente (MPA, 2012). De acordo com dados atuais, no Rio Grande do Sul, a produção de jundiá e outros peixes nativos representa 8% da produção

total de espécies cultivados, ficando atrás apenas das carpas (73%) e tilápia (19%) (PEIXE BR, 2018).

A espécie tem preferência por ambientes de águas lênticas, como lagos e poços fundos de rios com fundo de areia e lama, próximo às margens e vegetação (BALDISSEROTTO; RADÜNZ NETO, 2004), possui fácil adaptação ao cultivo intensivo, reprodução bem sucedida em cativeiro (DIEMER et al., 2011), boa eficiência alimentar e carne sem a presença de espinhos intramusculares (FRACALOSSI et al., 2004). No entanto, a espécie apresenta algumas características indesejáveis como a maturidade sexual precoce, a qual resulta no declínio da taxa de crescimento dos animais devido a boa parte da energia consumida ser destinada para os processos reprodutivos (GARCIA et al., 2017), ocasionando o crescimento heterogêneo da espécie.

Durante o estágio larval alimentam-se de zooplâncton, e na fase adulta são onívoros apresentando forte preferência por peixes, insetos, crustáceos, restos de vegetais e detritos orgânicos (BALDISSEROTTO; RADÜNZ NETO, 2004). Devido ao hábito onívoro tem a capacidade de se nutrir de uma grande variedade de alimentos (GOMIERO et al., 2007), apresentando potencial para o aproveitamento de dietas elaboradas com distintas fontes proteicas, animais e/ou vegetais.

Estudos realizados em sistemas de cultivo intensivos demonstraram que a combinação de fontes de origem animal (farinha de peixe, farinha de carne e osso) e origem vegetal (farelo de soja, de canola e girassol) proporcionaram melhor desempenho em juvenis de jundiá, em relação à utilização de uma única fonte proteica (GOULART et al., 2013; LAZZARI et al., 2006; LOVATTO et al., 2014, 2015,), demonstrando que a espécie não é totalmente dependente da farinha de peixe.

Contudo, estudos usando somente fontes proteicas vegetais em dietas para o jundiá ainda são pouco conclusivos, havendo necessidade de maior exploração do potencial dessas fontes diferenciadas na alimentação dessa espécie.

2 ARTIGO 1

Artigo científico intitulado “Extrusão do farelo de arroz para otimização do processo de concentração proteica por método químico enzimático” à ser submetido para revista LWT - Food Science and Technology e está formatado segundo as normas descritas no Guia dos Autores (Anexo A).

Extrusão do farelo de arroz para otimização do processo de concentração proteica por método químico enzimático

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Resumo

Esse estudo teve como objetivo avaliar o processo de extrusão do farelo de arroz desengordurado e desfitinizado (FADD) e sua influência na obtenção de concentrado proteico através de processo químico-enzimático. O FADD foi extrusado em equipamento de rosca única; temperatura na 1ª zona de aquecimento de 40ºC, na 2ª de 80ºC e na 3ª de 120ºC. O processo de concentração proteica foi realizado através de metodologia descrita por Loureiro et al. (2019), com modificações. Esse processo foi aplicado para o FADD e FADDextr. Dessa maneira, para os quatro produtos avaliados, FADD, FADDextr, CPFA e CPFAext foram aplicados três contrastes ortogonais: 1) FADD *versus* demais; 2) FADDext *versus* CPFA e CPFAext; 3) CPFA *versus* CPFAext. Houve diferença significativa na composição química dos produtos obtidos, em todos os contrastes. O processo de extrusão reduziu o conteúdo de proteína bruta do FADD, o que influenciou negativamente o processo de concentração proteica. FADD e FADDext apresentaram os maiores teores de amido disponível. A concentração proteica e a extrusão alteraram o conteúdo de todas as frações de fibra alimentar. O FADD apresentou o maior conteúdo de fibra insolúvel e menor conteúdo de fibra solúvel quando comparado aos demais produtos. Foi observado que a extrusão do FADD diminuiu a solubilidade proteica. Dessa maneira, conclui-se que a extrusão do FADD, na condição estudada, não foi capaz de melhorar a eficiência da extração da proteína pelo processo de concentração proteica utilizado, uma vez que acarretou na diminuição da proteína do FADD. A concentração proteica do FADD permite obtenção de um concentrado proteico com 42,3% de proteína, maior conteúdo de lipídeos, fibra solúvel e proteína solúvel.

Palavras chave: alfa amilase; amiloglucosidase; farelo de arroz desengordurado e desfitinizado; método físico; solubilidade proteica.

1. Introdução

O arroz (*Oryza sativa* L.) é o segundo *stample food* de maior produção mundial (Rajamoorthy, Rahim & Munusamy 2015), sendo utilizado como principal fonte de energia por mais de 50% da população mundial (Ariyama et al., 2012). O Brasil é o maior produtor do cereal fora do continente asiático, ocupando a 9° posição no ranking mundial (FAO, 2018), sendo o estado do Rio Grande do Sul responsável por 70% da produção nacional (CONAB, 2019).

No beneficiamento do arroz, o grão é descascado e polido, gerando como subproduto o farelo de arroz (FA), o qual corresponde a cerca de 9% do peso do grão (Lorenzett, Neuhaus & Schwab, 2012). O FA apresenta composição nutricional relevante, visto que é fonte de óleo, proteína, fibra e outros compostos funcionais (Sharif, Butt, Anjum & Khan, 2014). Apesar de seus benefícios nutricionais e perspectivas de uso, o FA é subutilizado (Wu, Li & Wu, 2020) principalmente devido à presença de lipases ativas, que aceleram a rancificação e a oxidação proteica do farelo, e à presença de ácido fítico. Estes fatores limitam sua utilização na nutrição humana e animal.

Tecnologias para extração de lipídios e ácido fítico já são consolidadas na cadeia produtiva arrozeira. A partir dessas extrações tem-se o subproduto Farelo de arroz Desengordurado e Desfitinizado (FADD), objeto de nosso estudo. O FADD apresenta reduzidos teores de ácido fítico, maior teor proteico (em torno de 18% de proteína bruta) (Loureiro et al., 2019) e perfil de aminoácidos com maior percentual de lisina (0,82%) (dados não mostrados), quando comparado à maioria dos cereais. Apesar disto, a utilização da proteína do FADD é limitada devido à presença de fibras (Francis, Makkar & Becker, 2001). Metodologias vêm sendo testadas para melhorar a qualidade nutricional deste produto através

de processos físicos, químicos e enzimáticos, com o propósito de reduzir o teor de fibras e melhorar a qualidade e solubilidade das proteínas.

Dentre os métodos físicos, a extrusão pode ser utilizada para auxiliar no processo de obtenção de concentrados proteicos vegetais. A extrusão provoca modificações na estrutura da matriz fibrosa e nas características físico-químicas de seus constituintes (Vasanthan, Jiang, Yeung & Li, 2002). Embora não seja por si só uma estratégia de concentração de nutrientes, pode facilitar a ação de agentes químicos e enzimáticos para melhorar o processo de concentração de nutrientes (Fellows, 2006; Vernaza, Chang & Steel, 2009). Além disso, é amplamente utilizada no processamento de produtos ricos em fibras, tais como o FADD e pode ser considerado um processo com baixa geração de resíduos (Lopes da Silva, Santos & Choupina, 2015).

Processos químicos e enzimáticos são, tradicionalmente, utilizados para obtenção de concentrados proteicos. Aumento na solubilidade proteica e redução no teor de fibras, com consequente aumento do conteúdo de proteína, são algumas das vantagens destes processos (Damodaran, Parkin, Fennema, 2010). Diante disso, o uso do processo de extrusão combinado aos processos químicos e enzimáticos de concentração proteica deve ser estudado para elucidar as modificações ocasionadas nas características químicas e funcionais dos concentrados proteicos.

De acordo com o exposto, o presente estudo teve como objetivo avaliar o processo de extrusão do FADD e sua influência na obtenção de concentrado proteico através de processo químico-enzimático em meio aquoso, avaliando suas características nutricionais e tecnológicas.

2. Materiais e métodos

2.1. *Matéria prima*

O FADD foi doado pela Indústria Gaúcha de Alimentos Ltda. (INGAL) Santa Maria, RS, Brasil. O FADD corresponde a uma massa úmida (pH 1,5, 42g/100g de matéria seca). Para o processamento, inicialmente o FADD foi seco em estufa de ar forçado à 50°C por 24 horas e moído em moinho de laboratório (Marconi-MA630/1) para obtenção de partículas menores que 600μm. Após a secagem o FADD foi acondicionado em embalagem de polietileno de baixa densidade e armazenado a -18ºC.

Para realização dos procedimentos, o FADD foi separado em duas partes amostrais, mantendo-se uma fração integral e outra fração à ser processada por extrusão.

2.2. *Processo de extrusão*

Para extrusão do FADD foi utilizada extrusora de rosca única com capacidade de produção semi-industrial (modelo RXPQ Labor 24, INBRAMAQ - Indústria de Máquinas Ltda, Ribeirão Preto - SP). As condições de operação para extrusão do FADD envolveram o uso de duas camisas helicoidais; rosca curta com taxa de compressão de 3:1, de uma saída, com taxa de compressão de 3,6:1 e relação comprimento/diâmetro (L/D) de 15,5:1; sub-trafila de orifícios de 5,5mm; trafila de um orifício de 3,7mm; temperatura na 1ª zona de aquecimento de 40ºC, na 2ª de 80ºC e na 3ª de 120ºC; velocidade de rotação da rosca: 206 RPM (100%); velocidade de alimentação: 90g/min; umidade inicial da amostra: 18,5%.

Os extrusados obtidos foram secos a 50ºC em estufa de secagem com circulação de ar forçado por 24 horas. Após, foram moídos em micro moinho (Marconi-MA630/1) e este

material foi submetido à concentração proteica.

2.3. *Concentração proteica do* FADD *in natura e extrusado*

Durante o processo de concentração proteica do FADD, buscou-se por produto que apresentasse ao menos duas vezes mais proteína bruta que o FADD. Após avalição dos processos de concentração proteica do FADD, considerou-se, factível a metodologia descrita por Loureiro et al. (2019), com modificações.

Primeiramente as amostras foram dispersas individualmente em meio aquoso, na proporção 1:10 (P/V) e misturadas por 5 minutos, usando agitador magnético com aquecimento (60ºC). Após a homogeneização, ajustou-se o pH do meio para 4,5 com NaOH 4N e adicionou-se a enzima amiloglucosidase (AMG 300L) na proporção de 2ml de enzima por litro de solução, permanecendo em incubação por 15 min. Posteriormente, o pH foi elevado para 6,0 com NaOH 4N à temperatura de 60ºC, sendo adicionada a enzima α-amilase (Termamyl 2X) na proporção de 2ml de enzima por litro de solução e a alíquota incubada por 30 min.

Elevou-se o pH para 11,0 com NaOH 4N sendo mantida a temperatura de 60ºC por 30 min para a solubilização da proteína extraída no processo anterior. Na última etapa, o pH do meio foi ajustado para 4,5 com HCl 2N, mantendo-se a temperatura de 60ºC por 30 min, sob agitação constante.

Após a incubação com as enzimas, as amostras foram submetidas a lavagens sequenciais. Primeiramente, as amostras foram filtradas (106 μm); as frações aquosas foram reservadas e as frações retidas na peneira foram homogeneizadas em meio aquoso na proporção 1:10 (P/V (com relação ao peso inicial da amostra), sob temperatura constante de 60ºC durante 20 min.

Após essa etapa, as amostras foram novamente filtradas (106 μm) e a frações retidas na peneira foram homogeneizadas em meio aquoso na proporção 1:5 (P/V) à 60ºC durante 20 min. As frações aquosas (filtrado) foram homogeneizadas na proporção final de 1:25 (P/V) e centrifugados a 2.500 RPM por 10 min.

Os sobrenadantes foram descartados e os centrifugados secos a 50ºC, em estufa de secagem com circulação de ar forçado por 24 horas. Após a secagem foram obtidos os concentrados proteicos: CPFA (obtido através do FADD sem extrusão) e CPFAext (obtido a partir do farelo de arroz extrusado).

2.4. *Rendimento e Composição química*

O rendimento (R%) dos concentrados proteicos foi calculado através da formula:

R%= [massa inicial do produto (g) x massa do concentrado proteico (g)]/ 100 (1)

Matéria seca, material mineral e proteína bruta (determinação do nitrogênio pelo método Micro Kjeldahl - N x 6.25, número 920.87) foram determinados de acordo com metodologias descritas pela AOAC (1995). A gordura residual foi extraída e quantificada por extração à frio (Bligh & Dyer, 1959).

2.4.1. *Determinação de elementos minerais*

As amostras de farelo de arroz foram moídas em micro-moinho e acondicionadas em envelopes plásticos hermeticamente fechados. As amostras foram encaminhadas e as análises dos nutrientes realizadas no Laboratório de Nutrição Mineral de Plantas, Departamento de

Ciência do Solo, Universidade de São Paulo (USP), Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Piracicaba– SP. A metodologia utilizada para análise dos nutrientes das amostras seguiram os padrões sugeridos por Malavolta, Vitti e Oliveira (1989) com uso de digestão nítrico-perclórica e, posterior leitura colorimétrica para P (método metavanadato de amônio) e espectrofotometria de absorção atômica para Ca, K e Mg.

2.4.2. *Determinação de amido disponível e resistente*

Para a determinação das frações do amido disponível (AD) e do amido resistente (AR) foi utilizado o método 996.11 1 AOAC (1995) (Protocolo P100SP), e suas modificações (protocolos P300SP, P100CP, P300CP e PTF), propostas por Walter, Silva e Perdomo (2005).

2.4.3. *Determinação da fibra dietética*

As determinações de fibra total (FT), fibra solúvel (FS) e insolúvel (FI) foram realizadas de acordo com o método enzímico-gravimétrico descrito pelo método 985.29 AOAC (1995).

2.5. *Capacidade de retenção de água e capacidade de retenção do óleo*

A capacidade de retenção de água e a capacidade de retenção de óleo das amostras foram determinadas conforme descrito por McConnell, Eastwood & Mitchell. (1974). Os resultados foram expressos em quantidade de água ou óleo pela amostra (g g^{-1}).

2.6. *Solubilidade proteica*

A solubilidade proteica foi determinada através do método de Morr et al. (1985). A concentração de nitrogênio solúvel foi determinada pelo método de micro-Kjeldahl (AOAC, 1995). A porcentagem de proteína solúvel foi calculada como: [(concentração proteica do sobrenadante (mg/mL) x 50) / (peso da amostra (mg) x concentração de proteína na amostra (%))] x 100.

2.7. *Análises estatísticas*

Os dados foram primeiramente analisados para identificação de *outliers* e submetidos à análise de variância pelo Teste F para comparação de medias, à 5% de probabilidade. Foram aplicados três contrastes ortogonais: 1) FADD *versus* demais grupos experimentais; 2) FADDext *versus* CPFA e CPFAext; 3) CPFA *versus* CPFAext.

3. Resultados e discussão

3.1. *Composição química e elementos minerais*

Os resultados do presente estudo mostram que houve diferença significativa na composição química dos produtos obtidos, em todos os contrastes (Tabela1).

Com relação ao teor de proteína bruta, para o contraste FADD *versus* demais grupos experimentais o CPFA apresentou o maior conteúdo de proteína bruta, seguido do CPFAext.

O processo de extrusão, nas condições realizadas no estudo, reduziu o conteúdo de proteína bruta do FADD, o que influenciou negativamente o processo de concentração proteica. Bertipaglia, de Melo, Sugohara, de Melo & Bertipaglia (2008) observaram redução

na fração proteica de soja e milho após o processo de extrusão à 120ºC. Esse mesmo comportamento foi observado em nosso estudo quando o FADD sofreu processo de extrusão. Essa diminuição na fração proteica pode ser decorrente da oxidação da proteína, ocasionada pela alta temperatura empregada no processo, que provoca modificações químicas nas cadeias laterais dos aminoácidos, tais como desaminação ou carboxilação (Damodaran et al., 2010). Além disso, o pH extremamente baixo do FADD (em torno de 1,5) pode ter influenciado negativamente o conteúdo proteico, como observado por Sørensen, Stjepanovic, Romarheim, Krekling, and Storebakken (2009), que afirma que condições combinadas de extrusão em pH muito baixo podem reduzir o teor de proteína.

No presente estudo, observou-se redução no conteúdo de proteína bruta, bem como na solubilidade proteica para o FADDext (Tabela 3), resultados que confirmam a desnaturação e oxidação proteica. A oxidação pode causar mudanças de conformação na estrutura secundária e terciária das proteínas, induzindo ligações cruzadas adicionais, as quais podem reduzir a solubilidade proteica e o conteúdo de proteína do produto (Estrada et al., 2018).

Como o processo de extrusão diminuiu o conteúdo de proteína bruta do FADD, consequentemente CPFAext apresentou menor conteúdo de proteína bruta em comparação ao concentrado proteico obtido através do farelo *in natura* (CPFA). Desta maneira, o contraste CPFA versus CPFACext confirma o maior conteúdo proteico do CPFA (42,39 ± 0,126 %PB).

Para o conteúdo de lipídios (Tabela 1) o maior teor foi encontrado para o CPFA. A concentração proteica provoca aumento no conteúdo de lipídios devido as interações lipídicas-proteicas e pela formação de lipoproteínas hidrofóbicas (Araújo, 2008).

Para FADDext o aumento do teor de lipídios em comparação ao FADD pode ter sido ocasionado pela alta temperatura durante a extrusão, uma vez que as células de gordura se fundem em gotículas de óleo e rompem a estrutura celular, melhorando assim a velocidade da extração de óleo (Dalbhagat, Mahato & Mishra, 2019). Esse processo ocorre em consequência

da combinação do cisalhamento com a alta pressão, resultando na expulsão de óleo (Sandrin, Caon, Zibetti & de Francisco, 2018).

Em relação às frações de amido, para o amido disponível (Tabela 1) FADD e FADDext apresentaram os maiores teores. Contudo observou-se que o processo de extrusão, nas condições estudadas, não foi capaz de elevar o conteúdo de amido disponível do farelo de arroz.

Para o contraste FADDext *versus* CPFA e CPFACext foi observado maior conteúdo de amido disponível no farelo extrusado, em relação aos concentrados proteicos. Este resultado já era esperado, uma vez que a concentração proteica químico-enzimática foi realizada com enzimas amilolíticas (amiloglucosidase e α- amilase), que agem nas ligações glicosídicas do amido, rompendo-as e acarretando diminuição no conteúdo de amido disponível.

Para o contraste CPFA *versus* CPFAext (Tabela 1), observou-se que o concentrado proteico obtido através do FADD apresentou menor conteúdo de amido disponível (4.79%) quando comparado ao concentrado proteico que foi obtido a partir do farelo extrusado (11.67%). Esse resultado demonstra que as combinações do processo de extrusão e da concentração proteica não agiram da forma esperada.

Durante o processo de extrusão observa-se maior solubilidade do amido, devido à degradação dos grânulos no decorrer do cozimento do produto (Gui, Gil & Ryu, 2012). Dessa forma, esperava-se que a combinação da extrusão com o processo químico enzimático de concentração proteica fosse capaz de diminuir o conteúdo de amido disponível do produto final. No entanto, outros fatores podem levar à degradação das moléculas de amido, como a velocidade de rotação do parafuso da extrusora, a qual possui relação direta com a força de cisalhamento gerada sobre os extrusados (Mościcki et al., 2012), melhorando assim a disponibilidade do amido.

Para o amido resistente, apenas o contraste FADDext *versus* CPFA and CPFAext (Tabela 1) não apresentou diferença significativa. Enquanto que para os outros contrastes foi observado diferença significativa, pelo aumento na quantidade de amido resistente do CPFA. Esse aumento pode ter ocorrido devido à maior concentração de amido resistente por peso total do produto, sendo inversamente proporcional ao conteúdo de amido disponível.

O amido resistente é a fração do amido que não irá fornecer glicose ao organismo, mas será fermentada no intestino grosso produzindo gases e ácidos graxos de cadeia curta. Por essa característica em particular, acredita-se que os efeitos do amido resistente possam ser comparáveis aos da fibra alimentar sendo normalmente considerado como um componente desta (Champ & Faisant, 1996).

Após o tratamento térmico e/ou de umidade, ocorre o rompimento e a gelatinização da estrutura do grânulo de amido. Quando esse gel esfria e retrograda o amido forma uma estrutura cristalina, insolúvel e resistente à ação enzimática (Walter, da Silva, & Perdomo, 2005). Nesse estudo, o processo de concentração proteica visou a dispersão da amostra em meio aquoso a elevadas temperaturas (45 à 60°C) o que pode ter acarretado em uma gelatinização incompleta e cristalização do amido, aumentando o conteúdo de amido resistente no CPFA.

Para obtenção do concentrado proteico desse estudo, inúmeros testes de combinação de pH, temperatura e tempo de incubação das enzimas foram realizados. Os melhores resultados foram obtidos com a utilização da amiloglucosidase e da amilase, respectivamente. A combinação dessas duas enzimas traz a vantagem da exoatividade da amiloglucosidase, que é capaz de perfurar poros profundos e estreitos nas moléculas do amido, bem como a endoatividade da α- amilase, que permite o alargamento dos poros das moléculas (Shariffa, Karim, Fazilah & Zaidul, 2009).

A amiloglucosidase desempenha um importante papel na hidrólise do amido *in natura*

por ter a capacidade de hidrolisar tanto ligações α-1,4 como α-1,6 (Norouzian, Akbarzadeh, Scharer, & Moo, 2006). Na parte inicial da hidrólise, as ações catalíticas das enzimas podem possibilitar a desintegração física da estrutura e, consequentemente, expor novos sítios suscetíveis à ação dessas duas enzimas (Robertson et al., 2006).

Autores já relatam que modificações adicionais na ordem de adição das enzimas podem aumentar a eficiência da hidrólise final do amido nativo (Yan & Zhengbiao, 2010). Em nosso estudo, a modificação na ordem de adição das enzimas possibilitou maior extração de proteína para obtenção do concentrado proteico, quando comparados ao método químico-enzimático anteriormente utilizado por Loureiro et al., (2019), sendo apresentado o melhor em conteúdo proteico e rendimento.

A concentração proteica e a extrusão alteraram o conteúdo de todas as frações de fibra alimentar ($P < 0,05$). O FADD apresentou o maior conteúdo de fibra insolúvel e menor conteúdo de fibra solúvel. Não foi encontrada diferença significativa na fibra total para o contraste FADDext *versus* CPFA and CPFAext (Tabela 1). O aumento de fibra total e fibra solúvel observado no CPFA podem ser decorrentes da ação enzimática durante o processo de concentração proteica. Hanmoungjai, Pyle & Niranjan (2001) também observaram maior conteúdo de fibra total em farelo de arroz submetido à concentração da proteína através de processo enzimático.

Nos contrastes CPFA *versus* CPFAext (Tabela1), foram observados aumento da fibra solúvel e a diminuição do conteúdo de fibra insolúvel. Analisando FADD pós-processamento, foi possível observar que CPFA apresentou os maiores conteúdos de fibra total e fibra solúvel. Já os menores conteúdos dessas frações foram encontrados nas amostras CPFAext e FADDext, respectivamente.

O aumento de fibra solúvel nas amostras extrusadas ocorreu devido à conversão da fibra insolúvel em fibra solúvel, a qual foi induzida devido à cocção e temperatura

empregadas no processo de extrusão (120ºC). Através da quebra das ligações covalentes e não covalentes que ocorre entre os carboidratos e as proteínas ligadas a fibra durante a extrusão, partículas menores e mais solúveis são geradas (Dang & Vasanthan, 2019). Resultados semelhantes foram observados no processo de extrusão para farelo de trigo (Andersson, Andersson, Jonsäll, Andersson & Fredriksson, 2017; Rashid, Rakha, Anjum, Ahmed & Sohail, 2015; Yan, Ye & Chen, 2015), farelo de aveia (Zhang, Bai, & Zhang, 2011) e farelo de arroz (Dang & Vasanthan, 2019).

Em relação ao conteúdo de matéria mineral (Tabela 2), o FADD apresentou menor percentual que os demais produtos analisados, demonstrando que tanto o processo de extrusão do farelo quanto a concentração proteica pela metodologia químico-enzimática elevam o conteúdo mineral do FADD. Sharma, Chauhan e Kuldeep Agrawal (2004) também encontraram aumento no conteúdo de matéria mineral para o farelo de arroz extrusado, em comparação ao farelo de arroz *in natura*. No entanto, os autores alegam que esse aumento ocorreu devido aos minerais contidos na água utilizada no processo de extrusão.

Resultados semelhantes foram relatados por autores que demonstraram que a extrusão (Ferreira & Arêas, 2010) e a concentração proteica (Gailord et al., 2010) elevaram o conteúdo de alguns macro elementos principalmente pela diminuição de fatores antinutricionais, como fibras e polissacarídeos não amiláceos.

Em nosso estudo também foi possível observar diferenças significativas no conteúdo dos macro elementos analisados (Tabela 2) em todos os contrastes avaliados ($P<0,05$). Desta forma, os resultados demonstram que a extrusão e a concentração proteica possuem a capacidade de alterar o conteúdo de P, Ca, K e Mg presentes no farelo de arroz.

Observou-se que a extrusão do FADD aumentou o conteúdo de P, Ca e K, contudo, houve diminuição no conteúdo de Mg. Por outro lado observou-se que a concentração proteica elevou o conteúdo de matéria mineral e diminuiu os macro elementos avaliados,

sendo essa diminuição mais proeminente para o CPFA (Tabela 2). O aumento da matéria mineral foi, possivelmente ocasionada, pelo aumento dos demais minerais presentes no FADD e não analisados (Fe, Zn, Mn, Cu, Na e S) no estudo. Modesti (2006) também observou comportamento semelhante em concentrados proteicos de folhas de mandioca (obtido por métodos físicos e químicos), onde os concentrados proteicos apresentaram redução de P, Ca, K e Mg e aumento para Fe, Cu e S.

3.2. *Análises tecnológicas*

Na Tabela 3 estão representados os resultados da capacidade de hidratação e capacidade de ligação à gordura. Não foram encontradas diferenças para capacidade de hidratação em nenhum dos contrastes avaliados. No entanto, para capacidade de ligação ao óleo houve diferenças significativas para os três contrastes avaliados ($P<0,05$). A maior capacidade de ligação ao óleo foi observada na amostra FADD. Esse resultado sugere a presença de uma grande quantidade de grupos hidrofóbicos em relação aos grupos hidrofílicos presentes na estrutura primária das proteínas (Subagio, 2006).

No entanto, a menor capacidade de ligação ao óleo foi observada para CPFAext, fato que pode estar relacionado à variação de absorção de óleo conforme o número de grupos hidrofóbicos (aminoácidos apolares) expostos na proteína (Dench, Rivas e Caygill, 1981), os quais estão geralmente localizados internamente, dificultando a capacidade de ligarem-se com a gordura (Lovatto et al., 2017). Resultado semelhante foi observado em resíduo de soja, onde a baixa capacidade de ligação ao óleo ocorreu pela alteração da estrutura da amostra, ocasionada por alta pressão de inchamento gerada pela extrusão e, posterior, tratamento enzimático (Qu et al., 2017)

Foi observado que a extrusão do farelo de arroz diminuiu a solubilidade proteica. O

processo de extrusão ocasiona desnaturação proteica devido ao calor empregado no produto, a qual acarreta na desestabilização das estruturas secundárias, terciárias ou quaternárias das proteínas (Haque, Aldred, Chen, Barrow & Adhikari, 2013). Além disso, pH, solventes orgânicos miscíveis em água e alguns solutos, podem levar à desnaturação proteica

A classificação mais utilizada para proteínas vegetais se dá pela solubilidade proteica. As proteínas são definidas como solúveis em água (albuminas), solúveis em soluções salinas (globulinas), solúveis em etanol (prolaminas), solúveis em soluções ácidas (glutelinas ácidas), e solúveis em soluções alcalinas (glutelinas básicas) (Osborne, 1924). É sabido que a proteína do farelo de arroz é composta por 60% de albumina, 27% de glutelina e prolamina e ainda 7% de globulina (Juliano, 1993). O conhecimento dos grupos proteicos auxilia na compreensão dos processos de extração e concentração proteica.

Em nosso estudo, observou-se que a combinação da concentração proteica e da extrusão, foi capaz de diminuir ainda mais a solubilidade proteica, uma vez que para o contraste CPFA *versus* CPFAext foi observada uma diminuição de 49,48% na solubilidade da proteína do CPFACext, quando comparado ao concentrado proteico obtido pelo farelo de arroz não extrusado.

Nesse trabalho, a avaliação da solubilidade proteica foi realizada sem testar diferentes faixas de pH, ou seja, a resposta sobre a solubilidade proteica está relacionada somente ao pH de cada produto. Os farelos de arroz na forma *in natura* ou extrusada (FADD e FADDext, respectivamente) apresentaram pH em torno de 1,5 e os concentrados proteico (CPFA e CPFAext) apresentaram-se em pH 4.5.

Essas informações possuem grande importância, uma vez que é possível observar que tanto o FADD quanto FADDext apresentaram maior solubilidade proteica do que seus concentrados proteicos (Tabela 3). Este resultado demonstra que em pHs extremamente baixos (em torno de 1,5) há melhor solubilidade da proteína do que em pHs mais elevados,

como o que ocorre na concentração proteica (pH final de 4,5). Resultados semelhantes foram encontrados por Rafe & Sadeghian, (2017) em farelo de arroz extrusado, onde a menor solubilidade proteica foi observada em pH 4,0.

Os resultados obtidos para solubilidade proteica estão de acordo com os resultados de Bedin, Netto, Bragagnolo e Taranto (2020), onde a menor solubilidade proteica foi encontrada em pH 5,0, independente do método de extração proteica avaliado no estudo (ultrassom, micro-ondas e extração alcalina). A redução da solubilidade proteica observada no CPFA e CPFACext já era esperada, uma vez que a maioria das proteínas apresenta menor solubilidade proteica no ponto isoelétrico (4,7), local no qual a quantidade de cargas elétricas das proteínas se iguala e não se repelem, obtendo-se uma carga líquida igual a zero (Phongthai, Limb & Rawdkuena, 2016). As interações hidrofóbicas tem a função de auxiliar as interações de proteína-proteína, resultando na diminuição da solubilidade, as quais disponibilizam condições favoráveis para a formação de agregados proteicos com grande diâmetro e alta densidade (Bedin, 2018).

As proteínas possuem papel muito importante nas propriedades estruturais e funcionais do farelo de arroz. A solubilidade das proteínas apresenta-se como um pré-requisito relevante para a funcionalidade das proteínas alimentares, além de ser um índice de potencial para uso de aplicações proteicas (Rafe & Sadeghian, 2017).

4. Conclusão

A extrusão do farelo de arroz desengordurado e desfitinizado, na condição estudada, não promoveu aumento na extração da proteína pelo processo de concentração proteica utilizado, quando comparado ao conteúdo proteico do CPFA.

O uso de processo químico enzimático, com adição das enzimas amiloglucosidade e α-

amilase, respectivamente, possibilita a concentração proteica do farelo de arroz na forma *in natura* e extrusada.

O concentrado proteico obtido a partir do farelo de arroz desengordurado e desfitinizado extrusado, possibilita a obtenção de produto com 33,46% de proteína bruta, maior conteúdo de macro elementos analisados (P, Ca, K e Mg), amido disponível e resistente e menor teor de fibra total.

A concentração proteica do farelo de arroz desengordurado e desfitinizado na forma *in natura* permite obtenção de um concentrado proteico com maior teor de proteína bruta (42,30%), lipídeos, fibra solúvel e proteína solúvel.

Sendo assim, considera-se que ambos concentrados proteicos apresentam potencial para serem utilizados como ingredientes proteicos funcionais.

Declaração de conflitos de interesse

O artigo é o trabalho original dos autores. Os autores declaram que não há conflitos de interesses financeiros ou pessoais que possam parecer influenciar o trabalho relatado neste artigo.

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Tabela 1

Composição química do farelo de arroz desengordurado e desfitinizado *in natura*, extrusado e de seus concentrados proteicos.

Fontes de variação	Materia Seca	Protein Bruta	Lipidios	Amido Disponível	Amido Resistente	Fibra Total	Fibra Insolúvel	Fibra Solúvel
	g/100g							
	.....FADD *versus* demais grupos experimentais.....							
FADD	94,48 ± 0,07	20,59± 0,02	2,67± 0,11	29,27 ± 0,10	1,14 ± 0,247	36,73± 0,67	35,18 ± 0,28	1,55 ± 0,39
FADDext	94,05 ± 0,06	18,04 ± 0,16	3,59 ± 0,04	27,64 ± 0,23	2,48 ± 0,202	32,83± 0,26	28,44 ± 0,20	4,38 ± 0,49
CPFA	91,75 ± 0,01	42,39 ± 0,13	4,77± 0,08	4,79 ± 0,30	1,27 ± 0,612	38,85± 0,56	30,43 ± 0,26	8,42 ± 0,30
CPFAext	94,57 ± 0,11	33,46 ± 0,88	3,91± 0,05	11,67 ± 3,03	1,46 ± 0,087	25,41± 0,06	19,37 ± 1,87	6,04 ± 1,86
Teste F	*	*	*	*	*	*	*	*
	..... FADDext *versus* CPFA and CPFACext.....							
Teste F	*	*	*	*	[b]NS	NS	*	*
	..... CPFA *versus* CPFA ext.....							
Teste F	*	*	*	*	*	*	*	*

Médias ± desvio padrão (n=3). Asterisco (*) representa diferenças significativas P < 0,05. FADD: Farelo de arroz desengordurado e desfitinizado; FADDext: Farelo de arroz desengordurado e desfitinizado que passou por processo de extrusão; CPFA: Concentrado proteico de farelo de arroz desengordurado e desfitinizado; CPFAext: Concentrado proteico de Farelo de arroz desengordurado e desfitinizado que passou por processo de extrusão.
NS: Não significativo.

Tabela 2

Matéria mineral e macro elementos do farelo de arroz desengordurado e desfitinizado *in natura*, extrusado e de seus concentrados proteicos.

Fontes de Variação[a]	Matéria Mineral	Fósforo	Cálcio	Potássio	Magnésio
			g/100g		
	.....FADD *versus* demais grupos experimentais.....				
FADD	5,45 ± 0,029	0,968	0,017	0,504	0,312
FADDext	5,77 ± 0,15	0,981	0,029	0,588	0,291
CPFA	5,90 ± 0,010	0,426	0,019	0,168	0,061
CPFAext	5,63 ± 0,010	0,718	0,080	0,392	0,138
Teste F	*	*	*	*	*
	..... FADDext versus CPFA and CPFACext……..				
Teste F	*	*	*	*	*
	..... CPFA *versus* CPFAext.....				
Teste F	*	*	*	*	*

Médias ± desvio padrão (n=3). Asterisco (*) representa diferenças significativas P < 0,05.
FADD: Farelo de arroz desengordurado e desfitinizado; FADDext: Farelo de arroz desengordurado e desfitinizado que passou por processo de extrusão; CPFA: Concentrado proteico de farelo de arroz desengordurado e desfitinizado; CPFAext: Concentrado proteico de Farelo de arroz desengordurado e desfitinizado que passou por processo de extrusão.

Tabela 3

Análises Funcionais do farelo de arroz desengordurado e desfitinizado *in natura*, extrusado e de seus concentrados proteicos.

Fontes de variação	C. Hidratação	C. Ligação ao óleo g/100g	Solubilidade proteica
	..... FADD *versus* demais grupos experimentais.....		
FADD	2,823 ± 0,027	2,342 ± 0,051	12,650 ± 3,41
FADDext	3,002 ± 0,010	2,131 ± 0,043	11,899 ± 0,023
CPFA	2,496 ± 0,285	1,912 ± 0,105	8,326 ±0,475
CPFAext	2,693 ± 0,456	1,729 ± 0,109	4,120 ±1,351
Teste F	NS[b]	*	NS
	..... FADDext versus CPFA and CPFACext.....		
Teste F	NS	*	*
	..... CPFA *versus* CPFAext.....		
Teste F	NS	*	*

Médias ± desvio padrão (n=3) Asterisco (*) representa diferenças significativas P < 0,05.
FADD: Farelo de arroz desengordurado e desfitinizado; FADDext: Farelo de arroz desengordurado e desfitinizado que passou por processo de extrusão; CPFA: Concentrado proteico de farelo de arroz desengordurado e desfitinizado; CPFAext: Concentrado proteico de Farelo de arroz desengordurado e desfitinizado que passou por processo de extrusão. NS: Não significativo.

3 ARTIGO 2

Artigo científico intitulado “Protein concentrate from rice bran residue in diets for silver catfish (*Rhamdia quelen*): effects on growth, biochemical parameters and activity of digestive enzymes” à ser submetido para a revista Animal Feed Science and Technology e está formatado segundo as normas descritas no Guia dos Autores (Anexo B).

Protein concentrate from rice bran residue in diets for silver catfish (*Rhamdia quelen*): effects on growth, biochemical parameters and activity of digestive enzymes

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Abstract

The present study aimed to evaluate the use of de-oiled and de-phytinized rice bran protein concentrate (DRBPC) in diets for silver catfish (*Rhamdia quelen*), as a partial substitute for protein derived from fishmeal (FM) and its effects on performance, activity of digestive enzymes, blood and liver metabolism. DRBPC was included to replace the following levels of crude protein from fishmeal in diets: (25, 35, 45 and 55%). Then five isoproteic and isocaloric diets were elaborated with three repetitions each. A total of 300 juveniles of silver catfish with an initial body weight of 10.28 ± 0.19 g were distributed into 15 polyethylene tanks (20 animals per tank) of 100-Liter each, connected to a thermo regulated water recirculation system. The animals were fed the experimental diets, for a period of 45 days, three times a day (9:00 a.m., 1:00 p.m. and 5:00 p.m.) until apparent satiety. The data were subjected to analysis of variance (ANOVA) and means were compared to the Tukey's Test ($p < 0.05$). At the end of the experimental study, it was observed that the inclusion of DRBPC from 45% reduced the weight gain, the specific growth rate and the relative weight gain. The decrease of total protein deposited was verified from the 35% inclusion of DRBPC in the animals' diet. The highest activity of the trypsin enzyme was observed in diet 35% of inclusion of DRBPC. No differences were observed for feed conversion rate, digestive somatic index, intestinal quotient, hepatic somatic index, protein efficiency rate, plasma biochemistry and hepatic parameters in fish fed different level of DRBPC. In view of these results, inclusion of DRBPC in up to 35% in the diet did not negatively affect growth performance, parameters plasmatic and liver, nutrient deposition, somatic parameters and digestive enzyme activity in silver catfish (*Rhamdia quelen*).

Keywords: plant protein concentrate, silver catfish, zootechnical performance, fishmeal.

1. Introduction

Annually, the global food waste is quantified at about 1.5 billion tons (Makkar, 2017), almost one third of the world production of food intended for human consumption (Thieme and Makkar, 2017). The same authors report that food waste negatively impacts the $ 750 billion economy, in addition to social and environmental impacts. These residues come from agro-industrial processing, such as leaves, bran, pies and crude oil. Their use is necessary, to avoid waste and favor the processing of food (Costa Filho et al., 2017).

A major challenge in animal production is the need for sustainable protein sources for feed (Hinchcliffe et al., 2019). Plant based protein have been the main protein sources due to the relatively low price and large production (Xie et al., 2016). On the other hand, plant ingredients have the anti-nutritional factors (ANFs) and have less palatability due to the high levels of non-soluble carbohydrates such as fiber and resistant starch (Daniel, 2018).

Modern aquaculture requires a reduction animal protein in the diet (Allam, et al., 2020) which can be used directly in the human diet. Actually, in aquafeeds the fishmeal is considered as the source of higher quality protein. Alternative source of protein can minimize the dependency on fishmeal by replacing as the main source of protein (Moniruzzaman et al., 2018). Previous studies have shown that there are several plant residues that have the potential to be included in the fish diet (Loureiro et al., 2019; Lovatto et al., 2018; Mo et al., 2016; Choi et al., 2016), as potential substitutes for fishmeal.

It is believed that the use of locally sourced food resources and low-cost protein sources are of great importance in aquafeeds industry, in order to minimize production costs (Güroy et al., 2013; Hardy, 2010). Rice is a cereal with its production widely spread throughout the world, being used as an energy source for 50% of the population. Brazil is the largest cereal producer outside the Asian continent, ranking ninth in the ranking (FAO, 2018).

Rice bran is the major by product generated during milling, which is further extracted for oil. The defatted residues of bran contain 15.4% protein (Hamada, 2000). By reducing the fiber content, the amino acid level and crude protein level also increased in the raw material (Palmegiano et al., 2006).

From the perspective of fish feed manufacturing, the production of omnivorous fish is a trend in aquaculture, due to the better acceptance of diets with different terrestrial ingredients, as well as the ease of food handling and acceptance of feed pellets. The silver catfish (*Rhamdia quelen*) is an omnivorous species with a high dietary protein requirement (Salhi et al., 2004). Due to the omnivorous habit and depending on availability, the species has the capacity to feed on a wide variety of foods (Gomiero et al., 2007).

Based on the above, the main objective of this study was to evaluate the use of de-oiled and de-phytinized rice bran protein concentrate (DRBPC) as a potential substitute for fish meal in the silver catfish (*Rhamdia quelen*) diet based on performance results of growth, blood and liver metabolism as well as digestive enzyme activity.

2. Material and methods

2.1. Preparation of rice bran protein concentrate

The protein concentration methodology was developed at the Laboratory of Fisheries, Federal University of Santa Maria (UFSM) and consisted of a chemical–enzymatic process. De-oiled and de-phytinized rice bran (DDRB) was obtained from the protein concentrate provided by INGAL- Indústria Gaúcha de Alimentos Ltda, Brazil. The DDRB is residue generated after extracting phytic acid for biofertilizer production.

For protein concentrate process, the DDRB were individually dispersed in an aqueous

medium, in the proportion 1:10 (W / V) and mixed for 5 minutes, using a magnetic stirrer with heating at 60ºC. After homogenization, the pH of the sample was raised to 4.5 with 4N NaOH and the 2 ml/L^{-1} enzyme amyloglucosidase (AMG 300L) was added, remaining in incubation for 15 min. Subsequently, the pH was increased to 6.0 with 4N NaOH at a temperature of 60ºC, with the enzyme α-amylase (Termamyl 2X) being added (2 ml/L^{-1}) and the aliquot incubated for 30 min.

The pH was raised to 11.0 with 4N NaOH being maintained at a temperature of 60ºC for 30 min for the solubilization of the protein extracted in the previous process. In the last step, the pH of the medium was adjusted to 4.5 with 2N HCl, maintaining a temperature of 60ºC for 30 min, under constant agitation. After incubation with the enzymes, the samples were subjected to sequential washes performed as described below: The samples were filtered (106 μm sieves) and the aqueous fractions were reserved. The fractions retained in the sieve were homogenized in an aqueous medium in the proportion 1:10 (W / V (in relation to the initial weight of the sample), under a constant temperature of 60ºC for 20 min.

After this stage, the samples were filtered again and the fractions retained in the sieve were homogenized in an aqueous medium in the proportion 1: 5 (W / V) at 60ºC for 20 min. The aqueous fractions (filtrate) were homogenized in the final ratio of 1:25 (P / V) and centrifuged at 2,500 rpm for 10 min. The supernatants were discarded and the centrifuges dried at 50ºC, in a drying oven with forced air circulation for 24 hours. After drying, the protein concentrates called de-oiled and de-phytinized rice bran protein concentrate DRBPC were obtained.

2.2. Diet preparation

The experimental diets were formulated to replace different levels of crude protein from fishmeal with crude protein from rice bran protein concentrate. Five isonitrogenous (370 g/kg crude protein) and isocaloric (13.4 MJ/kg) diets were formulated. The levels assessed were:

- 0DRBPC: Control diet, without inclusion of DRBPC. Fish meal (55,3%) and soy protein concentrate (60%) as protein sources in the diet.
- 25DRBPC: 250 g/kg crude protein from fishmeal was replaced by the DRBPC.
- 35DRBPC: 350 /kg crude protein from fishmeal was replaced by the DRBPC.
- 45DRBPC: 450 g/kg crude protein from fishmeal was replaced by the DRBPC.
- 55DRBPC: 550 g/kg crude protein from fishmeal was replaced by the DRBPC.

The experimental diets were prepared according to the crude protein requirements for silver catfish established by Meyer and Fracalossi (2004). The ingredients were ground, weighed and then manually mixed until homogeneous, then water was added and diets were extruded in an EX-MICRO Lab Micro extruder (Model Extrusora EX Laboratório, Exteec Máquinas, Campinas, Brazil), with production capacity of 15 kg of feed per hour. The extruded diets (4 mm) was dried in forced air stove for 24 hours at 50°C and stored at-18°C. Nutrient composition, formulation and amino acid profiles of diets are presented in Table 1.

151 *2.3. Fish, experimental condition and feeding*

152

153 The biological assay was conducted at the Laboratory of Fisheries of the Department
154 of Animal Science, Federal University of Santa Maria (UFSM)—RS, Brazil, after being
155 approved by UFSM's Ethics Committee on Animal Trials, process nº 9387290319.

156 The biological assay was performed in a water recirculating system consisting of two
157 biological filters with gravel, backwash system, UV filter sterilizer (GreenFreeTMUV-2
158 18W) and controlled temperature. The fishes were evenly distributed (20 animals per tank)
159 into 15 polyetilene tanks (100-Liter) with individual water inlets and outlets. Was utilized a
160 total of 300 silver catfish with initial mean weight of 10.28 ± 0.19 g and a length of
161 10.61 ± 0.09 cm.

162 The animals were conditioned to diets and to the experimental system for fifteen days
163 prior to experiment. During 45 days of the study, the fish were fed to apparent satiety, three
164 times a day (9:00 a.m., 1:30 p.m. and 5:00 p.m.).

165 The tanks were cleaned twice daily (8 a.m. and 3 p.m. for 45 days) to remove faeces.
166 During the experimental period, the water quality parameters were monitored by a
167 colorimetric kit (Alfakit®). These parameters are as follows: temperature of 24.97 ± 1.72°C,
168 dissolved oxygen: 6.44 ± 0.58; pH: 7.37 ± 0.22; total ammonia: 0.18 ± 0.08 mg/L; nitrite:
169 0.01 ± 0.01 mg/L; alkalinity: 43.330 ± 6.83 mg $CaCO_3$ L^{-1}; and hardness: 42.50 ± 7.58 mg
170 $CaCO_3$ L^{-1}. According to Baldisserotto and Silva (2004), these parameters are within the
171 optimum range for silver catfish *R. quelen* culture.

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176 *2.4 Sample collection and analytical methods*

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178 In the early and late 45-day experimental period, biometrics was performed to collect
179 individual data of the animals, all of which had fasted for 24 h and were anesthetized with
180 benzocaine (100 mg L^{-1}). Body weight (g) and total length of each animal was collected to
181 estimate the following:

182 Feed conversion rate (FCR): Total feed consumption (g) / [final fish weight (g) –
183 initial fish weight (g)]; Specific growth rate (SGR, % day): [(ln final body weight – ln initial
184 body weight)/period] x 100; Relative weight gain (% RWG): 100 x [final body weight (g) –
185 initial body weight (g)]/ initial body weight (g).

186 Three fish were randomly selected from each tank (nine animals per experimental
187 diet), and euthanized by overdose of benzocaine (250 mg L^{-1}) in accordance with the
188 American Veterinary Medical Association (AVMA, 2013) to determine protein retention:
189 Protein efficiency ratio (PER): Body weight gain (g) / protein intake (g); Total protein
190 deposited (TPD, g): [final body weight × (% final body protein / 100)] – [initial body weight
191 × (% initial body protein / 100)].

192 For calculation of PER, crude protein was determined by the micro-Kjeldahl method
193 (method 960.52) using the NX6.25 factor (AOAC, 1995)).

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195 *2.5. Plasma biochemistry, hepatic and digestive parameters assay*

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197 At the end of the experimental period, six fish of each experimental diet were
198 captured for analysis of plasmatic parameters. The animals fasted for 24 h. Blood samples
199 was quickly collected from the caudal vein using heparinized syringes. After the blood
200 sample collection, the fish were euthanized by overdose of benzocaine (250 mg L^{1})

(AVMA, 2013). Subsequently, the animals were eviscerated and the liver for removed to calculate the hepatosomatic index (HSI%) = (weight of the liver/ weight of the whole fish) x 100. The liver samples were frozen at -20 ° C for analysis of biochemical parameters.

The blood placed in refrigerated centrifuge tubes for plasma separation by centrifugation (1000 g, 10 min at room temperature). The plasma was stored and refrigerated (-20 °C) for analyzes of the albumin, total proteins, triglycerides and cholesterol, quantified by colorimetric commercial kit (Doles®, Doles Reagents and Laboratory Equipment Ltda., Goiania, State of Goias, Brazil).

To hepatic protein analysis, the samples were heated at 60 ºC with KOH and centrifuged (1000xg for 10 min). Supernatant was used to determine the total protein level according to the method described by Bradford (1976), using bovine serum albumin as standard.

To measure hepatic amino acids and Alanine aminotransferase (ALAT), liver samples were mechanically disrupted by adding 1 mL phosphate buffer 20 mM, pH 7.5 and the homogenate was centrifuged at 1000x*g* for 10 min. The neutral supernatant extract was used for amino acid colorimetric determination according to Spies (1957), using ninhydrin 1.5% in isopropyl alcohol as the color reagent. This neutral extract was used to measure alanine aminotransferase (ALAT) (EC 2.6.1.2). The enzymes were determined by using colorimetric procedures following the protocols described in the kits (Doles Reagents and Laboratory Equipment Ltda. Goiania, Goiás, Brazil). ALAT concentration was expressed as UI/mg tissue.

To quantify the hepatic ammonia, tissue samples were homogenized by adding 10% TCA and centrifuged (1000g for 10 min) for protein flocculation. Hepatic ammonia was measured according to the technique described by Verdouw et al. (1978) protocol after ammonia reaction with phenol and hypochlorite forming a blue-colored indophenol

compound.

In the analysis of digestive enzymes a 10 cm portion of the anterior intestine of fish was removed a frozen (-20º C) for assessing the trypsin and chymotrypsin digestive enzymes. Each portion was dissected into Petri dishes containing saline (0.9 % NaCl), to remove any remaining intestinal contents and then homogenized. The homogenization was performed with buffer (0.02 M Tris / 0.01 M phosphate, pH 7.5 in 50% glycerol) at 1:20 tissue: buffer ratio. We used Turrax tissue homogenizer (Marconi, Brazil, MA 102). The homogenates were centrifuged at 1200x g for 10 minutes and supernatants were used as source of enzymes.

Trypsin activity (E.C.3.4.21.4) was analyzed with α-*p*-toluenosulphonyl-L-arginine methyl ester hydrochloride (TAME) as substrate. The extracts were incubated for two minutes (25 ° C) in 2 ml of buffer (0.2 M Tris / 0.01 M $CaCl_2$), pH 8.1. Chymotrypsin activity (E.C.3.4.21.1) was analyzed with benzoyl tyrosine ethyl ester (BTEE) as substrate. Crude extracts were incubated for two minutes in one ml of buffer (0.1 M Tris / 0.1 M $CaCl_2$), pH 7.8. Both trypsin and chymotrypsin activities were assayed in duplicates and the enzymatic activities were read at 247 and 256 nm, respectively, according to protocols described by Hummel (1959). One unit of enzyme was defined as the amount of enzyme required to hydrolyze one µmol of substrate (TAME or BTEE) / min / mg of protein.

2.6. Statistical analysis

The data were checked for outlier existence. Statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA). The experimental design was completely randomized with five treatments and three replications. The data were subjected to analysis of variance (ANOVA) and means were compared to the Tukey's Test ($p < 0.05$).

3. Results

The use of DRBPC to replace fishmeal negatively influenced the animals' performance (Table 2). The final weight was reduced according to the degree of substitution of the protein source, being lower in those fishes who received the diet with 55% rice bran protein concentrate. For SGR and RWG the behavior was similar, but without significant difference between treatments 0DRBPC, 25DRBPC and 35DRBPC. The FCR did not differ significantly between treatments.

No statistical differences were observed for somatic index - DSI, IQ and HSI (Table 3) of fish that received diets with different levels of inclusion of DRBPC. For Total Protein Deposited-TPD (Figure 1A) Fish that received diets with 0DRBPC and 25DRBPC protein in place of FM fishmeal protein showed higher TPD content, followed by levels 35DRBPC, 45DRBPC and 55DRBPC. The results had a linear behavior. It is observed that the higher the level of replacement of fishmeal protein by DRBPC protein, the lower the deposition of protein in the carcass. However, no significant differences were observed for protein efficiency ratio - PER ($p = 0.157$) for the inclusion levels of the DRBPC evaluated (Figure 1B).

The inclusion of different levels of DRBPC in the diets did not result in significant differences in the plasma analyzes (albumin, total proteins, triglycerides and cholesterol) and liver (protein, free amino acids, ammonia and ALAT) performed at the end of the experimental period (Table 4).

Regarding the activity of digestive enzymes, it was observed that the inclusion of DRBPC in the diets caused significant changes ($P < 0.05$) for trypsin (Figure 2A). Increase ($P < 0.05$) activity of the trypsin enzyme was observed in fish fed with the 35DRBPC diet. For chymotrypsin, none significant difference ($P < 0.05$).

For ratio trypsin:chymotrypsin (Tr:Ch), an increase was observed for the 35% DRBPC diet ($P < 0.05$).

4 Discussion

The decrease in the performance of the animals as the protein substitution of fishmeal from protein of DRBPC increases can be caused by the low availability of nutrients from the diets offered, a fact also observed by Wu et al., (2000). With our results, it was possible to observe an inverse relationship between the growth of animals and the increase in partial replacement of fishmeal by DRBPC.

This explanation is in line with the results obtained by Güroy et al. (2013), Cai et al. (2018) and Abasubong et al. (2019), which support the hypothesis that the low growth performance of fish fed diets containing rice protein concentrate can be attributed to the availability of amino acids that compound this ingredient..

This decrease in animal performance has also been observed in other studies using vegetable protein sources as a substitute for fish meal in fish nutrition as corn protein concentrate for *Oreochromis niloticus* (Khalifa et al., 2018) and *Oreochromis* sp. (Ng et al., 2019), fermented cotton flour for *Acanthopagrus schlegelii* (Sun et al., 2015;), amaranth leaf protein concentrate for *Oreochromis niloticus* (Ngugi et al.,2017), rice protein concentrate for *Megalobrama amblycephala* (Cai et al., 2018; Abasubong et al., 2019), soy protein concentrate for *Platichthys stellatus* (Li et al., 2015), and *Acanthopagrus schlegelii* (Kalhoro et al., 2018) . These authors reported that the lowest performance indexes were observed in fish fed with partial or total inclusion of the vegetable protein source.

Possibly, the fiber content found in the CPFA influenced the availability of dietary amino acids, which affected fish performance. Whereas the ratio of fiber content in the diet

301 can influence the digestibility and absorption of nutrients (carbohydrates, proteins and lipids),
302 compromising the metabolism, digestion and zootechnical parameters of a given species
303 (Souza, 2016). According to Rodrigues et al., (2010), this influence of fibers can act on the
304 motility and time of gastrointestinal transit of food, changing the speed and time of gastric
305 emptying. In our study, a large difference was observed in the total dietary fiber content of the
306 diets, with variations from 5.9 to 17% (Table 1), which was influenced by the fiber content of
307 the DRBPC (38.85%) as the level of inclusion of the diet.

308 In the previous study carried out by our research group, for the first time de-oiled and
309 de-phytinized rice bran protein concentrate (26.8% of crude protein) was used to replace
310 fishmeal. That same study demonstrated that the 25% substitution level does not cause
311 changes in the animals' performance (Loureiro et al., 2019).

312 In the current study, either DRBPC was obtained from a different process. A protein
313 concentrate with a higher percentage of crude protein (42.3%) and consequently less
314 carbohydrate content was obtained and applied in fish diets. In this way, fishmeal replacement
315 levels greater than 25% were tested, in order to enhance the use of DRBPC. However, in the
316 current study, protein substitution levels of fishmeal above 35% demonstrated a decrease in
317 the animals' performance. Similar values observed by Ng et al. (2019) to replace fishmeal
318 with zea mays protein concentrate in diets for hybrids of red Tilápia (*Oreochromis sp*.),
319 Reporting that the best replacement level, without causing changes to the animals, it must be
320 between 25 and 33 %.

321 The somatic indexes can be changed according to the composition, bioavailability and
322 anti or pro-nutritional factors present in the diets (Leenhouwers et al., 2006, Baldisserotto,
323 2009). In the present study, there were no changes in the DSI, IQ and HSI, demonstrating that
324 the fish organism did not need to adapt to diets containing different levels of DRBPC.

The higher the level of substitution of fishmeal by DRBPC, the lower the deposition of protein in the fish carcass. This result shows that the animals were not able to use the protein received in the diet efficiently. For protein deposition in the carcass (TPD- Figure 1), the significant differences ($P < 0.05$) found were reflected in an inverse way to the animals' weight gain results (Table 2). Although the diets are isoproteic, the protein derived from DRBPC was not metabolized like the protein in fishmeal.

Although the predominant concern about the effects of various alternative plant proteins is on fish growth and feed efficiency, it is important to monitor the influence of diet on fish biochemical index, such as changes in liver metabolism (Vilhelmsson et al., 2004) and activities enzymatic (Krogdahl et al., 2003), which are also indicative of the use of the evaluated ingredients.

In the present study, no significant differences were observed in the plasma and liver parameters evaluated. However, a numeric increase in plasma albumin ($P = 0.636$) was observed in animals that received diets containing DRBPC. This behavior may have occurred due to the low use of protein resulting from DRBPC, considering that albumin acts as a reservoir of amino acids. However, this increase in serum albumin occurs when the availability of amino acids via diet is less than that required by the body (Santos et al., 2004). The maintenance of ammonia and hepatic ALT levels demonstrate that there was no increase in hepatic ammonia excretion with a decrease in fishmeal from diets, as well as maintenance of catabolism and protein anabolism.

Several factors can alter the production and activity of digestive enzymes in fish, such as eating habits, type of diet ingredient, among others (Pavasovic et al., 2007). In the present study, the increase in the activity of the trypsin enzyme in fish fed with DRBPC may be related to an attempt by the body to increase the digestibility of the protein, resulting in increased proteolytic activity (Lovatto et al., 2017).

Trypsin cleaves protein at the carboxyl side of basic amino acids, lysine and arginine (Stryer, 1988), which show higher digestibilities than other amino acids (Skrede et al., 1998). Chymotrypsin cleaves protein at the carboxyl side of aromatic amino acids phenylalanine, tyrosine, tryptophan, as well as of large hydrophobic residues such as methionine (Stryer, 1988).

Trypsin is the key protease activating other pancreatic proteases including chymotrypsin in fish (Sunde et al. 2001). Usually, it is expected that any factors that affect trypsin activity should also influence chymotrypsin activity in a similar way, since they are the dominating digestive proteases and their activities are related (Cara et al., 2007). In our study, no similar behavior was observed in the activity of trypsin and chymotrypsin, in the 35DRBPC diet, due to the increase for trypsin activity only. This increased activity of the trypsin enzyme is related to the increased secretion of proteolytic enzymes in an effort to increase the absorption of protein in portions of the intestine (Alarcón et al., 2001).

These results are in line with the increase in enzyme activity described by Penn et al. (2011) when using levels of pea protein concentrate in the diet of *Salmo salar* and Song et al., (2014) when using soy protein hydrolyzate in the diet of *Platichthys stellatus*. This situation may have occurred in fish that received a diet with 35% DRBPC.

Some authors propose the use of the trypsin:chymotrypsin (Tr:Ch) ratio as a better indicator of nutritional condition, since it might indicate to what extent chymotrypsin is activated by trypsin, and this in turn may indicate growth potential of the fish (Rungruangsak Torrissen and Male, 2000; Sunde et al., 2001). These authors suggest that the higher the Tr:Ch ratio, the higher the absorption and transport rate of essential amino acids for protein synthesis.

In our study, we observed that the increase in the Tr:Ch ratio occurred due to the increase in the trypsin enzyme activity of fish in the 35DRBPC diet as a possible alternative

to improve protein digestibility of the diet in question. Possibly, similar maintenance in chymotrypsin activity occurred because the increased expression of chymotrypsin is strongly associated with periods when there is a reduction in the growth rate of fish (Rungruangsak-Torrissen et. al, 2006), what was not observed in our study.

5 Conclusion

The results of this study showed that the a inclusion of up to 35% of DRBPC to replace the protein from fishmeal does did not negatively affect growth performance, parameters plasmatic and liver, nutrient deposition, somatic indices and activity of the digestive enzymes in silver catfish (*Rhamdia quelen*). These results indicated that of DRBPC can be considered to be an alternative protein ingredient to reduce the use of fishmeal in diets to aquaculture.

Conflict of interest statement

The article is the original work of the authors. The authors declare that there are no financial or personal conflicts of interest that may appear to influence the work reported in this article.

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Table 1

Ingredients, chemical composition and essential amino acid content of the experimental diets used during feeding trial for silver catfish.

Content	Experimental diets				
	Diets Code				
	0DRBPC	25DRBPC	35DRBPC	45DRBPC	55DRBPC
Ingredients (g/kg)					
DRBPC	0	138.0	183.0	235.3	287.7
Fish meal	400.0	294.5	260.0	220.0	180.0
SPC[a]	248.0	248.0	248.0	248.0	248.0
Starch	157.6	157.6	157.6	157.6	157.6
Soy oil	23.4	28.0	29.5	31.3	33.0
Mix vitamin/mineral[b]	30.0	30.0	30.0	30.0	30.0
Dicalcium phosphate	10.0	10.0	10.0	10.0	6.00
BHT[c]	0.1	0.1	0.1	0.1	0.1
Limestone calcitic	22.0	22.0	22.0	22.0	22.0
Inert[d]	108,9	71.8	59.8	45.7	35.6
Proximate composition (g/kg)					
Crude protein[e]	372.2	371.5	371.9	373.3	372.3
Crude lipid[e]	78.3	75.8	76.5	74.3	77.2
Dry matter[e]	933.3	942.6	935.2	936.5	935.9
Digestible energy [f] (MJ/kg)	13.4	13.4	13.4	13.4	13.4
Ash[e]	151.7	135.6	137.8	124.8	117.9
Total Dietary Fiber[e]	59.0	112.6	130.1	150.4	170.7
Calcium[g]	26.5	22.5	21.2	19.7	17.2
Total phosphorus[g]	13.0	11.2	10.6	9.9	8.5
Amino acids [h] (g/kg^{-})					
Lysine	26.0	24.0	23.0	23.0	22.0
Arginine	34.0	33.0	33.0	37.0	32.0
Threonine	19.0	19.0	19.0	18.0	18.0
Tyrosine	15.0	14.0	14.0	14.0	14.0
Valine	22.0	21.0	21.0	21.0	21.0
Methionine + cysteine	13.0	12.0	12.0	12.0	12.0
Isoleucine	18.0	18.0	18.0	18.0	18.0
Leucine	33.0	33.0	32.0	32.0	32.0
Phenylalanine	21.0	20.0	20.0	20.0	20.0
Histidine	10.0	10.0	10.0	10.0	10.0

[a] Soybean protein concentrate (60% crude protein).
[b] Composition of vitamin and mineral mixture : 300 mg, Ascorbic AC: 15,000 mg, Pantothenic Ac: 3,000 mg, Biotin: 0.06mg, niacin (B3): 9,000 mg Hill (B4): 103.500 mg, Vit.A: 1,000,000 IU, Vit B1:. 1,500 mg, Vit B2: 1:50 mg, Vit B6. 1,500 mg, Vit D3: 240 000 IU Vit. E: 10 000 mg, Vit K3:. 400 mg, Inositol: 10,000 mg Iron: 5.000mg, Manganese: 8.000 mg, Copper 1.000 mg Zinc: 14 000 mg Iodine: 45 mg, Cobalt: 60 mg, Selenium 60 mg, Magnesium: 5 mg, Mig Plus®.
[c] Butyl-hydroxy-toluene (antioxidant).
[d] Fine sand washed.[e] Analyzed – Fish Culture Laboratory (Laboratório de Piscicultura, UFSM, Brazil).
[f] Digestible Energy = [(CP * 23.61 MJ / kg * 0.9) + Fat * 39.82 MJ / kg * 0.85) + CSDN * 17.21 MJ / kg *0.50)] (Jobling, 1983).
[g] Calculated by analyzing the ingredients. [h] Calculated based on the composition of the raw materials.

Table 2

Growth index in silver catfish fed with increasing levels of rice bran protein concentrate (DRBPC).

Index	0DRBPC	25DRBPC	35DRBPC	45DRBPC	55DRBPC	P value
Body Weight (g)	27.31 ±6.05^{a}	24.59 ± 6.53^{ab}	23.73 ± 5.71^{abc}	21.46 ± 4.64^{bc}	20.84 ± 4.23^{c}	0.000
Feed conversion rate	0,98 ±0.06	1,06 ± 0.14	1,02 ± 0.29	1,05 ± 0.25	1,18 ± 0.09	0.128
Specific growth rate	22.27 ±0.21^{a}	19.35 ± 0.16^{ab}	18.67 ± 0.12^{ab}	16.14 ± 0.05^{b}	15.69 ± 0.16^{b}	0.002
Relative weight gain	173.22 ±26.49^{a}	139.35 ± 16.94^{ab}	131.93 ± 12.15^{ab}	106.76 ± 47.16^{b}	102.90 ± 14.55^{b}	0.003

Values given as mean ± standard deviation (n=3). Values with different letters differ significantly by Tukey test ($p<0.05$).

Table 3

Digestive somatic index, Intestinal quotient and hepatic somatic index values in silver catfish fed with increasing levels of rice bran protein concentrate (DRBPC).

Parameters	0DRBPC	25DRBPC	35DRBPC	45DRBPC	55DRBPC	P value
Digestive Somatic Index	1.585±0.22	1.698±0.23	1.755±0.27	1.622±0.27	1.741±0.37	0.78
Intestinal Quotient	0.975±0.22	1.061±0.16	1.144±0.24	1.075±0.36	1.145±0.56	0.90
Hepatic Index	1.390±0.29	1.976±0.42	1.731±0.44	1.681±0.45	1.831±0.69	0.32

Values given as mean ± standard deviation (n=9). Values with different letters differ significantly by Tukey test ($p<0.05$).

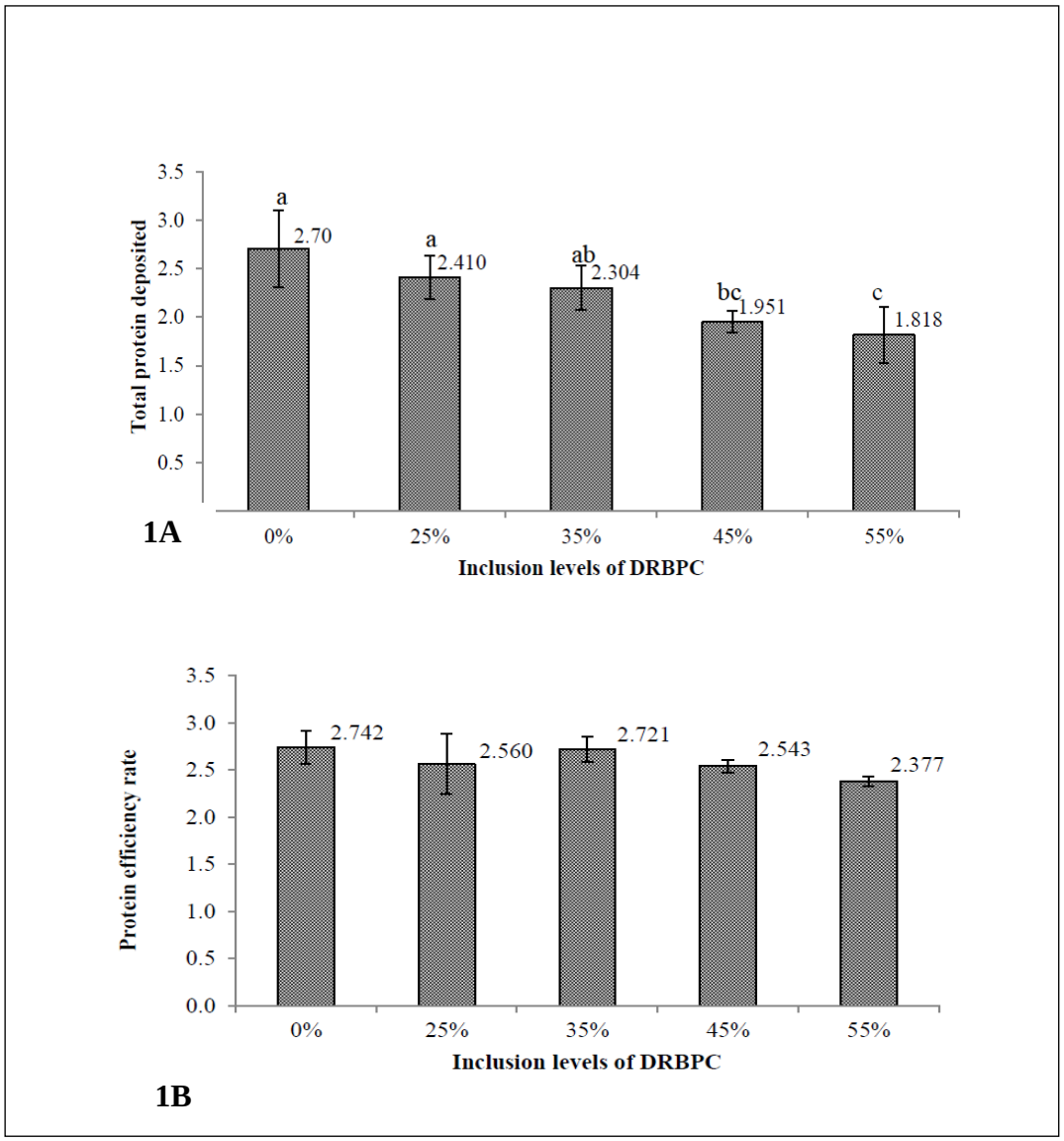


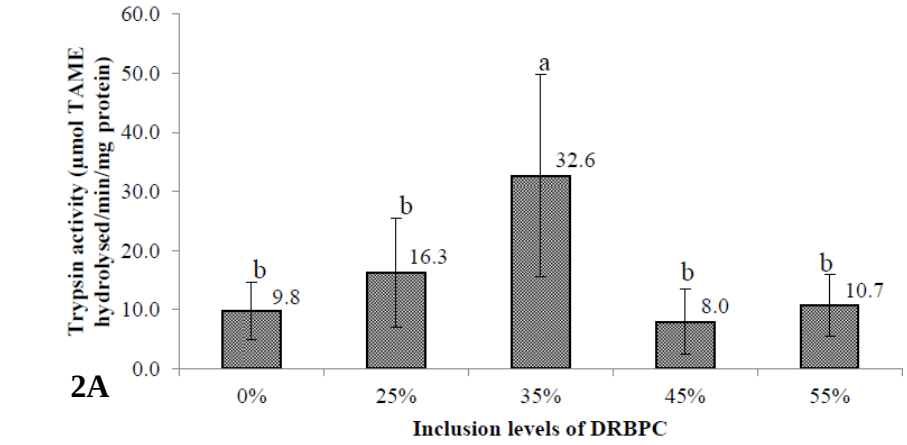
Fig. 1. Total protein deposited (A) and Protein efficiency rate (B) in silver catfish fed with increasing levels of rice bran protein concentrate (DRBPC). Values are given as mean ± standard deviation (n=9). Values with different letters differ significantly by Tukey test ($p<0.05$).

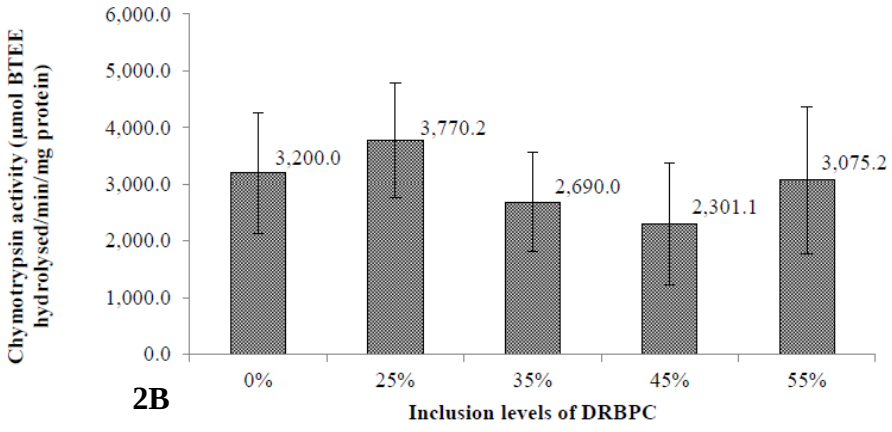
Table 4

Plasma biochemistry ($g.dL^{-1}$) and hepatic parameters values in silver catfish fed with increasing levels of rice bran protein concentrate (DRBPC).

	PARAMETERS	0DRBPC	25DRBPC	35DRBPC	45DRBPC	55DRBPC	p value
Plasma biochemistry (g.dL)	Albumin	0.70±0.13	1.05 ±0.36	0.86±0.38	0.93±0.52	1.05 ±0.44	0.636
	Total proteins	31.20 ±0.46	36,58±0.21	36,07±0.65	37,13±0.96	43,13±1.60	0.334
	Triglycerides	309.67±70.73	342.67±79.71	302.00±100.14	319.17±96.20	418. 60±109.98	0.377
	Cholesterol	127.17±21.24	143.40±21.76	188.17±86.29	118.60±25.20	181.80±34.71	0.744
Hepatic parameters	Protein mg/ g tec.	126.14±2.33	116.33±1.68	114.34±1.48	109.19±0.77	113.29±1.17	0.444
	Free Amino acids mM /g tec	7.74±1.27	6.48±0.89	7.62±1.66	6.355±1.57	5.91±1.26	0.113
	Ammonia mM /g tec	8.19±1.63	7.58±1.25	7.59±0.86	7.08±1.10	8.29±1.11	0.443
	ALAT UI/mg tec.	4.19±1.35	3.70±1.57	5.61±1.94	5.41±1.63	4.96±3.01	0.430

Values given as mean ± standard deviation (n=6). Values with different letters differ significantly by Tukey test ($p<0.05$)





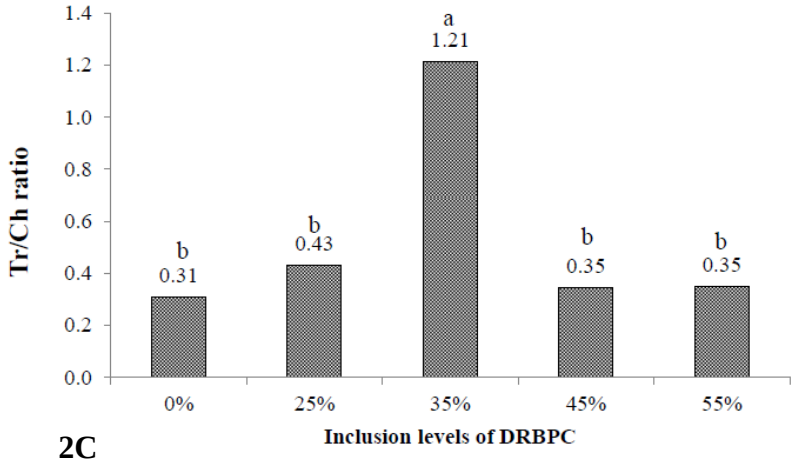


Fig. 2. Trypsin (A), chymotrypsin (B) activity and ratio trypsin:chymotrypsin (Tr:Ch) (C) in silver catfish fed with increasing levels of rice bran protein concentrate (DRBPC). Values given as mean ± standard deviation (n=6). Values with different letters differ significantly by Tukey test ($p<0.05$).

4 DISCUSSÃO GERAL

Atualmente, existe uma grande necessidade e expectativa pelo surgimento de novas fontes proteicas alternativas à farinha de peixe, principal fonte de proteína comumente usada na elaboração de dietas aquícolas. O rápido crescimento do setor e o aumento da demanda da população mundial por proteína animal de alta qualidade resultam na escassez desse ingrediente tornando-o mais caro e, consequentemente, elevando os custos com a alimentação dos animais.

Desde há muito tempo sabe-se da importância da farinha de peixe na alimentação de organismos aquáticos, devido à sua qualidade nutricional e aproveitamento metabólico por parte dos animais. Contudo, devido aos peixes e resíduos utilizados para produção de farinha de peixes terem possibilidade de uso direto na alimentação humana, há necessidade de substituições parcial ou total na dieta das espécies aquícolas.

As fontes proteicas de origem vegetal surgem como uma forte alternativa pois apresentam preços acessíveis e boa qualidade nutricional. Contudo, a maioria das fontes vegetais possuem aspectos negativos intrínsecos, que prejudicam o desempenho e a saúde dos peixes, como a presença de inibidores enzimáticos e alto de teor de fibras, entre outros.

Dentre as diversas fontes proteicas vegetais podemos ressaltar os resíduos e coprodutos agroindustriais, os quais são gerados em grandes quantidades e, geralmente, não possuem qualidade nutricional para atender as necessidades nutricionais dos peixes. Entretanto, existem diferentes maneiras de reduzir ou eliminar esses aspectos negativos e melhorar a qualidade nutricional destas fontes de proteína, por meio de processos físicos, químicos e enzimáticos, combinados ou não.

Em nosso estudo, a fonte proteica de origem vegetal utilizada foi o resíduo intitulado Farelo de Arroz Desengordurado e Desfitinizado (FADD), o qual foi submetido ao processo de extrusão com o objetivo de avaliar a eficiência do processo, sobre a melhora na extração da proteína pelo método de concentração proteica químico-enzimática em meio aquoso.

O CPFA apresentou o maior teor proteico (42,39% de proteína bruta) ($P<0,05$) dentre as amostras. O FADDext apresentou o menor teor de proteína bruta (Tabela 1-Artigo 1) e também baixa solubilidade proteica (Tabela 3-Artigo 1) quando comparado FADD, demonstrando que o processo de extrusão reduziu o teor de proteína e consequentemente, influenciou no processo de concentração proteica (CPFAext - 33,46% de proteína bruta). Esses resultados confirmam a ocorrência de desnaturação e oxidação proteica no FADDext, pois segundo Estrada et al. (2018) a oxidação pode ocasionar alterações na conformação das

estruturas secundárias e terciárias das proteínas, podendo causar a redução da solubilidade proteica e do teor de proteína bruta do ingrediente.

Outro fator que pode ter influenciado na redução do teor de proteína do FADDext é o pH extremamente baixo (em torno de 1,5) do FADD. Segundo Sørensen et al. (2009), a combinação da extrusão em pH muito baixo contribuem para a redução do teor proteico do ingrediente, devido à aceleração de desnaturação proteica e desdobramento nas moléculas proteicas, havendo perdas irreversíveis neste nutriente.

O maior conteúdo de lipídios observado no CPFA ocorreu pela exclusão da fração fibrosa durante o processo de concentração proteica, ocasionando um aumento do conteúdo de lipoproteínas (BERTIPAGLIA et al., 2008). Durante o processo de extrusão a alta temperatura faz com que as células de gordura se unam e formem gotículas de óleo, rompendo a estrutura celular e facilitando a velocidade da retirada de óleo (DALBHAGA; MAHATO; MISHRA, 2019), confirmando o aumento do conteúdo de lipídio do FADDext.

O processo de extrusão não influenciou no conteúdo de amido disponível do FADDext em relação ao FADD que foi maior ($P<0,05$) em comparação aos concentrados proteicos (Tabela1-Artigo 1). Esse resultado já era esperado, pois a metodologia de concentração proteica utiliza enzimas amidolíticas (amiloglucosidase e α- amilase) durante o processo, ocasionando a redução do conteúdo de amido disponível das amostras concentradas.

Entretanto, as reduções observadas nos concentrados proteicos foram contrárias às relatadas em outros estudos. De acordo com Gui, Gil e Ryu (2012), durante o processo de extrusão verifica-se maior solubilidade do amido, em consequência da degradação dos grânulos no decorrer do cozimento do produto. Dessa forma, era esperado que a combinação do processo de extrusão e a metodologia de concentração reduzissem ainda mais o conteúdo de amido disponível do CPFAext (11,67%), quando comparado ao CPFA (4,79%). Para amido resistente o maior conteúdo foi observado no CPFA (Tabela –Artigo 1), pois varia de acordo com o peso total da amostra sendo inversamente proporcional ao conteúdo de amido disponível.

As variáveis analisadas para fibra alimentar das amostras foram alteradas pelos processos de extrusão e concentração proteica (Tabela 1-Artigo1). A maior concentração ($P>0,05$) de fibra total (FT) e fibra solúvel (FS) observadas no CPFA pode ter ocorrido pelo uso das enzimas durante o processo de concentração proteica. Resultado que corrobora ao encontrado por Hanmoungjai, Pyle e Niranjan (2001), que relataram aumento do conteúdo de FT em farelo arroz ao concentrar a proteína por extração enzimática. Os menores conteúdos para essas variáveis foram observadas nas amostras extrusadas (Tabela 1-Artigo 1). No

entanto, a FS foi maior nas amostras extrusadas em comparação a *in natura*, devido a conversão da FI em FS, a qual foi influenciada pelo cozimento e temperatura (120ºC) em que as amostras foram submetidas durante o processo de extrusão.

Essa conversão também foi observada em outros estudos com a extrusão de farelo de trigo (ANDERSSON et al., 2017; RASHID et al., 2015; YAN; YE; CHEN, 2015), farelo de aveia (ZHANG; BAI; ZHANG, 2011) e farelo de arroz (DANG; VASANTHAN, 2019). Segundo Gualberto et al. (1997) a extrusão pode ocasionar uma reestruturação das frações das fibras, induzindo a um aumento no conteúdo de fibra solúvel a partir da insolúvel. A extrusão também resulta na quebra das ligações covalente e não covalente existentes entre os carboidratos e as proteínas ligadas a fibra, as quais irão gerar partículas menores e mais solúveis (DANG; VASANTHAN, 2019)

Em nosso estudo foi observado que a extrusão e a concentração proteica, quando combinadas ou não, possuem a capacidade de alterar o conteúdo de matéria mineral e dos macro elementos (Ca, P, K e Mg) presentes no farelo de arroz (Tabela 2 –Artigo 1). Resultados semelhantes foram descritos por Sharma, Chauhan e Kuldeep (2004), que observaram aumento no conteúdo de matéria mineral do farelo de arroz extrusado em comparação ao *in natura.* Para o conteúdo de macro elementos, foram encontradas alterações em outros estudos, onde os autores relataram que a extrusão (FERREIRA & ARÊAS, 2010) e a concentração proteica (GAILORD et al., 2010) elevam o conteúdo de alguns macro elementos, devido à redução de antinutrientes, como as fibras e polissacarídeos não amiláceos.

A maior capacidade de retenção de óleo (CRO) observada no FADD (Tabela 3-Artigo 1) pode ter sido ocasionada pela presença de uma grande quantidade de grupos hidrofóbicos em relação aos grupos hidrofílicos presentes na estrutura primária das proteínas (SUBAGIO, 2006) da amostra. O CPFAext apresentou a menor CRO (Tabela 3-Artigo 1), resultado que pode ter ligação com a quantidade de grupos hidrofóbicos expostos na proteína e a capacidade de absorver o óleo (DENCH; RIVAS; CAYGILL, 1981). Qu et al. (2017) observaram comportamento semelhante ao extrusar resíduo de soja, onde a estrutura da amostra sofreu modificação devido à alta pressão de inchamento gerada pelo processo e extrusão e, posterior tratamento enzimático.

A redução da solubilidade proteica após o processo de extrusão do FADD (Tabela 3 – Artigo 1) demonstrou que provavelmente a extrusão acarretou na desnaturação das proteínas pelo tratamento térmico empregado. Diversos fatores podem ter causado a desnaturação como por exemplo o calor, pH, solventes orgânicos miscíveis em água, solutos entre outros. A leve

desnaturação proteica (temperaturas menores que 100 ºC) pode facilitar a digestão (Ljøkje et al., 2004), permitindo melhor acesso das enzimas proteolíticas a proteína desnaturada desdobrada do que a proteína nativa (Cheftel, 1979). Contudo, um aumento subsequente da temperatura combinado a outros fatores como mudanças no pH podem reduzir a taxa de digestão de proteínas por bloquear os locais de ataque enzimático (Papadopoulos, 1989).

Tanto o FADDext quanto CPFAext apresentaram reduzida solubilidade proteica, sendo a combinação da extrusão seguida da concentração proteica capaz de reduzir ainda mais a solubilidade proteica (Tabela 3-Artigo 1).

O conteúdo de proteína bruta das amostras foi a variável que definiu a escolha do processo para obtenção do concentrado proteico. Dessa maneira, o CPFA apresentou 42,3% de proteína bruta (Tabela 1-Artigo 1) e 36% de rendimento, sendo o processo e produto escolhido para o ensaio biológico com jundiás. A partir dessa avaliação, a proteína advinda do CPFA foi utilizada como ingrediente proteico na dieta dos peixes, como substituto da proteína advinda da farinha de peixe, em diferentes níveis de inclusão.

A proteína do CPFA foi então empregada como substituta da proteína da farinha de peixe em diferentes níveis (zero, 25, 35, 45 e 55%) de inclusão da dieta dos peixes. Assim, constatou-se que utilização do CPFA a partir no nível de substituição de 35% influenciou negativamente no desempenho dos animais, de acordo com os índices de crescimento avaliados (Tabela 2-Artigo 2). A piora do desempenho dos animais foi, com ou sem diferenças significativas ($P<0,05$), acompanhada pelo nível de inclusão do CPFA na dieta, obtendo-se uma relação inversa entre os índices de crescimento e a substituição parcial da farinha de peixes pelo CPFA. O mesmo padrão foi observado para a Deposição de proteína corporal (Figura 1A-Artigo 2).

Possivelmente, o conteúdo de fibras ainda contidas no CPFA tenham influenciado no desempenho dos peixes. Uma vez que a relação do conteúdo de fibra na dieta pode influenciar na digestibilidade e absorção dos nutrientes (carboidratos, proteínas e lipídios), comprometendo o metabolismo, a digestão e os parâmetros zootécnicos de uma determinada espécie (SOUZA, 2016). Segundo Rodrigues et al. (2010), essa influência das fibras pode atuar na motilidade e no tempo de trânsito gastrointestinal do alimentos, alterando a velocidade e o tempo de esvaziamento gástrico. Em nosso estudo, foi observada grande diferença no conteúdo de fibra alimentar total das dietas obtendo-se variações de 5,9 a 17% (Tabela 1-Artigo 2), a qual foi influenciada pelo conteúdo de fibra do CPFA (38,85%) (Tabela 1-Artigo 1) conforme o nível de inclusão. Uma vez que somente os níveis de farinha de peixe e CPFA variaram entre as dietas teste (Tabela 1-Artigo 2).

De acordo com Raskovic et al. (2011), devido as fibras possuírem a capacidade de influenciar a absorção dos nutrientes, conforme o tipo (insolúvel e solúvel) e nível de inclusão, as mesmas podem causar modificações nas estruturas e órgãos associados ao sistema digestório. Estudos com jundiá utilizam até 10% de fibra alimentar nas dietas dos peixes (RODRIGUES et al., 2012; ADORIAN et al., 2015).

Rodrigues et al. (2012) demonstraram que ingredientes com elevados conteúdos de fibra alimentar total e fibra insolúvel (polpa cítrica e farelo de trigo) apresentaram baixa digestibilidade aparente da proteína (DAP) para o jundiá (34,4% DAP para polpa cítrica e 58.8% DAP para farelo de trigo). Ressalta-se que essa baixa digestibilidade ocorreu em dietas contendo menos de 10% de fibra alimentar total.

Em nosso estudo, o CPFA apresentou elevados níveis de fibra insolúvel (30,43%), fator que pode ter influenciado na disponibilidade e aproveitamento proteico. Segundo Souza (2016), o conteúdo de fibra presente na dieta compromete o desempenho dos peixes, pois possui relação direta com a digestão e absorção dos nutrientes. Fibras insolúveis aumentam a velocidade do trânsito gastrointestinal, reduzindo o tempo de digestão e, consequentemente, o uso de nutrientes pelos peixes (HETLAND et al. 2004; KROGDAHL et al. 2005)

A maior atividade da enzima tripsina observada nos peixes alimentados com a dieta contendo 35% de CPFA (Figura 2A-Artigo 2), pode estar relacionada com a tentativa do organismo em aumentar a digestibilidade da proteína, ocasionando o aumento da atividade proteolítica (LOVATTO et al., 2017) para melhorar a absorção de proteína nas porções do intestino (ALARCÓN; GARCÍA-CARREÑO; NAVARRETE, 2001).

Alguns autores propuseram o uso da razão tripsina:quimotripsina (Tr:Ch) como um melhor indicador do estado nutricional, pois pode indicar até que ponto a quimotripsina é ativada pela tripsina, o que, por sua vez, pode indicar potencial de crescimento do peixe ou se o aumento na atividade da tripsina pode estar relacionado a melhora na digestibilidade e absorção dos nutrientes (SUNDE et al., 2001).

5 CONCLUSÃO GERAL

Com base nos resultados obtidos, é possível concluir que:

- O processo de extrusão do FADD *in natura*, na condição estudada, não foi capaz de melhorar a extração da proteína junto ao processo de concentração proteica utilizado;
- O concentrado proteico obtido a partir do FADD (CPFA) apresentou maior teor de proteína bruta (42,39%) em sua composição, sendo escolhido como ingrediente a ser incluído na dieta de jundiás;
- Apesar do CPFAext (obtido a partir do FADD extrusado) apresenta teor de proteína bruta (33,46%) menor que o CPFA, No entanto, o produto apresenta maior conteúdo de minerais (P, Ca, K e Mg), amido disponível e resistente e menor teor de fibra total, quando comparado aos CPFA. Dessa maneira, sugere-se estudos futuros com a inclusão deste ingrediente na dieta de jundiás e outras espécies de peixes;
- A substituição da proteína da farinha peixe em até 35% de CPFA em dietas de jundiás (*Rhamdia quelen*), não afeta negativamente os parâmetros de crescimento, metabólicos, deposição de nutrientes e enzimáticos dos animais.
- O CPFA pode ser considerado um ingrediente proteico alternativo a farinha de peixe em dietas aquícolas;
- Novos estudos podem ser conduzidos buscando avaliação da digestibilidade aparente e a inclusão do CPFA na dieta de outras espécies de peixes.

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ANEXO A – Normas da revista LWT - Food Science and Technology - Artigo 1

Introduction

LWT - Food Science and Technology is an official journal of the Swiss Society of Food Science and Technology (SGLWT/SOSSTA) and the International Union of Food Science and Technology (IUFoST).

LWT - Food Science and Technology is an international journal that publishes innovative papers in the fields of food chemistry, biochemistry, microbiology, technology and nutrition. The work described should be innovative either in the approach or in the methods used. The significance of the results either for the science community or for the food industry must also be specified. Contributions that do not fulfil these requirements will not be considered for review and publication. Submission of a paper will be held to imply that it presents original research, that it has not been published previously, and that it is not under consideration for publication elsewhere.

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Essentials to ensure fast handling of Research papers and Short communications

• Manuscript-text must be saved as either a MS Word, Word Perfect, RTF, TEX or Plain ASCII file. Continuous line numbering must be added and the text must be double spaced.

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• Highlights must be included (a summary of your main achievements in 3-5 bullet points no more than 85 characters each).

• Figures and tables must be submitted as separate files and are clearly labeled.

• The international system of units (SI units) must be used only.

• If analytical data are reported in tables and/or figures: Number of replications should be mentioned in the legend or a footnote and standard error or other evidence of reliability of data must be given.

• Your Cover letter should explain the novelty of the research presented, that your paper presents original research, that it has not been published previously and that it is not under consideration for publication elsewhere.

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• Please note that this list is not extensive and purely highlights the most important aspects of a submission. For full details on all article types please refer to the online Guide for Authors at https://www.elsevier.com/journals/lwt-food-science-and-technology/0023-6438/guide-for-authors.

Types of paper

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Review articles. These concise reviews should present a focused aspect on a topic of current interest or an emerging field. They are not intended as comprehensive literature surveys covering all aspects of the topic, but should include all major findings and bring

together reports from a number of sources. They should aim to give balanced, objective assessments by giving due reference to relevant published work, and not merely present the prejudices of individual authors or summarise only work carried out by the authors or by those with whom the authors agree. Undue speculation should also be avoided. These reviews will receive priority in publication.

The reviews may address pertinent issues in food science, technology, processing, nutritional aspects of raw and processed foods and may include nutraceuticals, functional foods, use of "omics" in food quality, food processing and preservation, and food production.
Topics to be covered should be at the cutting edge of science, well thought out, succinct, focused and clear. Ideally, the review should provide a view of the state of the art and suggest possible future needs and trends.
All articles will be subjected to peer review process.

Submit an abstract of the proposed review to the Editor in Chief (Professor Rakesh Singh), rsingh@uga.edu for consideration prior to preparing the full length manuscript. Abstract of the proposed work should include the following:
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ANEXO B – Normas da revista Animal Feed Science and Technology - Artigo 2

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