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Patrícia Inês Mombach

HIDROLISADOS PÉCTICOS NA DIETA DE TILÁPIA DO NILO E JUNDIÁ: IMPLICAÇÕES NUTRICIONAIS E POTENCIAL PREBIÓTICO

Santa Maria, RS
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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Zootecnia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutora em Zootecnia**.

Orientadora: Profª. Drª. Leila Picolli da Silva

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Endereço: Avenida Roraima, n. 1000, Bairro Camobi, Santa Maria, RS. CEP: 97105-900
Fone (55) 3220 8365; E-mail: patimombach@hotmail.com

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Leila Picolli da Silva, Dra. (UFSM)
(Presidente/Orientadora)

Carla Cristina Zeppenfeld, Dra. (UFSM)

Luciano de Oliveira Garcia, Dr. (FURG)

Fernanda Rodrigues Goulart Ferrigolo, Dra. (UFSM)

Alexandra Pretto, Dra. (UNIPAMPA)

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“Nenhum obstáculo é grande demais quando confiamos em Deus”
(Aristóteles)

RESUMO

HIDROLISADOS PÉCTICOS NA DIETA DE TILÁPIA DO NILO E JUNDIÁ: IMPLICAÇÕES NUTRICIONAIS E POTENCIAL PREBIÓTICO

AUTORA: Patrícia Inês Mombach
ORIENTADORA: Leila Picolli da Silva

A obtenção de novos prebióticos a partir de resíduos agroindustriais visa potencializar o aproveitamento destes subprodutos, além de trazer benefícios à nutrição animal. Assim, objetivou-se produzir e caracterizar hidrolisados pécticos a partir de bagaço de maçã e casca de maracujá, e investigar seu potencial prebiótico para alevinos de tilápia do Nilo (*Oreochromis niloticus*) e jundiá (*Rhamdia quelen*). Na primeira fase realizou-se a extração da pectina dos resíduos, seguida do processo de hidrólise química. Os resíduos e seus respectivos hidrolisados foram caracterizados quanto aos teores de matéria seca, fibra alimentar total, fibra solúvel, fibra insolúvel, cinzas, proteína bruta e gordura. Os hidrolisados também foram analisados quanto ao perfil de monossacarídeos e propriedades físico-químicas. Na segunda fase foram realizados dois ensaios biológicos (tilápia do Nilo – 42 dias e jundiá – 49 dias) para a avaliação de cinco dietas teste, sendo uma dieta controle e as demais com adição de hidrolisados pécticos (2,5 ou 5 g/kg) de bagaço de maçã ou casca de maracujá. Ao final dos períodos experimentais foram coletados dados e material biológico para avaliação do desempenho, parâmetros plasmáticos, hepáticos, enzimáticos, histológicos, composição corporal e avaliação do conteúdo intestinal dos peixes. Os resultados foram submetidos à teste de normalidade, seguido por análise de variância, sendo as médias dos tratamentos comparadas por análise de contrastes ortogonais ao nível de 5% de significância. O bagaço de maçã e a casca de maracujá apresentaram-se promissores para extração de pectina, resultando em hidrolisados com elevada concentração de fibra solúvel. Na análise dos monossacarídeos, o hidrolisado de bagaço de maçã apresentou quantidades semelhantes de glicose-xilose-ácido galacturônico (14,7; 13,5 e 16,11%). O hidrolisado de bagaço de maçã demonstrou elevada capacidade de ligar-se à água, característica que apresenta-se reduzida no hidrolisado de casca de maracujá. A produção de ácidos graxos de cadeia curta à nível intestinal foi influenciada pelos hidrolisados pécticos, sendo que os alevinos de tilápia do Nilo apresentaram maior produção de ácido acético, com influência na concentração de glicose hepática. Os alevinos de jundiá apresentaram maior produção de ácido butírico, relacionada com a contagem de células caliciformes à nível intestinal. Para a tilápia do Nilo, a suplementação com hidrolisado de casca de maracujá (2,5 ou 5 g/kg) proporcionou menor teor de gordura corporal, garantindo um alimento mais saudável e com maior estabilidade de prateleira. Os hidrolisados pécticos causaram alterações nos parâmetros histológicos intestinais, onde os alevinos de tilápia do Nilo obtiveram maiores benefícios com a suplementação do hidrolisado de casca de maracujá (2,5 e 5 g/kg), que garantiu maior renovação celular, maior capacidade de absorção de nutrientes e manutenção da integridade intestinal, e os alevinos de jundiá apresentaram maior contagem de células caliciformes, com a suplementação de 2,5 g/kg de hidrolisado de bagaço de maçã. Os hidrolisados pécticos de bagaço de maçã e casca de maracujá, adicionados na dieta de alevinos de tilápia do Nilo e jundiá possibilitaram a obtenção de resultados satisfatórios, no entanto, mais estudos devem ser realizados com intuito de definir níveis que possibilitem maiores ganhos na nutrição de peixes.

Palavras-chave: Aditivos prebióticos. Hidrolisado de bagaço de maçã. Hidrolisado de casca de maracujá. Nutrição de peixes.

ABSTRACT

PECTIC HYDROLYSATES IN THE DIET OF NILE TILAPIA AND SILVER CATFISH: NUTRITIONAL IMPLICATIONS AND PREBIOTIC POTENTIAL

AUTHOR: Patrícia Inês Mombach
ADVISOR: Leila Picolli da Silva

The acquisition of new prebiotics from agroindustrial wastes aims to enhance the use of these byproducts, besides bringing benefits to animal nutrition. The objective of this study was to produce and characterize pectic hydrolysates from apple pomace and passion fruit peel and to investigate their prebiotic potential for Nile tilapia (*Oreochromis niloticus*) and silver catfish (*Rhamdia quelen*). In the first phase the pectin extraction from the residues was carried out, followed by the chemical hydrolysis process. The residues and their respective hydrolyzates were characterized as dry matter, total dietary fiber, soluble fiber, insoluble fiber, ash, crude protein and fat content. Hydrolysates were also analyzed for monosaccharide profile and physicochemical properties. In the second phase, two biological tests (Nile tilapia - 42 days and silver catfish - 49 days) were carried out to evaluate five test diets, one control diet and the other with addition of pectic hydrolysates (2.5 or 5 g/kg) apple pomace or passion fruit peel. At the end of the experimental periods, data and biological material were collected for performance evaluation, plasmatic, hepatic, enzymatic and histological parameters, body composition and evaluation of the intestinal contents of the fish. The results were submitted to the normality test, followed by analysis of variance, and the means of the treatments were compared by analysis of orthogonal contrasts at the 5% level of significance. The apple pomace and the passion fruit peel were promising for pectin extraction, resulting in hydrolysates with a high concentration of soluble fiber. In the analysis of the monosaccharides, the apple pomace hydrolyzate showed similar amounts of glucose-xylose-galacturonic acid (14.7, 13.5 and 16.11%). The apple pomace hydrolyzate showed high capacity to bind to water, a characteristic that is reduced in the passion fruit peel hydrolyzate. The production of short chain fatty acids at the intestinal level was influenced by the pectic hydrolysates, and the Nile tilapia juveniles presented higher production of acetic acid, with an influence on the hepatic glucose concentration. The silver catfish juveniles presented higher production of butyric acid, related to the count of goblet cells at intestinal level. For Nile tilapia, supplementation with passion fruit peel hydrolyzate (2.5 or 5 g/kg) provided lower body fat content, ensuring a healthier food with greater shelf stability. Pectic hydrolysates caused alterations in intestinal histological parameters, where Nile tilapia juveniles obtained greater benefits with the supplementation of passion fruit peel hydrolyzate (2.5 and 5 g/kg), which ensured a greater cell renewal, a higher absorption capacity of nutrients and maintenance of intestinal integrity, and silver catfish juveniles presented higher goblet cell counts with the supplementation of 2.5 g/kg of apple pomace hydrolyzate. The pectic hydrolysates of apple pomace and passion fruit peel, added to the diet of Nile tilapia and silver catfish juveniles allowed for satisfactory results, however, further studies should be carried out in order to define levels that allow greater gains in fish nutrition.

Keywords: Prebiotic additives. Apple pomace hydrolyzate. Passion fruit peel hydrolyzate. Fish Nutrition.

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1 INTRODUÇÃO

Impulsionada pelo aumento na demanda por alimentos de alta qualidade nutricional, reflexo do crescimento populacional, a produção mundial de pescado vem apresentando crescimento médio de 3,2% ao ano nas últimas cinco décadas (FAO, 2014). O mercado consumidor prioriza alimentos saudáveis, preferencialmente produzidos sem uso de antibióticos como promotores de crescimento, pois o seu uso continuado favorece o aparecimento de cepas bacterianas resistentes no meio criatório, podendo afetar a saúde dos consumidores (MONTAGNE; PLUSKE; HAMPSON, 2003; MOTA et al., 2005; RAMOS et al., 2014). Estas constatações demonstram a importância de desenvolver pesquisas visando a obtenção de promotores alternativos, efetivos no equilíbrio da microbiota do trato gastrointestinal (SILVA; NÖRNBERG, 2003) e que não deixem resíduos na carcaça e no meio ambiente, tais como os prebióticos. Este grupo complexo de polissacarídeos não amiláceos e oligossacarídeos, resistente à digestão enzimática no trato gastrointestinal de animais não ruminantes, promove o crescimento e atividade fermentativa de bactérias benéficas no trato digestório do hospedeiro (GIBSON; ROBERFROID, 1995). Como resultado do efeito prebiótico são relatados: maior crescimento das populações microbianas benéficas, modificações nas características anatômicas do trato digestório promovendo aumento na área de absorção da mucosa intestinal, geração de ácidos graxos voláteis usados como fonte de energia, melhora geral nas condições luminais, atuação benéfica sobre o sistema imune e, melhora no desempenho animal (GIESE et al., 2011; SONG et al., 2014).

Em paralelo ao crescimento da aquicultura, o Brasil se desenvolve notavelmente no setor agroindustrial, onde a produção e processamento de frutas vem se destacando. A produção de maçã em grande escala no Brasil teve início nos anos 60 e atualmente o país apresenta produção expressiva concentrada na Região Sul, sendo o Rio Grande do Sul o segundo maior Estado produtor, responsável por 46,6% da produção nacional na safra de 2012 (IBGE, 2016). Aproximadamente 30% da produção nacional de maçãs é destinada ao processamento para obtenção de sucos, geléias e fermentados, gerando como principal subproduto o bagaço de maçã, que representa entre 20 a 40% do total de maçãs processadas (COELHO, 2007; NOGUEIRA et al., 2005). O rendimento e a composição do bagaço são dependentes da tecnologia empregada na extração do suco, sendo que este subproduto pode apresentar até 18% de pectina em sua composição (NOGUEIRA et al., 2005). Geralmente, o bagaço de maçã é utilizado na alimentação de bovinos, onde deve ser empregado com moderação porque pode causar alterações na fermentação ruminal, ou é simplesmente

dispensado no solo, apresentando riscos de contaminação ambiental (VILLAS BOAS; ESPÓSITO, 2000).

O maracujá é produzido em regiões de clima tropical e subtropical, sendo o Brasil o maior produtor mundial, com produção média anual de 900 mil toneladas (IBGE, 2012). Seu cultivo está basicamente voltado para a indústria de sucos e polpas (ZERAIK et al., 2010), gerando grande quantidade de resíduos na forma de cascas e sementes. A casca de maracujá ainda é pouco explorada, mas representa uma fonte promissora de fibra dietética, principalmente de pectina (14 a 23%) (YAPO, 2009).

De acordo com o exposto, é notável que os grandes investimentos das agroindústrias de processamento de frutas no aumento da sua capacidade produtiva, vêm gerando grandes quantidades de subprodutos e resíduos. Embora os esforços venham sendo remetidos à uma tecnologia limpa, com o gerenciamento adequado da biomassa residual produzida, grande parte deste material ainda é subutilizado, considerado sem valor econômico e fonte potencial de contaminação ambiental (COELHO; WOSIACKI, 2010). Assim, são necessários estudos de aplicabilidade, a fim viabilizar a sustentabilidade das cadeias produtivas. Com o desenvolvimento de processos adequados, é possível aproveitar diversos componentes dos resíduos de frutas, tais como fibras e substâncias antioxidantes (GULLÓN et al., 2011; MOURE et al., 2006; NABARLATZ; EBRINGEROVÁ; MONTANÉ, 2007;).

Neste cenário, substâncias pécticas que compõem a fibra alimentar solúvel de vários resíduos de frutas surgem como alternativa promissora por apresentarem composição químico estrutural diferenciada (MOHNEN, 2008). Sabe-se que entre as substâncias prebióticas, os oligossacarídios não digestíveis de cadeia linear são fermentados mais extensivamente e por um maior número de espécies bacterianas do que os de cadeia ramificada (VAN LAERE et al., 1997), o que influencia diretamente na proporção entre os ácidos graxos voláteis (AGVs) produzidos. Esta constatação é explicada pela variabilidade e seletividade fermentativa das populações microbianas do trato digestório, que pode ser moldada com o uso direcionado de substratos de distintas ações prebióticas. Neste contexto, os oligossacarídeos produzidos a partir da hidrólise da pectina têm sido apontados como uma nova classe de prebióticos capazes de exercer efeitos benéficos (GÓMEZ et al., 2014; GULLÓN et al., 2013; HOLCK et al., 2011). Sabe-se que os hidrolisados pécticos possuem caráter ácido, pela presença do ácido galacturônico, o que lhes confere características e efeitos fisiológicos diferenciados (CHEN et al., 2013). Porém, a maioria das investigações ainda concentra-se nas fases de produção e caracterização, uma vez que diferentes matérias-primas, métodos de extração e hidrólise da pectina são empregados (GULLÓN et al., 2013).

Entre as inúmeras espécies criadas na piscicultura, a tilápia do Nilo (*Oreochromis niloticus*) destaca-se pelo ciclo de cultivo curto e adaptação aos sistemas de criação. No Brasil, representa aproximadamente 47% do total de peixes produzidos (IBGE, 2016). Novos prebióticos podem potencializar o desempenho destes animais, considerando que a Tilápia possui grande apelo comercial e a otimização de sua criação é imprescindível.

O jundiá (*Rhamdia quelen*), espécie nativa da região Sul do País, é considerado um modelo biológico regional, e apresenta carne saborosa ausente de espinhos intramusculares, promovendo sua popularização entre produtores e consumidores (CARNEIRO; MIKOS, 2005). Ainda são poucos os trabalhos que apontam o efeito dos prebióticos na nutrição desta espécie, os quais podem melhorar a saúde e promover efeitos benéficos no desempenho dos peixes (ADORIAN et al., 2015; ADORIAN et al., 2016; GOULART et al., 2017; GOULART et al., 2018).

Para a piscicultura, são necessários estudos sobre os efeitos biológicos e metabólicos de hidrolisados pécticos, sobretudo abordando o caráter prebiótico destes constituintes alimentares. Este fato leva a uma ampla possibilidade de estudos, onde fontes agroindustriais subutilizadas ou descartadas no meio ambiente poderão ser racionalmente exploradas como matéria prima para obtenção de agentes que auxiliam na melhora do desempenho animal, manutenção da saúde e na segurança alimentar do pescado ofertado ao mercado consumidor.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Produzir e caracterizar hidrolisados pécticos obtidos a partir de resíduos agroindustriais e investigar seu potencial prebiótico quando adicionados na dieta de alevinos de tilápia do Nilo (*Oreochromis niloticus*) e jundiá (*Rhamdia quelen*).

1.1.2 Objetivos específicos

- Extrair a pectina de bagaço de maçã e casca de maracujá e gerar informações sobre eficiência de obtenção dos constituintes pécticos;

- Produzir hidrolisados pécticos pelo processo de hidrólise ácida e caracterizar o produto quanto a sua variabilidade química e propriedades físico-químicas;

- Adicionar os hidrolisados pécticos em duas concentrações (2,5 ou 5 g/kg) na dieta de alevinos de tilápia do Nilo (*Oreochromis niloticus*) e jundiá (*Rhamdia quelen*) e avaliar os efeitos sobre o desempenho produtivo, parâmetros metabólicos, composição corporal, conteúdo intestinal e morfometria intestinal.

Os estudos apresentados nesta tese foram realizados em duas fases. Na primeira etapa foram conduzidas atividades para obtenção eficiente de concentrados e hidrolisados pécticos, analisando a variabilidade química e propriedades físico-químicas das frações potenciais para uso como agentes prebióticos. Na segunda etapa os hidrolisados pécticos foram testados quanto ao seu potencial prebiótico, em ensaios biológicos com alevinos de tilápia do Nilo e jundiá.

Os resultados estão apresentados na forma de artigos científicos, onde o Artigo 1 aborda a "Obtenção e caracterização de hidrolisados pécticos a partir de resíduos agroindustriais". No Artigo 2, "Otimização do uso da biomassa produzida na fruticultura associado à produção de pescado", são apresentados os resultados e discussões referentes à avaliação dos efeitos da suplementação dos hidrolisados pécticos na dieta de alevinos de tilápia do Nilo. O Artigo 3 contempla o uso de "Hidrolisados pécticos na nutrição do jundiá (*Rhamdia quelen*)".

2 ARTIGO 1

OBTENÇÃO E CARACTERIZAÇÃO DE HIDROLISADOS PÉCTICOS A PARTIR DE RESÍDUOS AGROINDUSTRIAIS

OBTAINING AND CHARACTERIZING OF PECTIC HYDROLYSATES FROM AGROINDUSTRIAL WASTE

Patrícia Inês Mombach[1]; Taida Juliana Adorian[1]; Dirleise Pianesso[1]; Giovani Bressiani Pedroso[2]; Ayrton Figueiredo Martins[2]; Leila Picolli da Silva[1]

[1]Departamento de Zootecnia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, CEP: 97105-900, Brazil.
[2]Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, CEP: 97105-900, Brazil.

RESUMO - A intensa atividade agroindustrial no setor de produtos de origem vegetal vem gerando grandes quantidades de subprodutos e resíduos, como bagaços e cascas de frutas. Estes resíduos da extração de sucos ou polpas, muitas vezes subutilizados, acabam gerando passivos ambientais. Para que a biomassa residual seja transformada em produtos com valor nutricional e tecnológico, o presente estudo foi desenvolvido com o objetivo de gerar informações sobre a obtenção de compostos pécticos, variabilidade química e propriedades físico-químicas de hidrolisados obtidos a partir de bagaço de maçã e casca de maracujá. Primeiramente, os resíduos de frutas foram processados para obtenção do resíduo em pó. A pectina foi extraída em meio termo-aquoso, sendo realizados testes afim de determinar a proporção peso:volume que permite o maior rendimento de extração. Após realizou-se o processo de hidrólise química da pectina. O bagaço de maçã e casca de maracujá, e seus respectivos hidrolisados foram caracterizados quanto aos teores de matéria seca, fibra alimentar total, fibra solúvel, fibra insolúvel, cinzas, proteína bruta e gordura. Os hidrolisados também foram analisados quanto ao perfil de monossacarídeos, capacidade de hidratação, capacidade de ligação à gordura e capacidade de ligação ao cobre. Entre os resíduos agroindustriais avaliados, a casca de maracujá apresentou maior rendimento de extração de pectina (17,22%), seguida do bagaço de maçã (12,41%). A análise química demonstrou aumento de 133,17% e 121,40% na fração solúvel da fibra nos hidrolisados de bagaço de maçã e casca de maracujá, em relação à sua matéria prima de origem. Na análise dos monossacarídeos, o hidrolisado de bagaço de maçã apresentou quantidades semelhantes de glicose-xilose-ácido galacturônico (14,7; 13,5 e 16,11%), enquanto o hidrolisado de casca de maracujá apresentou maior teor de ácido galacturônico (13,7%), seguido de glicose (8,5%) e xilose (4,7%). Em relação as características físico-químicas, o hidrolisado péctico de bagaço de maçã demonstrou elevada capacidade de ligar-se à água, característica que apresenta-se reduzida no hidrolisado de casca de maracujá. A partir destas informações conclui-se que os hidrolisados pécticos, poderão exercer efeitos diferenciados de acordo com a fonte de origem.

Palavras-Chave: Biomassa residual. Hidrolisado de bagaço de maçã. Hidrolisado de casca de maracujá. Nutrição.

ABSTRACT - The intense agroindustrial activity in the vegetable products sector has been generating large amounts of by-products and residues, such as bagasse and fruit peels. These waste from the extraction of juices or pulps, often underutilized, end up generating environmental liabilities. For this residual biomass to be transformed into products with nutritional and technological value, the present study was developed with the objective of generating information on the obtaining of pectic compounds, chemical variability and physicochemical properties of hydrolysates obtained from apple pomace and passion fruit peel. First, the fruit residues were processed to obtain the powdered residue. The pectin was extracted in thermo-aqueous medium, being carried out tests in order to determine the ratio of weight:volume that allows the highest yield of extraction. After the chemical hydrolysis process of the pectin was carried out. The apple pomace and passion fruit peel and their respective hydrolysates were characterized as dry matter, total dietary fiber, soluble fiber, insoluble fiber, ash, crude protein and fat. The hydrolysates were also analyzed for monosaccharide profile, hydration capacity, fat binding capacity and copper binding capacity. Among the agroindustrial wastes evaluated, the passion fruit peel presented a higher yield of pectin extraction (17.22%), followed by apple pomace (12.41%). The chemical analysis showed an increase of 133.17% and 121.40% in the soluble fraction of the fiber in the hydrolysates of apple pomace and passion fruit peel, in relation to its raw material of origin. In the analysis of the monosaccharides, the apple pomace hydrolyzate presented similar amounts of glucose-xylose-galacturonic acid (14.7, 13.5 and 16.11%), while the passion fruit peel hydrolyzate had a higher content of galacturonic acid (13.7%), followed by glucose (8.5%) and xylose (4.7%). Regarding the physico-chemical characteristics, the pectic hydrolysis of apple pomace showed a high capacity to bind to water, a characteristic that is reduced in the passion fruit peel hydrolyzate. From this information it is concluded that the pectic hydrolysates may exert different effects according to the source of origin.

Keywords: Residual biomass. Apple pomace hydrolyzate. Passion fruit peel hydrolyzate. Nutrition.

1 INTRODUÇÃO

O Brasil é um país com notável capacidade produtiva e vem se destacando pela sua intensa atividade agroindustrial no setor de produtos de origem vegetal. Porém, o aumento exponencial do processamento de matéria prima vegetal gera grandes quantidades de subprodutos e resíduos (COELHO; WOSIACKI, 2010). Uma análise consciente dessa evolução produtiva, demonstra que a exploração da biomassa residual (folhas, palhadas, farelos, cascas, bagaços, etc) gerada pela agroindustrialização ainda é muito deficitária. Este fato leva a considerável desperdício produtivo, uma vez que o material descartado ou inadequadamente explorado, além de gerar grandes passivos ambientais, leva consigo densidade massiva de nutrientes e compostos bioativos com alto potencial de aplicação nutricional e tecnológica.

A produção de suco de frutas é um dos exemplos deste desperdício produtivo, gerando em média, 40% de massa residual (COELHO, 2007; GERON, 2007). Este fato é

alarmante, principalmente considerando que o Brasil é o terceiro maior produtor mundial de frutas, destinando 70% desta produção à indústrias de extração de suco ou polpa (IBGE, 2016). Neste cenário, o desenvolvimento de processos visando aumentar a eficiência de utilização da biomassa agroindustrial remanescente, assume vital importância para garantir a sustentabilidade ambiental e menor desperdício produtivo da industrialização de frutas.

Cascas e bagaços de maracujá e maçã são ricos em pectinas (MOURE et al., 2006; NABARLATZ; EBRINGEROVÁ; MONTANÉ, 2007; GULLÓN et al., 2011), macromoléculas complexas e heterogêneas que estão entre os componentes da parede celular dos vegetais. Suas características estruturais são diversificadas de acordo com a fonte, mas com predominância de ácido galacturônico nas cadeias principais e de monossacarídeos neutros nas laterais (ROUND et al., 2010; WIKIERA et al., 2015; ZHANG et al., 2015). Pela abundância e baixo custo, sua caracterização estrutural e aplicações vem sendo estudadas, com o intuito de ampliar sua utilização como ingrediente prebiótico na nutrição animal. Os estudos com hidrolisados pécticos tem demonstrado sua efetiva ação prebiótica, mas com variações na resposta biológica devido a sua diversidade molecular, normalmente imposta pela matéria prima de origem (CHEN et al., 2013; GÓMEZ et al., 2014; GULLÓN et al., 2011).

Para que a biomassa residual resultante do processamento de maçã e maracujá seja explorada na geração de promotores de saúde animal, o presente estudo foi desenvolvido com vistas a gerar informações sobre eficiência de obtenção de compostos pécticos, variabilidade química e propriedades físico-químicas das frações potenciais para uso como agentes prebióticos.

2 MATERIAIS E MÉTODOS

2.1 AMOSTRAS

O bagaço de maçã (BM) (*Pyrus malus*, var. Gala e Fuji) foi doado pela indústria de processamento de suco Grupo Fischer. A casca de maracujá amarelo (CM) (*Passiflora edulis f. flavicarpa*) foi obtida após remoção completa da polpa. Os resíduos foram lavados em água corrente, triturados em cortador elétrico (Metvisa, CUT 2.5, 1650 rpm) e secos em estufa a 50°C durante 24-48 horas. Os materiais secos foram moídos em micro moinho (27000 rpm/60 segundos - Marconi, MA-630/1) para a obtenção do resíduo em pó, com tamanho médio de partícula de 0,3 mm.

2.2 EXTRAÇÃO DA PECTINA E CÁLCULO DE RENDIMENTO

A pectina foi extraída em meio termo-aquoso, sob temperatura de 100ºC durante 1 hora, seguindo a metodologia proposta por Calliari (2015), com modificações. Foram realizados testes com o intuito de obter as proporções peso:volume (amostra:água) para o maior rendimento de extração de pectina das fontes (Figura 1). Para o bagaço de maçã utilizou-se a proporção peso:volume de 3:97 e para a casca de maracujá, 4:96. Após resfriamento, a solução foi centrifugada (3500 rpm/10 min) e ao sobrenadante foi adicionado etanol 96% na proporção de 1:1 para precipitação e recuperação da pectina (repouso de 24h / 5ºC), que foi submetida a secagem em estufa com circulação de ar (50ºC / 48 horas) e moída (tamanho médio de partícula de 0,3 mm). O rendimento de extração foi obtido pela relação entre o peso da pectina extraída e o peso da matéria-prima inicial, seguindo a fórmula:

$$\text{Rendimento (\%)} = \frac{\text{pectina extraída (g)}}{\text{matéria-prima inicial (g)}} \times 100$$

2.3 HIDRÓLISE DA PECTINA

Os concentrados pécticos obtidos a partir de bagaço de maçã e casca de maracujá foram dissolvidos em solução de HCl 0,25 N, na proporção amostra(g):solução(mL) de 1:50, em temperatura de 60ºC, sob agitação constante por 2 horas (MOURA, 2015). Para interromper a hidrólise foi adicionado KOH 0,9N em quantidade suficiente para atingir a neutralização da solução (pH 7,0). O material neutralizado foi seco em estufa com circulação de ar (50ºC / 12 horas). Após secagem o material foi moído em micro moinho a 0,3mm, obtendo-se os hidrolisados pécticos.

2.4 CARACTERIZAÇÃO DOS RESÍDUOS E DOS HIDROLISADOS PÉCTICOS

O bagaço de maçã e a casca de maracujá, bem como os hidrolisados pécticos obtidos a partir destas fontes (HBM e HCM, respectivamente) foram caracterizados quanto aos teores de matéria seca (método 925.45b – AOAC, 1995), cinzas (método 923.03 – AOAC, 1995), proteína bruta (método 960.52 – AOAC, 1995), fibra alimentar total, fibra insolúvel e fibra solúvel (método 991.43 – AOAC, 1995). A gordura foi extraída e quantificada de acordo com o método de Bligh e Dyer (1959).

Os hidrolisados também foram analisados quanto à capacidade de hidratação, capacidade de ligação à gordura (WANG; KINSELLA, 1976) e capacidade de ligação ao cobre (McBURNEY; VAN SOEST; CHASE, 1983). O perfil de monossacarídeos dos hidrolisados pécticos foi determinado por Cromatografia Líquida de Alta Eficiência acoplado ao detector de índice de refração (HPLC-RID), de acordo com metodologia descrita por Sluiter et al. (2006).

3 RESULTADOS E DISCUSSÃO

A literatura científica tem demonstrado que as metodologias e as matérias primas utilizadas alteram o rendimento de extração da pectina (CALLIARI, 2015; GOULART, 2015; MOMBACH, 2015; MOURA, 2015). Em nosso estudo, optamos pela extração dos constituintes pécticos em meio termo-aquoso, que pode ser menos eficiente quando comparado à métodos de extração química, mas apresenta as vantagens de baixo custo e garantia de seguridade ambiental (ARTHEY; ASHURST, 1997). Na extração termo-aquosa obtivemos máxima eficiência extrativa das pectinas de casca de maracujá e bagaço de maçã em proporções distintas de matéria prima:água (Figura 1), demonstrando que os fatores intrínsecos de cada fonte (diversidade estrutural e de arranjo molecular da parede celular, manejo e ambiente de cultivo e de processamento) interferem na reatividade da matéria prima e no rendimento de extração. Entre os dois resíduos agroindustriais, a casca de maracujá apresentou maior rendimento de extração de pectina (Figura 2), com maior rentabilidade extrativa (17,22%) obtida usando 25% a menos de água (4:96) quando comparado a melhor rentabilidade para o bagaço de maçã (12,41% na proporção 3:97). Com extração química em meio ácido (HCl 0,1 N) da pectina de casca de maracujá, Moura (2015) obteve rentabilidade semelhante aos resultados encontrados no presente estudo. Já Yapo (2009), utilizando ácido nítrico a 0,3M, obteve rendimento de 13,9% de pectina para a mesma matéria prima. A comparação dos estudos demonstra que a extração termo-aquosa é promissora, uma vez que possibilita rentabilidade extrativa semelhante a extração química, mas com custo reduzido e menor geração de resíduos poluentes.

A análise química (Tabela 1) demonstrou que a fração solúvel da fibra aumentou em 133,17% e 121,40% nos hidrolisados de bagaço de maçã e casca de maracujá, em relação às respectivas fontes de origem. As pectinas solúveis hidrolisadas apresentam fermentabilidade intensificada à nível intestinal (VAN LAERE et al., 1997) quando comparada as respectivas

cadeias moleculares íntegras, pois permitem maior acessibilidade microbiana, potencializando sua ação prebiótica (HOLCK et al., 2011; GULLÓN et al., 2013; GÓMEZ et al., 2014).

O elevado teor de matéria mineral observado nos hidrolisados foi resultante do processo de neutralização da reação hidrolítica, onde a reação entre KOH e HCl gerou o sal KCl, que cristalinizou na secagem das amostras. Embora seja um constituinte exógeno, este sal não causa efeitos deletérios ao organismo, desde que seja obedecida a quantidade pré-estabelecida, considerando a exigência destes minerais pelos animais suplementados. A concentração e hidrólise das pectinas causou diminuição efetiva nos teores de proteína e gordura em relação as matérias primas iniciais, especialmente quando observado a gordura presente no bagaço de maçã (16,72%), que reduziu a menos de 1% no seu respectivo hidrolisado (Tabela 1).

A análise monomérica dos hidrolisados pécticos (Tabela 2) comprova a diversidade estrutural das fontes utilizadas e a heterogeneidade deste polissacarídeo. O hidrolisado de bagaço de maçã apresentou quantidades semelhantes de glicose-xilose-ácido galacturônico (14,7; 13,5 e 16,11%, respectivamente), enquanto o hidrolisado de casca de maracujá apresentou maior teor de ácido galacturônico (13,7%), seguido de glicose (8,5%) e xilose (4,7%). Os teores de ácido galacturônico dos hidrolisados pécticos analisados em nosso estudo apresentaram-se inferiores à resultados da literatura, para outras fontes extrativas (casca de limão - 69,6%, de casca de maracujá - entre 68,4 e 71,9%, de abóbora - 75 a 78% e de casca de soja - 68 a 72%) (CUI; CHANG, 2014; KALAPATHY; PROCTOR, 2001; YAPO, 2009). As diferenças entre resultados, além de refletir a variabilidade dos resíduos agroindustriais testados, também é atribuído a variações nos procedimentos metodológicos, desde a fase de extração da pectina até a obtenção do hidrolisado.

Na nutrição animal, o conhecimento das propriedades físico-químicas dos ingredientes é relevante para definir sua influência na tecnologia de processamento da ração e na ação fisiológica sobre o trato digestório. Processos de aquecimento, hidrólise física ou hidrólise química podem afetar significativamente a capacidade de hidratação das fontes. Os resultados do estudo demonstram que o hidrolisado péctico de bagaço de maçã possui aproximadamente 70 vezes mais capacidade de ligar-se à água quando comparado ao hidrolisado de casca de maracujá (Figura 3). A capacidade de retenção de água influi sobre o volume, a viscosidade e textura da dieta, além de alterar a digestão e absorção de nutrientes, principalmente gorduras (FIETZ; SALGADO, 1999; GUNNESS; GIDLEY, 2010; RUBIO-SENENT et al., 2015).

A capacidade de ligação à gordura depende das propriedades de superfície da fonte, da sua densidade, espessura e da natureza hidrofóbica das partículas (LÓPEZ et al., 1997). Em

nosso estudo, os resultados encontrados para a capacidade de ligação a gordura foram semelhantes entre os hidrolisados pécticos de bagaço de maçã (0,83 g de óleo/g de amostra) e casca de maracujá (0,86 g de óleo/g de amostra) (Figura 3). Estes valores são considerados baixos quando comparados à resultados da literatura para concentrados pécticos de outras fontes. Moura (2015) avaliou a capacidade de ligação à gordura para os concentrados pécticos de casca de soja (2,73 g de óleo/g de amostra), casca de maracujá (4,05 g de óleo/g de amostra) e bagaço de laranja (2,97 g de óleo/g de amostra). Rubio-Senent et al. (2015) extraíram pectinas de bagaço de azeitona e obtiveram um concentrado com alta capacidade de absorção de gordura (6,17 g de óleo/g de amostra). De maneira geral, ingredientes com maior capacidade de ligação à gordura poderão diminuir o acesso da lipase pancreática ao substrato, reduzindo a absorção deste nutriente no trato digestório. Aplicando estes resultados na nutrição animal, é desejável uma baixa capacidade de ligação à gordura, pois do contrário o aporte energético poderá ser prejudicado, considerando que as dietas são formuladas para atender as exigências dos animais por fase de desenvolvimento.

A capacidade de ligação ao cobre é um indicativo da capacidade de ligação à cátions, usada para indicar a natureza iônica e grau de interação com sítios de ligação ativos das fontes (KAHLON; SMITH, 2007). Em nosso estudo, os hidrolisados de bagaço de maçã (0,017 g de Cu/g de amostra) e casca de maracujá (0,016 g de Cu/g de amostra) apresentaram baixa capacidade de ligação ao cobre (Figura 3). Moura (2015) obteve resultados mais elevados para esta característica em pectinas parcialmente hidrolisadas de casca de soja, casca de maracujá e bagaço de laranja (> 0,06 g de Cu/g de amostra). Sabe-se que polissacarídeos e ologissacarídeos pécticos podem agir como quelantes de metais, seja pela presença de seus grupamentos ácidos com alta afinidade por cátions, ou pela substituição das moléculas de água da esfera de solvatação de cátions pelos grupos hidroxila do polissacarídeo (CHEN; MCCLEMENTS; DECKER, 2010). Visando a suplementação de hidrolisados pécticos na nutrição animal, são desejáveis baixas capacidades de ligação ao cobre pois, elevada CLCu pode reduzir a disponibilidade de minerais catiônicos, essenciais para o desenvolvimento dos animais. Além disso, fibras com elevada CLCu possuem maior capacidade de quelação dos sais biliares no trato digestório, incrementando sua excreção fecal e reduzindo sua participação na digestão das gorduras, bem como, reduzindo sua reabsorção pelo epitélio intestinal (LEE et al., 2002). Esse fato leva a menor eficiência de uso das gorduras para suprir as demandas energéticas dos animais.

4 CONCLUSÃO

O bagaço de maçã e a casca de maracujá são fontes promissoras para extração de pectina, sendo que a casca de maracujá apresentou maior rendimento (17,22%). Os hidrolisados pécticos de bagaço de maçã e casca de maracujá apresentaram composição química e características físico-químicas diferenciadas. O hidrolisado de casca de maracujá apresentou maiores variações no teor dos monossacarídeos presentes. Já o hidrolisado de bagaço de maçã apresentou maior capacidade de hidratação, o que pode influenciar na tecnologia de processamento da ração e na ação fisiológica sobre o trato digestório. Visando a geração de promotores de desempenho e saúde animal, é evidente a necessidade de avaliação *in vivo* dos hidrolisados de bagaço de maçã e casca de maracujá, pois poderão apresentar resultados biológicos distintos devido a fatores intrínsecos a cada fonte.

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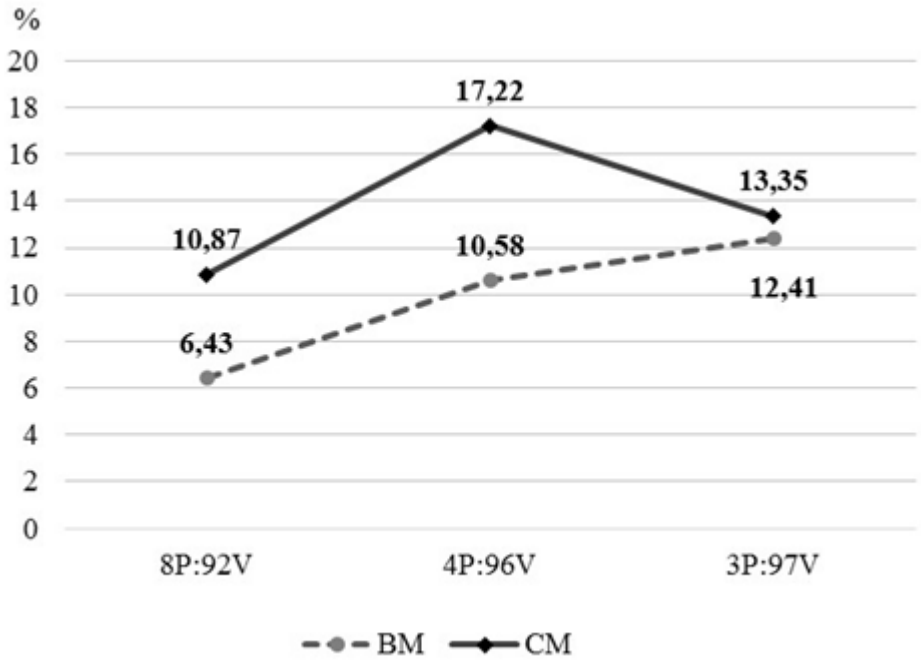
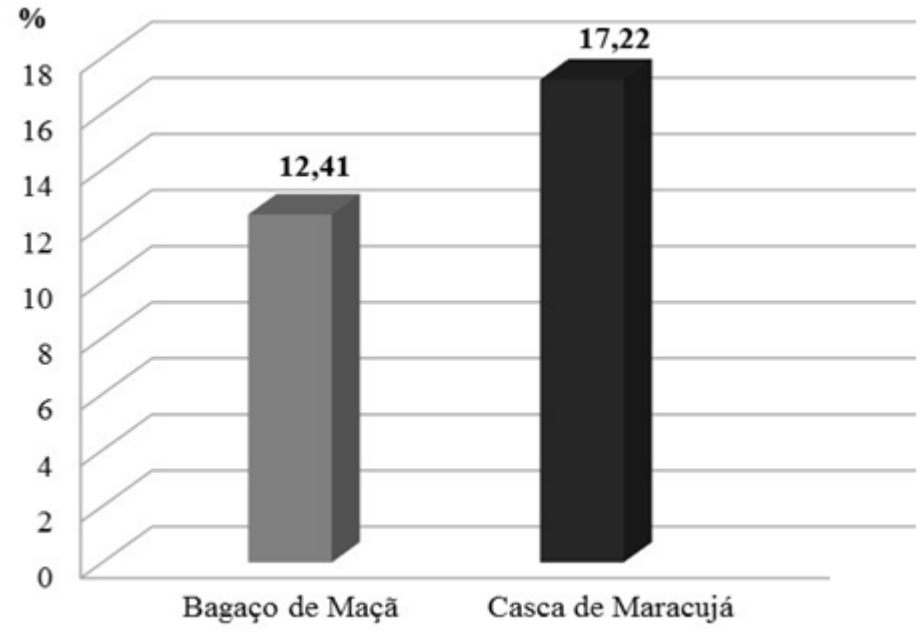
Figura 1 – Testes para avaliar o rendimento de extração de pectina a partir do bagaço de maçã (BM) e da casca de maracujá (CM)



Fonte: Elaborada pela autora.
P:V = proporção peso:volume.

Figura 2 – Rendimento de extração de pectina de resíduos de frutas



Fonte: Elaborada pela autora.

Tabela 1 – Composição nutricional dos resíduos e hidrolisados pécticos de bagaço de maçã e casca de maracujá

Composição química (%)	Resíduos		Hidrolisados pécticos	
	Bagaço de maçã	Casca de maracujá	HBM	HCM
Matéria seca	95,71 ± 0,05	91,77 ± 0,57	95,74 ± 0,27	95,42 ± 0,58
Fibra alimentar total	83,18 ± 3,21	64,56 ± 3,64	39,47 ± 0,39	40,14 ± 0,74
Fibra alimentar solúvel	16,13 ± 1,01	17,80 ± 1,69	37,72 ± 0,97	39,41 ± 0,44
Fibra alimentar insolúvel	67,05 ± 2,20	46,76 ± 1,94	1,75 ± 0,57	0,73 ± 0,29
Proteína bruta	6,11 ± 0,45	4,44 ± 0,01	1,33 ± 0,02	2,41 ± 0,02
Gordura	16,72 ± 0,83	1,88 ± 0,31	0,75 ± 0,07	0,40 ± 0,04
Cinzas	1,46 ± 0,13	5,84 ± 0,12	52,92 ± 0,04	54,54 ± 0,33

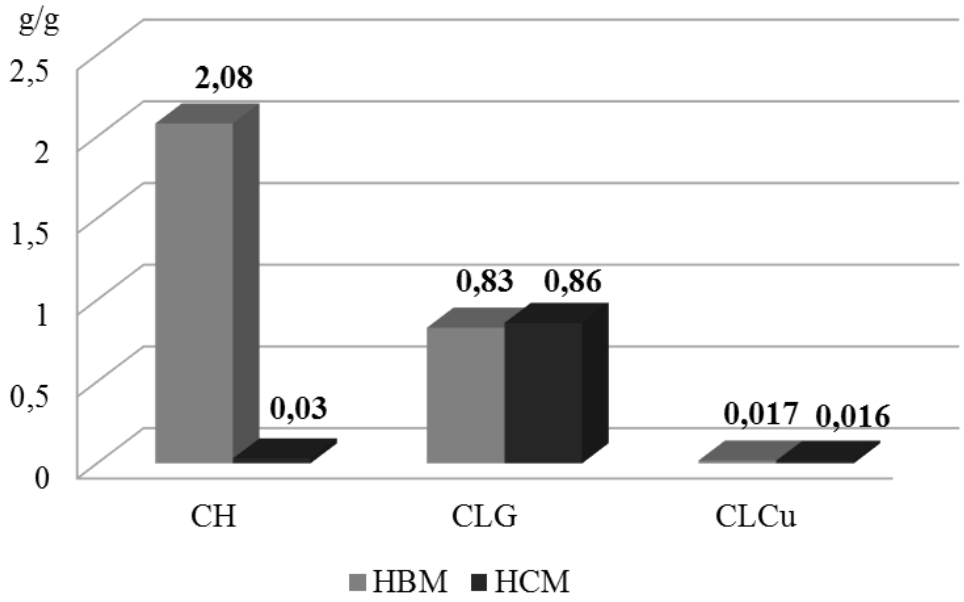
Fonte: Elaborada pela autora.
HBM: hidrolisado de bagaço de maçã; HCM: hidrolisado de casca de maracujá.

Tabela 2 – Composição química (%) dos hidrolisados pécticos, via HPLC-RID

Hidrolisado Péctico	Composição							
	Glc	Xil	Ara	Gal	Ram	Man	Fru	AGal
HBM	14,70	13,50	0,00	0,00	0,00	0,00	0,00	16,11
HCM	8,55	4,75	0,00	0,00	0,00	0,00	0,00	13,70

Fonte: Elaborada pela autora.
HBM: hidrolisado de bagaço de maçã; HCM: hidrolisado de casca de maracujá; Glc: glicose; Xil: xilose; Ara: arabinose; Gal: galactose; Ram: ramnose; Man: manose; Fru: frutose; AGal: ácido galacturônico.

Figura 3 – Propriedades físico-químicas dos hidrolisados pécticos extraídos de bagaço de maçã e casca de maracujá



Fonte: Elaborada pela autora.
HBM: hidrolisado de bagaço de maçã; HCM: hidrolisado de casca de maracujá; CH: Capacidade de hidratação (g água/ g amostra); CLG: Capacidade de ligação à gordura (g óleo/ g amostra); CLCu: Capacidade de ligação ao cobre (g Cu/g de amostra).

3 ARTIGO 2

Optimization of the use of biomass produced in fruit farming associated with fish production[**]

Patrícia Inês Mombach[1,*]; Taida Juliana Adorian[1]; Dirleise Pianesso[1]; Bruno Bianch Loureiro[1]; Fernanda Rodrigues Goulart[1]; Luiza Loebens[2]; Mariane Bittencourt Fagundes[3]; Roger Wagner[3]; Leila Picolli da Silva[1]

[1]Department of Animal Science, Federal University of Santa Maria, Santa Maria, Brazil.
[2]Department of Biology, Federal University of Santa Maria, Santa Maria, Brazil.
[3]Department of Food Science and Technology, Federal University of Santa Maria, Santa Maria, Brazil.

*Corresponding author:
Phone: 55 (55) 3220-8365
Fax: 55 (55) 3220-8240
E-mail address: patimombach@hotmail.com

Abstract

The obtaining of food additives from agroindustrial waste aims at enhancing the use of the available biomass, besides bringing benefits to animal nutrition. The objective of the work was to investigate the prebiotic potential of pectic hydrolysates extracted from apple pomace and passion fruit peel, added to the diet of Nile tilapia (*Oreochromis niloticus*). The pectin was extracted from agroindustrial residues and the pectic hydrolysates were obtained. The biological assay introducing pectic hydrolysates in the diet of Nile tilapia (initial weight 3.9 ± 0.67 g) lasted 42 days. Five experimental diets were evaluated, one of them a control diet and the others with the addition of pectic hydrolysates (2.5 or 5 g/kg) to replace part of the cellulose. Firstly, the fish were weighed, measured, allocated to experimental units and fed three times a day until apparent satiation. At the experimental endpoint at day 42, the animals fasted for 18 hours and then subjected to biometrics for data collection, and fish were removed to determine hepatic, enzymatic, body composition, histological parameters and to assess intestinal contents. The study was based on a completely randomized experimental design with a 2x2 factorial arrangement, including an additional treatment, thus totaling 5 treatments with 4 replicates. The results were subjected to a normality test, followed by analysis of variance, and the means of the treatments were compared by analysis of orthogonal contrasts ($p<0.05$). The pectic hydrolysates obtained from

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apple pomace and passion fruit peel, added to the diet of Nile tilapia, allowed to obtain satisfactory results related to liver, histological, enzymatic, carcass and intestinal contents. However, further studies should be carried out to define the adequate amounts of pectic prebiotics supplementation, aiming at analyzing the effects on Nile tilapia growth and alimentary efficiency, as well as to elucidate their mechanisms of action.

Keywords Additives; Apple pomace; Aquaculture; Passion fruit peel; Pectic hydrolysates; Sustainability

1 Introduction

Food production systems need to be more efficient to meet the demand of the world's growing population, which is expected to reach 9.7 billion people by 2050 (FAO 2017). Nonetheless, this productive increase will not be easily achieved with the current agricultural matrix, which has caused intense soil, water and biodiversity degradation over the last years. The paradigm shift regarding food production should be guided by new technologies that permit a better use of the available biomass, reducing waste and ensuring more sustainable production patterns.

Fruit industrialization is responsible for a waste disposal that can exceed 40% of the total biomass, causing massive wastages of nutrients and bioactive compounds that have been little explored as to their nutritional action and function. It is well known that the apple pomace and the passion fruit peel are potential sources of pectin extraction, which can be hydrolyzed, generating non-digestible oligosaccharides (Chen et al. 2013) that exert a prebiotic action on animal health and performance. Seen in these terms, our research group has been working with residues from fruit industrialization to prospect new prebiotic additives for fish nutrition (Goulart et al. 2018; Goulart et al. 2017).

Aquaculture is considered one of the most efficient activities for a large-scale expansion of the high-quality foods production in the next years, since it allows to push the boundaries of agriculture for the use of flooded areas, which are still little explored in the production of animal protein. This activity has been growing exponentially in Brazil, with an expected increase of 104% in fish production by 2025 (FAO 2016). Currently, about 80% of Brazilian aquaculture production covers freshwater fish, and tilapia (*Oreochromis niloticus*) accounts for 38% of the total produced in the country (IBGE 2016).

In aquaculture, prebiotic additives have been studied as they can replace the use of antibiotics, bringing beneficial effects on the performance and/or animal health (Merrifield et al. 2010; Ringo et al. 2010). However, pectic hydrolysates still lack scientific evidence on their biological properties and the dose required to exert prebiotic effects on fish nutrition.

Based on the above, the present study aimed at investigating the prebiotic potential of pectic hydrolysates extracted from apple pomace and passion fruit peel, added at two concentrations (2.5 and 5 g/kg) to the diet of Nile tilapia juveniles (*Oreochromis niloticus*).

2 Materials and methods

The study was conducted in the Laboratory of Fisheries of the Department of Animal Science, Federal University of Santa Maria (UFSM) – RS, Brazil, after approval by the UFSM Ethics Committee on Animal Research and Experimentation under protocol 7941021015.

2.1 Pectin extraction and obtainment of pectic hydrolysates

The apple pomace was obtained after industrial juice extraction. The passion fruit peel was obtained after manual pulping.

Pectin was extracted from apple pomace and passion fruit peel in an aqueous medium at the concentration of 3:97 and 4:96 (sample/water), respectively, at a temperature of 100 °C for 1 hour. After the mixture has cooled down, it was centrifuged (3500 rpm/10 min) and to the supernatant was added 96% ethanol in a ratio of 1:1, for the precipitation of the pectin. After pectin precipitation (rested 24h/5 °C), it was separated from the alcohol and put in the oven with controlled air circulation at 50°C for 48 hours. After drying, it was ground in a micro mill for 60 seconds, obtaining particles of an average size of 0.3 mm.

To obtain the hydrolysates, the pectin was dissolved in 0.25 N HCl solution, in a 1:50 rate of sample (g):solution (mL), at 60 °C, under continuous stirring for 2 hours. To suspend the hydrolysis, KOH was added for neutralization (pH 7.0). The neutralized material was dried at 50 °C for 12 hours, using an oven with forced air circulation. The pectic hydrolysates were obtained after the material was milled using the micro mill (Moura 2015).

2.2 Experimental design, treatments and diet preparation

The study was based on a completely randomized experimental design with a 2x2 factorial arrangement with an additional treatment, totaling 5 treatments with 4 replicates. Therefore, five experimental diets were assessed, one of them a control diet and to the others was added pectic hydrolysates to replace the part of the cellulose. The hydrolysates were obtained from two different sources (AP – apple pomace and PFP – passion fruit peel) and added at two levels (2.5 or 5 g/kg) to the diet. The experimental diets were formulated to be isocaloric (12.96 MJ/kg of metabolizable

energy) and isoproteic (31% CP) basically composed of fish meal, corn starch and soy protein concentrate (SPC 60%).

To prepare the diets, the ingredients were ground (590 μm), weighed, mixed until complete homogenization, water was added until the mass reached the appropriate point and after the pelletization process was carried out. The pellets were oven dried for 24h at a temperature of 50 °C. After drying, the feedstuffs were ground and sieved to obtain granules that allow food intake of the fish. The composition of moisture, crude protein, ash, (AOAC 1995), fat (Bligh and Dyer 1959), and the physicochemical properties (hydration capacity, fat binding capacity (Wang and Kinsella 1976) and copper binding capacity (McBurney et al. 1983)) was analyzed in the diets.

2.3 Biological assay

The assay was performed in a water recirculating system, consisting of a motor pump (1 hp) and 20 tanks (125 L) with individual water inlet and outlet valves and two biological filters with crushed stone. A total of 500 Nile tilapia juveniles with an initial average weight of 3.9 ± 0.67 g were used. The fish underwent a seven-day adaptation period in the experimental units (25 fish/tank) and were fed three times a day until apparent satiation during the 42 days of the experimental period.

2.4 Quality of water

Pipe cleaning and siphoning of residues from the experimental units were performed twice a day, followed by a renewal of 10% of the system water. In the water of the culture, the temperature was measured on a daily basis with a mercury bulb thermometer re (24.94 ± 1.07ºC). Weekly, were measured by colorimetric kits (Alfa-Tecnoquímica) the dissolved oxygen (6.27 ± 0.58 mg/L), pH (6.91 ± 0.38), ammonia (0.37 ± 0.14 mg/L), nitrite (0.34 ± 0.14 mg/L), alkalinity (36 ± 7.87 mg $CaCO_3$/L) and hardness (29.7 ± 16.44 mg $CaCO_3$/L).

2.5 Data collection and assessed variables

Two weighing biometrics and an individual measurement of the animals (initial and at 42 days) were carried out aiming at keeping up with the zootechnical performance. Before each biometry, fish were fasted for 18 hours and sedated with benzocaine at the concentration of 190 mg per liter of water (Okamura et al. 2010). In the final biometry, fish were collected in order to determine hepatic, enzymatic, body composition and histological parameters and to evaluate intestinal contents. These animals were subjected to euthanasia by benzocaine overdose (250 mg/L) (AVMA 2013). The following parameters were evaluated:

Growth and food efficiency parameters: Using data obtained concerning weight (g), total length (from the forward end of the head to the end of the caudal fin – cm) and consumption, the following variables were calculated: daily weight gain (g): (final weight – initial weight)/total of experiment days, and apparent feed conversion: (total consumption)/(final biomass – initial biomass).

Carcass parameters: Eight fish/treatment were analyzed for moisture contents (method 925.45b – AOAC 1995), ash (method 923.03 – AOAC 1995), crude protein (método 960.52 – AOAC 1995) and fat (Bligh and Dyer 1959). Based on these results, the body nutrient retention was calculated:

- Protein Deposition in the Body (PDB): [Fw * (%FCPB100)] – [Iw * (%ICPB/100)];

- Fat Deposition in the Body (FDB): [Fw * (%FFB/100)] – [Iw * (%IFB/100)];

Where: Fw = final weight; Iw = initial weight; FCPB = final crude protein in the body; ICPB= initial crude protein in the body; FFB = Final fat in the body; IFB = Initial fat in the body.

Histological parameters: The anterior intestine of four fish per treatment was collected and prepared for light microscopy. Histological samples were fixed in 10% formalin and stored in 70% ethanol, then they were subjected to the routine histological technique, according to the method described by Gressler et al. (2016). Applying the routine histological technique, the material was dehydrated in an ascending series of ethanol (70% - 99% alcohol) and embedded in methacrylate glycol resin (Technovit 7100). Afterwards, using a rotary microtome, 2μm slices were obtained (LEICA RM2245) and subsequently stained with hematoxylin-eosin. For morphological examination, the slices were observed through an optical microscopy (ZEISS PrimoStar, AxioCam ERc5s), registered and analyzed using ZEN LITE software (Carl Zeiss). At each repetition villus height, epithelium thickness and muscle layer thickness were estimated using ImageJ® software. The slices were thoroughly examined to determine the presence of histopathological alterations. Goblet cells were counted in 500 μm of villus.

Enzymatic parameters: The digestive tract of eight fish per treatment was measured and the intestine was separated. The intestinal contents were discarded and the respective organ was homogenized in buffer solution pH 7.0, using Potter-Elvehjem homogenizer. The supernatants obtained after centrifugation were used in the assays as an enzymatic source to determine trypsin and chymotrypsin activity (Hummel 1959).

Hepatic parameters: The livers of eight fish/treatment were used to quantify levels of glucose, glycogen (Park and Johnson 1949) and protein (Bradford 1976).

Gut contents parameters: The gut contents were collected to determine the presence of short-chain fatty acids (SCFA) by gas chromatography, according to the method proposed by Bianchi et al. (2011). For this purpose, the fish were previously fed the experimental diets and subjected to euthanasia by benzocaine overdose (250 mg/L), in accordance with the American Veterinary Medical Association (AVMA 2013). The samples of gut contents used to determine the presence of SCFA

were collected after sectioning the intestines, stored in sterile plastic tubes and kept at -20 °C until analysis.

2.6 Statistical analysis

The results were subjected to the normality test, followed by analysis of variance. The means of the treatments were compared by analysis of orthogonal contrasts at a 5% level of significance.

3 Results and discussion

The dietary inclusion of prebiotic additives is associated with tangible benefits on animal metabolism, performance or health. In the present study, the Nile tilapia juveniles reached the end of the experimental period with a mean weight of 50.0 ± 8.3 g and a mean total length of 13.4 ± 0.7 cm. During the 42 days of experiment, the animals obtained 1.1 ± 0.1 g of daily weight gain and showed an apparent feed conversion of 1.0 ± 0.1. Although no statistical superiority was observed in the performance of the animals fed diets containing pectic hydrolysates in relation to the control diet, it must be pointed out that the fish grew healthy, without requiring the use of antimicrobials, ensuring greater food security for the consumer market. According to Olano-Martin et al. (2002), pectic hydrolysates, characterized by their content of lower molar mass pectins, present great prebiotic potential. In a study conducted by Moura (2015), the inclusion of partially hydrolyzed pectin found in passion fruit peel in Wistar rats diets, at the same concentrations tested in this research, caused weight gain in relation to the animals fed control diet. However, Nile tilapia juveniles did not respond in the same way, indicating that the use of pectic hydrolysates and the determination of the appropriate amount of nutritional supplementation aiming at prebiotic effects on fish performance still require further investigation. It is hoped that by defining the proper dose levels for this species, the use of pectic hydrolysates fulfills its role in the rational use of available resources, helping to mitigate environmental impacts and prioritizing a clean and sustainable production.

The centesimal composition of the Nile tilapia juveniles showed a higher body crude protein content and a higher protein deposition in the control fish in comparison with the treatment group, when 2.5 g/kg of passion fruit peel was used (Table 2). Similarly, Grisdale-Helland et al. (2008) found a higher crude protein content in the body composition of the control group of Atlantic salmon (*Salmo salar*) supplemented with prebiotics MOS (mannan oligosaccharides) or GOS (galacto-oligosaccharides). The evaluation of body composition reflects the quality of the final product (fish), which depends on the nutritional characteristics of the ingredients that compose the diet. This study observed that the fat content and fat deposition in the body were lower in treatments using 2.5 and 5 g/kg of passion fruit peel when compared to the control group (Table 2). This fact indicates that

supplementation with pectic hydrolysates of passion fruit peel up to 5 g/kg can lead to fish with lower fat contents, which it would be desirable for a greater shelf stability (lower fat contents for oxidative events) and a better health for customers.

Through the metabolic profile of the fish it is possible to identify the physiological state of the animal and its relationship with the nutrients in the diet. When dealing with metabolic parameters, it is known that prebiotics influence glucose and fatty acids metabolism in mammalian liver (Roberfroid et al., 2010). However, for fish, information on hepatic responses derived from prebiotic use is still scarce. The modulation of glucose metabolism observed in mammals fed diets supplemented with prebiotics is mainly related to the production of SCFA on the gut microbiota, and its hepatic use for gluconeogenesis and inhibition of cholesterol synthesis (Roberfroid et al. 2010). The present research found that the use of 5 g/kg of hydrolysate obtained from apple pomace caused the elevation of glucose concentrations in the liver of Nile tilapia juveniles (Figure 2). The explanation for this result may be related to the production of SCFA, particularly the production of acetic acid, which was produced in a higher amount in comparison with the control treatment (Figure 3). Acetic acid, the main product of the fermentative degradation of pectic chains, is absorbed in the epithelium and directed towards the liver, from where it can be directed towards the peripheral tissues (Wenzel 2012). According to Jeney et al. (1997), the increase in glucose levels may also be an indicator of stress, which results in increased levels of cortisol and epinephrine, causing gluconeogenesis and glycogenolysis in the liver, and thus resulting in hyperglycemia in order to satisfy the increased energy requirements during stress, allowing the body to react to the stressor. However, in the case of animals fed diets containing prebiotics, these changes may not be considered significant, since prebiotics reflect the reduction of endogenous stress events.

The gastrointestinal tract is one of the main routes of infection in fish and its histomorphology directly affects the microbial community. Several studies have reported the effects of prebiotics on the on the increased number and size of villi, creating a larger absorptive area (Ringo et al. 2010; Zhou et al. 2009). In the present study, villus height was higher in treatments with 2.5 and 5 g/kg of hydrolysates obtained from passion fruit peel in comparison with the control treatment (Table 2), indicating an increase in the absorptive capacity. The thickness of the intestinal epithelium was higher than the control groups in treatments with 5 g/kg of hydrolysates from apple pomace and 2.5 and 5 g/kg hydrolysates from passion fruit peel (Table 2), ensuring a greater epithelial integrity. By associating these data, one can affirm that the greater capacity of nutrient absorption reflects positively upon the maintenance of the intestinal integrity (Dimitroglou et al. 2011). The counting of goblet cells of the gut of Nile tilapia juveniles was higher in treatments with 2.5 g/kg of hydrolysates from apple pomace and 2.5 and 5 g/kg of hydrolysates from passion fruit peel (Table 2). Goblet cells are important immunological indicators, since they secrete mucus that covers the intestinal epithelium, creating a protective barrier against antigens, toxins and digestive enzymes in the intestinal lumen, as well as reducing the population of undesirable bacteria in contact with the epithelial surface (Gaudier

et al., 2009). Anguiano et al. (2013) studied the intestinal histomorphology and digestive enzymes of red drum (*Sciaenops ocellatus*) fed diets containing different prebiotics (MOS, FOS, TOS and GroBiotic®) and concluded that the best nutrient digestibility in response to prebiotic supplementation was related to changes in the height of the villi and enterocytes, reflecting on the health condition of the host.

The digestive efficiency of animals is closely related to the ability to secrete enzymes acting in the hydrolysis of food polymers. There is little information on the direct influence of prebiotics on the activity of digestive enzymes trypsin and chymotrypsin, which are responsible for the hydrolysis of proteins in the gut. Goulart et al. (2017) evaluated the prebiotic potential of different dietary fiber concentrates in the dietary habits of silver catfish (*Rhamdia quelen*) and observed that the animals supplemented with pectin showed a reduced activity of the enzymes trypsin and chymotrypsin in contrast to the animals fed the control diet. This can be explained by the fact that pectin has a high water-binding capacity, increasing digesta viscosity (Brito et al. 2008). Therefore, the addition of this polysaccharide to the fish diet may have a negative effect, making the interaction between enzyme and substrate difficult and reducing nutrient uptake by the intestinal mucosa (Gomes et al. 2016). However, lower molar mass pectic hydrolysates tend to behave differently in relation to the integral molecules, since they have a reduced capacity to bind to water, resulting in a lower viscosity. In this study, the treatment with 5 g/kg of apple pomace hydrolysates promoted greater trypsin and chymotrypsin activity in the digestive tract, while the other treatments did not show any difference in relation to the control group (Table 2), confirming that the interaction between enzyme-substrate was not hindered to the point of limiting the enzymatic action in the treatments supplemented with pectic hydrolysates.

Regarding the intestinal aspects, the fermentative activity of the beneficial microbial populations whose development was stimulated by the consumption of prebiotics, results in the generation of short chain fatty acids (SCFA). The main SCFAs produced are acetate, propionate and butyrate, which can be absorbed and metabolically used by the intestinal mucosa as a source of energy, besides having metabolic and physiological influences that reflect on animal health, absorption and deposition of fat, cholesterol metabolism and proliferation of epithelial cells (Guillon and Champ 2000). In our study, acetic acid production was higher in the treatments with 5 g/kg of apple pomace hydrolysate and 2.5 g/kg of passion fruit peel hydrolysate in comparison with the control group (Figure 3). According to Wenzel (2012), acetic acid is the main SCFA resulting from the fermentative process of pectin. The production of propionic acid remained constant between treatments (Figure 3). Due to the detection limit of the equipment, it was not possible to quantify the butyric acid concentration. In a work performed on Wistar rats, a higher production of short chain fatty acids was observed in the cecum, mainly propionic and butyric acids, and a more pronounced effect was observed between 2.5 and 5 g/kg of passion fruit peel (Moura 2015). The proliferation of beneficial microbial populations and an increased fermentative activity busts the production of organic acids in

the gastrointestinal tract, which inhibit the development of pathogenic bacteria, improving luminal integrity and triggering a positive effect on the immune system (Freitas et al. 2014).

4 Conclusion

The supplementation with passion fruit peel hydrolyzate (2.5 or 5 g/kg) provides the fish with a lower fat content, ensuring a healthier food with greater shelf stability. The passion fruit peel hydrolyzate, at both levels supplemented, also provided greater benefits related to intestinal histological parameters, guaranteeing greater cell renewal, greater capacity of nutrient absorption and maintenance of intestinal integrity. The supplementation with 5 g/kg of apple pomace hydrolyzate resulted in increased activity of trypsin and chymotrypsin enzymes. This treatment also provided a higher level of hepatic glucose, which may be related to the greater production of acetic acid at the intestinal level. However, further studies should be carried out in order to define the appropriate amounts of pectic supplementation, aiming at generating positive effects on growth and food efficiency of Nile tilapia, as well as to clarify their mechanisms of action.

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Conflict of interest The authors declare that they have no conflict of interest

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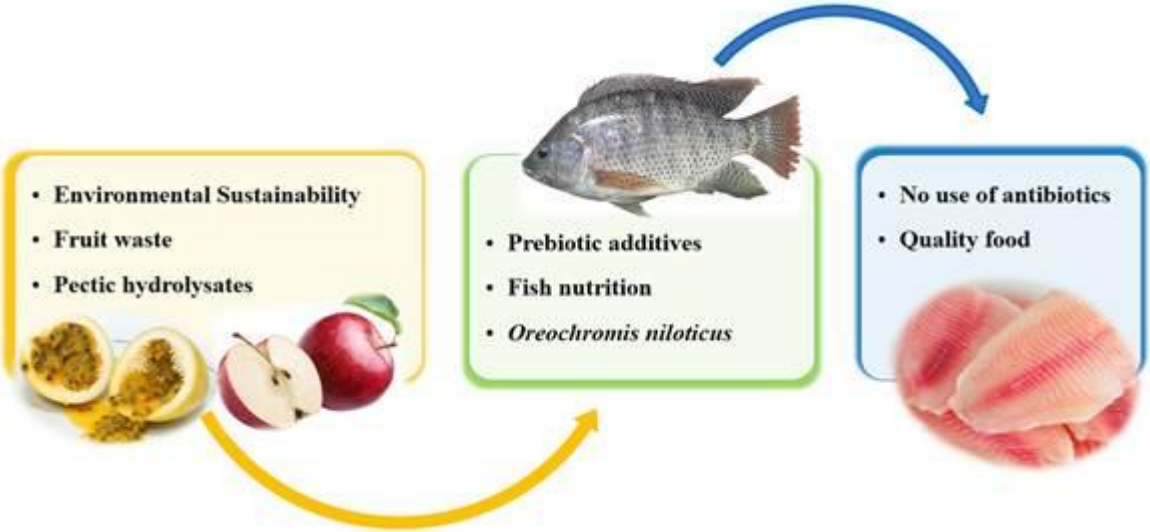

408

409 **Fig. 1** Sustainable food production from the integration between fruit farming and aquaculture

Table 1 Dietary formulations and proximate composition of the experimental diets (g/kg)

Ingredients	Diets				
	Control	Apple pomace hydrolysate		Passion fruit peel hydrolysate	
		2.5	5	2.5	5
Fish meal[1]	280	280	280	280	280
Maize starch	305	305	305	305	305
Cellulose	60	57.5	55	57.5	55
SPC 60%[2]	220	220	220	220	220
Apple pomace hydrolysate	0	2.5	5	0	0
Passion fruit peel hydrolysate	0	0	0	2.5	5
Soybean oil	30	30	30	30	30
Vitamin and mineral mixture[3]	30	30	30	30	30
Sodium chloride	5	5	5	5	5
Inert[4]	69.8	69.8	69.8	69.8	69.8
BHT[5]	0.2	0.2	0.2	0.2	0.2
Diets composition					
Moisture[6]	36.1	38.70	39.0	42.1	47.8
Crude protein[6]	315.8	318.2	316.7	319.3	330.4
Digestible energy (MJ/kg)[7]	12.96	12.96	12.96	12.96	12.96
Fat[6]	65.7	64.9	64.0	65.0	65.8
Ash[6]	182.0	174.4	178.8	178.5	179.9
Ca[8]	16.7	16.7	16.7	16.7	16.7
P[8]	9.0	9.0	9.0	9.0	9.0
Physicochemical properties					
HC (g water/ g sample)[6/9]	1.95	2.22	2.16	2.15	1.87
FBC (g oil/ g sample)[6/10]	1.36	1.37	1.40	1.37	1.37
CBC (mg Cu/ g sample) [6/11]	9.31	9.68	9.42	8.87	9.2

[1]Waste flour tilapia – Copisces/Paraná,RS, Brazil;
[2]Soy protein concentrate, 60% CP;
[3]Vitamin and mineral mixture – composition/kg of product: Folic acid: 299.88 mg; Ascorbic acid: 15000.12 mg; Pantothenic acid: 3000.10 mg; Biotin: 0.06 mg; Niacin (B3): 9000.32 mg; Hill (B4): 103500.00 mg; Vitamin A: 1,000,000. IU; Vitamin B1: 1500.38 mg; Vitamin B2: 1500.00 mg; Vitamin B6: 1500.38 mg; Vitamin D3: 240000.00 IU; Vitamin E: 10000.00 mg; Vitamin K3: 400.00 mg; Inositol: 9999.92 mg; Iron: 6416.80 mg; Manganese: 8000.40 mg; Copper: 1000.00 mg; Zinc: 13999.50 mg; Iodine: 45.36 mg; Cobalt: 60.06 mg; Selenium: 60.30 mg; Magnesium: 5.10 mg; Chloride: 2.30%; Sulfur: 0.01%;
[4]Inert= sand;
[5]Antioxidant butylated hydroxytoluene;
[6]Composição analisada (Laboratório de Piscicultura/UFSM);
[7]ED= energia digestível calculada: [(%Proteína bruta * 5.65 * 0.85) + (%Gordura * 9.4 *0.9) + (Carboidratos * 4.15 * 0.7)] (Jobling, 1983);
[8]Valores calculados com base na composição dos ingredientes;
[9]HC= Hydration capacity;
[10]FBC= Fat binding capacity;
[11]CBC= Copper binding capacity.

Table 2 Centesimal composition, body nutrient deposition, histological parameters and enzymatic activity of Nile tilapia fed with diets with inclusion of pectic hydrolysates[1]

Treatments	Moisture (%)	CP (%)	Fat (%)	Ash (%)	PDB (%)	FDB (%)	VH (µm)	ET (µm)	MLT (µm)	GCC (500 µm)	Trypsin[2]	Chymotrypsin[3]
Control	74.2 ± 0.4	14.8 ± 1.5	7.7 ± 0.6	4.4 ± 1.1	6.8 ± 0.7	3.6 ± 0.3	569.3 ± 12.9	43.6 ± 1.0	68.5 ± 1.6	6.7 ± 0.9	3.9 ± 0.9	4.5 ± 0.9
2.5 AP	73.7 ± 0.6	13.8 ± 0.6	7.8 ± 0.3	4.2 ± 0.8	6.5 ± 0.4	3.8 ± 0.2	610.7 ± 58.8	49.1 ± 7.0	56.7 ± 5.0	10.0 ± 1.8	3.8 ± 0.8	4.6 ± 0.9
5 AP	74.0 ± 0.5	13.7 ± 1.0	7.9 ± 0.8	4.3 ± 0.3	6.4 ± 0.5	3.8 ± 0.5	450.9 ± 68.2	60.3 ± 2.9	45.5 ± 2.2	7.0 ± 0.8	4.6 ± 1.1	5.3 ± 1.3
2.5 PFP	74.3 ± 1.9	13.4 ± 0.7	6.5 ± 0.4	4.7 ± 0.5	6.0 ± 0.4	2.9 ± 0.2	712.2 ± 55.4	63.6 ± 9.0	38.2 ± 8.5	9.7 ± 1.7	3.8 ± 0.8	4.1 ± 0.5
5 PFP	74.8 ± 1.2	14.2 ± 0.1	6.6 ± 0.9	4.3 ± 0.4	6.5 ± 0.2	3.1 ± 0.4	779.6 ± 23.3	88.4 ± 2.6	46.1 ± 1.4	11.0 ± 0.8	3.4 ± 0.7	4.0 ± 0.4
Orthogonal contrasts												
C x 2.5 AP	ns	ns	ns	ns	ns	ns	ns	ns	0.002	0.003	ns	ns
C x 5 AP	ns	ns	ns	ns	ns	ns	0.004	0.001	0.000	ns	0.050	0.014
C x 2.5 PFP	ns	0.044	0.013	ns	0.029	0.008	0.001	0.000	0.000	0.005	ns	ns
C x 5 PFP	ns	ns	0.028	ns	ns	0.027	0.000	0.000	0.000	0.000	ns	ns

[1]Values expressed as mean ± standard deviation. [2]Trypsin: µmol TAME hydrolysed/min/mg protein; [3]Chymotrypsin: mmol BTEE hydrolysed/min/mg protein. The results were compared by orthogonal contrasts ($p<0.05$); ns: not significant ($p>0.05$). AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate; C: control; CP: crude protein; PDB: protein deposition in the body; FDB: fat deposition in the body; VH: villus height; ET: epithelium thickness; MLT: muscle layer thickness; GCC: goblet cells counts.

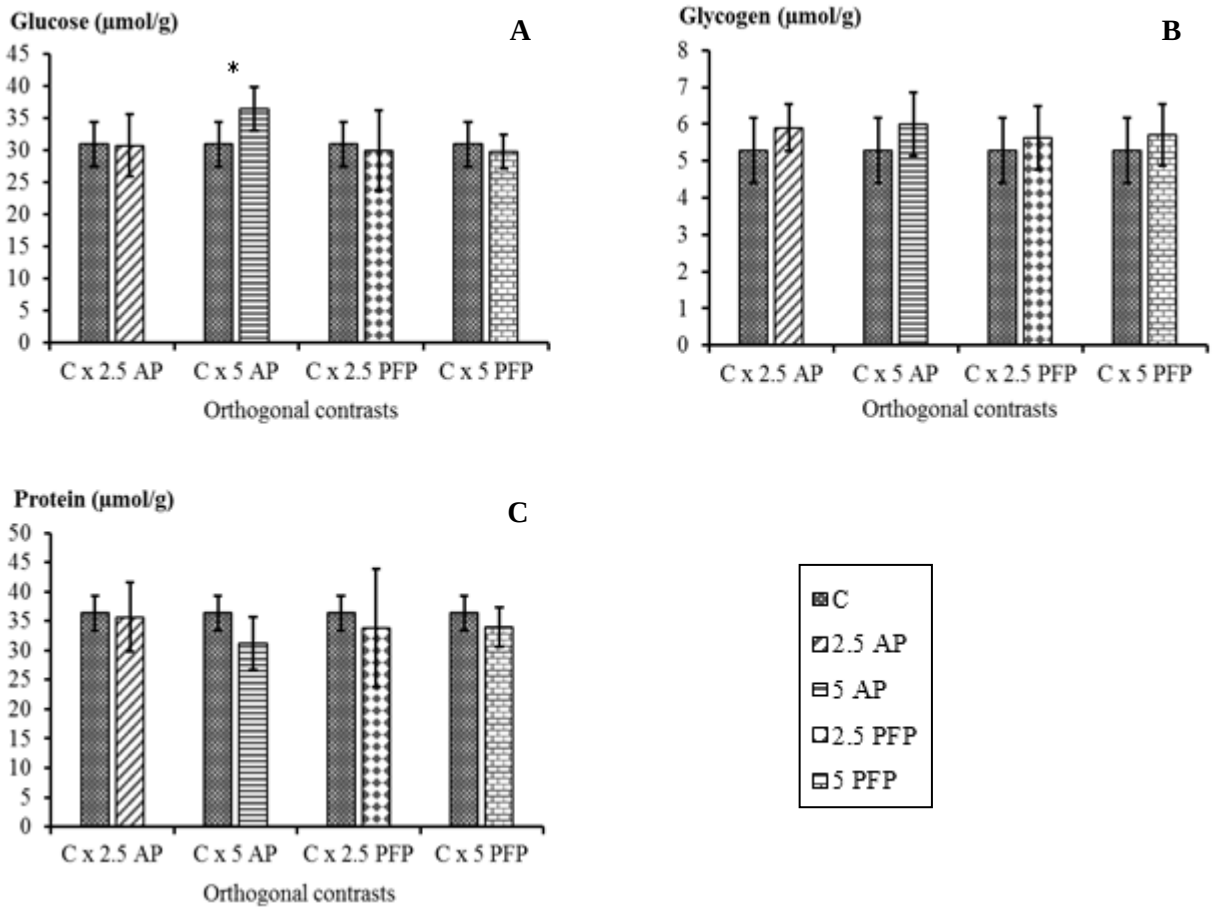

Fig. 2 Hepatic parameters of Nile tilapia fed diets with inclusion of pectic hydrolysates. Values expressed as mean ± standard deviation. C: control; AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate

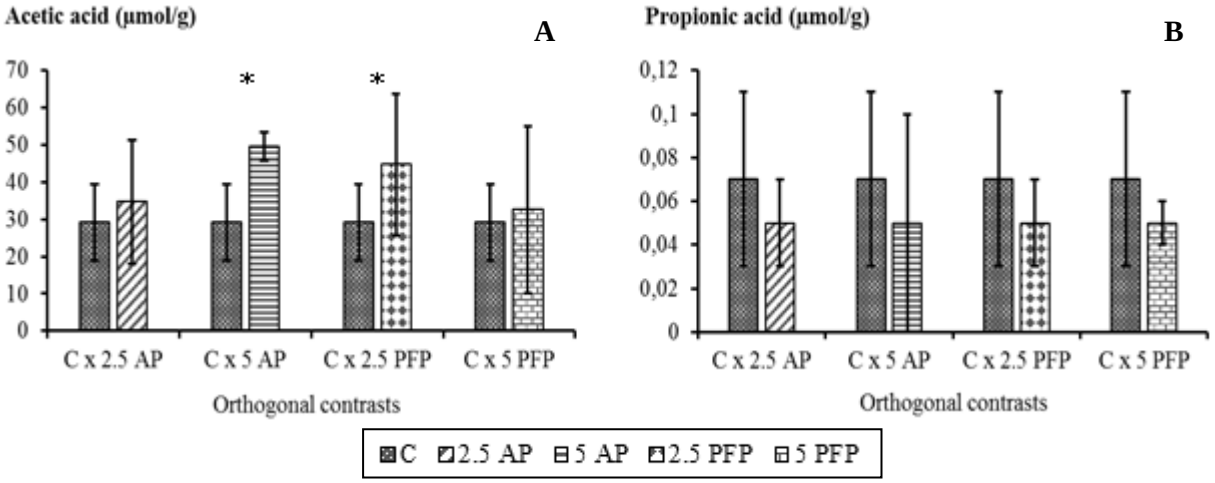

Fig. 3 Production of SCFA in the intestine of Nile tilapia fed diets with inclusion of pectic hydrolysates. Values expressed as mean ± standard deviation. C: control; AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate.

4 ARTIGO 3

Pectic hydrolysates in the diet of silver catfish (*Rhamdia quelen*)[**]

Fermentable dietary fiber in fish nutrition

Patrícia Inês Mombach[a,*]; Taida Juliana Adorian[a]; Dirleise Pianesso[a]; Thaís Soares dos Santos[a]; Joziane Soares de Lima[a]; Silvino Sasso Robalo[a]; Leila Picolli da Silva[a]

[a]Departamento de Zootecnia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, CEP: 97105-900, Brazil.

*Corresponding author:

Phone: 55 (55) 3220-8365 Fax: 55 (55) 3220-8240

E-mail: patimombach@hotmail.com

Abstract

World demand for food is growing and, concomitantly, the use of agroindustrial waste requires optimization. Thus, the aim of this study was to investigate the prebiotic potential of pectin hydrolysates extracted from agroindustrial waste (apple pomace - AP and passion fruit peel - PFP) and added to the diet of silver catfish (*Rhamdia quelen*) juveniles. Pectin was extracted from agroindustrial waste and pectin hydrolysates were obtained after hydrolysis. A 49-day biological assay was conducted by including pectin hydrolysates in the diet of silver catfish juveniles (initial average weight of 9.4 ± 1.4g). Five test diets (isocaloric and iso-

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protein diets) were evaluated: one diet served as control while the other four were added pectin hydrolysates (2.5 and 5 g/kg) to replace some of the cellulose. First the fish were weighed, measured, allocated in experimental units and fed three times a day until apparent satiation. At 49 experimental days, the fish were fasted for 18 hours, and biometric data and biological material were then collected to determine performance, plasma, liver and histological parameters as well as evaluate intestinal contents. The experimental design was completely randomized in a 2x2 factorial arrangement with an additional treatment, in a total of 5 treatments with 4 replicates. The results underwent the normality test, followed by analysis of variance, and treatment means were compared by orthogonal contrast analysis at the 5% level of significance. The inclusion of 2.5 g/kg of apple pomace hydrolysates resulted in greater production of butyric acid, increased thickness of the muscular layer and a higher goblet cell count in the intestine. The inclusion of 5 g/kg of apple pomace hydrolysates led to greater concentration of liver protein. Further studies are needed to broaden the knowledge about the use of these additives in the diet of silver catfish and establish levels that allow greater gains for the species.

KEYWORDS: Additives, apple pomace hydrolyzate, aquaculture, passion fruit peel hydrolyzate

1 | INTRODUCTION

Aquaculture is the most promising industry in the supply of high-quality animal protein, but production system intensification has resulted in greater exposure of animals to stressors, which leads to the emergence of infectious diseases that cause major economic losses (Chen et al., 2014; Pohlenz & Gatlin, 2014). For a long time, antibiotics have been administered to minimize these problems in fish farming. However, indiscriminate use of antibiotics has suppressed the immune system of animals, disseminated drug-resistant pathogens and posed

major environmental risks (Allameh et al., 2015; Brogden et al., 2014). An alternative to antibiotics is prebiotic additives, which are environmentally safe and can be used as tools to reduce economic losses resulting from disease outbreaks in fish farming (Cyrino et al., 2010). Prebiotics are nondigestible ingredients formed by a combination of non-starch polysaccharides which serve as a substrate for some colon bacteria, thereby beneficially modulating the native microbiota of the host, strengthening the immune system and improving the performance and health of animals (Bach Knudsen, 2001; Montagne, Pluske & Hampson, 2003; Theuwissen & Mensink, 2008; Silva & Nörnberg, 2003).

There has been ongoing research on new sources of prebiotics, and results point to a possible beneficial integration between productive chains. This is clear when we consider the growing world demand for processed foods, which produces a large amount of waste biomass and results in significant environmental liabilities. In the case of fruit processing, waste (peel and pomace) can be used as raw material for synthesis of new prebiotics (pectic hydrolysates), which are recently discovered alternatives for studies on safe additives (Moura, 2015). However, they are not fully explored in fish nutrition. This integrative approach can not only reduce the impact of the fruit processing industry on the environment but also streamline the responsible and sustainable cultivation of aquatic organisms.

Therefore, the objective of this study was to investigate the prebiotic potential of pectin hydrolysates extracted from agroindustrial waste (apple pomace - AP and passion fruit peel - PFP) and added at two concentrations (2.5 or 5 g/kg) to the diet of silver catfish (*Rhamdia quelen*).

2 | MATERIAL AND METHODS

The study was conducted in the Laboratory of Fisheries, Department of Animal Science, Federal University of Santa Maria (UFSM) - RS, Brazil, after being approved by UFSM

Ethics Committee on Animal Experiments, under protocol number 7941021015.

2.1 | Pectin extraction and pectin hydrolysates

Apple pomace was obtained after mechanical extraction of the juice. Passion fruit peel was collected after hand pulping.

Pectin was extracted from apple pomace and passion fruit peel in an aqueous solution at a concentration of 3:97 and 4:96 (sample:water), respectively, under a temperature of 100ºC for 1 hour. After cooling, the mixture was centrifuged (3500 rpm/10 min and ethanol 96% at a 1:1 ratio was added to the supernatant for pectin precipitation. After precipitation (rest of 24h/5ºC), pectin was separated from alcohol and taken to an air circulation oven at 55º C for 48 hours. After drying, it was micro-milled for 20 seconds, which resulted in an average particle size of 0.3 mm. This extraction procedure was adapted from the methodology described by Calliari (2015).

To obtain protein hydrolysates, pectin was dissolved in a 0.25 N solution of HCl in a 1:50 sample (g):solution (mL) ratio at 60ºC under constant agitation for 2 hours. Hydrolysis was interrupted by adding KOH for neutralization (pH 7.0) (Moura, 2015). The neutralized material was dried at 50ºC in a forced air circulation oven for 12 hours. After that, the material was micro-milled to produce pectin hydrolysates.

2.2 | Experimental design, treatments and preparation of diets

The experimental design was completely randomized in a 2x2 factorial arrangement with additional treatment, in a total of 5 treatments with 4 replicates. Therefore, five test diets were evaluated. There was a control diet while the other diets were added pectic hydrolysates to replace some of the cellulose. The hydrolysates were obtained from two distinct sources (AP -

apple pomace and PFP- passion fruit peel) and added at two levels (2.5 or 5 g/kg) in the diet. The experimental diets were formulated to be isocaloric diets (13.49 MJ kg^{-1} of metabolizable energy) and iso-protein diets (38% CP) (Meyer & Fracalossi, 2004), composed basically of fish meal, corn starch and soy protein concentrate (SPC 60%) (Table 1).

To prepare the diets, the ingredients were milled (590μm), weighed, and mixed to complete homogenization. Water was added until the dough reached the appropriate point. Pelletizing was then performed. The pellets were dried in a forced air circulation oven for 24h at a temperature of 50 °C. After drying, the feeds were ground and sieved to obtain granules that could be swallowed by fish. The composition of moisture, crude protein, ash, (AOAC 1995), fat (Bligh and Dyer 1959), and the physicochemical properties (hydration capacity, fat binding capacity (Wang and Kinsella 1976) and copper binding capacity (McBurney et al. 1983)) was analyzed in the diets.

2.3 | Biological assay

The assay was conducted in a recirculating aquaculture system, consisting of a pump (1 hp), 20 tanks (280 L) with single inlet and outlet of water and two biological filters made with crushed stone. The experiment used 600 silver catfish juveniles with initial average weight of 9.4 ± 1.4g. The fish went through a seven-day adaptation period in experimental units (30 fish/tank) and were fed three times a day to apparent satiation for a 49-day assay period.

2.4 | Water quality

Piping and syphons were cleaned twice a day in the experimental units, followed by renewal of 10% of the water system. In the water of the culture, the temperature was measured on a daily basis with a mercury bulb thermometer re (24.94 ± 1.07ºC). Weekly, were measured by

colorimetric kits (Alfa-Tecnoquímica) the dissolved oxygen (7.24 ± 0.73 mg/L), pH (7.63 ± 0.35), ammonia (0.27 ± 0.18 mg/L), nitrite (0.14 ± 0.12 mg/L), alkalinity (46 ± 12.47 mg $CaCO_3$/L) and hardness (31.5 ± 23.46 mg $CaCO_3$/L).

2.5 | Data collection and analysis of variables

Two biometric assessments were performed (at 1 and 49 days) to monitor fish performance. The fish were measured and weighed individually. The fish were fasted for 18 hours before each biometric assessment, and then benzocaine sedation was used at a concentration of 100 mg/L of water. In the final biometry, fish were collected in order to determine performance, plasmatic, hepatic and histological parameters and to evaluate intestinal contents. These animals were subjected to euthanasia by benzocaine overdose (250 mg/L) (AVMA 2013). The following parameters were evaluated:

Performance parameters: Based on weight and length measurements as well as analysis of feed consumption, the following data collected: final weight: FW(g); daily weight gain (g/day): DWG=(final weight - initial weight)/day; specific growth rate (%/day): SGR=(ln (final weight) - ln (initial weight)/day)*100; apparent feed conversion: AFC=(total consumption)/(final biomass - initial biomass); and survival (%). The following measurements were also made: weight of gutted fish, weight and length of the digestive tract, liver weight and visceral fat weight. Based on these results, the following parameters were calculated: carcass yield (%): CY=(weight of gutted fish with heads and gills/whole fish weight)*100; somatic digestive index (%): SDI=(digestive tract weight/whole fish weight)*100; hepatosomatic index (%): HSI=(liver weight/whole fish weight)*100; visceral fat index (%): VFI=(visceral fat weight/whole fish weight)*100; and gut quotient: GQ=(digestive tract length/fish length).

Plasma Parameters: blood samples from eigth fish/treatment were collected with the use of syringes with anticoagulants (heparin), through a puncture in the caudal vein. Blood was centrifuged (3000 rpm/10 min), and the plasma was used to determine the level of albumin, cholesterol, glucose, total proteins and triglycerides using commercial colorimetric kits (Labtest®), alkaline phosphatase with a commercial kit (Doles®) and total immunoglobulins according to the method described by Hoseinifar et al. (2015). This method consists in the precipitation of total immunoglobulins by adding polyethylene glycol 12% and incubation for 120 minutes at 4ºC. Protein content was subsequently determined with a commercial kit (Labtest®). The difference in protein content before and after precipitation of immunoglobulin molecules corresponds to total immunoglobulin content.

Hepatic parameters: The livers of eigth fish/treatment were used to quantify levels of glucose, glycogen (Park & Johnson, 1949) and protein (Bradford, 1976).

Histological parameters: Fragments were collected of the intestine (10 cm) of four fish/treatment. The samples were fixed in 10% formalin and then dehydrated in alcohol (70%), cleaned with xylene and paraffin-embedded. After solidification, the blocks were cut to prepare the slides. Villus height, epithelium thickness, muscular layer thickness and goblet cell count in six villi were estimated in each replicate.

Evaluation of intestinal contents: At the end of the experimental period, intestinal contents were collected from eigth fish/treatment and analyzed for production of short-chain fatty acids (acetic, propionic and butyric acids) through gas chromatography according to the method proposed by Bianchi et al. (2011).

2.6 | Statistical analysis

The results were submitted to the normality test, followed by analysis of variance. Treatments means were compared by orthogonal contrast analysis at the 5% level of significance.

3 | RESULTS

3.1 | Performance parameters

The fish in the control treatment showed higher final weight and daily weight gain when compared to fish of the treatments with 5 g/kg of apple pomace hydrolysates and 2.5 g/kg of passion fruit peel hydrolysates (Table 2). Specific growth rate of silver catfish juveniles was smaller in the treatment with 2.5 g/kg of passion fruit peel hydrolysates when compared to the control treatment (Table 2). Apparent feed conversion was also more efficient in the control treatment when compared to the treatment with 2.5 g/kg of passion fruit peel hydrolysates (Table 2). There was no difference among treatments for the variables survival (Table 2), carcass yield and digestive rates (Table 3).

3.2 | Plasma parameters

The treatments tested in this study did not alter the plasma parameters evaluated in silver catfish juveniles, except for total immunoglobulins, which were lower in the treatment with 2.5 g/kg of apple pomace hydrolysates in comparison to the control treatment (Table 4).

3.3 | Liver parameters

Liver glucose levels were not influenced by the treatments (Table 5). The fish of the treatments with 2.5 and 5 g/kg of passion fruit peel hydrolysates showed lower glycogen reserves in comparison to the fish of the control treatment (Table 5). The levels of protein in the liver were higher in fish in the treatment with 5 g/kg of apple pomace hydrolysates when compared to the control treatment (Table 5).

3.4 | Histological parameters

There were no differences among the treatments for villus height (Table 6). Epithelium thickness and goblet cell count were higher in the treatment with 2.5 g/kg of apple pomace hydrolysates, while muscular layer thickness was lower than this treatment in comparison to the control treatment (Table 6).

3.5 | Assessment of intestinal contents

Production of acetic acid in the intestine of silver catfish juveniles was not changed between treatments (Figure 1A). Production of propionic acid was higher in fish of the control treatment when compared to the treatment with 5 g/kg of apple pomace hydrolysates (Figure 1B). On the other hand, the production of butyric acid was higher in the treatment with 2.5 g/kg of apple pomace hydrolysates when compared to treatment without inclusion of pectin hydrolysates (Figure 1C).

4 | DISCUSSION

It is known that the use of prebiotics leverages the productive performance of fish, hence it is a tool for optimization of cultivation. Efficacy of using these additives is proven by stronger intestinal beneficial microbiota, improvements in immune response, increase in the area of intestinal absorption and higher survival rates after challenges against pathogens, which culminates in positive effects on animal performance (Buentello, Neill & Gatlin, 2010; Li & Gatlin, 2005; Silva & Nörnberg, 2003; Zheng et al. 2011; Zhou, Buentello & Gatlin, 2010). Contrary to this perspective, our study demonstrated that the addition of pectin hydrolysates, depending on the source of origin and concentration in the diet, negatively influenced or showed no effect on fish performance (Tables 2 and 3). A large number of studies with new

prebiotics also reported inconclusive effects in initial tests. González-Félix et al. (2018) worked with the commercial prebiotic GroBiotic®-A for *Totoaba macdonaldi* but they did not find any effect of supplementation on fish performance. The authors suggested higher level tests should be made for prebiotics in the diet to define appropriate dosing. Burr, Gatlin and Hume (2009) did not find any effect on the growth of *Sciaenops ocellatus* fed diets supplemented with 1% of GroBiotic®-A or inulin for 8 weeks. These authors point out the initial stage of fish development (initial weight of 2.6 g) as a possible cause of results, because those fish would still be establishing a microbial community of the gastrointestinal tract. These studies have shown that several factors inherent to the product and the animals influence the effect of prebiotics, even those previously consolidated for some species. Considering that our study is the first which uses pectin hydrolysates in the diet of silver catfish, we suggested that other tests should be carried out to establish effective doses for the species.

For other animal species, the use of pectin hydrolysate showed beneficial effects on performance, when the levels in use were equal to or higher than those tested in our study. Moura (2015) evaluated the inclusion of partially hydrolyzed pectin of passion fruit peel in the diet of Wistar rats at levels of 2.5; 5; 7.5 and 10 g/kg and found that, regardless of the level being tested, the addition of pectin hydrolysates increased consumption by approximately 50% in comparison to the control diet, which was directly reflected on higher body weight gain.

In addition to the species and levels of inclusion, cultivation conditions may also have influenced the results. According to Silva and Nörnberg (2003), the stress level of animals can influence the biological response measured by the addition of prebiotics in the diet. If animals are under environmentally favorable conditions, they are believed to have reached equilibrium. Thus, with or without the provision of prebiotics, responses may be very similar.

However, when animals are under unfavorable conditions (e.g. stress resulting from handling, high densities, presence of pathogens, sudden changes in water quality, etc.), the beneficial action of prebiotic supply may be more significant.

Prebiotics can strengthen the metabolic system without, however, causing direct effects on performance. These changes are often found in plasma, where the components are routinely used as indicative of stress and resistance to bacterial infections (Maita, 2007). For example, immunoglobulins found in plasma are involved in systemic immunity, and they play a central role in the maintenance of homeostasis (Salinas, Zhang & Sunyer, 2011). Phosphatases, which are enzymes of the innate immune system of fish, act as antimicrobial agents because of their hydrolytic capacity (Bates et al., 2007; Beck & Peatman, 2015). Albumin, the most abundant protein in plasma, has the important function of transport, mainly of hormones and fatty acids. Since total proteins are considered more stable components and few factors from the diet can affect their levels in the blood (Maita, 2007). However, it has been reported that the action of prebiotics may cause an increase in the levels of plasma protein in fish. In a study carried out by Goulart et al. (2018), the animals that received a diet with 1% of pectin showed higher levels of albumin and total protein in comparison to the diet without addition of pectin. Andrews et al. (2011) also found a significant increase in the levels of albumin and total protein in the serum of *Labeo rohita* fed with mannanoligosaccharides in comparison to the control group. These results may be associated with a more efficient immune response (Maita, 2007). The levels of cholesterol and plasma triglycerides are also indicators of the prebiotic effect, because the reduction in the levels of plasma lipid components of fish is associated with poorer fish health. A study conducted by Maita et al. (1998) with yellowtail (*Seriola quinqeradiata*) reported a reduction in total cholesterol in animals that had lower resistance to infestation by *Lactococcus garvieae*. In addition, lower plasma glucose levels are associated with lower stress levels in animals, which can be used as

an indication of the prebiotic effect (Gaggia, Mattarelli & Biavati, 2010). However, in our study, pectin hydrolysates at the tested levels did not show promising effects on the plasma variables (Table 4). This finding suggests that the inclusion of pectin hydrolysates in the diet of silver catfish needs further research for satisfactory results at the plasma level.

In fish nutrition, the action of prebiotics in the liver is still not completely clear. It is known that additives with a prebiotic effect can promote effects on glucose metabolism in mammals (Roberfroid et al., 2010). In our study, liver glucose levels were not influenced by the treatments, but we found lower reserves of glycogen in the liver of silver catfish that received diets with 2.5 and 5 g/kg of passion fruit peel hydrolysates (Table 5). This result may be due to the fact that glucose levels are kept stable by liver glycogen, which suggests that diets which contain passion fruit peel hydrolysates had lower levels of glucose, which is kept stable by glycogen. The liver is responsible for the synthesis of components associated with the innate and adaptive immune response of animals, which include total circulating proteins, which are acknowledged to be important for the immune system (Andrews et al., 2011). In our study, liver protein showed a greater concentration in the fish treatment with 5 g/kg of apple pomace hydrolysates (Table 5). This result indicates the prebiotic potential of this source at the respective level of inclusion, because a higher concentration of liver protein, in the long term, tends to reflect the levels of plasma proteins, which have a strong relationship with fish immunity (Adorian et al., 2016).

The addition of prebiotics is intended to help maintain the integrity of the intestinal mucosa of fish and their nutrient absorption capacity. In addition, the integrity of the intestinal membrane should be maintained to avoid possible infections caused by opportunistic microorganisms (Brumano & Gattás, 2009; Ferreira, 2012). An assessment of the intestinal histology of silver catfish showed increased epithelium thickness and goblet cell count in fish in the treatment with 2.5 g/kg of apple pomace hydrolysates, while muscular layer thickness

was lower in the same treatment when compared to the control treatment (Table 6). According to Gaudier et al. (2009), goblet cells are important immunological indicators, because they secrete mucus that covers the intestinal epithelium, thus creating a protective barrier against antigens, toxins and digestive enzymes that exist in the intestinal lumen, in addition to reducing the population of undesirable bacteria in direct contact with the epithelial surface. Based on this information, it can be claimed that the inclusion of 2.5 g/kg of apple pomace hydrolysates promoted greater cell renewal, thus ensuring better epithelial integrity and less propensity to diseases. According to Brumano and Gattás (2009), the use of prebiotics in animal nutrition improves the intestinal membrane, hence villi can remain uniform and keep the same size. As a result, there is an increase in nutrient absorption capacity, and animal performance can thus be improved. However, in our study, there was no influence of pectin hydrolysates on villus size. González-Félix et al. (2018) also did not find an effect of supplementation of commercial prebiotics and probiotics on the folding width, enterocyte height or microvillus height in the intestine of *Totoaba macdonaldi*.

Fermentative growth and activity of beneficial microbial populations generate short-chain fatty acids, which are related to prebiotic effects. As a result, there is a decrease in pH of the gastrointestinal tract, thus inhibiting the development of pathogenic bacteria, improvement in the lumen and anatomy of the gastrointestinal tract, in addition to positive effects on the immune system and animal performance (Freitas, Rabello & Watanabe, 2014; Silva & Nörnberg, 2003). In our study, there was no effect of treatments on the production of acetic acid in the intestine of silver catfish juveniles (Figure 1A). The production of propionic acid was higher in fish of the control treatment when compared to treatment with 5 g/kg of apple pomace hydrolysates (Figure 1B). According to Roediger and Moore (1982), propionic acid is converted into glucose in the liver; however, in our study, liver glucose levels (Table 5) remained equivalent between the treatments. On the other hand, the production of butyric

acid was higher in the treatment with 2.5 g/kg of apple pomace hydrolysates when compared to treatment without inclusion of pectin hydrolysates (Figure 1C). In accordance with Pascoal and Watanabe (2014), butyric acid is directly involved in the growth and multiplication of epithelial cells, thus increasing absorption capacity. This can be confirmed in our results, since the treatment with greater production of butyric acid (2.5 g/kg of apple pomace hydrolysates) also presented increased muscular layer thickness and goblet cell count in the intestine (Table 6).

5 | CONCLUSION

At the end of this study, some promising results were found:

- The inclusion of 2.5 g/kg of apple pomace hydrolysates resulted in greater production of butyric acid and higher goblet cell count in the intestine, indicating greater cell renewal and ensuring better epithelial integrity;

- The inclusion of 5 g/kg of apple pomace hydrolysates promoted greater liver protein concentration; in the long term, it tends to reflect plasma protein levels, which have a strong relationship with fish immunity.

Based on information reported in the literature, it is known that pectin hydrolysates have a prebiotic potential. Considering that our study is the first which uses pectin hydrolysates in the diet of silver catfish, there should be further research with assays to establish doses that would allow more gains in the cultivation of the species.

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350

351 **CONFLICT OF INTEREST**

352 The authors declare that they have no conflict of interest.

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TABLE 1 Dietary formulations and proximate composition of the experimental diets (g/kg)

Ingredients	Diets				
	Control	Apple pomace hydrolysate		Passion fruit peel hydrolysate	
		2.5	5	2.5	5
Fish meal[1]	414	414	414	414	414
Maize starch	201	201	201	201	201
Cellulose	60	57.5	55	57.5	55
SPC 60%[2]	203	203	203	203	203
Apple pomace hydrolysate	0	2.5	5	0	0
Passion fruit peel hydrolysate	0	0	0	2.5	5
Soybean oil	31	31	31	31	31
Vitamin and mineral mixture[3]	30	30	30	30	30
Sodium chloride	5	5	5	5	5
Inert[4]	55.8	55.8	55.8	55.8	55.8
BHT[5]	0.2	0.2	0.2	0.2	0.2
Diets composition					
Moisture[6]	43.2	36.8	45.0	47.0	49.0
Crude protein[6]	389.2	389.0	391.1	383.7	391.3
Digestible energy (MJ/kg)[7]	13.49	13.49	13.49	13.49	13.49
Fat[6]	80.1	79.1	79.7	78.5	78.9
Ash[6]	191.6	197.6	201.0	201.1	196.4
Ca[8]	23.3	23.3	23.3	23.3	23.3
P[8]	10.5	10.5	10.5	10.5	10.5
Physicochemical properties					
HC (g water/ g sample)[6/9]	2.22	2.39	2.35	2.18	2.02
FBC (g oil/ g sample)[6/10]	1.31	1.35	1.32	1.35	1.31
CBC (mg Cu/ g sample) [6/11]	12.14	12.19	12.36	11.75	12.12

[1]Waste flour tilapia – Copisces/Paraná,RS, Brazil;
[2]Soy protein concentrate, 60% CP;
[3]Vitamin and mineral mixture – composition/kg of product: Folic acid: 299.88 mg; Ascorbic acid: 15000.12 mg; Pantothenic acid: 3000.10 mg; Biotin: 0.06 mg; Niacin (B3): 9000.32 mg; Hill (B4): 103500.00 mg; Vitamin A: 1,000,000. IU; Vitamin B1: 1500.38 mg; Vitamin B2: 1500.00 mg; Vitamin B6: 1500.38 mg; Vitamin D3: 240000.00 IU; Vitamin E: 10000.00 mg; Vitamin K3: 400.00 mg; Inositol: 9999.92 mg; Iron: 6416.80 mg; Manganese: 8000.40 mg; Copper: 1000.00 mg; Zinc: 13999.50 mg; Iodine: 45.36 mg; Cobalt: 60.06 mg; Selenium: 60.30 mg; Magnesium: 5.10 mg; Chloride: 2.30%; Sulfur: 0.01%;
[4] Inert= sand;
[5]Antioxidant butylated hydroxytoluene;
[6]Composição analisada (Laboratório de Piscicultura/UFSM);
[7]ED= energia digestível calculada: [(%Proteína bruta * 5.65 * 0.85) + (%Gordura * 9.4 *0.9) + (Carboidratos * 4.15 * 0.7)] (Jobling, 1983);
[8]Valores calculados com base na composição dos ingredientes;
[9]HC= Hydration capacity;
[10]FBC= Fat binding capacity;
[11]CBC= Copper binding capacity.

514 **TABLE 2** Growth parameters and feed efficiency of silver catfish juveniles fed diets with
515 pectic hydrolysates[1]

Treatments	FW(g)	DWG (g)	SGR (%/day)	AFC	S%
Control	46.48 ± 14.33	0.79 ± 0.07	3.36 ± 0.13	1.06 ± 0.05	100.0 ± 0.0
2.5 AP	43.73 ± 14.40	0.70 ± 0.04	3.09 ± 0.04	1.12 ± 0.03	100.0 ± 0.0
5 AP	42.47 ± 12.85	0.68 ± 0.09	3.07 ± 0.26	1.17 ± 0.13	100.0 ± 0.0
2.5 PFP	39.25 ± 12.64	0.61 ± 0.11	2.91 ± 0.31	1.33 ± 0.17	100.0 ± 0.0
5 PFP	43.32 ± 14.62	0.70 ± 0.07	3.15 ± 0.17	1.19 ± 0.05	99.1 ± 1.8
Orthogonal contrasts					
C x 2.5 AP	ns	ns	ns	ns	ns
C x 5 AP	0.032	0.051	ns	ns	ns
C x 2.5 PFP	0.000	0.005	0.008	0.002	ns
C x 5 PFP	ns	ns	ns	ns	ns

516 [1]Values expressed as mean ± standard deviation. The results were compared by orthogonal contrasts ($p<0.05$);
517 ns: not significant ($p>0.05$). AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate; C: control; FW:
518 final weight; DWG: daily weight gain; SGR: specific growth rate; AFC: apparent feed conversion; S: survival.

519 **TABLE 3** Yield and digestive indices of silver catfish juveniles fed diets with inclusion of
520 pectic hydrolysates[1]

Treatments	CY (%)	SDI (%)	SHI (%)	VFI (%)	IQ
Control	87.53 ± 0.73	3.36 ± 0.37	1.73 ± 0.16	1.33 ± 0.47	1.13 ± 0.24
2.5 AP	87.03 ± 1.36	3.56 ± 0.72	1.73 ± 0.20	1.56 ± 0.73	1.16 ± 0.16
5 AP	88.07 ± 3.94	3.37 ± 0.58	1.62 ± 0.41	1.34 ± 0.64	1.21 ± 0.20
2.5 PFP	87.38 ± 1.77	3.62 ± 0.47	1.61 ± 0.16	1.40 ± 0.75	1.17 ± 0.32
5 PFP	87.39 ± 1.51	3.63 ± 0.59	1.87 ± 0.20	1.49 ± 0.60	1.13 ± 0.15
Orthogonal contrasts					
C x 2.5 AP	ns	ns	ns	ns	ns
C x 5 AP	ns	ns	ns	ns	ns
C x 2.5 PFP	ns	ns	ns	ns	ns
C x 5 PFP	ns	ns	ns	ns	ns

521 [1]Values expressed as mean ± standard deviation. The results were compared by orthogonal contrasts ($p<0.05$);
522 ns: not significant ($p>0.05$). AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate; C: control; CY:
523 carcass yield; SDI: somatic digestive index; SHI: somatic hepato index; VFI: visceral fat index; IQ: intestinal
524 quotient.

525 **TABLE 4** Plasma parameters of silver catfish juveniles fed diets with inclusion of pectic hydrolysates[1]

Treatments	ALB (g/dL)	CHOL (mg/dL)	GLU (mg/dL)	TPRO (g/dL)	TRIG (mg/dL)	ALKP	TIG
Control	0.80 ± 0.14	126.75 ± 21.19	47.25 ± 11.34	3.30 ± 0.41	354.38 ± 75.40	19.14 ± 4.27	1.89 ± 0.39
2.5 AP	0.86 ± 0.25	115.63 ± 25.82	49.75 ± 10.94	3.11 ± 0.44	367.63 ± 107.1	16.47 ± 5.40	1.51 ± 0.47
5 AP	0.91 ± 0.43	138.00 ± 33.95	42.50 ± 11.78	3.37 ± 0.41	426.75 ± 156.2	18.48 ± 6.92	1.85 ± 0.24
2.5 PFP	0.80 ± 0.30	127.00 ± 19.78	50.88 ± 11.23	3.23 ± 0.36	329.13 ± 84.98	15.43 ± 4.16	1.74 ± 0.32
5 PFP	0.90 ± 0.23	129.88 ± 15.82	51.38 ± 11.33	3.35 ± 0.36	413.50 ± 109.5	21.32 ± 8.25	1.81 ± 0.26
Orthogonal contrasts							
C x 2.5 AP	ns	ns	ns	ns	ns	ns	0.034
C x 5 AP	ns	ns	ns	ns	ns	ns	ns
C x 2.5 PFP	ns	ns	ns	ns	ns	ns	ns
C x 5 PFP	ns	ns	ns	ns	ns	ns	ns

526 [1]Values expressed as mean ± standard deviation. The results were compared by orthogonal contrasts ($p<0.05$); ns: not significant ($p>0.05$). AP: apple pomace hydrolysate;
527 PFP: passion fruit peel hydrolysate; C: control; ALB: albumin; CHOL: cholesterol; GLU: glucose; TPRO: total proteins; TRIG: triglycerides; ALKP: alkaline phosphatase;
528 TIG: total immunoglobulins.

529 **TABLE 5** Liver parameters of silver catfish juveniles fed diets containing pectic
530 hydrolysates[1]

Treatments	Glucose (µmol/g)	Glycogen (µmol/g)	Protein (mg/g)
Control	31.26 ± 6.11	7.13 ± 0.71	47.82 ± 5.10
2.5 AP	30.84 ± 4.64	6.58 ± 0.77	51.56 ± 3.21
5 AP	31.90 ± 8.65	6.69 ± 0.44	52.68 ± 3.52
2.5 PFP	31.96 ± 4.07	6.42 ± 0.81	49.69 ± 4.32
5 PFP	29.12 ± 7.10	5.90 ± 0.32	51.25 ± 2.43
Orthogonal contrasts			
C x 2.5 AP	ns	ns	ns
C x 5 AP	ns	ns	0.016
C x 2.5 PFP	ns	0.034	ns
C x 5 PFP	ns	0.000	ns

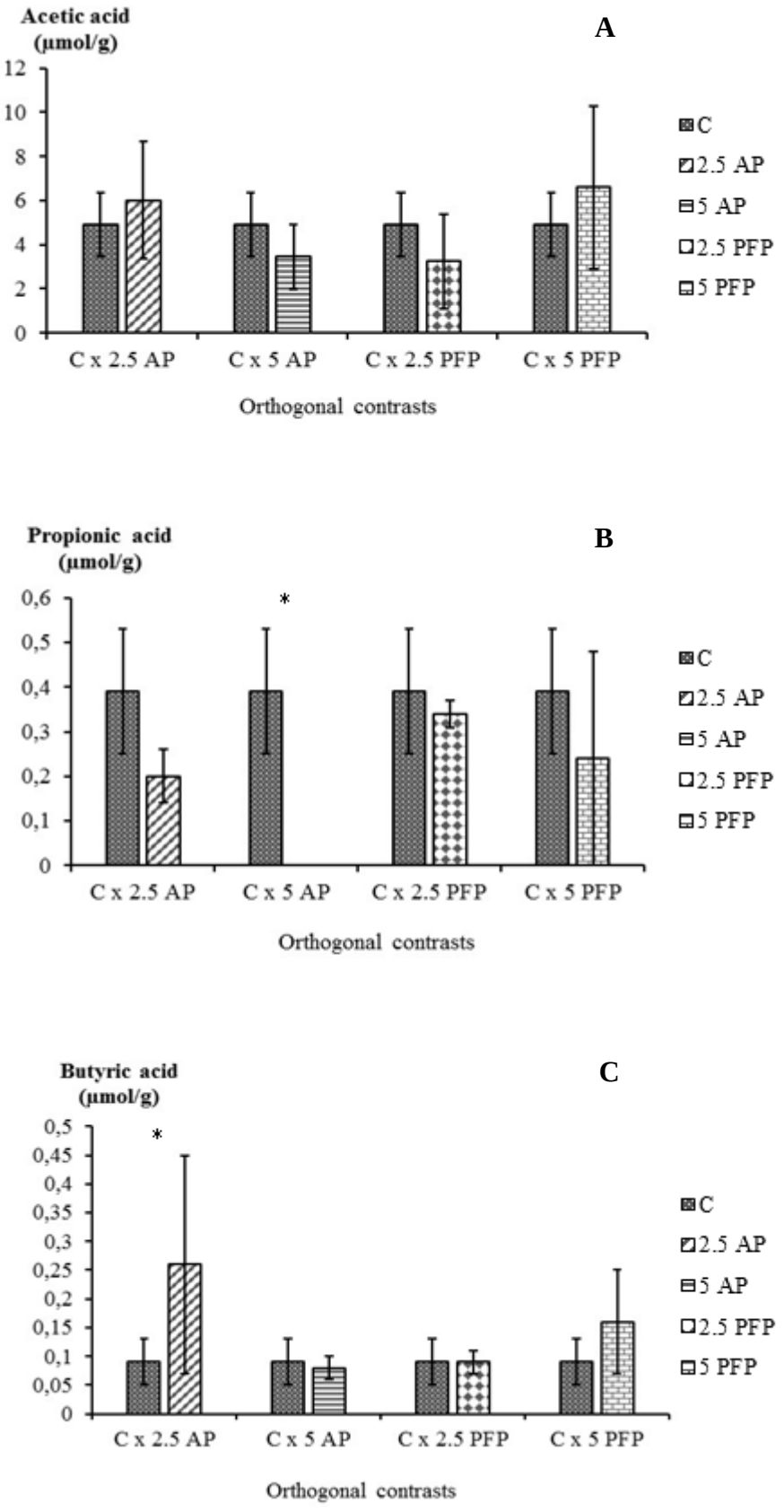
531 [1]Values expressed as mean ± standard deviation. The results were compared by orthogonal contrasts ($p<0.05$);
532 ns: not significant ($p>0.05$). AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate; C: control.

533 **TABLE 6** Intestinal histological parameters of silver catfish juveniles fed diets with inclusion
534 of pectic hydrolysates[1]

Treatments	VH (µm)	ET (µm)	TML (µm)	GC (em 500 µm)
Control	669.49 ± 80.12	95.34 ± 25.34	49.93 ± 3.97	20.00 ± 1.83
2.5 AP	685.87 ± 105.0	123.43 ± 26.57	34.93 ± 4.57	25.50 ± 2.08
5 AP	718.61 ± 50.01	118.84 ± 7.44	51.06 ± 7.29	17.50 ± 4.51
2.5 PFP	762.36 ± 27.11	107.46 ± 15.62	46.28 ± 1.69	18.75 ± 1.71
5 PFP	641.72 ± 29.99	94.30 ± 4.41	52.72 ± 2.47	20.75 ± 2.22
Orthogonal contrasts				
C x 2.5 AP	ns	0.046	0.000	0.011
C x 5 AP	ns	ns	ns	ns
C x 2.5 PFP	ns	ns	ns	ns
C x 5 PFP	ns	ns	ns	ns

535 [1]Values expressed as mean ± standard deviation. The results were compared by orthogonal contrasts ($p<0.05$);
536 ns: not significant ($p>0.05$). AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate; C: control; VH:
537 villus height; ET: epithelium thickness; TML: thickness of muscle layer; GC: goblet cells count.

FIGURE 1 Production of short chain fatt acids in the intestine of silver catfish juveniles fed diets containing pectic hydrolysates. Values expressed as mean ± standard deviation. C: control; AP: apple pomace hydrolysate; PFP: passion fruit peel hydrolysate.



5 DISCUSSÃO GERAL

Diante da necessidade mundial de produção de alimentos em quantidade e com qualidade, na aquicultura o tradicional uso de antibióticos como promotores de crescimento tem sido questionado em função dos seus efeitos residuais. Como alternativa, tem se buscado aditivos prebióticos que fortalecem a microbiota benéfica do trato digestório, reduzindo a susceptibilidade à doenças e refletindo positivamente sobre o desempenho dos peixes (RINGO et al., 2010). Paralelamente à intensificação produtiva, é necessário primar por sistemas de geração de alimentos minimamente poluidores, com foco na sustentabilidade. Nos últimos anos o intenso crescimento do setor agroindustrial, onde destaca-se a cadeia de produção e processamento de frutas, vem gerando grande quantidade de biomassa com considerável potencial nutricional, mas que ainda necessita de estudos de avaliação e aplicabilidade. Neste sentido, esta tese foi desenvolvida com o propósito de avaliar hidrolisados pécticos obtidos a partir de bagaço de maçã e casca de maracujá e seus efeitos na nutrição de peixes.

As biomassas residuais do processamento de maçã e maracujá apresentaram-se promissoras para extração de pectina e produção de hidrolisados pécticos. No entanto, o rendimento de extração foi variável de acordo com a fonte de origem (12,41% para o bagaço de maçã e 17,22% para a casca de maracujá). A concentração da pectina possibilitou aumento de mais de 100% da fração solúvel da fibra nos hidrolisados em relação à sua matéria prima, comprovando a eficiência do processo. Embora alguns estudos citem a intensa fermentabilidade das pectinas pela microbiota intestinal, torna-se necessário ressaltar que a composição monomérica destes polissacarídeos varia de acordo com a matéria prima de origem, refletindo-se diretamente sobre o fortalecimento de algumas populações microbianas e seus produtos correlatos. A análise monossacarídica dos hidrolisados de bagaço de maçã e casca de maracujá demonstrou a heterogeneidade da pectina e sua diversidade estrutural, de acordo com a fonte de obtenção. O hidrolisado de bagaço de maçã apresentou quantidades equivalentes dos monômeros identificados (glicose, xilose e ácido galacturônico), enquanto o hidrolisado de casca de maracujá apresentou maior teor de ácido galacturônico, seguido de glicose e xilose. Assim, entende-se que estes dois hidrolisados pécticos, mesmo quando incluídos em níveis iguais na dieta, poderão causar respostas biológicas distintas devido as suas particularidades químico-estruturais, refletindo-se em seu modo de ação.

A fermentabilidade intestinal das substâncias pécticas gera AGCC, aos quais são atribuídos efeitos promotores de crescimento animal (HOLCK et al., 2011; GULLÓN et al., 2013; GÓMEZ et al., 2014). Normalmente, os principais AGCC produzidos (acetato,

propionato e butirato) são absorvidos e utilizados como fonte de energia. De acordo com Wenzel (2012), o ácido acético é o principal AGCC resultante do processo fermentativo da pectina. De fato, no estudo com alevinos de tilápia do Nilo, os tratamentos com 5 g/kg de hidrolisado de bagaço de maçã e 2,5 g/kg de hidrolisado de casca de maracujá proporcionaram maior produção de ácido acético quando comparados ao tratamento controle. Estes resultados indicam que as populações microbianas benéficas tiveram o seu desenvolvimento estimulado pelo consumo dos hidrolisados, aumentando sua atividade fermentativa. A maior concentração de glicose no fígado dos alevinos de tilápia do Nilo suplementadas com 5 g/kg de hidrolisado de bagaço de maçã, também pode ser atribuída a elevação na produção de ácido acético. Para peixes, as informações de respostas hepáticas relacionadas ao uso de prebióticos ainda são escassas. Mas para mamíferos suplementados, a modulação do metabolismo da glicose está relacionada com a produção de AGCC à nível intestinal e sua utilização hepática para gliconeogênese (ROBERFROID et al. 2010).

Os alevinos de jundiá responderam distintamente quanto à produção de ACGG, com diferença na produção de ácido butírico entre os tratamentos, com maior produção observada para animais suplementados com 2,5 g/kg de hidrolisado de bagaço de maçã, quando comparado ao tratamento sem inclusão de hidrolisados pécticos. De acordo com Pascoal e Watanabe (2014) o ácido butírico está diretamente envolvido no crescimento e multiplicação das células epiteliais, aumentando a capacidade absortiva. Isto pode ser confirmado em nossos resultados, pois o tratamento com maior produção de ácido butírico, apresentou também maior contagem de células caliciformes à nível intestinal.

O fígado é responsável pela síntese de componentes associados à resposta imune inata e adaptativa dos animais, dos quais fazem parte as proteínas circulantes totais, de reconhecida importância relacionada ao sistema imunológico (ANDREWS et al., 2011). Analisando os resultados hepáticos dos alevinos de jundiá, observou-se diferença nos níveis de proteína, onde o tratamento com 5 g/kg de hidrolisado de bagaço de maçã apresentou maior concentração deste constituinte. Este resultado indica o potencial prebiótico da fonte no respectivo nível de inclusão, pois a maior concentração de proteína hepática, a longo prazo, se refletirá nos níveis de proteínas plasmáticas, que apresentam forte relação com a imunidade dos peixes (ADORIAN et al., 2016).

As características físico-químicas (capacidade de hidratação, capacidade de ligação a gordura e capacidade de ligação ao cobre) são relevantes para aplicabilidade e ação fisiológica dos hidrolisados. Os resultados do estudo demonstraram que o hidrolisado péctico de bagaço de maçã possui a maior capacidade de ligar-se à água, enquanto esta característica

apresentou-se reduzida no hidrolisado de casca de maracujá. A maior capacidade de hidratação pode causar aumento no volume e na viscosidade do conteúdo intestinal, reduzindo a taxa de absorção de nutrientes (FIETZ; SALGADO, 1999) e refletindo-se negativamente sobre o desempenho dos animais. No entanto, em nossos estudos com tilápia do Nilo não foram observadas diferenças entre os tratamentos para as variáveis de desempenho avaliadas. Para os alevinos de jundiá, não é possível afirmar que os resultados de desempenho foram diretamente influenciados pela capacidade de hidratação dos hidrolisados. Estes resultados podem ser explicados pelos níveis de suplementação dos hidrolisados, que foram baixos, de modo que a capacidade de hidratação não causou alterações consideráveis na dieta ao ponto de limitar sua digestão e absorção.

A capacidade de ligação a gordura e capacidade de ligação ao cobre apresentaram-se baixas e semelhantes entre os hidrolisados pécticos, indicando atuação análoga quando forem incluídos em dietas. De maneira geral, a baixa capacidade de ligação à gordura é desejável na nutrição de peixes, pois as dietas são formuladas para atender as exigências do animal. Se esta característica físico-química se apresentar elevada, poderá reduzir a absorção da gordura no trato digestório, causando um déficit energético no metabolismo do animal. A capacidade de ligação ao cobre é indicativo da capacidade de ligação à íons catiônicos. No metabolismo dos animais a elevação na CLCu ocasionada pela adição de fibra, pode ser indicativo de aumento de quelação dos sais biliares, com reflexos negativos sobre a digestão das gorduras e sobre a circulação entero-hepática (LEE et al., 2002).

O trato gastrointestinal é uma das principais vias de infecção em peixes e sua histomorfologia afeta diretamente a comunidade microbiológica. Em nosso estudo com alevinos de tilápia do Nilo, foram observados efeitos dos tratamentos sobre altura de vilosidades (2,5 e 5 g/kg hidrolisado de casca de maracujá); espessura do epitélio (5 g/kg de hidrolisado de bagaço de maçã e 2,5 e 5 g/kg de hidrolisado de casca de maracujá) e contagem de células caliciformes (2,5 g/kg de hidrolisado de bagaço de maçã e 2,5 e 5 g/kg de hidrolisado de casca de maracujá). Associando estas informações, é possível afirmar que a maior capacidade de absorção de nutrientes e a secreção de muco pelas células caliciformes reflete positivamente na manutenção da integridade intestinal, reduzindo as infecções bacterianas. Os alevinos de jundiá também apresentaram algumas alterações a nível intestinal em resposta a suplementação dos hidrolisados na dieta, como: maior espessura de epitélio, maior contagem de células caliciformes e menor espessura de camada muscular (2,5 g/kg de hidrolisado de bagaço de maçã), indicando maior renovação celular, garantindo melhor integridade epitelial e menor propensão à doenças.

Dependendo da fonte de origem, da concentração na dieta e da espécie a suplementação dos hidrolisados apresentou resultados diferentes sobre o desempenho dos peixes. No entanto, os peixes cresceram saudáveis, sem necessidade de uso de antimicrobianos, o que garante maior seguridade alimentar ao mercado consumidor. De acordo com Olano-Martin, Gibson e Rastall (2002), os hidrolisados pécticos, caracterizados como pectina de menor massa molar, apresentam grande potencial prebiótico. Em trabalho realizado por Moura (2015), a inclusão de pectina de casca de maracujá parcialmente hidrolisada incluída nas dietas de ratos *Wistar*, nas mesmas concentrações testadas no presente estudo, promoveu maior ganho de peso em relação aos animais alimentados com a dieta controle. No entanto, os alevinos de tilápia do Nilo e jundiá não responderam da mesma forma, o que indica que o uso dos hidrolisados pécticos e a definição de níveis adequados de suplementação visando efeitos prebióticos no desempenho de peixes, ainda carecem de mais investigações.

O presente estudo foi direcionado à duas espécies de peixes, que embora sejam classificadas como onívoras quanto ao hábito alimentar, possuem características comportamentais, anatômicas e fisiológicas peculiares. A tilápia aceita bem ingredientes vegetais na sua dieta e apresenta ciclo de cultivo relativamente curto, devido ao seu crescimento acelerado, quando em condições criatórias adequadas. O jundiá é uma espécie mais seletiva, principalmente quanto a fonte proteica da dieta, tendo preferência por ingredientes de origem animal. Apesar de ser uma espécie onívora, apresenta características anatômicas do trato digestório que assemelham-se as espécies carnívoras. Os estudos de uso deste bagre para cultivo comercial são recentes, portanto, vários aspectos de seu metabolismo digestivo ainda não estão completamente elucidados.

Os resultados obtidos neste estudo demonstraram a necessidade de se ter especial cuidado na aplicação de prebióticos para promoção de saúde e desempenho de peixes. A fonte de origem do prebiótico será determinante para as ações benéficas, porém, cada espécie, de acordo com suas peculiaridades, responderá de forma distinta quando suplementada com estes novos aditivos. O cultivo na piscicultura e na aquicultura de maneira geral, conta com uma infinidade de espécies cultivadas, cada uma com características e exigências próprias. Portanto, para que um novo prebiótico seja difundido comercialmente, este necessita ter seus efeitos comprovados para diversas espécies. Dessa forma, fontes e níveis adequados de prebióticos deverão ser definidos considerando a espécie e fase criatória, de modo a não incorrer em erros que possam diminuir a importância do uso destes aditivos na nutrição animal.

6 CONCLUSÕES GERAIS

Com base nos resultados obtidos neste trabalho, pode-se concluir que:

- As biomassas residuais do processamento de maçã e maracujá apresentaram-se promissoras para extração de pectina, resultando em hidrolisados pécticos com elevada concentração de fibra solúvel;
- O hidrolisado de bagaço de maçã apresentou quantidades semelhantes de glicose-xilose-ácido galacturônico (14,7; 13,5 e 16,11%), enquanto o hidrolisado de casca de maracujá apresentou maior teor de ácido galacturônico (13,7%), seguido de glicose (8,5%) e xilose (4,7%), comprovando a diversidade estrutural da pectina, de acordo com a fonte de origem.
- O hidrolisado de bagaço de maçã apresentou maior capacidade de hidratação, o que pode influenciar na tecnologia de processamento da ração e na ação fisiológica sobre o trato digestório;
- A produção de AGCC à nível intestinal foi influenciada pelos hidrolisados pécticos, sendo os alevinos de tilápia do Nilo apresentaram maior produção de ácido acético, com influência na concentração de glicose hepática. O alevinos de jundiá apresentaram maior produção de ácido butírico, relacionada com a espessura de camada muscular e contagem de células caliciformes à nível intestinal;
- Para a tilápia do Nilo, a suplementação com hidrolisado de casca de maracujá (2,5 ou 5 g/kg) proporcionou menor teor de gordura corporal, garantindo um alimento mais saudável e com maior estabilidade de prateleira.
- Os hidrolisados pécticos causaram alterações nos parâmetros histológicos intestinais. Os alevinos de tilápia do Nilo obtiveram maiores benefícios com a suplementação do hidrolisado de casca de maracujá (2,5 e 5 g/kg), que garantiu maior renovação celular, maior capacidade de absorção de nutrientes e manutenção da integridade intestinal. Já os alevinos de jundiá apresentaram maior contagem de células caliciformes, indicando maior renovação celular, com a suplementação de 2,5 g/kg de hidrolisado de bagaço de maçã;
- Dependendo da fonte de origem, da concentração na dieta e da espécie, a suplementação dos hidrolisados apresenta resultados diferentes, indicando que o uso dos hidrolisados pécticos e a definição de níveis adequados de suplementação visando efeitos prebióticos no desempenho de peixes, ainda carecem de mais investigações.

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ANEXOS

ANEXO A – NORMAS PARA PUBLICAÇÃO NA REVISTA AGRONOMY FOR SUSTAINABLE DEVELOPMENT

MAJOR GUIDELINES OVERVIEW

Key instructions are summarized in the following table:

	RESEARCH ARTICLE	REVIEW ARTICLE	META-ANALYSIS
Sections	1. Introduction 2. Materials and methods 3. Results and discussion 4. Conclusion	Contents 1. Introduction 2. First section... X. Conclusion	1. Introduction 2. Materials and methods 3. Results and discussion 4. Conclusion
Pages number	< 15 pages Times 11, 1.5 spacing	unlimited	< 15 pages Times 11, 1.5 spacing
Font and spacing	Times 11 ; 1.5 spacing		
References	< 30 ; must contain the DOI	must contain the DOI	must contain the DOI
Abstract	< 300 words Structured in 3 parts: 1. Background/issues/hypothesis 2. Experimental 3. Results/novelty	< 300 words Structured in 2 parts: 1. Background/issues 2. Major advances	< 300 words Structured in 3 parts: 1. Background/issues/hypothesis 2. Experimental 3. Results/novelty
Cover letter	Must explain the novelty	Must explain the interest	Must explain the novelty
Figures and tables	5 maximum including 2 tables max.	unlimited	5 maximum including 2 tables max.
Color photo	Mandatory in the introduction		
Figure captions	> 3 sentences		
Figure format	Y axis title horizontal; no symbol legend		

TYPES OF ARTICLES

Agronomy for Sustainable Development publishes three types of papers: *Research articles, Review articles and Meta-analyses*. The findings should be located at the interface of Agriculture and Sustainable Development: see Aims and Scope for specific topics.

SUBMISSION PROCESS

Agronomy for Sustainable Development only accepts online submission, at the following address: http://www.editorialmanager.com/asde

Authors must justify that their manuscript fit the journal Aims and Scope. Therefore, they must select the classification item(s) corresponding to the main topic of their manuscript.

The manuscript must be accompanied by a cover letter containing the article title, the full first name (no initial) and last name of all the authors, a paragraph describing the claimed novelty of the findings versus current knowledge, and a list of six suggested, international reviewers (title, name, postal address, Email address). The suggested reviewers must have no conflict of interest with the authors; they should not be co-authors of previous publications co-signed by the authors.

The ORCID identifier is mandatory for the corresponding author.

EDITORIAL PROCESS

Upon submission, articles enter the preselection process. At that stage, the general quality of the manuscript and its compliance with scope and author instructions are evaluated by the Managing Editor and the Editors in chief. The articles pre-selected are then assigned to an Associate Editor and at least two external reviewers, in a single blind process.

The Associate Editor submits his/her decision to the Managing Editors, which communicates a final decision to the authors. When revisions are requested, the authors are asked to answer point by point to each reviewer comment. The revised manuscript returns to the same Associate Editor and is eventually evaluated again by the same or by alternative reviewers. Upon acceptance of the manuscript, the journal requests that the authors provide a short post on their article, that will be published in the journal blog (http://ist.blogs.inra.fr/agronomy/). The purpose of this post is to convert the main research information into easily accessible language in order to be understandable by the largest possible audience. This post must be accompanied by a relevant photo in landscape format. At the production stage, author should carefully examine the article proofs. No major corrections such as change in authorship will be accepted at this stage.

REQUIRED FORMAT FOR RESEARCH ARTICLES

General

Research articles should report the results of original research. The material should not have been previously published or submitted for publication elsewhere. Research articles should focus on one major discovery supported by 2-4 results.

Novelty

The novelty, or difference, of the major finding versus current knowledge should be clearly explained in:

- the cover letter to the Editor-in-Chief;
- the abstract;
- the end of the Results and Discussion section;
- the Conclusion section.

To explain the novelty, authors should first state what is already known (current knowledge), then state what is the added value of the main finding. Novelty claims should be made in an affirmative way, using for instance “Here we show for the first time that ...”, or “This is the first...” Only articles that show an outstanding added value will be sent for in-depth evaluation.

English

All manuscripts should be written in high-quality American English. Non-English native authors should seek appropriate help from English-writing professionals before submission. The journal may ask authors to provide a certificate from an English language proofreading service, ensuring correct grammar and typographical error corrections (i.e., punctuation, spelling, inconsistencies…) to help authors present a clear and scientific message.

Sections

The manuscript should contain the following items (in the same order):

- article title
- full first and last names of authors with an asterisk “*” highlighting the corresponding author; postal addresses; e-mail address of the corresponding author
- Abstract (less than 300 words)
- List of keywords (maximum 10)
- Introduction
- Materials and methods (including subsections - 2.1, 2.2...)
- Results and discussion (including topical subsections - 3.1, 3.2...)
- Conclusion

- Acknowledgments
- Declaration on conflict of interest
- References

Statement of data availability (see in section 11. below what is expected here)
Other sections such as annexes and appendices are not accepted.
Separated "results" and "discussion" sections are not accepted.

General presentation
The text length of research articles is limited to 15 pages, excluding figures, tables and references. The number of literature references is limited to 30. All text should be written in a concise and integrated way, by focusing on major points, findings, breakthrough or discoveries, and their broad significance. All running text should be in Times 11 or Times New Roman 11, with 1.5 line spacing. Figure and table captions must be self-explanatory and they should be written in Times 10 or Times New Roman 10. Lines, as well as every page of the manuscript, including the title page, references, tables, etc. should be numbered.

Title
The title of research articles should be concise and informative and focused on the main scientific discovery.

Abstract
The research articles abstract of less than 300 words should report concisely on the main scientific breakthrough. The abstract should not contain abbreviations nor literature references. The abstract is structured in three parts: the first part summarizes the Introduction section, it thus gives the background, the global and specific issues, and the hypothesis (about 3-4 sentences). The second part abstracts the Experimental section, it thus gives a brief overview of the experiments or surveys (about 2-3 sentences). The third part abstracts the Results and discussion section, it thus gives: the 1-2 major results using precise trends and data, then the interpretation of those results, then the claimed novelty of those results versus current knowledge, then the basic or applied benefits of those results for sustainable agriculture. Novelty claims should be made in an affirmative way, using for instance “Here we show that ...”, “Here we demonstrate that ...” or “This is the first...”

Abbreviations
In general abbreviations should be avoided in the main text because they decrease article readability and impact. Only 1-2 common abbreviations such as DNA or LED are accepted in the main text. When their use is essential, abbreviations must be explained when they first appear in the text. . Abbreviations in figures, tables and equations are accepted only if there is not enough space to write full words. Here, abbreviations should be explained in figure and table captions, or after equations.

Footnotes
Footnotes in the running text and in tables are not accepted. Table footnotes should be included in the table caption.

Units
Data description in the text, tables and figures should follow the International System of Units, as it is the most widely used system of measurement. The choice of another system of units may be tolerated if it is explained and argued clearly.

REQUIRED FORMAT FOR REVIEW ARTICLES
For review articles please follow the general instructions for research articles, with the following exceptions:

- The page number may surpass 15;
- The figure and table numbers are not limited.

- The title should end by ". A review"
- The abstract of less than 300 words should contain two parts: the first part should give general and global issues, then specific and scientific issues in about 5-6 sentences. The second part should start by, e.g., "Here we review… The major points are the following: 1)… 2)…". Those points are the major advances demonstrated in the article by literature analysis. The reader should clearly understand the added value of those advances.
- The first section of the article should be "1. Introduction", and the last section "X. Conclusion". All sections and sub-sections should be numbered. At the end of each section, authors are advised to propose a concise view of the novelty described and/or the main research hypotheses addressed by the reviewed knowledge.
- A Contents should be inserted after the list of keywords, before the introduction section.

REQUIRED FORMAT FOR META-ANALYSES

For meta-analyses, please follow the general instructions for research articles, with the following exceptions:

The title should end by ". A meta-analysis"

An additional section "References of the meta-analysis" should be inserted after the "References" section

Meta-analyses should meet the following criteria[1]:

- The procedure used to select papers from scientific databases should be explained,
- Individual data should be weighted according to their level of precision when possible,
- Site-year variability of the results should be analyzed from an agronomic point of view, to identify relevant explanatory variables,
- Efforts should be made to check for the publication bias and confounding effects.

REFERENCES

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1993).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list. Reference list entries should be alphabetized by the last names of the first author of each work. The DOI should be indicated when available.

- Journal article

Eden M, Gerke HH, Houot S (2017) Organic waste recycling in agriculture and related effects on soil water retention and plant available water: a review. Agron Sustain Dev 37 (2):21. doi:10.1007/s13593-017-0419-9

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

Lamichhane JR, Durr C, Schwanck AA et al. (2017) Integrated management of damping-off diseases. A review. Agron Sustain Dev 37 (2):25. doi:10.1007/s13593-017-0417-y

- Article by DOI

Coqueret V, Le Bot J, Larbat R et al. (2017). Nitrogen nutrition of tomato plant alters leafminer dietary intake dynamics. J Insect Physiol. doi:10.1016/j.jinsphys.2017.04.002

- Book

Mengel K, Kirkby EA (1987) Principles of plant nutrition. International Potash Institute, Bern

- Book chapter

García-Tejero I.F., Durán-Zuazo V.H., Muriel-Fernández J.L. et al. (2011) Water and Sustainable Agriculture. In: Water and Sustainable Agriculture. SpringerBriefs in Agriculture. Springer, Dordrecht, pp. 1-94

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

Alloush GA (1990) The mechanism of mobilization of iron from soil minerals in the rhizosphere of Cicer arietinum L. Dissertation, University of Leeds

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see http://www.issn.org/services/online-services/access-to-the-ltwa/

For authors using EndNote, an output style that supports the formatting of in-text citations and reference list is available at: http://endnote.com/downloads/style/agronomy-sustainable-development. The authors should check very carefully that references cited in the text are in match with the reference list; and that all references in the list are really cited in the text. The accuracy of references should also be carefully checked.

ARTWORK (TABLES AND FIGURES)

Color figure in the introduction section

For both research and review articles, the introduction must contain one figure including 1-2 color photos. The photo(s) should reveal the main topic of the article to a wide audience.

Number of tables and figures

For research articles, the number of tables plus figures is limited to 5, including a maximum of 2 tables and the introduction color figure. For articles at the interface with social sciences, a higher number of tables and figures may be tolerated, if duly justified by the authors in the cover letter. For review articles, there is no limitation of tables/figures number.

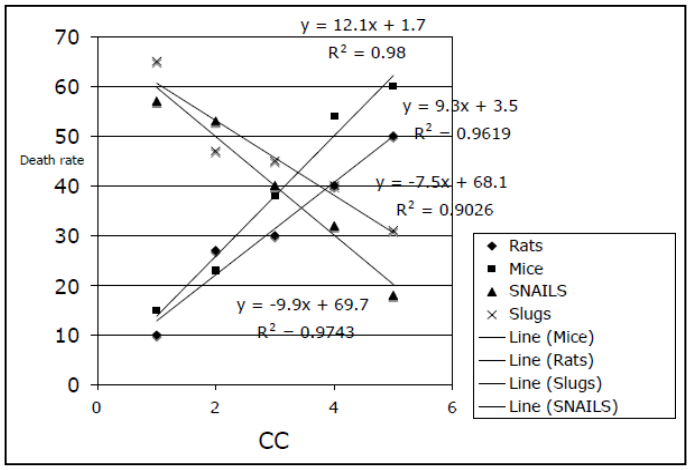
Colors

Color illustrations are accepted at no charge both for the electronic version and the printed version of the journal.

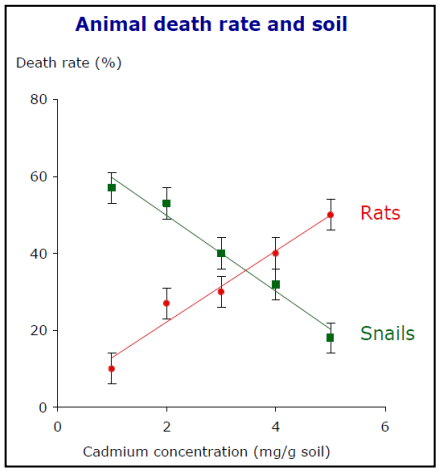
Format

The titles of figure and axes should be bold.
The Y-axis title should be written horizontally at the above-left of the graph, when possible. Preferably, a graph should contain a maximum of 3 curves.
Symbol legends are not accepted; the name of a curve should be written in the graph, beside the corresponding curve, using arrows if necessary.
Regression equations should not appear on the graph, but rather at the end of the caption

WRONG



RIGHT



Authors are encouraged to use contrasting colours (red, blue, green…) to increase the readability of the figures.

Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
Do not use background lines
All lines should be at least 1 pt wide.

Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.) and the placement of letters in the figure parts should be consistent throughout the paper (i.e. preferably top left)

Captions

A "scheme" or "photo" should be named "figure". Figure captions should be self-explanatory and must contain a brief description of the main scientific point of the figure, using 1–2 well thought sentences: a figure should be almost understandable without reading the main body text of the article. The characters should be in Times or Times New Roman with an appropriate size to be readable after 50% reduction.
Do not refer to colors in the captions in case readers print in black and white

Resolution and quality

Figures and tables should be of high quality.

- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Combination artwork should have a minimum resolution of 600 dpi
- Halftones should have a minimum resolution of 300 dpi

Figure Placement and Size
Tables and figures should be uploaded as separated files at the submission stage. Their place in the manuscript should be clearly indicated by authors.
When preparing your figures, size figures to fit in the column width.
The figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.

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In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to- speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (color-blind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

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Supplementary materials should not be used to support the author main conclusion. ASD does not allow data, graphs, schemes, photos, short tables and common figures as supplementary materials. ASD allows only two categories of supplementary materials: videos (V) and audios
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- Supply all supplementary material in standard file formats.
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It is possible to collect multiple files in a .zip or .gz file.

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In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

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RESEARCH DATA POLICY

ASD Data policy
Following a Springer Nature initiative, Agronomy for Sustainable Development adopted a standardised research data policy: the Research Data Policy Type 2.
Specifically, a submission to ASD implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non- commercial purposes, without breaching participant confidentiality. ASD strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers.
We encourage authors to ensure that their datasets are deposited in publicly available repositories (where available and appropriate) whenever possible. The selection of the repository is the authors' responsibility. ASD does not accept datasets as supplementary material.

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The journal encourages authors to provide a statement of data availability in their article. Data availability statements should include information on where data supporting the results reported in the article can be found, including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. Data availability statements can also indicate whether data are available on request from the authors and where no data are available, if appropriate.
Data Availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

- The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]
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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Authors must disclose all relationships or interests that could have direct or potential influence or impart bias on the work. Although an author may not feel there is any conflict, disclosure of relationships and interests provides a more complete and transparent process, leading to an accurate and objective assessment of the work. Awareness of a real or perceived conflicts of interest is a perspective to which the readers are entitled. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is

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- Financial support for attending symposia
- Financial support for educational programs
- Employment or consultation
- Support from a project sponsor
- Position on advisory board or board of directors or other type of management relationships
- Multiple affiliations
- Financial relationships, for example equity ownership or investment interest
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The corresponding author will include a summary statement in the text of the manuscript in a separate section before the reference list, that reflects what is recorded in the potential conflict of interest disclosure form(s).

See below examples of disclosures:

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Conflict of Interest: The authors declare that they have no conflict of interest.

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- A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. “salami-publishing”).
- No data have been fabricated or manipulated (including images) to support your conclusions
- No data, text, or theories by others are presented as if they were the author’s own (“plagiarism”). Proper acknowledgements to other works must be given (this includes material

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- If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be placed with the article or in severe cases complete retraction of the article will occur. The reason must be given in the published erratum or retraction note. Please note that retraction means that the paper is maintained on the platform, watermarked "retracted" and explanation for the retraction is provided in a note linked to the watermarked article.
- The author’s institution may be informed.

ANEXO B – NORMAS PARA PUBLICAÇÃO NA REVISTA AQUACULTURE NUTRITION

1. SUBMISSION

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

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at

http://mc.manuscriptcentral.com/anu

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2. AIMS AND SCOPE

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

Aquaculture Nutrition publishes papers which strive to:

- increase basic knowledge of the nutrition of aquacultured species and elevate the standards of published aquaculture nutrition research
- improve understanding of the relationships between nutrition and the environmental impact of aquaculture
- increase understanding of the relationships between nutrition and processing, product quality, and the consumer.
- help aquaculturalists improve their management and understanding of the complex discipline of nutrition
- help the aquaculture feed industry by providing a focus for relevant information, techniques, tools and concepts.

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