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ALIMENTAÇÃO DO BEIJUPIRÁ (*Rachycentron canadum* Linnaeus, 1766) CULTIVADO COM RESÍDUOS DO PROCESSAMENTO DE CAMARÃO

CAROLINA NUNES COSTA-BOMFIM

Recife - PE

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Tese apresentada ao Programa de Pós-graduação em Oceanografia da Universidade Federal de Pernambuco como requisito para obtenção do título de Doutor em Oceanografia.

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RESUMO

Na presente tese foi analisada a utilização de resíduos do processamento de camarão como ingrediente em dietas para o beijupirá *Rachycentron canadum*, espécie de peixe marinho nativo da costa brasileira. O conhecimento sobre o uso de resíduos de crustáceos em dietas para peixes marinhos é inicialmente apresentado com o objetivo de definir o plano de fundo em que esta tese se insere. O primeiro estudo, além do desenvolvimento da metodologia a ser aplicada nos estudos subsequentes, demonstra que a frequência diária de fornecimento do alimento (1, 2, 3, 4 e 6 refeições/dia) não afeta o desempenho produtivo (ganho de peso, consumo, conversão alimentar, taxa de crescimento específico e sobrevivência) de beijupirás com peso superior a 110 g. Os estudos posteriores se basearam na produção, por meio de autólise enzimática, de um hidrolisado de camarão bruto (SPH), o qual, após centrifugação, resultou em um sobrenadante (hidrolisado de camarão centrifugado, CSPH) e um precipitado (carotenoproteína). Estes três produtos foram caracterizados quanto à composição centesimal e perfis de aminoácidos e de ácidos graxos. Foram estimados, ainda, os coeficientes de digestibilidade aparente (CDA) do CSPH para o beijupirá. Posteriormente, foram analisados o desempenho produtivo, composição nutricional (proteína bruta, lipídios totais, extrato não nitrogenado, cinzas, umidade, aminoácidos e ácidos graxos), taxa de eficiência proteica, taxa de eficiência econômica e índice de lucro econômico de juvenis de beijupirás alimentados com dietas com níveis crescentes de CSPH (0, 12, 24 e 36% da proteína das dietas). Também foram determinadas as atividades enzimáticas digestivas proteolíticas do ceco, estômago e intestino destes animais. SPH, CSPH e carotenoproteína apresentaram alto conteúdo proteico e perfil de aminoácidos similar à farinha de peixe, principal ingrediente proteico em dietas para peixes marinhos. O CDA da proteína do CSPH para juvenis de beijupirás foi superior a 90%. Os beijupirás alimentados com dietas com 12% CSPH apresentaram maior desempenho produtivo e menor custo. As maiores atividades enzimáticas proteolíticas foram registradas nos peixes alimentados com a dieta contendo 24% CSPH. Diante desses resultados, recomenda-se que a inclusão de CSPH em dietas para o beijupirá não exceda 12% do conteúdo total de proteína bruta.

Palavras-chave: Piscicultura marinha; nutrição; proteína; ingrediente alternativo; resíduos de camarão.

ABSTRACT

We assessed the use of shrimp processing wastes as an ingredient in diets for cobia (*Rachycentron canadum*), a marine fish native to the Brazilian coast. The current knowledge on the use of crustacean processing wastes in the nutrition of marine fish is initially presented with the aim of defining the background in which this thesis falls. The first study tested the methodology to be implemented in the following trials and, at the same time, we were able to show that feeding 1, 2, 3, 4 or 6 daily meals had no effect on performance parameters (weight gain, feed intake, feed conversion ratio, specific growth rate and survival) of cobia juveniles weighing more than 110 g. The subsequent studies were based on the production, by enzymatic autolysis, of a shrimp protein hydrolyzate (SPH), which, after centrifugation, resulted in a supernatant (centrifuged shrimp protein hydrolyzate; CSPH) and a precipitate (carotenoprotein). These products were characterized in terms of proximal composition and amino acid and fatty acid profiles. The apparent digestibility coefficients (ADC) of CSPH for cobia were also estimated. Subsequently, we analyzed the performance, nutritional composition (crude protein, total lipids, nitrogen-free extract, ash, moisture, and amino acid and fatty acid profiles), protein efficiency ratio, economic efficiency ratio and the index of economic profit of juvenile cobia fed diets with increasing levels of CSPH (0, 12, 24 and 36% in protein diets). The proteolytic digestive enzyme activities of the cecum, stomach and intestine of these animals were also determined. SPH, CSPH and carotenoprotein presented high protein content and an amino acid profile similar to fish meal, the main protein ingredient used in diets for marine fish. The ADC of protein from CSPH for juvenile cobia was higher than 90%. Cobia fed diets with 12% CSPH had a higher growth performance. The major proteolytic enzyme activities were observed in cobia fed the diet containing 24% CSPH. Based on these results, it is recommended that the inclusion of CSPH in diets for cobia juveniles should not exceed 12% of the total crude protein content.

Key-words: Marine fish farming; nutrition; protein; alternative ingredients; shrimp waste.

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INTRODUÇÃO

A aquicultura destaca-se como um importante setor de produção de alimentos nos últimos anos. Sua contribuição alcançou 41% do total de pescados disponíveis mundialmente para consumo humano em 2010, além de apresentar uma taxa anual de crescimento nos últimos 40 anos em torno de 6,5%, o que é superior à taxa de crescimento da população mundial (FAO, 2012). A produção aquícola no Brasil vem apresentando um crescimento acima do observado pelos outros setores da pecuária, como a avicultura, suinocultura e bovinocultura. Devido a grande diversidade de espécies no Brasil, condizente com o nosso potencial hidrográfico, a aquicultura brasileira tem a possibilidade de se tornar uma das principais do mundo (MPA, 2011).

Entre os setores da aquicultura, a piscicultura marinha tem crescido significativamente nos últimos anos, alcançando uma produção mundial de 1,8 milhões de t em 2010 (FAO, 2012). No Brasil, o cultivo de peixes marinhos também apresenta um enorme potencial, diante do extenso litoral brasileiro de aproximadamente 8 mil km, e por possuir uma grande diversidade de peixes marinhos e estuarinos, tanto de águas quentes como frias (Baldisserotto & Gomes, 2010). Embora registros indiquem a existência da criação de peixes marinhos no litoral de Pernambuco desde o início do Século XVII (Von Ihering, 1932), a produção atual da piscicultura marinha é incipiente, com um registro de produção de apenas 49 toneladas em 2009, através do cultivo do beijupirá (*Rachycentron canadum*) em mar aberto no litoral de Pernambuco (MPA, 2011).

O beijupirá é uma espécie marinha que tem sido indicada como de grande potencial para deslanchar a piscicultura marinha no Brasil (Cavalli & Hamilton, 2007; Cavalli et al., 2011). Trata-se de uma espécie pelágica, migratória e que se adequa a temperaturas entre 22 e 32ºC, sendo encontrada em todo o litoral brasileiro. O beijupirá apresenta um crescimento rápido, atingindo 4-6 kg em um ano, alta sobrevivência e baixa conversão alimentar, além de um bom valor de mercado. É uma espécie carnívora, que possui preferência alimentar por crustáceos, além de peixes e outros invertebrados (Shaffer & Nakamura, 1989; Chou et al., 2001).

Como a maioria das espécies carnívoras, o beijupirá exige um alto teor de proteína bruta em suas dietas. Chou et al. (2001) estimaram que o crescimento máximo dessa espécie ocorre com dietas contendo 44,5% de proteína bruta. A farinha de peixe é a fonte proteica mais utilizada em dieta de organismos aquáticos, principalmente

carnívoros, por apresentar boa qualidade nutricional, como alta digestibilidade, bom perfil de aminoácidos e outros nutrientes essenciais, como ácidos graxos poli-insaturados. Entretanto, com a redução da pesca por captura nos últimos anos e a falta de sustentabilidade, principalmente pela destinação de produtos pesqueiros de menor valor para a produção de farinha de peixe (NRC, 2011), surge a necessidade de se buscar fontes proteicas alternativas à farinha de peixe, que sejam economicamente viáveis e que atendam às necessidades nutricionais dos peixes.

Nesse sentido, o presente trabalho teve como objetivo analisar o hidrolisado proteico produzido a partir de resíduos do processamento do camarão como fonte proteica alternativa em dietas para o beijupirá, avaliando o desempenho produtivo, digestibilidade, composição nutricional e as atividades enzimáticas digestivas do beijupirá.

REVISÃO DE LITERATURA

Ao longo do tempo, a criação de organismos aquáticos, ou aquicultura, evoluiu de uma atividade de pequena escala e de baixa tecnologia para operações intensivas consideradas como importantes empreendimentos para obtenção de divisas externas através da exportação (Beveridge & Little, 2002), mas também como estratégia fundamental na perspectiva da segurança alimentar. Atualmente, a aquicultura é um dos sistemas de produção de alimentos com maior taxa de crescimento no mundo, o que coloca esta atividade em foco pela grande oportunidade de produção de alimentos, geração de postos de trabalho e desenvolvimento de negócios (Howarth, 1996). Em 2010, a aquicultura contribuiu com 41% do total de 148 milhões de toneladas de pescados produzidos mundialmente (FAO, 2012). No Brasil, a aquicultura foi responsável por aproximadamente 38% do total de 1,25 milhões de toneladas de pescados produzidos em 2009 (MPA, 2011).

A piscicultura marinha, por sua vez, tem sido um dos setores da aquicultura mundial apresenta uma das maiores taxas de crescimento (FAO, 2012). Enquanto a aquicultura como um todo apresenta uma taxa anual de crescimento de 8,8% nos últimos dez anos, a piscicultura marinha cresceu 9,3% ao ano no mesmo período (FAO, 2012). Apesar da inexpressiva produção no Brasil, não há como negar o enorme potencial para o desenvolvimento dessa atividade no nosso país, principalmente pelo

seu extenso litoral e grande diversidade de espécies marinhas e estuarinas, tanto de águas quentes como frias (Cavalli & Hamilton, 2007; Baldisserotto & Gomes, 2010; MPA, 2011). Entre as espécies nativas consideradas como tendo potencial para a aquicultura no Brasil estão as tainhas (*Mugil liza*, *M. platanus* e *M. curema*), os robalos (*Centropomus undecimalis* e *C. parallelus*), o linguado (*Paralichthys orbignyanus*), os lutjanídeos (*Lutjanus analis* e *L. synagris*), a arabaiana (*Seriola rivoliana*), a carapeba (*Eugerres brasilianus*), os serranídeos (*Epinephelus* sp.), o beijupirá (*Rachycentron canadum*), entre outras (Cavalli & Hamilton, 2007; Baldisseroto & Gomes, 2010).

Entre estas espécies, o beijupirá (*R. canadum*) se destaca por suas características biológicas e de adaptação às atividades de aquicultura. Único representante da família Rachycentridae, o beijupirá é uma espécie nerítica e epipelágica, de hábito natatório ativo, devido à ausência da vesícula gasosa, e de comportamento migratório. A espécie distribui-se naturalmente em todo o litoral do Brasil, sendo, portanto, encontrada em águas tropicais e subtropicais (Shaffer & Nakamura, 1989; Brown-Peterson et al., 2001). No ambiente natural, o beijupirá pode alcançar comprimento máximo em torno de 200 cm e um peso de 68 kg (Shaffer & Nakamura, 1989). De hábito predador, inclui na sua dieta o zoobentos e o nécton, alimentando-se, preferencialmente, de peixes e caranguejos, embora possa eventualmente consumir bivalves (Meyer et al., 1996; Arendt et al., 2001). De forma diferente do registrado em estudos nos Estados Unidos, Peregrino et al. (2005) encontraram que o principal item alimentar consumido na costa de Pernambuco foram os peixes ósseos, com baixíssima ocorrência de crustáceos. Os resultados deste estudo indicam também que a ocorrência e a alimentação do beijupirá na costa pernambucana estão associadas à presença de recifes ao longo do litoral, os quais abrigam espécies residentes, de baixo deslocamento.

Taiwan e China são os únicos países produtores que constam nas estatísticas oficiais da aquicultura (FAO, 2012). A aquicultura desta espécie teve início em 1995 com apenas 3 toneladas produzidas em Taiwan, embora em 2004 tenha alcançado cerca de 5.000 toneladas (Kaiser & Holt, 2005). Além de Taiwan e da China, o beijupirá também é criado comercialmente no Vietnam, onde sua produção em 2008 foi estimada em 1.500 t (Nhu et al., 2011). Existem também relatos da aquicultura desta espécie nos Estados Unidos (Weirich et al., 2004), México (Segovia-Valle et al., 2006), Ilhas Réunion (Gaumet et al., 2007), Japão (Nakamura, 2007), Indonésia (Wahjudi & Michel, 2007), Porto Rico, Tailândia, Irã, República Dominicana, Bahamas, Martinica, Panamá (Benetti et al., 2008), Emirados Árabes Unidos (Yousif et al., 2009), Colômbia,

Singapura, Belize (FAO, 2012), e Índia (Gopakumar et al., 2011). No Brasil, existem projetos de criação de *R. canadum* nos estados de São Paulo, Rio de Janeiro, Bahia, Pernambuco e Rio Grande do Norte (Cavalli et al., 2011).

O grande interesse na aquicultura desta espécie se deve principalmente a sua alta taxa de crescimento (Liao & Leaño, 2007), pois é capaz de alcançar um peso médio entre 4 e 6 kg em um ano de criação (Arnold et al., 2002), e entre 8 e 10 kg em 16 meses (Su et al., 2000; Liao et al., 2004). Além disso, o beijupirá também apresenta uma série de outras características favoráveis à aquicultura, incluindo a facilidade para desovar em cativeiro (Caylor et al., 1994; Arnold et al., 2002; Faulk & Holt, 2006), relativa tolerância às variações de salinidade (Faulk & Holt, 2006), resposta positiva à vacinação (Lin et al., 2006), adaptabilidade ao confinamento e aceitação de dietas extrusadas (Craig et al., 2006), e carne de excelente qualidade (Liao et al. 2004; Craig et al., 2006, Liao & Leaño, 2007).

A aquicultura do beijupirá, assim como de outras espécies carnívoras, se baseia no fornecimento de dietas com alto conteúdo proteico. Os peixes geralmente apresentam uma maior exigência de proteína nas dietas quando comparado aos animais terrestres, devido a menor exigência em energia, a qual se justifica pela forma pecilotérmica da vida aquática (Kaushik & Seiliez, 2010). Segundo o NRC (2011), a exigência em proteína digestível da maioria das espécies de peixe marinho é superior à 36%. No caso específico do beijupirá, Chou et al. (2001) estimaram que o nível ideal de proteína bruta nas dietas para juvenis desta espécie seria 44,5%. Ingredientes de origem marinha, principalmente a farinha de peixe, são os mais utilizados por serem ótimas fontes de nutrientes. A farinha de peixe possui um teor de proteína variando entre 54 a 72% (Furuya, 2010; NRC, 2011), sendo rico em nutrientes essenciais, como os aminoácidos metionina e lisina, e os ácidos graxos poli-insaturados EPA (ácido eicosapentanóico) e DHA (ácido docosahexanóico). Além disso, a farinha de peixe tem alta digestibilidade, que garante uma boa conversão alimentar e com isso a redução de resíduos nitrogenados no ambiente de cultivo, além de alta palatabilidade (Watanabe, 2002; Hardy, 2008; NRC, 2011). Por outro lado, os níveis de minerais, assim como as demais características das farinhas de peixe, variam amplamente de acordo com a origem, que pode ser a partir de pequenos peixes pelágicos inteiros ou a partir dos resíduos da indústria de beneficiamento (Hardy & Barrows, 2002; NRC, 2011).

De modo geral, a farinha de peixe compreende entre 20 e 60% da composição das dietas utilizadas na alimentação de peixes criados em cativeiro (Watanabe, 2002).

Entretanto, a farinha de peixe é um ingrediente de oferta limitada e demanda crescente (Davis & Arnold, 2000; Naylor et al., 2000; Tacon & Metian, 2008). A variação da disponibilidade e constante flutuação nos preços deste insumo podem afetar seriamente a sustentabilidade e a rentabilidade da aquicultura de espécies carnívoras (Naylor et al., 2000; Olvera-Novoa et al., 2002). Em 2008, por exemplo, das 115 mil toneladas de peixes produzidas mundialmente, 67% foi destinada à produção de farinha e óleo de peixe. Com relação à utilização da farinha de peixe, Tacon & Metian (2008) estimam que as dietas para peixes marinhos consomem cerca de 18% da farinha de peixe produzida mundialmente.

Outra questão importante é que a produção de farinha de peixe causa forte pressão de pesca sobre espécies forrageiras, ocasionando sobrepesca e até a depleção de alguns desses estoques, o que resultaria na redução de alimento para as espécies em níveis tróficos superiores (Naylor et al., 2000). Em virtude disso, El-Sayed (1999) defende a insustentabilidade de se utilizar a farinha de peixe como principal fonte de proteína em dietas aquáticas. A substituição da farinha de peixe por outras fontes de proteína também serviria, portanto, para amenizar a pressão sobre os estoques pesqueiros, além de contribuir para a redução dos custos de produção.

A utilização de fontes proteicas alternativas à farinha de peixe, que tenham menor custo e que promovam o crescimento dos peixes, seria vantajosa tanto para a indústria de rações como para os aquicultores. Felizmente, vários estudos com fontes alternativas vêm sendo desenvolvidos (Watanabe, 2002; Gatlin et al., 2007; NRC, 2011). Na avaliação de ingredientes para uso em dietas aquícolas, Glencross et al. (2007) ressaltam a necessidade de inicialmente caracterizar a composição química, bem como avaliar a digestibilidade. O grau de digestibilidade, ou assimilação dos nutrientes pelos peixes, irá influenciar a produção de efluentes, podendo impactar o ambiente (Glencross et al., 2007; Tacon & Forster, 2003). A identificação de fontes proteicas alternativas à farinha de peixe deve também considerar a diminuição dos impactos econômicos e ambientais e, ao mesmo tempo, atender às exigências nutricionais das espécies cultivadas.

A utilização de ingredientes de origem vegetal, como farelo e concentrados de soja (Burr et al., 2012), farinha de glúten de milho (Sarker et al., 2012), farinha de trigo e de glúten de trigo (Torstensen et al., 2008; Valente et al., 2011), farelo de canola, sendo esses obtidos a partir de sementes de oleaginosas, grãos e legumes (NRC, 2011), vem sendo considerada. Entretanto, além de não atenderem às necessidades nutricionais

dos peixes carnívoros, as fontes proteicas de origem vegetal possuem outros fatores que dificultam sua utilização (Hardy, 2008; Krogdahl et al., 2010). Entre estes pode-se citar fatores antinutricionais, como inibidores de protease, a presença de ácido fítico, que reduz a disponibilidade do zinco, além de uma menor digestibilidade da proteína. O uso de ingredientes alternativos também varia em função do custo, nível de proteína, perfil de aminoácidos, presença de fatores antinutricionais e outros fatores limitantes quanto ao nível de substituição (Hardy, 2008).

Além dos ingredientes de origem vegetal, produtos oriundos de animais terrestres também são utilizados em substituição à farinha de peixe. Entre estes temos a farinha de sangue, farinha de carne e ossos, farinha de penas e a farinha de vísceras de aves (Quartaro et al., 1998; Burr et al., 2012), que podem possuir menor custo quando comparadas a outras fontes proteicas, principalmente as de origem vegetal, mas são considerados potenciais substitutos por apresentarem maior similaridade com a farinha de peixe, devido à composição de aminoácidos, minerais, fosfolipídios e colesterol. Além disso, são livres de fatores antinutricionais e não possuem organismos geneticamente modificados (Yu, 2008; NRC, 2011). Entretanto, esses produtos podem conter uma composição variável, além de apresentarem alguns riscos, como contaminação bacteriana e vetor de doenças, como observado em bovinos (febre aftosa e “mal da vaca louca”), sendo seu uso atualmente limitado na Europa (Campestrini, 2005; NRC, 2011).

Dietas sem farinha de peixe, ou seja, contendo apenas ingredientes alternativos, como concentrado proteico de soja e canola, proteína oriunda de animais terrestres, farinha de krill, aminoácidos sintéticos e alimentos com poder atrativo, normalmente resultam em maiores custos de produção do que quando se utilizam dietas contendo farinha de peixe (NRC, 2011). Entretanto, o aumento do preço da farinha de peixe observado nos últimos anos tem mudado um pouco esse contexto. Nesse caso, têm-se sugerido o uso de baixos níveis de inclusão de farinha de peixe (5 a 20%) em dietas para peixes marinhos (NRC, 2011).

Um possível substituto da farinha de peixe são os rejeitos do próprio processo de industrialização de pescado, cuja disponibilidade vem crescendo em consonância com o incremento da produção de peixes e camarões em cativeiro, o que resulta no aumento do descarte de resíduos do beneficiamento destes produtos (Fanimo et al., 2000). Muitas vezes estes resíduos são depositados sem nenhum tratamento prévio ou depuração, gerando um problema ambiental (Knorr, 1991; Vazquez & Murado, 2008), existindo a

necessidade do aperfeiçoamento de sistemas de aplicação e gerenciamento destes resíduos. Nesse sentido, Maia et al. (1998) destacam a importância de se utilizar a matéria-prima em toda sua extensão, recuperando os subprodutos e evitando a formação do próprio resíduo. Infelizmente, a tecnologia sobre a utilização de resíduos das indústrias de pescado atualmente disponível não se demonstra economicamente atrativa em vista do elevado investimento inicial. O uso de aterros sanitários e lagoas de tratamento de efluentes, por exemplo, não são alternativas recomendáveis, devido ao odor desagradável que provocam nas áreas costeiras ou continentais (Lustosa Neto, 1994).

Uma alternativa viável para estes resíduos seria a obtenção de produtos obtidos por meio de tratamentos biotecnológicos, que podem ser incorporados como ingredientes na formulação de dietas de organismos aquáticos, tais como farinhas, hidrolisados proteicos e silagens (Gildberg & Stenberg, 2001; Forster, 2008). Estes produtos surgem como alternativa inclusive como forma de reduzir o impacto causado pela imobilização destes resíduos no ambiente (Arvanitoyannis & Kassaveti, 2008). Desta forma, ao serem adicionados às dietas, estes ingredientes poderiam reduzir custos ao mesmo tempo em que manteriam ou até mesmo melhorariam a qualidade nutricional e a atratividade das dietas destinadas à alimentação animal.

Os resíduos do processamento do camarão, por exemplo, que são constituídos principalmente pela cabeça e representam de 34 a 45% do peso vivo (Barrat & Montano 1986), são normalmente descartados como resíduo. Knorr (1991) estimou a produção mundial de resíduos de crustáceos em 1,2 milhões de toneladas por ano. No entanto, como a produção mundial de camarões oriundos da pesca e aquicultura recentemente atingiu seis milhões de toneladas (FAO, 2012), é razoável estimar que pelo menos dois milhões de toneladas de resíduos de processamento de camarão estejam disponíveis anualmente em todo o mundo. No Brasil, a produção de camarões pela aquicultura foi de 65.188 t (MPA, 2011), o que permite estimar a disponibilidade de 22.160 t de resíduos por ano.

Apesar do baixo valor comercial, os resíduos do processamento de camarão são identificados como fonte proteica animal de qualidade devido ao seu perfil nutricional. Possui níveis relativamente altos (40 a 65%) níveis de proteína bruta (Fanimo et al., 2000; Gildberg & Stenberg, 2001; Halver & Hardy, 2002; Heu et al., 2003) e lipídios totais (6,2 a 12,8%) (Hertrampf & Piedad-Pascual, 2000). Apresentam também altos teores de carotenóides, como a astaxantina, a qual, adicionada às dietas de salmonídeos,

confere a coloração avermelhada característica do filé, funcionando como atrativo ao consumidor final (Ogawa et al., 2007; NRC, 2011). Os resíduos de camarão também possuem alto teor de quitina e quitosana (Subasinghe, 1999; Synowiecki & Al-Khateeb, 2000, Cahu et al., 2012), que possuem efeito imunoestimulatório, atuando na prevenção de doenças em peixes (Golapakannan & Arul, 2006; Sakai, 1999).

Em vista dessas qualidades, os resíduos de camarão vêm sendo estudados como possíveis ingredientes para dietas, principalmente dos organismos aquáticos, na forma de farinha, mantendo a qualidade na composição nutricional, como alto teor de proteína (Fanimo, 2000). Entretanto, o uso da farinha de camarão em peixes como tilápia (Guimarães et al., 2008), pode afetar negativamente o desempenho, devido a baixa digestibilidade da quitina, que é um aminopolissacarídeo indigestível para a maioria dos peixes (Shiau & Yu, 1999).

Outra forma de aproveitamento destes resíduos é a silagem (Fagbenro & Bello-Olusoji, 1997), que consiste em uma forma de preservação da matéria prima pela adição de ácidos orgânicos ou inorgânicos (silagem ácida), de microorganismos (silagem biológica) ou de enzimas (silagem enzimática) (Borghesi, 2004).

O hidrolisado proteico, outra forma de aproveitamento, difere da silagem por sua preservação ocorrer após o processo de hidrólise (NRC, 2011). Este processo consiste na degradação das proteínas e, consequentemente, em uma maior digestibilidade para os peixes (Berge & Storebakken, 1996). A presença de moléculas de baixo peso molecular e aminoácidos livres, por sua vez, estimulam o consumo pelos peixes, garantindo aos hidrolisados o poder de estimulantes alimentares (Rungruangsak & Utne, 1981; NRC, 2011). O fornecimento de hidrolisados em dietas possibilita o consumo destes resíduos, como fonte proteica de qualidade nutricional, por espécies que não possuem habilidade na digestão do resíduo de camarão na forma de farinha. Hidrolisados de camarões e peixes são relatados como potenciais substitutos à farinha de peixe em dietas para peixes (Fagbenro & Jauncey, 1993; Plascencia-Jatomea et al., 2002;. Hevroy et al., 2005), pois asseguram um maior consumo de ração e um subsequente aumento no crescimento dos peixes devido ao efeito atrativo e composição nutricional (Berge & Storebakken, 1996). Além da qualidade nutricional, os hidrolisados também contêm compostos que estimulam a resposta imune não específica em peixes (Bogwald et al., 1996; Gildberg et al., 1995; Murray et al., 2003; Liang et al., 2006). Frações de baixo peso molecular podem agir como imunomoduladores, aumentando a atividade dos macrófagos de peixes (Bogwald et al., 1996; Gildberg et al., 1995). Assim, a

suplementação de dietas com hidrolisados de camarão pode aumentar o crescimento dos peixes, bem como aumentar a resistência a doenças.

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CAPÍTULO 1

The effect of feeding on growth performance of cobia

(*Rachycentron canadum*) juveniles

The effect of feeding on growth performance of cobia (*Rachycentron canadum*) juveniles

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Abstract

World aquaculture production of cobia is gradually increasing. Since feeding is the major operational expense in cobia farming, proper feed management must be exercised as it affects productive parameters and environmental quality. This study assessed the impact of feeding frequency on growth, survival, feed intake, condition factor and size heterogeneity of cobia under laboratory conditions. Juveniles (mean weight of 110 g)

were hand-fed a commercial diet containing 47.4% crude protein and 8.5% lipids for 60 days. The same amount of feed was offered daily, but divided in 1, 2, 3, 4 or 6 meals. Groups of 8 fish were randomly distributed in twenty 500 L tanks that were continuously supplied with filtered seawater at 5 L. min^{-1}. None of the parameters associated with growth performance (survival, weight gain, specific growth rate, feed intake, condition factor and size variation) showed any significant differences between treatments. Although under the conditions of the present study feeding frequency had no effect on the growth performance of cobia juveniles, in commercial farming operations, where large numbers of fish are kept within a single rearing structure, fish behavior during feeding may lead to aggressive interactions. Under these conditions, it is difficult to ensure that all cobia are fed to satiation and thus it is usual to provide two or more meals per day. Therefore, although the present results indicate that for an individual cobia the provision of more than one daily meal had no significant effect on growth performance, further investigations on the effects of feeding frequency are warranted under field conditions.

Keywords: Growth, feed management, feeding, cobia.

Introduction

Cobia (*Rachycentron canadum*) is a marine finfish species with emerging potential for aquaculture. This species presents several characteristics that turn it into a natural candidate for mariculture: easiness of spawning in captivity and high fecundity (Franks et al., 2001; Arnold et al., 2002), established larviculture protocols (Holt et al., 2007), capacity for rapid growth rates (Chou et al., 2001), amenability to a variety of

rearing techniques and culture systems, adaptability to commercially available aquafeeds and a high quality white flesh (Liao et al., 2004; Liao and Leaño, 2007). As a result, production of cobia in the past decade has gradually increased in tropical and subtropical areas of the world. In 2009, a total of 31,926 Mt of cobia were harvested from aquaculture farms (FAO, 2011). Main producing countries are China, Taiwan and Vietnam (FAO, 2011; Nhu et al., 2011), but attempts to rear cobia have also been reported in the USA, La Réunion Island, Japan, Indonesia (Liao and Leaño, 2007), United Arab Emirates (Yousif et al., 2009), Belize, Panama, Brazil, Mexico, Dominican Republic, Martinique, Puerto Rico, Bahamas, Thailand, Iran (Benetti et al., 2010), Colombia, Singapore (FAO, 2011) and India (Gopakumar et al., 2011).

Feeding is considered the most expensive operational cost in cobia farming (Sanches et al., 2008; Miao et al., 2009). So far, however, little work has been carried out to establish proper feed management practices, despite its potential to reduce both economical and environmental pressure in marine fish culture operations. For instance, it is well established that feeding frequency plays a crucial role on fish performance (Elliott, 1975; Murai and Andrews, 1976; Jobling, 1983; Tung and Shiau, 1991; Thomassen and Fjaera, 1996; Johansen and Jobling, 1998; Wang et al., 1998; Liu and Liao 1999; Sanches and Hayashi, 2001; Schnaittacher et al., 2005) and ultimately on the economic viability of fish farms (Başçinar et al., 2007), yet this aspect has not been properly considered for cobia (Chen and Liao, 2007; Fraser and Davies, 2009).

Feed management is known to affect not only productive parameters, but it also influences environmental quality (Lovell, 2002). Although there is a trend towards the establishment of open ocean farms, at present most cobia farming operations are carried out in floating cages placed in protected areas. Under these conditions, net pen operations directly release waste and uneaten food into the marine environment, which

may impact water quality and change the chemical and biological structure of the sediment. Alongi et al. (2003) and Tacon and Forster (2003) agree that environmental impact may occur if feeding regimes are inappropriately employed.

Based on work with other marine finfish species, it is hypothesized that feeding frequency will affect the growth performance of cobia. The present study was therefore designed to determine the number of daily feeding sessions that results in maximum growth of juvenile cobia under laboratory conditions.

Material and Methods

Cobia juveniles weighing around 100 g were obtained from a private hatchery (Aqualider Maricultura S.A., Ipojuca, PE, Brazil). Eight fish were stocked into each of twenty 500 L flow-through circular tanks. Tanks were supplied with a continuous flow (approximately 5 L/min) of sand-filtered seawater and continuous aeration. Water temperature, salinity and dissolved oxygen were monitored in each tank daily using a multi-parameter (YSI 556 - Yellow Springs Instruments, USA), while concentrations of total ammonia and nitrite were determined every three days with commercial kits (Labcon tests – Alcon, Brazil).

Before initiation of the experiment, the fish were conditioned for one week and fed twice daily (at 0700 h and 1700 h) to apparent satiation a commercial diet (Socil Evialis, São Lourenço da Mata, PE, Brazil). Analysis of the diet (AOAC, 1990) indicated that it contained 47.4 % crude protein, 8.5% lipids and 9.4% ash. After conditioning, all fish were pooled and those of similar size were visually selected, weighed and measured. Mean (± SE) initial weight and total length were 109.7 g (± 0.9)

and 24.84 cm (± 0.03), respectively. Photoperiod regime was natural and the diurnal cycle lasted from sunrise at 0530 h and sunset at 1730 h over the course of the experiment. First and last meals were offered at 0700 h and 1700 h, respectively.

The experiment lasted 60 days during which period the fish were hand-fed the same commercial diet as in the conditioning period. The experimental design consisted of five treatments with four replicates. All treatments received the same daily amount of feed (3% of fish biomass per day; Liao et al., 2004, Xiao et al., 2010), but divided in one, two, three, four or six meals. Feed consumption was monitored and recorded at each feeding. Dead fish, if any, were removed daily from the tanks and weighed. Tanks were scrubbed and siphoned every other week. Tanks were covered to reduce both fish losses from jumping and the incidence of direct light.

Every 15 days, four fish from each tank were anesthetized with 5 ppm clove oil (AQUI-S, Bayer S.A., Chile) and weighed individually. At the end of the experimental period, all fish were counted. Survival, weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), condition factor (K) and apparent feed intake (FI) were determined. The following formulae were used to assess these parameters:

WG (%) = (final weight – initial weight) x (initial weight)/100

FCR = (dry feed fed) / (wet weight gain)

SGR (%/day) = [ln (final weight) x ln (initial weight)] / (number of days) x 100

$K = [(\text{weight}) / (\text{length})^3] \times 1000$

FI (% body weight/day) = 100 x [average individual feed intake x (initial weight/final weight)$^{0.5}$)] / number of days

All data are reported as mean ± standard error (SE). Analysis of variance (ANOVA) was applied to determine statistical differences between treatments. Analysis of the data was based on normality assumptions of ANOVA. Tukey's multiple range test was used to examine differences between treatments whenever significant differences were detected by ANOVA at a probability level of 5%.

Results

Temperature, dissolved oxygen and salinity levels were 28.2ºC (± 0.01), 6.71 mg/L (± 0.04) and 38.7 (±0.04), respectively. Total ammonia-nitrogen was 0.28 mg/L (± 0.02), while no nitrite was detected. Water quality variables throughout this study were therefore considered within ranges suitable for cobia development.

One replicate of the treatment with three daily meals was lost due to the lack of water flow overnight. Parameters of growth performance are summarized in Table 1, while the variation of the mean weight of cobia juveniles during 60 days is depicted in Figure 1. Survival ranged from 93.3 to 100% and no significant differences between treatments were observed (P = 0.42). Initial and final weights, WG and SGR were not significantly different between treatments (P = 0.19, 0.09, 0.08 and 0.08, respectively).

The condition factor (K) ranged from 6.9 to 7.4 at the beginning of the trial, but it increased significantly to 8.0-8.4 after 60 days (P = 0.02). Again, however, no differences were observed between treatments (P = 0.14 and 0.48, respectively). FCR and apparent feed intake were also not significantly different between treatments (P = 0.35 and 0.56, respectively).

Discussion

The results in present study are contrary to the hypothesis that cobia juveniles would grow faster if they were fed a commercial diet more frequently. We found that cobia from all treatments grew at the same rate. None of the parameters associated with growth performance (survival, final weight, WG, SGR, FCR and K) showed any significant differences among treatments. There is only one study available on the effect of feeding frequency on cobia juveniles. Rombenso et al. (2009) reported similar survival and growth when cobia with an initial size of 3 g were reared in 1 m^3 cages and fed 3, 6 or 9 daily meals. However, they did not investigate the effects of feeding cobia less frequently than three times per day so the effect of feeding cobia juveniles once or twice daily under field conditions remains to be examined. In a study with larval cobia, Nhu (2009) found no significant differences in growth when a weaning diet was offered continuously (from 0600 h till 1800 h) or divided into 4 or 7 daily meals for 15 days. This author found, however, that mortality due to cannibalism was lower in the continuous feeding regime, but there were no differences between 4 or 7 meals per day. Cannibalism is promoted by differences in fish size and among other factors it may be affected by feed availability (Goldan et al., 1997; Wang et al., 1998). In other finfish species, increasing feeding frequency has been demonstrated to control size variation and thus reduce mortality due to cannibalism as well as the stress and labor costs related to grading (Dou et al., 2000; Goldan et al., 1997; Wang et al., 1998). In this study, however, size variation was not significantly affected by feeding frequency and no significant differences in survival were observed.

The condition factor (K) increased from the beginning to the end of this study. The significant increase in K indicates that fish probably received enough food during the experimental period (Thomassen and Fjaera, 1996). Chuang et al. (2010) found that

the condition factor of 6-7 kg cultured cobia ranged from 12.3 to 13.3, and was significantly higher than those of wild cobia (K = 9.6) from Taiwanese waters. Lipid levels were also higher in the flesh of cultured cobia. In this regard, Benetti et al. (2010) reported that cultured cobia usually present excessive intra-peritoneal fat and abnormally large livers, and their bodies are shorter and fatter than wild-caught fish. These morphological patterns may be related to an increased feed intake and lower swimming and feed activities of cultured fish in comparison to their wild counterparts (Christiansen and Jobling, 1990; Boisclair and Tang, 1993; Chuang et al., 2010). Our results, however, indicate that feeding frequency had no significant effect on the condition factor of cobia juveniles.

The effects of feeding frequency on fish growth are also related to the size of the stomach, since species with smaller stomachs require more frequent feeding to achieve maximum growth (Pillay and Kutty, 2005). In nature, cobias are known to be voracious feeders, often ingesting whole preys (Shaffer and Nakamura, 1989). Carnivorous fish such as cobia are morphologically capable of ingesting large preys as they distend their stomachs to increase storage capacity. This allows them to be satiated after a single, large meal. On the other hand, omnivorous and herbivorous fish have comparatively smaller stomachs, but longer intestines. It is therefore commonplace that higher weight gains are observed when several daily meals are offered to omnivorous fish, as has been observed for tilapia (Tung and Shiau et al., 1991; Sanches and Hayashi, 2001; Riche et al., 2004).

The present results suggest that there is no benefit in feeding cobia larger than 110 g more than once daily. However, in practical farming operations, cobias may be fed more than once per day. During weaning, cobias are fed manually to satiation 5 to 6 times daily (Liao et al., 2004) or as many as 10 times a day (Nguyen et al., 2011). In

grow-out within sea cages, cobia may be fed once a day and 6 days a week (Liao et al., 2004) or twice a day (Benetti et al., 2010), while in recirculation aquaculture systems dividing feeding in several daily sessions is preferred as a way to avoid peaks of oxygen demand and ammonia excretion by fish. The discrepancy between the present results and the current practices in some cobia farms may be explained by differences in fish size and management practices. In commercial cobia farming operations, a large number of fish are maintained within a single rearing structure and it is quite common that the behavior of cobia during the feeding period leads to aggressive interactions. Under these conditions, it is also difficult to ensure that all the fish are fed to satiation. It is therefore common to use a fixed ration, and offer two or more meals per day. This would provide a better opportunity for smaller, less aggressive fish to obtain food (Schnaittacher et al., 2005) and consequently fish of more uniform sizes are produced (Wang et al., 1998). Unfortunately, little is known about size hierarchy and social dominance among cobia under practical farming conditions. Work with the gilthead sea bream (*Sparus auratus*) has shown that a linear dominance hierarchy is established in groups of less than 10 fish (Goldan et al., 2003; Montero et al., 2009), and that aggressive interactions occur during feeding (Karplus et al., 2000; Goldan et al., 2003). Montero et al. (2009) found that this type of aggressive interaction is more pronounced when the number of individuals in the group is small, with a linear hierarchy more easily established in groups of five animals compared to groups of 10 animals. *S. aurata*, however, is a schooling fish, which contrasts to cobia, a species that is usually solitary or found in groups of 2-8 individuals (Shaffer and Nakamura, 1989).

Another possible explanation for the lack of significance in cobia growth when an increased number of daily meals is offered may be due to the food passing too rapidly through the digestive tract. With a brief residence time, there may not be enough time to

efficiently digest and absorb the feed. This would decrease the effectiveness of the digestion and assimilation processes (Liu and Liao, 1999). Furthermore, repeated feeding throughout long periods of the day may increase swimming activity of the fish and hence lead to higher energy expenditure and negatively affect growth rates (Johansen and Jobling, 1998). Earlier work with other species of finfish indicates a relationship between gastro-intestinal evacuation rate and the establishment of optimal feeding frequency regimen (Elliot, 1975; Gwither and Grove, 1981; Grove et al., 1985). Unfortunately, no work has as yet defined this aspect in cobia.

The present results suggest that there is no benefit in feeding cobia juveniles larger than 110 g more frequently than once daily. Therefore, it may be possible to reduce feeding frequency in cobia farms without adversely affecting survival, growth rate and size variation, thereby improving profitability through decreased labor costs as well as facilitating offshore grow-out operations. This possibility, however, warrants further testing under practical field conditions.

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Table 1. Mean (± SE) performance parameters of cobia (*Rachycentron canadum*) juveniles fed a commercial diet under different feeding frequencies for 60 days.

	Number of daily meals					*P* value
	One	Two	Three	Four	Six	
Survival (%)	97.5 (± 2.5)	100.0 (± 0.0)	93.3 (± 3.3)	95.0 (± 2.9)	97.5 (± 2.5)	0.42
Initial weight (g)	112.48 (± 1.95)	110.96 (± 0.72)	109.13 (± 2.98)	108.43 (± 1.81)	107.08 (± 0.66)	0.19
Final weight (g)	273.97 (± 10.11)	303.10 (± 5.85)	292.25 (± 8.89)	277.62 (± 10.45)	271.40 (± 6.35)	0.09
Weight gain (%)	143.51 (± 7.36)	173.16 (± 4.86)	168.18 (± 10.78)	156.04 (± 8.59)	153.46 (± 5.56)	0.08
SGR[b] (%/day)	1.48 (± 0.05)	1.67 (± 0.03)	1.64 (± 0.07)	1.56 (± 0.06)	1.55 (± 0.04)	0.08
Initial K	7.4 [A] (± 0.1)	7.1 [A] (± 0.2)	7.2 [A] (± 0.1)	7.2 [A] (± 0.1)	6.9 [A] (± 0.1)	0.14
Final K	8.2 [B] (± 0.2)	8.4 [B] (± 0.1)	8.0 [B] (± 0.3)	8.2 [B] (± 0.1)	8.1 [B] (± 0.0)	0.48
FCR[c] (g fed / g gained)	1.77 (± 0.01)	1.55 (± 0.05)	1.63 (± 0.09)	1.75 (± 0.12)	1.79 (± 0.11)	0.35
Feed intake (% body weight/day)	2.69 (± 0.07)	2.69 (± 0.03)	2.77 (± 0.04)	2.82 (± 0.13)	2.87 (± 0.10)	0.56
Initial CV (%)	12.0 (± 1.6)	7.2 (± 0.9)	7.3 (± 0.4)	9.1 (± 2.3)	13.0 (± 2.6)	0.14
Final CV (%)	15.2 (± 2.5)	13.6 (± 1.4)	15.5 (± 3.0)	15.4 (± 3.4)	21.3 (± 3.8)	0.42

[a]Capital superscript letters indicate significant differences within columns over time ($P<0.05$), comparison between Initial K and Final K.

[b]SGR: Specific growth rate; [c]FCR: feed conversion ratio.

CAPÍTULO 2

Composition and digestibility for cobia (*Rachycentron canadum*) of a protein hydrolysate produced by autolysis of shrimp processing discards

Composition and digestibility for cobia (*Rachycentron canadum*) of a protein hydrolysate produced by autolysis of shrimp processing discards

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Key words*:* shrimp by-product, hydrolysate, amino acids, lipids.

Abstract

The conversion of wastes from fisheries processing industries into valuable products may generate additional revenue for the industry at the same time that disposal costs and environmental problems are reduced. In this study, shrimp discards were ground

and submitted to enzymatic digestion. After 2 h, the enzymes were inactivated and the solid tissues and the crude hydrolysate (SPH) were separated by centrifugation. A supernatant (centrifuged shrimp protein hydrolysate, CSPH) and a precipitate (carotenoprotein) were produced. The chemical composition of SPH, CSPH, carotenoprotein and of a commercially available fish meal were determined and compared to assess their feasibility as ingredients in aquaculture feeds. The *in vivo* digestibility of CSPH for cobia in protein was 0.91. SPH, CSPH and carotenoprotein presented high protein contents and their amino acid profiles were comparable to fish meal. In addition, protein and lipids from CSPH were highly digestible by cobia. These products may therefore serve as ingredients in aquaculture feeds.

Introduction

One of the various environmental problems that take place in coastal areas is the disposal of large volume of wastes generated by fisheries processing industries. These discards may be dumped into the sea or other environmentally sensitive areas without any previous treatment or depuration (Knorr 1991; Vazquez & Murado 2008). Processing techniques for seafood discards are therefore needed to convert underutilized, nutrient-rich organic wastes into valuable and thus marketable products. The conversion of these discards may generate an opportunity of additional revenue for the industry at the same time that disposal costs and environmental problems are considerably reduced (Arvanitoyannis & Kassaveti 2008).

The most widely applied technique to process seafood processing wastes is fish meal production. World production of fish meal in recent decades has fluctuated around 6 million tons, and this is considered insufficient to cover the increasing demand of the

aquaculture industry (Tacon & Metian 2008). Knorr (1991) estimated annual world production of crustacean wastes at 1.2×10^6 tons. However, world production of both captured and farmed shrimp have recently reached six million tons (FAO 2012). Since the discards of shrimp processing plants are constituted mainly of heads, which accounts for approximately 34-45% of the whole shrimp weight (Barrat & Montano 1986), it is reasonable to estimate that at least 2.4 million tons of shrimp processing discards would be annually available worldwide for conversion into marketable end-products.

Shrimp processing wastes are known to have a good nutritional profile. Crude protein content may range from 40% (Halver & Hardy 2002) to 65% (Fanimo *et al.* 2000; Heu *et al.* 2003), while lipid levels vary between 6.2 and 12.8% (Hertrampf & Piedad-Pascual 2000). On the other hand, shrimp wastes contain a relatively high content of chitin (Subasinghe 1999; Synowiecki & Al- Khateeb 2000), a major component of crustaceans that is not digested by some fish species (Shiau & Yu 1999). One way to overcome this shortcoming is the use of enzyme-regulated hydrolysis. This process avoids the extremes of chemical and physical treatments usually applied in fish/shrimp meal production and thus minimizes undesirable reactions that could destroy valuable components (Dumay *et al.* 2004). The production of fish protein hydrolysates has therefore drawn large interest in the last few years (Berge & Storebakken 1996; Bezerra, 2000; Gildberg & Stenberg, 2001; Murray *et al.* 2003; Nilsang *et al.* 2005; Liang *et al.* 2006) as it is considered to be more predictable, reproducible and gentler in separating soluble nitrogen compounds than other processing techniques such a fish silage.

Shrimp and fish hydrolysates have been reported as potential fish meal replacement in aquaculture feeds (Fagbenro & Jauncey 1993; Plascencia-Jatomea *et al.* 2002;

Hevrøy *et al.* 2005). Shrimp hydrolysates have been used in aquaculture feeds ensuring a high feed intake, and a subsequent increase in fish growth due to their attractive effect and nutritional composition (Berge & Storebakken 1996). In addition to their nutritional quality, hydrolysates also contain compounds that stimulate the nonspecific immune response in cultured fish (Bogwald *et al.* 1996; Gildberg *et al.* 1995; Murray *et al.* 2003; Liang *et al.* 2006). Low molecular weight peptide fractions from hydrolysates may act as immunomodulators, enhancing the activity of fish macrophages through increased oxidative burst and morphological cell reactions (Bogwald *et al.* 1996; Gildberg *et al.* 1995). Hence, supplementing aquaculture feeds with shrimp hydrolysates may improve the growth performance of cultured fish as well as increase their resistance to diseases.

In assessing ingredients for use in aquaculture feeds, Glencross *et al.* (2007) stress the need to initially characterize their chemical composition as well as evaluate their digestibility. In this study, the content of major components (dry matter, crude protein, total lipids and ash), amino acids and fatty acids of three fractions obtained by enzymatic autolysis of cultured shrimp (*Litopenaeus vannamei*) discards (shrimp protein hydrolysate – SPH, centrifuged shrimp protein hydrolysate – CSPH and carotenoprotein) were determined and compared to a commercially available fish meal. Moreover, apparent digestibility coefficients for dry matter, crude protein and total lipids of diets containing SPH were determined. Hatchery-reared cobia (*Rachycentron canadum*) juveniles were used as test animals.

Materials and Methods

Production of the hydrolysate

Processing discards (mainly heads) of the Pacific white shrimp (*L. vannamei*), the most widely cultivated shrimp species in the world, were obtained from a processing plant (Netuno Alimentos S.A.) in Recife, Brazil. Shrimp discards were packed in plastic bags and stored at -18ºC until use.

The enzymatic autolysis process used to obtain the SPH, CSPH and carotenoprotein was adapted from Bezerra (2000). Shrimp discards were thawed at room temperature, ground and each kg was mixed to one litre of distilled water ($w.v^{-1}$). The blend was then submitted to digestion in a water bath (40 ± 2ºC, 2 h) under light agitation. After that, the enzymes were inactivated by heating (100ºC, 10 min) and the solid tissues and the crude hydrolysate (SPH) were separated by centrifugation (10,000 x g) for 10 min. This process produced a supernatant (centrifuged shrimp protein hydrolysate, hence named CSPH) and a precipitate (carotenoprotein or sediment with astaxanthin). All fractions were stored at -18 ºC until use. Each procedure was made in triplicate (n = 3).

Chemical analysis

The chemical composition (moisture, crude protein, total lipid, ash content, amino acids and fatty acids) of SPH, CSPH, carotenoprotein and a commercially available fish meal was analytically determined. Fish meal produced from Peruvian anchovy (*Engraulis ringens*) was purchased from Corporación Pesquera Inca (COPEINCA), Lima, Peru.

Moisture, crude protein (Kjeldahl procedures) and ash contents were determined following AOAC (1990), while total lipids were determined according to Bligh-Dyer (1959). All analyses were made in triplicate.

Fatty acid composition was analytically verified by gas chromatography (Varian CP 3800) equipped with a flame ionization detector (GC-FID), and a WAX (25 m x 0.25

mm x 0.2 µm) column, with a flow rate of 1.3 mL min^{-1} of Helium. The GC conditions were as follows: injector and detector temperature 280ºC (initial oven temperature, 150ºC increased to 230ºC), with three ramps and a total program run was 90 min. Fatty acids were identified by comparison with the retention time (RT) of the peaks of the samples with standard RT (Sigma, St Louis, MO, USA). Quantification was performed by standardization of peak areas.

The composition of amino acids was determined after acid hydrolysis (6N HCl for 24 h at 100ºC) preparation of the hydrolysate for chromatography, i.e. drying, re-drying and derivatisation. The derivatives were separated by high performance liquid chromatography (HPLC) by a commercial laboratory using an automatic amino acid analyzer equipped with a column (White *et al.* 1986). The content of amino acids was presented as mg g^{-1} of protein. The indispensable amino acid index (IAAI) was estimated according to Hardy & Barrows (2002):

$$IAAI = (Arg\text{-}TI/Arg\text{-}Egg) + (His\text{-}TI/His\text{-}Egg) + \cdots + (Val\text{-}TI/Val\text{-}Egg) \times 100$$

where "Arg-TI" is the content of arginine in the test ingredient (TI), and "Arg-Egg" is the content of arginine in hen's whole-egg protein. The amino acid profile of whole-egg protein was obtained from NRC (1993).

The molecular weight (MW) of CSPH was determined with polyacrylamide gel electrophoresis (SDS-PAGE) using a 4% ($w.v^{-1}$) stacking gel and a 12.5% ($w.v^{-1}$) separating gel (Laemmli, 1970). The gels were stained for protein overnight in 0.01% ($w.v^{-1}$) Coomassie Brilliant Blue. The background of the gel was destained by washing in 10% ($v.v^{-1}$) acetic acid. MW of the CSPH band was estimated using standard (BIORAD SDS-PAGE) myosin (198.84 kDa), β-galactosidase (115.7 kDa), bovine

serum albumin (96.74 kDa), ovalbumin (53.54 kDa), carbonic anhydrase (37.13 kDa), soybean trypsin inhibitor (29.13 kDa), lysozyme (19.54 kDa) and aprotinin (6.91 kDa).

Apparent digestibility

Apparent digestibility coefficients (ADC) were measured using an indirect method. Two experimental diets were prepared (Table 1). The reference diet was formulated to fulfil the known nutritional requirements of cobia (*Rachycentron canadum*) (Fraser & Davies, 2009; NRC, 2011), while the test diet contained 70% of the reference diet and 30% SPH. Chromic oxide (Cr_2O_3) was included in the test diet as inert marker at a concentration of 0.5%. ADC of crude protein, total lipid and dry matter were estimated from the average of three replicates of each diet according to Austreng (1978): ADC (%) = 100- [100 x (% indicator in diets x % nutrient in faeces) x (% nutrient in diets x % indicator in faeces)$^{-1}$].

Juveniles cobia obtained from a private hatchery (Aqualider Maricultura, Pernambuco, Brazil) were acclimated to the experimental conditions for 7 days and hand-fed a commercial diet (Socil Evialis, Pernambuco, Brazil). Fish were pooled and those of similar size were visually selected and weighed. Six groups of 15 fish (38.6 g ± 1.3) were randomly restocked into 300 L cylindro-conical fibreglass tanks supplied with a continuous flow of sand-filtered seawater and continuous aeration. Fish were then fed the reference diet containing the indicator for seven days (Sakomura & Rostagno 2007). After that, fish were fed the reference and test diets to visual satiety twice daily (0830 h and 1630 h). Thirty minutes after each meal, tanks and faecal columns were cleaned with a brush to remove residual feed and faeces in the system. Collection of faeces started the third day after feeding the experimental diets and was carried out twice daily using settlement method (Zhou *et al.* 2004). Plastic bottles (250 mL) were immersed

into ice to minimize bacterial degradation. The content of the bottles was immediately filtered with Whatman #1 filter papers and stored at -18ºC. Samples of faeces from each tank and diets were pooled and dried in an air-convection drier at 55ºC and stored at -18ºC for subsequent analyses. Moisture, crude protein, total lipids and ash contents were determined. Chromic oxide content of diets and faeces were determined by flame atomic absorption spectrophotometry (Spectro Analytical instruments Inc., Pennsylvania, USA).

Water temperature, salinity, pH and dissolved oxygen were monitored daily in each tank with a multi-parameter (Hanna HI9828, Hanna Instruments, Rhode Island, USA), while concentrations of total ammonia and nitrite were determined every three days with commercial test kits (Labcon, Alcon, Brazil). Water quality variables remained stable: mean (± SD) temperature, salinity, pH and dissolved oxygen (DO) levels were 29.3ºC (± 0.1), 35.8 (± 0.2), 7.84 (± 0.01) and 6.08 mg L^{-1} (± 0.16), respectively. Total ammonia levels remained below 0.01 mg L^{-1}. No nitrite was detected.

All data were submitted to Student's *t*-test. A 5% level of probability was chosen in advance to sufficiently demonstrate a statistically significant difference. Results are presented as means ± standard deviation (SD).

Results

The initial step of the enzymatic process used in the present work resulted in the production of the SPH, which, after centrifugation, generated a soluble fraction (CSPH) and an insoluble one (Carotenoprotein). For each kilogram of shrimp discards, the mean amount of SPH produced was 1,426.7 mL (± 142.9), or 1,454.2 g (± 101.6).

Centrifugation of the SPH produced an average (n = 3) of 79.4% CSPH and 20.6% carotenoprotein.

The proximate composition of SPH, CSPH, carotenoprotein and the Peruvian fish meal are presented in Table 2. The fish meal contained more than 760.0 g kg^{-1} crude protein, 116.5 g kg^{-1} total lipids and the ash content was around 120.0 g kg^{-1}. SPH had a proximate composition comparable to that fish meal. The content of crude protein in SPH (797.8 g kg^{-1}) was higher than in the fish meal, while lipid levels of SPH and fish meal were quite similar (116.2 and 116.5 g kg^{-1}, respectively). The lipid content in CSPH was 54.3 g kg^{-1}, however, was much lower in comparison to SPH and fish meal. Carotenoprotein had the highest lipid content 507.6 g kg^{-1} (Table 2). Ash content in SPH, CSPH and carotenoprotein ranged from 31.7 to 86.0 g kg^{-1}. The hydrolysis profile of CSPH may be visualized in Figure 1. CSPH had a molecular weigh lower than 29 kDa.

The amino acid profiles of SPH, CSPH, carotenoprotein and the fish meal are also presented in Table 2. The essential amino acids (EAA) with the highest contents in SPH and CSPH were arginine and lysine, while leucine and phenylalanine were the most abundant in the carotenoprotein fraction. Lysine levels in SPH, CSPH, carotenoprotein and fish meal were 71.3, 75.6, 61.3 and 73.6 mg g^{-1} of protein, respectively. Levels of methionine in SPH, CSPH and carotenoprotein from this study (27.6, 29.5 and 2.9 mg g^{-1} of protein, respectively) were lower than in the fish meal (39.7 mg g^{-1} of protein). Tryptophan was not detected in CSPH and relatively low levels were found in SPH. Content of tryptophan in the carotenoprotein was higher (8.8 mg g^{-1} of protein) than in the fish meal. Among all fractions analyzed in the present work, the most abundant non-EAA was glutamic acid. Comparatively higher levels of taurine were found in SPH, CSPH and carotenoprotein than in fish meal (respectively 16.2, 18.5 and 9.7 mg g^{-1} of

protein versus only 0.7 mg g^{-1} of protein in fish meal. Estimates of IAAI for SPH, CSPH and carotenoprotein were 921.0, 926.0 and 913.0 g kg^{-1}, respectively (Table 2). These values are considerably higher than the IAAI estimated for the fish meal (768.0 g kg^{-1}).

Levels of fatty acids in the different fractions are summarized in Table 3. Comparatively higher levels of n-3 highly unsaturated fatty acids (HUFA) are observed in the carotenoprotein and the fish meal. Levels of eicosapentaenoic acid (20:5*n*-3) in the caroprotein and fish meal were quite similar (85.3 vs. 85.1 g kg^{-1}), while docosahexaenoic acid (22:6*n*-3) varied from 96.5 g kg^{-1} in the carotenoprotein to 144.6 g kg^{-1} in the fish meal.

Mean ADC for crude protein, total lipids and dry matter of the test diet, containing CSPH were significantly higher than in the reference diet with fish meal (Table 4).

Discussion

The development of cost-effective processes to convert seafood processing discards into marketable products may not only create an alternative source of income for the industry, but also reduce possible environmental problems (Ruttanapornvareesakul *et al.* 2006; Arvanitoyannis & Kassaveti 2008; Vazquez & Murado 2008). The enzymatic process used here was based on the autolysis of shrimp processing wastes (Bezerra 2000). Since this procedure makes no use of commercial enzymes, final costs may be significantly reduced. This agrees with Cao *et al.* (2009) who stated that commercial enzymes are a major operational expense when enzymatic hydrolysis methods are applied.

The high quality of the Peruvian fish meal used in this study was confirmed analytically. According to Cho *et al.* (1985), high quality fish meals employed in salmon diets should contain at least 680.0 g kg^{-1} crude protein and the ash content should be less than 130.0 g kg^{-1}. These quality standards were thus fully met by the fish meal used here. SPH had a proximate composition comparable to that fish meal, although SPH present high content of moisture, different of fish meal. However, the lipid content in CSPH was much lower in comparison to SPH and fish meal. On the other hand, carotenoprotein presented high content of lipids. Similar results were found by Nilsang *et al.* (2005) in a fish hydrolysate produced with commercial enzymes. These authors explained that the lower lipid content in CSPH is a result of centrifugation that separates not only the insoluble protein fraction, but also lipids. Likewise, Gildberg & Stenberg (2001) found that about 600.0 g kg^{-1} of the extractable lipids were recovered in the sediment fraction (carotenoprotein) after centrifugation of a shrimp crude protein hydrolysate.

Relatively high levels of fatty acids were found in the different fractions obtained here. This is particularly true for the carotenoprotein fraction, which, as mentioned earlier, concentrated lipids after centrifugation. The dietary essentiality of *n*-3 HUFA for marine fish and shrimp has long been recognized (Sargent *et al.* 2002). It is therefore vital to provide sufficient amounts of these fatty acids if the aquaculture of marine fish and shrimp is to succeed. In the present study, carotenoprotein had levels of *n-3* HUFA, especially 20:5*n*-3, was similar to the fish meal, while SPH had levels of 22:6*n*-3 as high as the fish meal. Therefore, SPH and carotenoprotein may be considered good sources of these nutrients. No *n*-3 HUFA was detected in CSPH and thus it may not be considered as a source of these nutrients in aquaculture feeds.

One limiting factor in using shrimp processing discards as an ingredient in aquaculture feeds is their high ash content. Fagbenro & Bello-Olusoji (1997) found that raw minced shrimp heads may contain as much as 255.0 g kg^{-1} ash, while waste of the shrimp *Pandalus borealis* contained 260.0 g kg^{-1} ash (Gildberg & Stenberg 2001). The content of ash in SPH, CSPH and carotenoprotein from this study were therefore much lower than the content usually found in raw shrimp processing discards. The procedure applied here, specifically the separation of solid tissues after hydrolysis, reduced the amount of ash to levels lower than those usually found in commercially available fish meals. The major component of ash in shrimp tissues is calcium carbonate, to which chitin is usually encrusted. Chitin is abundant in shrimp tissues as it is the main component of the cell walls of crustaceans. Although some fish species have the capability to digest chitin through endogenous chitinolytic enzymes (Gutowska *et al.* 2004), high chitin levels may limit the use of shrimp discards and their by-products due to low digestibility (Fagbenro & Bello-Olusoji 1997; Shiau & Yu 1999; Plascencia-Jatomea *et al.* 2002).

The enzymatic hydrolysis clearly promoted protein digestion as a MW lower than 29 kDa was estimated for CSPH. In a similar study, Hevrøy *et al.* (2005) found that most polypeptides in a fish protein hydrolysate produced after hydrolyzing whole minced herring were smaller than 10 kDa. This indicates the predominance of short chain polypeptides and free amino acids in the composition of these hydrolysates. A high content of amino acids and short chain peptides was also observed by Santos (2008), when the variation of MW of a hydrolysate produced from shrimp processing discards was analyzed over time. After 30 min, the hydrolysate had a MW varying between 90 and 20 kDa, while after 120 minutes MW was lower than 20 kDa. The content of free amino acids and short chain peptides in hydrolysates usually indicates a

high nutritional value for food or as nitrogen source (Synowiecki & Al-Khateeb 2000; Gildberg & Stenberg 2001; Ruttanapornvareesakul *et al.* 2006).

The essential amino acids (EAA) with the highest content in SPH and CSPH were arginine and lysine, while leucine and phenylalanine were the most abundant in the carotenoprotein fraction. On the other hand, tryptophan was not detected in CSPH, and, since relatively low levels were found in SPH, it might be considered a limiting EAA for fish. Gildberg & Stenberg (2001) and Bueno-Solano *et al.* (2009) cited that the most abundant EAA were lysine and leucine and that no tryptophan was detected. These authors reported histidine as a possible limiting EAA in carotenoprotein, and methionine in the liquid hydrolysate. In carotenoprotein produced by lactic acid fermented and non-fermented shrimp waste, Armenta & Guerrero-Legarreta (2009) found high levels of leucine and lysine as well as aspartic and glutamic acids. Among all fractions analyzed in the present work, the most abundant non-EAA was glutamic acid, which concurs with the findings of Gildberg & Stenberg (2001) and Cao *et al.* (2009). Simpson *et al.* (1997) used trypsin and chymotrypsin hydrolysis and produced shrimp hydrolysates with high levels of alanine, proline, glycine, and arginine, which are considered important components in determining flavour in crustaceans. A protein hydrolysate derived from the lactic fermentation of shrimp by-products (Bueno-Solano *et al.* 2009) and a shrimp hydrolysate produced by autolysis (Cao *et al.*, 2009) resulted in products with high nutritional content. Similar results were related by Plascencia-Jatomea *et al.* (2002) that produced shrimp protein hydrolysate by lactic fermentation and found high amounts of arginine, lysine, leucine and threonine; again, tryptophan was not detected. The lactic fermentation also made possible the separation of chitin.

According to Hardy & Barrows (2002), an accurate method to predict the nutritional value of prospective feed ingredients in terms of amino acid composition is the IAAI,

which is the ratio of the indispensable amino acids in the test ingredient divided by the indispensable amino acids in whole-egg protein, a reference pattern of amino acids (NRC 2011). Estimates of IAAI for SPH, CSPH and carotenoprotein were well above 900.0 g kg^{-1} and hence considerably higher than for the Peruvian fish meal (768.0 g kg^{-1}). Based on the EAA profile of ingredients commonly used as protein sources in aquaculture feeds reported by Hertrampf & Piedad-Pascual (2000), the estimated IAAI for anchovy fish meal, hydrolyzed fish protein concentrate, shrimp head meal, shrimp head silage and soybean meal would be 886.0, 806.0, 635.0, 743.0 and 782.0 g kg^{-1}, respectively. Comparison of these IAAI to those of SPH, CSPH and carotenoprotein indicates that the three fractions obtained by autolysis of cultured *L. vannamei* discards also have relatively higher IAAI. This indicates the high content of essential amino acids in SPH, CSPH and carotenoprotein, and confirms the value of these ingredients as potential source of amino acids in aquaculture feeds.

All fish species require the same ten EAA for normal growth and metabolic functions (NRC 2011). Methionine and lysine are usually the first limiting EAA in ingredients used in the formulation of fish diets. Supplementation of methionine and lysine may therefore be needed so that fish growth will not be compromised (Halver & Hardy 2002; NRC 2011). Levels of methionine in SPH, CSPH and carotenoprotein from this study were lower than in the fish meal. Therefore, SPH, CSPH and carotenoprotein may not be considered important sources of methionine. On the other hand, lysine levels in SPH and CSPH were quite similar to the fish meal (71.3, 75.6 and 73.6 mg g^{-1} of protein, respectively). As the nutritional requirements for lysine in commonly reared fish species range from 40 to 86 mg g^{-1} of dietary protein (NRC 2011), SPH and CSPH hold potential as sources of lysine in aquaculture feeds.

Among the non-essential amino acids, taurine has been shown to have a positive effect on growth and feed efficiency of several fish species (Park *et al.* 2002; Gaylord *et al.* 2007; Lunger *et al.* 2007). The supplementation of taurine has been found to be particularly important when plant-based diets are offered to carnivorous fish, as their growth is often reduced when fish are fed meal-based diets (Gaylord *et al.* 2007; Lunger *et al*. 2007). Given the comparatively higher levels of taurine in SPH, CSPH and carotenoprotein than in fish meal, all three fractions produced here may serve as potential sources of taurine, but, in view of the higher levels, SPH and CSPH are the most likely candidates.

Apparent digestibility coefficients of crude protein and total lipid of the test diet exceeded 0.85 and were higher than in the reference diet, and also higher than the ADC values of several ingredients previously reported for cobia (Zhou *et al.* 2004). This indicates that protein and lipid from CSPH were well digested by cobia. An increase in the ADC of protein with increased dietary inclusion of fish protein hydrolysate was also observed in Atlantic salmon by Hevrøy *et al.* (2005). Likewise, rainbow trout had higher digestibility of protein from fish silage protein in comparison to protein in fish meal (Stone *et al.* 1989). The higher digestibility of CSPH in comparison to the fish meal based diet is probably due to the high content of short peptides and free amino acids. Protein hydrolysis increases its solubility and the supply of shorter peptides and free amino acids results in higher digestibility (Berge & Storebakken 1996).

Conclusion

The autolysis of shrimp processing discards was effective in obtaining high quality nutritional products that may be used as ingredients in aquaculture feeds. The three different fractions produced from shrimp processing discards (SPH, CSPH and

carotenoprotein) contained high protein contents and amino acid profiles that were comparable to a high-quality fish meal. CSPH may also be a suitable source of n-3 HUFA. In addition, protein and lipids from CSPH were highly digestible by cobia juveniles.

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Table 1 - Formulation and proximate composition (mean ± SD) of the experimental diets used to assess the apparent digestibility coefficient for cobia (*Rachycentron canadum*) of the centrifuged shrimp protein hydrolysate (CSPH).

Ingredients (*g kg*$^{-1}$)	Reference diet	Test diet
Fish meal [a]	607.0	220.0
CSPH	0.0	300.0
Wheat flour	206.0	261.8
Fish oil [a]	65.0	90.0
Vitamin and mineral premix [b]	20.0	20.0
Ascorbic acid monophosphate	3.0	3.0
Cellulose	93.8	100.0
Cr_2O_3	5.0	5.0
BHT[c]	0.2	0.2
Proximate composition		
Crude protein	39.6 ± 0.2	36.5 ± 1.0
Total lipids	14.1 ± 0.2	14.0 ± 0.1
Ash	10.6 ± 0.1	8.6 ± 0.1
Dry matter	96.4 ± 0.1	93.7 ± 0.1

[a] Pesquera Pacific Star S.A., Chile.
[b] Contains, as ingredient per kg (DSM Produtos Nutrionais, Jaguaré, Brazil): Vitamin A 1,000,000 UI; Vitamin D3 312,500 UI; Vitamin E 18750 UI; Vitamin K3 1,250 mg; Vitamin B12,500 mg; Vitamin B6 1875 mg; Vitamin B12 3,75 mg; Nicotinic acid 12,500 mg; Pantothenic acid 6,250 mg; Biotin 125 mg; Folic acid 750 mg; Vitamin C (Ascorbyl monophosphate) 31,250 mg; Cholin 50,000mg; Inositol 12,500 mg; Cu 625 mg; Zn 6,250 mg; Mn 1,875 mg; Se 12,5 mg; I 62,5 mg; Co 12,5 mg; BHA 1,520 mg; Ethoxyquin 1,605 mg; Fe 6,250 mg.
[c] Butylated hydroxytoluene.

Table 2 - Mean proximate composition (g kg^{-1} DW), amino acid profile (mg g^{-1} of protein) and indispensable amino acid index (IAAI - estimated in relation to the profile of whole egg protein - NRC, 1983, g kg^{-1}) for shrimp protein hydrolysate (SPH), centrifuged shrimp protein hydrolysate (CSPH) and carotenoprotein obtained by autolysis of shrimp (*Litopenaeus vannamei)* processing discards, and the Peruvian fish meal.

	SPH	CSPH	Carotenoprotein	Fish meal
Proximate composition				
Dry matter	79.8	74.9	236.6	891.9
Crude protein	797.8	894.2	460.9	762.8
Lipid	116.2	54.3	507.6	116.5
Ash	86.0	51.6	31.7	120.6
Essential amino acids				
Arginine	76.2	79.3	60.4	67.8
Histidine	24.3	24.0	29.2	29.7
Isoleucine	43.8	44.3	44.8	40.1
Leucine	68.1	70.1	70.1	69.2
Lysine	71.3	75.6	61.3	73.6
Methionine	27.6	29.5	29.0	39.7
Phenylalanine	48.6	44.3	65.2	39.8
Threonine	38.9	38.7	38.9	43.0
Tryptophan	4.9	0.0	8.8	5.8
Valine	56.7	57.2	55.5	46.2
Non-essential amino acids				
Aspartic acid	98.9	97.8	106.1	90.3
Glutamic acid	147.5	155.0	124.6	134.0
Serine	42.1	40.6	43.8	42.6
Glycine	74.6	77.5	60.4	82.5
Alanine	61.6	62.7	49.7	64.5
Proline	63.2	68.3	48.7	48.6
Tyrosine	53.5	22.1	125.6	29.6
Cystine	16.2	18.5	10.7	15.9
Taurine	16.2	18.5	9.7	0.7
IAAI	921.0	926.0	913.0	768.0

Table 3 - Mean (n=2) concentration of fatty acids (g kg^{-1} wet basis) of shrimp protein hydrolysate (SPH), centrifuged shrimp protein hydrolysate (CSPH) and carotenoprotein obtained by autolysis of shrimp (*Litopenaeus vannamei)* processing wastes, and a commercially available Peruvian fish meal.

	SPH	CSPH	Carotenoprotein	Fish meal
14:0	n.d.	n.d.	6.9	41.5
15:0	n.d.	n.d.	20.4	n.d.
15:1	n.d.	n.d.	7.4	n.d.
16:0	235.3	287.3	195.1	201.8
16:1*n*-7	n.d.	n.d.	24.1	57.1
17:0	n.d.	n.d.	19.0	6.3
17:1*n*-5	n.d.	n.d.	12.1	n.d.
18:0	77.8	200.6	76.5	57.9
18:1*n*-9 cis	219.0	347.2	200.2	205.6
18:1*n*-9 trans	44.7	n.d.	35.3	35.7
18:2*n*-6 cis	194.3	164.9	100.1	89.9
18:3*n*-6	n.d.	n.d.	3.9	n.d.
18:3*n*-3	18.9	n.d.	9.8	12.4
20:0	n.d.	n.d.	26.0	n.d.
20:1*n*-9	n.d.	n.d.	15.0	26.9
20:2*n*-6	23.6	n.d.	26.3	5.0
20:4*n*-6	56.6	n.d.	30.2 ± 0.36	10.2
20:5*n*-3	n.d.	n.d.	85.3 ± 0.65	85.1
22:0	n.d.	n.d.	5.3 ± 0.11	10.6
22:1*n*-9	n.d.	n.d.	3.2 ± 0.06	n.d.
23:0	n.d.	n.d.	1.2 ± 0.17	n.d.
22:6*n*-3	120.0	n.d.	96.5 ± 1.23	144.6
24:1*n*-9	n.d.	n.d.	4.0 ± 0.06	n.d.
Σ Saturates	313.1	487.9	350.3 ± 4.39	311.8
Σ Monounsaturates	263.7	347.2	297.4 ± 6.44	334.8
Σ (*n*-6)[1]	274.5	164.9	160.5	105.1
Σ (*n*-3)[2]	120.0	n.d.	181.8	229.7

n.d. = not detected; [1] ∑ (*n*-6) ≥ 18:2*n*-6; [2] ∑ *n*-3 ≥ 20:3*n*-3

Table 4 - Apparent digestibility coefficients (ADC) for crude protein, crude lipid and dry matter of cobia fed experimental diets

Diets	ADC		
	Crude protein	Crude lipid	Dry matter
Reference (Fish meal)	0.55^{b} (± 0.01)	0.51^{b} (± 0.02)	0.31^{b} (± 0.00)
Test (CSPH)	0.91^{a} (± 0.01)	0.86^{a} (± 0.01)	0.75^{a} (±0.00)
P value	0.0000	0.0001	0.0000

Values are mean (± SE). Different superscript letter in same column indicate significant differences ($P < 0.05$).

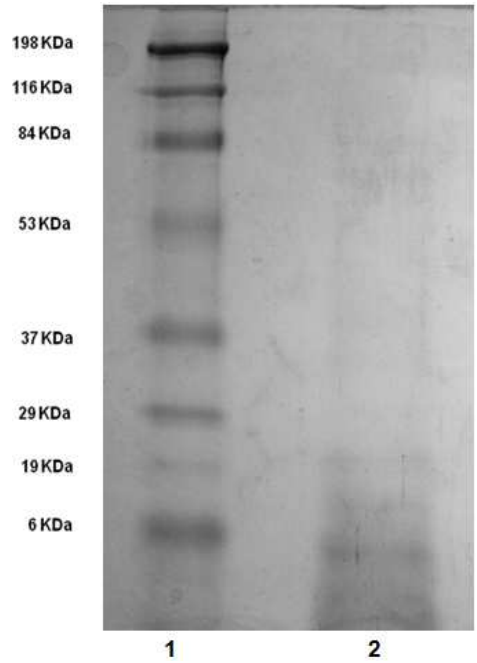

Figure 1 - Polyacrylamide gel electrophoresis (SDS-PAGE) of centrifuged shrimp protein hydrolysate (CSPH). Lane 1: molecular weights of standard protein markers (myosin 198.84 kDa, β-galactosidase 115.7 kDa, bovine serum albumin 96.74 kDa, ovalbumin 53.54 kDa, carbonic anhydrase 37.13 kDa, soybean trypsin inhibitor 29.13 kDa, lysozyme 19.54 kDa, and aprotinin 6.91 kDa); Lane 2: CSPH.

CAPÍTULO 3

Growth, feed efficiency and nutritional composition of juvenile cobia (*Rachycentron canadum*) fed diets containing increasing levels of shrimp protein hydrolysate

Growth, feed efficiency and nutritional composition of juvenile cobia (*Rachycentron canadum*) fed diets containing increasing levels of shrimp protein hydrolysate

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Abstract

A shrimp protein hydrolysate (CSPH) was tested as replacement of fish meal (FM) in diets for juvenile cobia. The effects of increasing dietary levels of SPH on the survival, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed intake (FI) of cobia were evaluated. Four isoproteic and isoenergetic diets were formulated so that 0% (Control), 12%, 24% or 36% of the fish meal content was replaced by SPH in protein diet. Each diet was fed to triplicate groups of fish with initial body weight of 11.93 g in 500-L cylindrical fiberglass tanks. Water quality variables (temperature, salinity, pH, dissolved oxygen, ammonia and nitrite) remained within recommended levels for rearing cobia. Survival, WG, SGR, FCR, PER and FI ranged among 90 and 100%, 40.2 g and 56.5 g, 4.72 and 6.06% day^{-1}, 1.11 and 1.54, 0.96 and 1.32, and 14.3 and 16.9 g, respectively. Survival and FI were not affected by the dietary treatments, but fish fed the diet containing

12% CSPH had highest WG, SGR, FCR and PER. Higher inclusion of CSPH lowered the cost of the diets. In conclusion, the present study demonstrated that up to 12% of the FM in cobia diets can effectively be replaced by CSPH without any negative consequences to feed utilization and fish performance. At this inclusion level, diets would cost an estimated 5.6% less.

Key words: hydrolysate; protein source; replacement; fish meal; amino acids; feed cost.

Introduction

The aquaculture of marine fish represents only 3.1% (or 1.8 million tons) of world aquaculture production, but its average annual growth rate in the period 1990 to 2010 was 9.3%, which is higher than most sectors of the aquaculture industry (FAO, 2012a). Given the increasing consumer demand for higher trophic-level species (FAO, 2012a), the production of marine fish from aquaculture is likely to increase further. A major constraint for the development of the farming of carnivorous fish species is the dependence on fish meal (FM), the major protein source used in aquaculture diets. FM is derived from wild-captured fish that are exploited at their maximum sustainable limit. Global production of FM in recent decades has oscillated around six million tons, which is deemed insufficient to cover the growing demand of the aquaculture industry (Tacon & Metian 2008). Thus, it is essential to minimize FM use in aquaculture diets and/or to identify viable substitutes to FM.

Shrimp processing discards are considered an alternative protein source for aquaculture diets (Hertrampf & Piedad-Pascual 2000). Global production of penaeid shrimps is gradually increasing and is currently estimated at around six million tons (FAO, 2012a). Since processing discards make up approximately 34-45% of the whole shrimp weight (Barrat & Montano 1986), no less than two million tons of shrimp wastes would be available every year.

The potential of hydrolysates as replacement for FM in aquaculture diets has long been recognized (Berge & Storebakken, 1996; Hertrampf and Piedad-Pascual, 2000; Refstie et al., 2004). Hydrolysates are obtained by the enzymatic hydrolysis of fish by-products, and contain a high content of soluble low molecular weight peptides and free amino acids (Hertrampf and Piedad-Pascual, 2000). Inclusion of hydrolysates in fish diets generally increases growth and feed utilization, and promotes nonspecific immunity through the enhancement of the activity of fish macrophages (Bogwald et al., 1996; Refstie et al., 2004; Hevroy et al., 2005; Liang et al., 2006). Supplementing aquaculture feeds with hydrolysates may therefore not only improve growth performance of fish but also increase their resistance to diseases.

In a previous study (Costa-Bomfim et al., in press), was demonstrated that the autolysis of shrimp processing wastes produces a high quality protein hydrolysate that was well digested by cobia (*Rachycentron canadum*), an emerging species in the aquaculture scenario with a global production in 2010 estimated at 40,768 tons (FAO, 2012b). Here, we evaluated the growth performance, proximate composition, amino acid and fatty acid profiles of cobia juveniles fed graded dietary levels of a shrimp protein hydrolysate in replacing FM.

Material and methods

Experimental diets

Four isocaloric, isonitrogenous diets (Table 1) were formulated to fulfill the nutritional requirements of cobia (Fraser & Davies, 2009; NRC, 2011). The diets contained increasing levels of shrimp protein hydrolysate (CSPH), which was obtained through the autolysis of shrimp (*Litopenaeus vannamei*) processing discards (Costa-Bomfim et al., in press). Inclusion levels of CSPH in the diets were based on Refstie et al. (2004) and Liang et al. (2006). A diet containing no CSPH was used as control. Experimental diets were therefore named control, 12, 24 and 36 according to the inclusion level of CSPH (0, 12, 24 and 36%, respectively).

CSPH was added in replacing FM in protein diet. Graded levels of fish oil and wheat flour were included to balance dietary lipid and energy levels, respectively. All ingredients were thoroughly mixed and oil was added to the dry mixture. Distilled water was then added and the blend was pelletized through a 4 mm dye. Pellets were dried at 55ºC for 24 h and stocked at -20ºC until use. The proximate composition, and the amino acid and fatty acid profiles of the experimental diets is presented in Tables 1 and 2.

2.2 Experimental conditions

Juvenile cobias obtained from a private hatchery (Aqualider Maricultura, Pernambuco, Brazil) were acclimated to the experimental conditions for a week. During this period, they were fed a commercial diet containing 44% protein and 8% lipids (Laguna, Socil Evialis, Brazil). Fifteen 500 L fiberglass tanks were each randomly stocked with 10 fish (11.93 ± 1.03 g) and three replicated tanks were assigned a dietary treatment. Fish were hand-fed the experimental diets to apparent satiation twice daily (08:00 and 16:00) during 30 days. The amount of diet consumed was recorded daily for each tank.

Throughout the trial, the tanks were supplied with sand-filtered seawater at a flow of about 260 L h^{-1}. Water temperature, salinity, pH, dissolved oxygen, and pH were monitored daily with a Hanna HI9828 multiparameter (Hanna Instruments, USA), while total ammonia and nitrite concentrations were estimated every three days using commercial test kits (Labcon, Alcon, Brazil). Water quality was maintained within ranges deemed appropriate for cobia: mean (± SD) temperature = 28.6 ± 0.9ºC; salinity = 35.7 ± 1.2, pH = 7.77 ± 0.25, dissolved oxygen = 6.06 ± 0.79 mg L^{-1}; total ammonia = 0.007 ± 0.005 mg L^{-1}; and no nitrite was detected.

2.3 Evaluation criteria

Analysis of body composition was carried out in 20 fish sampled at the beginning of the trial, and 10 fish from each tank (total of 30 fish per treatment) at the end of the trial. These fish were individually weighed, euthanized, and muscle tissues were sampled and preserved at -20ºC until analysis.

Growth parameters were calculated as follows:

Survival (%) = 100 x (final number of fish) x $(10)^{-1}$

Weight gain (WG, g) = final body weight – initial body weight

Specific growth rate (SGR, % day^{-1}) = 100 x (ln final weight – ln initial weight) x $(days)^{-1}$

Protein efficiency ratio (PER) = weight gain x (protein intake, g)$^{-1}$

Feed conversion ratio (FCR) = (feed intake, g) x (body weight gain, g)$^{-1}$

Feed intake (FI, % body weight day^{-1}) = 100 x [average individual dry matter feed x (average individual weight x final individual weight) ^{0,5}/days]

Economic parameters of experimental diets were calculated following Martínez-Llorens et al. (2007):

Economic efficiency ratio (ECR, Kg $fish^{-1}$) = (feed intake, kg) x (feed cost, kg $^{-1}$) x (weight gain, kg)$^{-1}$

Economic profit index (EPI, fish $^{-1}$) = (final weight, kg fish $^{-1}$) x (fish sale price, kg $^{-1}$) – (ECR, kg $fish^{-1}$) x (weight increase, kg).

The farm gate price for cobia was considered to be US$ 5.41 kg $^{-1}$ according to Cavalli et al. (2011).

2.4 Chemical analysis

Samples of diets and fish muscle tissues were analyzed in triplicate for proximate composition and fatty acid profile. Moisture, crude protein (Kjeldahl procedure) and ash contents were determined following AOAC (2000), while total lipids were determined

according to Bligh-Dyer (1959). Nitrogen free extract was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids and ash from 100%. Estimation of gross energy values of the diets was based on energy values of 5.65 kcal.g^{-1} for crude protein, 4.15 kcal.g^{-1} for nitrogen free extract and 9.40 kcal.g^{-1} for lipids.

Fatty acid composition was analytically verified by gas chromatography (Varian CP 3800) equipped with a flame ionization detector (GC-FID), and a WAX (25 m x 0.25 mm x 0.2 µm) column, with a flow rate of 1.3 mL min^{-1} of Helium. The GC conditions were as follows: injector and detector temperature 280ºC (initial oven temperature, 150ºC increased to 230ºC), muscle with three ramps, a total program run was 90 min. Fatty acids were identified by comparison with the retention time (RT) of the peaks of the samples with standard RT (Sigma, USA). Quantification was performed by addition of an internal standard (C 23:0 Sigma, USA) to the extracted lipids. The calculations were performed according to the following equation and expressed in mg g^{-1} of sample.

Concentration (mg g^{-1}) = $A_{FA} . M_{PI} . f_{AG} / A_{PI} . M . K$

A_{FA} = area of fatty acid methyl ester in the sample chromatogram;

M_{PI} =weight of internal standard added to the sample (mg);

F_{FA}= conversion factor;

A_{IS} = area of internal standard fatty acid methyl ester in the chromatogram;

M = mass of the sample (mg);

K = correction factor.

The composition of amino acids was determined after acid hydrolysis (6N HCl for 24 h at 100ºC), preparation of the hydrolysate for chromatography, i.e. drying, re-drying and derivatization. The derivatives were separated by high performance liquid chromatography (HPLC) by a commercial laboratory using an automatic amino acid analyzer equipped with a column according to White et al. (1986).

2.5 S*tatistical analysis*

One-way analysis of variance (ANOVA) and *a posteriori* Tukey's multiple range test were used to identify differences at the 5% significance level. The Dunnett test was used to identify differences between fish sampled at the beginning (control) and termination of the feeding trial.

Results

Experimental diets had similar protein and lipid contents that ranged from 43.11 to 43.97% and from 13.40 to 14.37%, respectively (Table 1). The amino acid composition of the experimental diets is also presented in Table 1. Concentrations of essential amino acids such as arginine (7.86 - 8.89 g 100 g^{-1} protein) and leucine (6.85 - 7.29 g 100 g^{-1}) increased with higher inclusion levels of CSPH in the diets, while levels of histidine, isoleucine, phenylalanine and lysine decreased. Methionine and tryptophan presented similar concentrations in all diets, as most of the non-essential amino acids. Exceptions were glutamic acid and taurine, which increased at higher dietary inclusion levels of CSPH. Although the crude lipid content was not different between diets (Table 1), the sum of saturated fatty acids and (*n*-3) PUFA presented no significant variation within the different diets (Table 2). On the other hand, monounsaturated fatty acids and (*n*-6) PUFA increased with higher dietary inclusion of CSPH.

Regardless of dietary treatment, cobia juveniles presented a rapid growth. For instance, fish fed diet 36, which resulted in the numerically lowest final body weight (BW) (51.9 g - Table 3), increased 4.35 times in comparison to the initial BW. Overall, the experimental diets had a significant effect on the performance of juvenile cobia (Table 3). Final BW, WG, SGR and PER were higher in cobia fed diet 12 than in fish fed diets 24 and 36, but not significantly

different to fish fed the control diet. FCR was significantly lower when feeding diet 12 than diet 24, but not significantly different when compared the control or diet 36. There were no differences in FI and survival between cobia fed the different experimental diets.

There were no significant differences in moisture, total lipids and ash contents in cobia muscle tissues between the experimental groups, but crude protein contents were lower in the muscle of cobia fed diets 24 and 36. (Table 4). The amino acid profile of muscle tissue is also presented on the Table 4. Lysine was the highest essential amino acid found in the muscle of the cobia fed experimental diets, with values ranging from 7.62 to 9.74 g 100 g^{-1} in protein. The lowest value was methionine in tissue muscle of cobia fed diet 24. In general, amino acid contents in muscle tissue of fish fed the experimental diets for 30 days were similar to initial fish. Although dietary taurine content increased with higher CSPH inclusion levels, no increase in muscle tissue levels was detected.

In general, fatty acid composition in tissue muscle of fish fed experimental diets presented lower contents in comparison with initial fish (Table 5). Despite diets presented increased fatty acids contents according to inclusion levels of CSPH, fish fed diets 12 and 24 had highest levels of saturated, monounsaturated, (*n*-6) PUFA and (*n*-3) HUFA in their muscle (Table 5).

The economic parameters of the experimental diets are presented in Table 6. Higher dietary levels of CSPH resulted in a reduction of the cost of the diets, but ECR and EPI presented no significant difference between diets.

Discussion

Water quality variables showed no significant differences among treatments and were within levels considered compatible with the development of cobia (Shaffer & Nakamura,

1989; Sun et al., 2006; Rodrigues et al.; 2007). The quality of the environmental conditions are also confirmed by the high survival and growth rates of fish in the present study. SGR in this study varied between 4.72 and 6.06% day^{-1}, which is higher than reported in several other studies with cobia (Craig et al., 2006; Lunger et al., 2007b; Silva Jr. et al., 2009; Salze et al., 2010; Mach & Nordvelt, 2011; Ren et al., 2011; Trushenski et al., 2011, 2012). The results in present study, however, are closer to the SGR estimated by Resley et al. (2006), between 4.70 and 5.40% day^{-1}, and Lunger et al. (2007a), who found it to vary from 2.00 to 5.13% day^{-1}.

The composition of all experimental diets is in accordance with the nutritional requirements of cobia (Fraser & Davies, 2009; NRC, 2011). Crude protein content of the diets ranged between 43.11 to 43.97% and is similar to the optimal level (44.5%) recommended by Chou et al. (2001), while concentrations of lysine, methionine and arginine in diets containing CSPH were higher than required by cobia (Fraser & Davies, 2009; Ren et al., 2012). Overall, the amino acid profile of the diets is comparable to the control diet that had FM as the main protein source (Watanabe, 2002; Refstie et al., 2004, Espe et al., 2006). The composition of diets containing CSPH agrees with Refstie et al. (2004) in that the nutritional value of the diets or the fish protein products are dependent of the raw materials as well as on the hydrolysation process. As reported by Costa-Bomfim et al. (in press), the enzymatic autolysis of shrimp discards resulted in a CSPH with a balanced amino acid profile that was highly digestible for cobia.

Studies using fish protein concentrate based on autolysed fish silage or hydrolysates in diets resulted in an improvement in FI (Plascencia-Jatomea et al., 2002; Refstie et al., 2004) and this is attributed to the attractive and stimulant properties of the free amino acids and low molecular weight peptide fractions (Rungruangsak & Utne, 1981). In the present study, however, FI was not affected by the increasing levels of CSPH. Similar results were reported

for turbot (Oliva-Teles et al., 1999), red drum (Li et al., 2004) and Atlantic salmon (Hevrøy et al., 2005).

The inclusion of 12% CSPH in the diet enhanced growth (WG and SGR) and feed utilization (FCR and PER) when compared to diets containing higher CSPH levels, but results were comparable to the control diet that had FM as its main protein source. Similarly, Mach & Nortvedt (2011) found that different dietary levels of silage protein had an effect on growth and feed utilization efficiency of juvenile cobia, with an inclusion of 13% fish silage being recommended for cobia diets. Experiments on Atlantic salmon (Espe et al., 1999; Refstie et al., 2004; Hevrøy et al., 2005) and tilapia (Plascencia-Jatomea et al., 2002) have shown that the inclusion of comparatively higher levels of fish silage or hydrolysates also reduced fish growth. In a study on cobia, diets based on raw fish or crabs were better utilized and thus supported growth better than diets containing fish or crab silage (Mach et al., 2010). According to Geiger (1947), this may be associated to an excess of free amino acids in the intestinal tract, which may negatively affect their absorption. In deficiency, a surplus of free amino acids may cause an imbalance in the relationship among the amino acids and their metabolism due to specific number of transporters in the intestine (Dabrowski & Guderly, 2002; Pezzato et al., 2005; Kotzamanis et al., 2007). Considering the concentration of plasma free amino acids in Atlantic salmon, Hevroy et al. (2005) concluded that amino acids from hydrolysates are absorbed earlier and nonsynchronously, and may thus be more prone to be catabolized than in fish fed less solubilized protein. Furthermore, the utilization of amino acids is related to the metabolic demands of the organism and occurs in a slower fashion, according to the breakdown of the protein that occurs in two steps (Geiger, 1947; NRC, 2011), or may be due to a lower usage of the newly synthesized protein (Espe et al., 1999). In this sense, Kaushik & Seilez (2010) stressed the need for precautions with the supply of free amino acids in fish diets.

Dietary concentrations of arginine, leucine glutamic acid and taurine increased with higher inclusion levels of CSPH, while levels of histidine, isoleucine, phenylalanine and lysine decreased. This had no apparent effect on the amino acid profile of muscle tissue as it was found to be homogenous regardless of dietary treatments. The most abundant amino acids in cobia muscle were glutamic acid, aspartic acid, lysine and leucine, while taurine was the least abundant amino acid in the muscle tissues. In previous results (Costa-Bomfim et al., in press) indicated high levels of taurine in CSPH, which explains the comparatively higher levels of taurine in diets with CSPH. Taurine is generally found as the most prominent free amino acid in cobia (Shiau, 2007; Chuang et al., 2010), particularly in the dark meat and viscera (Shiau, 2007), which concurs with its role in fish osmoregulation (Van Waarde, 1988). High levels of taurine are also often found in FM and in other animal by-products (Lunger et al., 2007). Though not considered an essential amino acid for fish, supplementation of taurine at 5 g kg^{-1} in a diet containing high levels of plant protein resulted in increased WG, feed efficiency and palatability of diets for cobia (Lunger et al., 2007). While the present study was not designed to analyze the supplementation of taurine, the inclusion of CSPH at its highest level (36%) resulted in an indirect supplementation of 1.3 g kg^{-1} of taurine, which had no apparent effect on growth of cobia juveniles.

The present study demonstrated that up to 12% of the FM in cobia diets can effectively be replaced by CSPH without any negative consequences to feed utilization and fish performance. Furthermore, the replacement of FM by 12% CSPH will reduce an estimated 5.6% of the cost of the diet. Given that feed cost is the major expenditure in cobia culture (Miao et al., 2009; Huang et al., 2011), this is particularly important for the further development of the aquaculture of this species.

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Table 1. Formulation (g $100g^{-1}$), proximate composition (% dry matter) and amino acid profile (g $100g^{-1}$ protein) of the experimental diets.

	Diets			
	0	12	24	36
Formulation				
Fish meal [a]	60.70	52.90	45.10	37.30
Shrimp protein hydrolysate	0	6.07	12.15	18.22
Wheat flour	20.60	21.20	21.80	22.40
Fish oil [b]	6.50	7.00	11.13	11.76
Vitamin and mineral premix[c]	2.00	2.00	2.00	2.00
Ascorbic acid monophosphate[d]	3.00	3.00	3.00	3.00
Cellulose[e]	9.88	10.51	12.15	18.22
BHT[f]	0.02	0.02	0.02	0.02
Proximate composition				
Crude protein	43.97	43.77	43.29	43.11
Crude lipid	13.40	14.05	13.93	14.37
Ash	12.66	12.22	11.44	10.56
Nitrogen free extract	29.97	29.96	31.34	31.96
Gross energy	4,987.7	5,037.0	5,055.9	5,112.8
Essential amino acids				
Arginine	7.86	8.24	8.31	8.89
Histidine	3.36	3.11	3.00	2.72
Isoleucine	4.13	3.65	4.10	3.75
Leucine	6.92	7.01	6.85	7.29
Phenylalanine	4.52	4.34	4.46	4.20
Lysine	7.33	7.31	6.99	5.06
Tryptophan	0.96	0.85	0.96	0.80
Threonine	3.45	3.79	3.50	3.23
Valine	5.76	5.17	5.78	5.26
Methionine	4.49	4.78	4.24	4.63
Non essential amino acids				
Aspartic acid	9.84	9.51	9.83	9.55
Glutamic acid	15.74	15.75	16.27	16.58
Serine	3.53	4.04	3.55	4.09
Glycine	8.44	8.60	8.17	8.86
Alanine	6.86	6.59	6.74	6.69
Proline	5.35	5.22	5.92	7.35
Tyrosine	3.31	3.24	2.83	2.80
Cystine	1.19	1.32	1.13	1.14
Taurine	1.28	1.30	1.36	1.41

[a] Corporación Pesquera Inca, Lima, Peru.

[b] Pesquera Pacific Star S. A., Chile.

[c] Contains, as ingredient per kg (DSM Produtos Nutrionais, Jaguaré, Brazil): Vitamin A 1,000,000 UI; Vitamin D3 312,500 UI; Vitamin E 18750 UI; Vitamin K3 1,250 mg; Vitamin B12,500 mg; Vitamin B6 1875 mg; Vitamin B12 3,75 mg; Nicotinic acid 12,500 mg; Pantothenic acid 6,250 mg; Biotin 125 mg; Folic acid 750 mg; Vitamin C (Ascorbyl monophosphate) 31,250 mg; Cholin 50,000mg; Inositol 12,500 mg; Cu 625 mg; Zn 6,250 mg; Mn 1,875 mg; Se 12,5 mg; I 62,5 mg; Co 12,5 mg; BHA 1,520 mg; Ethoxyquin 1,605 mg; Fe 6,250 mg.

[d] DSM Nutritional Products, Basel, Switzerland.

[e] Rhoster (São Paulo, Brazil).

[f] Butylated hydroxytoluene.

Table 2. Mean (± SD) fatty acid composition (mg g^{-1}) of the experimental diets (n=2).

	Diets			
	0	12	24	36
14:0	11.36 ± 0.12^{d}	13.53 ± 0.33^{c}	14.48 ± 0.24^{b}	16.17 ± 0.18^{a}
16:0	40.92 ± 0.78	40.78 ± 35.70	72.95 ± 1.34	83.58 ± 0.0
16:1*n*-7	15.34 ± 0.53^{b}	17.84 ± 0.26^{ab}	19.79 ± 1.34^{a}	21.22 ±1.47^{a}
18:0	10.54 ± 5.84	15.63 ± 0.15	18.02 ± 0.04	13.33 ± 8.15
18:1*n*-9	61.27 ± 0.98^{c}	68.49 ± 0.87^{c}	74.84 ± 0.62^{b}	86.34 ± 2.26^{a}
18:2*n*-6	30.54 ± 0.20^{d}	34.67 ± 0.85^{c}	36.85 ± 0.51^{b}	45.38 ± 1.0^{a}
18:3*n*-6	1.17 ± 0.01^{c}	1.38 ± 0.0^{b}	1.41 ± 0.03^{b}	1.92 ± 0.03^{a}
18:3*n*-3	2.65 ± 0.01^{c}	2.80 ± 0.03^{bc}	3.18 ± 0.08^{ab}	3.52 ± 0.12^{a}
20:0	0.88 ± 0.03	0.86 ± 0.0	0.87 ± 0.0	1.10 ± 0.08
20:1*n*-9	4.08 ± 0.08^{b}	4.06 ± 0.02^{b}	4.69 ± 0.10^{a}	4.81 ± 0.12^{a}
20:2*n*-6	1.15 ± 0.01^{b}	0.56 ± 0.79^{b}	1.21 ± 0.0^{b}	1.78 ± 0.05^{a}
20:3*n*-6	0.93 ± 0.0	0.47 ± 0.67	0.97 ± 0.0	1.09 ± 0.13
20:4*n*-6	3.05 ± 0.10^{c}	3.28 ± 0.16^{bc}	3.43 ± 0.0^{b}	3.77 ± 0.01^{a}
20:5*n*-3	21.72 ± 0.08^{b}	22.75 ± 0.01^{ab}	24.65 ± 0.74^{ab}	24.20 ± 1.10^{a}
22:6*n*-3	25.07 ± 0.01	25.31 ± 0.82	25.79 ± 0.53	25.78 ± 1.41
24:1*n*-9	1.20 ± 0.0	1.28 ± 0.39	1.28 ± 0.0	1.24 ± 0.01
Σ Saturated	64.39 ± 30.6	99.55 ± 7.4	106.5 ± 3.1	116.6 ± 9.8
Σ Monounsaturated	89.59 ± 2.64^{c}	101.6 ± 4.86^{bc}	111.9 ± 2.2^{ab}	124.7 ± 3.7^{a}
Σ (*n*-6)[2]	35.91 ± 0.06^{b}	39.22 ± 3.09^{b}	41.06 ± 4.5^{b}	53.50 ± 2.62^{a}
Σ (*n*-3)[3]	49.44 ± 0.07	50.23 ± 1.4	55.42 ± 1.34	52.68 ± 0.94

Different superscript letters within rows represent significant differences ($P < 0.05$)

[1] nd = not detected

[2] Σ (*n*-6) ≥ 18:2*n*-6

[3] Σ (*n*-3) ≥ 18:3*n*-3

Table 3. Mean (± SD) final body weight (BW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed intake (FI) of juvenile cobia (*Rachycentron canadum*) fed diets containing increasing levels of shrimp hydrolysate for 30 days.

	Diets				*P-value*[1]
	0	12	24	36	
Survival	93.3 ± 11.5	96.7 ± 5.7	100.0 ± 0.0	90.0 ± 10.0	0.51
Final BW (g)	65.0 ± 7.4^{ab}	67.4 ± 4.4^{a}	53.3 ± 4.7^{b}	51.9 ± 2.3^{b}	0.011
WG (g)	53.3 ± 7.6^{ab}	56.5 ± 5.5^{a}	40.4 ± 5.2^{b}	40.2 ± 3.0^{b}	0.012
SGR (% day^{-1})	5.72 ± 0.5^{ab}	6.06 ± 0.5^{a}	4.72 ± 0.4^{b}	4.97 ± 0.4^{ab}	0.029
FCR	1.11 ± 0.1^{ab}	1.04 ± 0.1^{a}	1.49 ± 0.3^{ab}	1.54 ± 0.03^{b}	0.015
PER	1.25 ± 0.2^{ab}	1.32 ± 0.1^{a}	0.96 ± 0.1^{b}	0.96 ± 0.07^{b}	0.017
FI	15.9 ± 3.8	16.9 ± 0.8	14.3 ± 1.3	14.8 ± 1.5	0.51

[1] Different superscript letters within rows represent significant differences ($P < 0.05$).

Table 4. Mean (± SEM) proximate composition (g $100g^{-1}$; n=3) and amino acid content (g $100g^{-1}$ protein) of the muscle tissue of cobia (*Rachycentron canadum*) sampled at the beginning of the trial and after feeding diets containing increasing levels of shrimp protein hydrolysate for 30 days.

	Initial	Diets			
		0	12	24	36
Proximate composition[1]					
Moisture	21.4 ± 0.6^{B}	22.5 ± 0.6	22.9 ± 1.2	24.0 ± 4.3^{A}	22.9 ± 0.3
Crude protein	17.8 ± 2.6	18.5 ± 3.6^{a}	18.9 ± 4.9^{a}	16.8 ± 1.2^{b}	17.5 ± 5.0^{ab}
Total lipids	1.41 ± 0.2^{B}	2.63 ± 0.02^{A}	2.09 ± 1.0^{A}	2.25 ± 0.8^{A}	2.65 ± 0.1^{A}
Ash	1.9 ± 0.6^{A}	1.39 ± 0.5^{B}	1.32 ± 0.4^{B}	1.32 ± 0.1^{B}	1.38 ± 0.0^{B}
Essential amino acids					
Arginine	6.26	5.70	5.78	5.30	5.69
Histidine	2.42	2.93	2.62	2.01	2.55
Isoleucine	5.00	4.90	4.84	4.89	4.78
Leucine	7.78	7.98	8.00	6.18	8.08
Phenylalanine	4.07	3.99	4.00	3.60	3.99
Lysine	8.81	9.74	9.49	7.62	9.41
Tryptophan	0.90	1.21	1.09	1.08	1.12
Threonine	3.99	4.29	4.30	4.22	4.36
Valine	5.54	5.75	5.63	6.28	5.64
Methionine	5.15	3.08	3.16	0.41	3.30
Non essential amino acids					
Aspartic acid	9.89	10.55	10.62	9.32	10.37
Glutamic acid	14.97	15.35	15.81	21.88	15.68
Serine	3.36	3.74	3.80	3.96	3.99
Glycine	5.97	5.00	5.14	4.79	5.21
Alanine	5.81	5.70	5.83	8.75	5.90
Proline	3.70	3.48	3.56	4.33	3.62
Tyrosine	3.51	3.89	3.90	3.30	3.88
Cystine	2.86	2.22	1.93	1.54	1.91
Taurine	nd	0.50	0.49	0.51	0.53

nd = Not detected

[1] Different subscript letters within each row indicate significant difference ($P < 0.05$)

[2] Capital letters indicate difference of each treatment between initial fish.

Table 5. Mean (± SEM) fatty acid composition (mg g^{-1}) of the muscle tissue of cobia (*Rachycentron canadum*) sampled at the beginning of the trial and after feeding diets containing increasing levels of shrimp protein hydrolysate for 30 days (n = 2).

	Diets				
	Initial	0	12	24	36
14:0	19.05 ± 1.55	10.11 ± 0.59^{b}	17.72 ± 1.77^{a}	16.35 ± 1.48^{a}	9.31 ± 0.61^{b}
16:0	154.14 ± 0.78	50.68 ± 0.78^{b}	98.08 ± 0.78^{a}	93.20 ± 0.78^{a}	56.81 ± 0.78^{b}
16:1*n*-7	35.88 ± 2.92	11.67 ± 5.87	27.59 ± 2.35	25.29 ± 1.57	nd
17:0	2.57 ± 0.01	1.02 ± 0.0	1.97 ± 0.18	1.68 ± 0.0	nd
18:0	56.48 ± 6.05	9.45 ± 5.22	27.84 ± 2.69	18.74 ± 9.56	16.74 ± 1.56
18:1*n*-9	213.01 ± 21.43	52.64 ± 5.81^{b}	106.35 ± 7.52^{a}	104.40 ± 5.44^{a}	63.36 ± 4.25^{b}
18:2n-6	200.48 ± 19.20	26.95 ± 2.49^{b}	53.75 ± 4.20^{a}	55.65 ± 4.39^{a}	34.06 ± 1.84^{b}
18:3*n*-6	2.28 ± 0.17	1.00 ± 0.11	2.04 ± 0.15	2.13 ± 0.50	1.11 ± 0.22
18:3*n*-3	17.41 ± 1.60	2.18 ± 0.23^{b}	4.0 ± 0.24^{a}	4.07 ± 0.40^{a}	2.20 ± 0.01^{b}
20:1*n*-9	6.98 ± 0.89	2.81 ± 0.26^{b}	5.09 ± 0.10^{a}	5.43 ± 0.61^{a}	2.68 ± 0.09^{b}
20:2*n*-6	3.98 ± 0.62	1.01 ± 0.07	1.59 ± 0.44	1.83 ± 0.83	nd
20:3*n*-6	1.95 ± 0.90	0.67 ± 0.08	1.40 ± 0.0	1.48 ± 0.31	nd
20:4*n*-6	6.53 ± 0.80	2.32 ± 0.24^{c}	5.75 ± 0.66^{a}	5.58 ± 0.30^{ab}	3.35 ± 0.0^{bc}
20:5*n*-3	20.89 ± 1.69	14.92 ± 1.60^{b}	26.57 ± 1.24^{a}	24.70 ± 0.47^{a}	14.75 ± 0.0^{b}
22:6*n*-3	30.55 ± 1.90	16.13 ± 0.89^{c}	36.28 ± 3.15^{a}	35.70 ± 1.72^{ab}	23.01 ± 0.0^{bc}
24:1*n*-9	1.51 ± 0.22	0.58 ± 0.03^{c}	1.40 ± 0.0^{ab}	1.55 ± 0.0^{a}	nd
Σ Saturated	232.2 ± 22.02	60.63 ± 8.9^{b}	128.38 ± 10.5^{a}	130.11 ± 2.7^{a}	82.55 ± 6.6^{b}
ΣMonounsaturated	283.1 ± 27.5	75.34 ± 13.2^{b}	154.86 ± 10.6^{a}	145.79 ± 3.7^{a}	74.36 ± 4.9^{b}
(*n*-6)[2]	215.2 ± 21.7	31.95 ± 2.6^{b}	62.97 ± 4.8^{a}	66.67 ± 6.3^{a}	36.85 ± 4.4^{b}
(*n*-3)[3]	68.9 ± 5.2	33.24 ± 2.7^{b}	66.94 ± 4.52^{a}	64.48 ± 2.6^{a}	39.97 ± 0.0^{b}

Different subscript letters within each row indicate significant differences ($P < 0.05$) [1]nd= Not detected

[2]Σ (n-6) ≥ 18:2*n*-6

[3]Σ (n-3) ≥ 18:3*n*-3

Table 6. Mean (± SEM) cost (US$ kg^{-1}), economic efficiency ratio (ECR; US$ kg^{-1}) and economic profit index (EPI; % day^{-1}) of the experimental diets (n = 3).

	Diets				
	0	12	24	36	*P*-value
Cost	2.50	2.36	2.22	2.07	
ECR	1.26 ± 0.12	1.17 ± 0.20	1.63 ± 0.32	1.42 ± 0.19	0.13
EPI	0.28 ± 0.03	0.30 ± 0.03	0.23 ± 0.03	0.23 ± 0.02	0.26

CAPÍTULO 4

Atividades proteolíticas em juvenis de beijupirá (*Rachycentron canadum*) alimentados com dietas contendo níveis crescentes de hidrolisado proteico de camarão

Atividades proteolíticas em juvenis de beijupirá (*Rachycentron canadum*) alimentados com dietas contendo níveis crescentes de hidrolisado proteico de camarão

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Resumo

O conhecimento sobre as enzimas digestivas no trato digestório do peixe pode auxiliar na compreensão da fisiologia digestiva e, desta forma, possibilita formular dietas adequadas às necessidades nutricionais de cada espécie. O objetivo deste estudo foi determinar a atividade das enzimas proteolíticas no estômago, cecos pilóricos e intestino de juvenis de beijupirás alimentados durante 30 dias com dietas contendo níveis de 0, 12, 24 e 36% de hidrolisado proteico de camarão (HPC), além de uma dieta comercial usada como controle externo. Após 30 dias de cultivo, os peixes foram sacrificados e os tecidos (estômago, ceco pilórico e intestino) retirados para preparo dos extratos brutos. Foram realizados ensaios enzimáticos (atividade inespecífica alcalina, inespecífica ácida/pepsina, tripsina, quimiotripsina, e leucino aminopepetidase). Independente da dieta, a maior atividade proteolítica foi registrada no estômago. O ceco pilórico apresentou, de modo geral, maiores atividades proteolíticas alcalinas, sendo superior às atividades registradas no intestino. As maiores atividades proteolíticas foram registradas nos peixes alimentados com a dieta 24%. O fornecimento de dietas com HPC proporcionou diferença nas atividades proteolíticas do trato

digestório dos beijupirás alimentados com o controle externo. A inclusão do HPC nas dietas afetou as atividades digestivas proteolíticas dos beijupirás. As maiores atividades proteolíticas foram registradas no estômago, ceco pilórico e intestino dos beijupirás alimentados com a dieta contendo 24% de HPC, sugerindo que o excesso de aminoácidos livres pode ter induzido ao aumento das atividades enzimáticas proteolíticas.

Palavras-chave: fonte proteica alternativa; peixe marinho; peptidases.

Proteolytic activities in juvenile cobia (*Rachycentron canadum*) fed diets containing increasing levels of shrimp protein hydrolysate

Abstract

Knowledge of digestive enzymes in the digestive tract of the fish may help understanding the digestive physiology, and enables the formulation of diets according to each species' nutritional requirement. The objective of this study was to determine the activity of proteolytic enzymes in the stomach, pyloric caeca and intestine of juvenile cobia fed for 30 days with diets containing levels of 0, 6, 12 and 18% of shrimp protein hydrolysate (CSPH). A commercial diet was used as an external control. After 30 days of culture, fishes were sacrificed, tissues (stomach, pyloric caeca and intestine) were removed for preparation of the crude extracts. Enzyme assays were performed (activity nonspecific alkaline, nonspecific acid / pepsin, trypsin, chymotrypsin, leucine aminopepetidase). Regardless of diet, the higher proteolytic activity was recorded in the stomach. The pyloric caeca showed generally higher alkaline proteolytic activities, being higher than the activity detected in the intestine. The major proteolytic activities were recorded in fish fed diet 12%. Feeding diets with shrimp protein hydrolysate provided the difference in proteolytic activities of the digestive tract cobia

fed with external control. The inclusion of HPC in the diets affected the digestive proteolytic activities in cobia. The major proteolytic activities were recorded in the stomach, pyloric caeca and intestine of cobia fed diet containing 12% SPH, suggesting that the excess of free amino acids may have led to an increased proteolytic enzyme activity.

Key-words: enzymes; proteolytic; shrimp hydrolysate; cobia

Introdução

O beijupirá (*Rachycentron canadum*) é uma espécie marinha que vem apresentando destaque na aquicultura mundial (Craig et al., 2006; Fines & Holt, 2010). Dentre as características que favorecem sua produção em cativeiro estão alta taxa de crescimento (Liao et al., 2004), podendo atingir 4-6 Kg em um ano (Chou et al., 2001), conversão alimentar aparente variando entre 1,5 e 1,8 (Chou et al., 2004) e alta sobrevivência (Chou et al., 2001). O beijupirá é uma espécie de hábito alimentar carnívoro, que possui preferência alimentar por crustáceos, além de peixes e outros invertebrados (Shaffer & Nakamura, 1989; Chou et al., 2001).

Apesar dos resultados promissores obtidos com essa espécie, e expansão do seu cultivo em cativeiro, informações sobre as exigências nutricionais ainda são incipientes (Chou et al., 2001; Craig et al., 2006; Fraser & Davies, 2009). Sendo importante ressaltar que o conhecimento sobre as necessidades nutricionais de uma espécie é fundamental para se obter um bom desempenho produtivo no cultivo intensivo, assim como, evitar doenças, reduzir os impactos no ambiente e dos custos de produção devido ao desperdício (Pezzatto et al., 2009; Trichet, 2010; NRC, 2011). Segundo Miao et al. (2009), em Taiwan os custos com ração correspondem a 46,1 % do custo total na produção do beijupirá. E para implantação de um

cultivo de beijupirá no Brasil, Domingues (2012) encontrou que o custo com ração pode variar de 32 a 59 % do total operacional.

Além dos ensaios de crescimento e de digestibilidade, com os ingredientes a serem testados, considerados estudos primordiais para determinação das exigências nutricionais dos peixes (Glencross et al., 2007; NRC, 2011). A determinação das atividades enzimáticas digestivas e o conhecimento das enzimas presentes no trato digestório do peixe possibilitam responder questões sobre a fisiologia digestiva, auxiliando na resolução dos problemas nutricionais em peixes (Hidalgo et al., 1999), assim como, nos processos digestivos e suas limitações, sendo importante na formulação das dietas (Costa-Bomfim et al., Capítulo 3; NRC, 2011), pois a digestão e absorção dos nutrientes depende da atividade das enzimas digestivas (Silva et al., 2010). Com exceção da quitinase e quitobiase (Fines & Holt, 2010), informações sobre atividades enzimáticas digestivas no beijupirá ainda são ausentes na literatura.

A rápida expansão da aquicultura, de modo geral, gerou um aumento na demanda pela farinha de peixe como ingrediente proteico, pela alta qualidade nutricional, sendo necessário buscar alternativas (Watanabe, 2002; Davies et al., 2009; Pezzatto et al., 2009). Nesse sentido, o aproveitamento dos resíduos do processamento do pescado, que possuem um grande valor nutricional, torna-se uma alternativa para a alimentação animal. Hidrolisados de peixe e camarão têm sido relatados como possíveis substitutos à farinha de peixe na alimentação de organismos aquáticos (Fagbenro & Jauncey, 1993; Plascencia-Jatomea et al., 2002; Hevrøy et al., 2005), apresentando alta digestibilidade e desempenho produtivo em juvenis de beijupirá (Costa-Bomfim et al., in press; Costa-Bomfim et al., Capítulo 3). Nesse propósito, o presente estudo teve como objetivo determinar a atividade das enzimas proteolíticas no estômago, ceco pilórico e intestino de juvenis de beijupirás alimentados com dietas contendo níveis crescentes de hidrolisado proteico de camarão.

Material e métodos

Dietas experimentais

Quatro dietas experimentais foram formuladas de acordo com as exigências nutricionais do beijupirá (NRC, 2011), contendo níveis crescentes de inclusão de 0, 6, 12 e 18% de hidrolisado proteico de camarão (HPC) em substituição à farinha de peixe, e uma dieta comercial utilizada como controle externo. A composição centesimal das dietas experimentais foi analisada seguindo metodologia da AOAC (2000) (Tabela 1).

O hidrolisado proteico de camarão foi obtido por autólise enzimática, utilizando descartes da indústria camaroneira (*Litopenaeus vannamei*). Os resíduos foram devidamente triturados e misturados com água destilada (1:1). A mistura foi submetida à hidrólise em banho térmico durante 2h, 40ºC, sob leve agitação. Em seguida, a mistura foi aquecida (100ºC, 10 min), a carapaça foi devidamente filtrada. O liquido obtido foi posteriormente centrifugado (10.000 x g) por 10 min. A partir do sobrenadante produzido foi obtido o hidrolisado proteico de camarão (HPC) (Cahu et al., 2012).

Delineamento experimental

Juvenis de beijupirás obtidos de uma empresa privada (Aqualider, Pernambuco, Brasil) foram aclimatados durante sete dias com ração comercial para peixes marinhos. Após aclimatação, grupos de dez peixes (11,93 ± 1,03 g) foram aleatoriamente distribuídos em 15 tanques de fibra de vidro de 500 L, com abastecimento de água salgada com um fluxo contínuo diário médio de 260 L h^{-1}. Os peixes foram alimentados com as cinco dietas durante 30 dias, em grupos de triplicata, sendo fornecidas em duas vezes diárias. Durante todo o período experimental, variáveis como temperatura (28,6 ± 0,9 ºC), salinidade (35,7 ± 1.2), oxigênio dissolvido (6,06 ± 0,79 mg L^{-1}) e pH (7,77 ± 0,25), foram registrados diariamente com o auxílio de um multiparâmetro Hanna HI9828 (Hanna Instruments, USA). Amônia

(0,007 ± 0,005 mg L^{-1}) e nitrito (não detectado) foram registrados a cada três dias utilizando teste comercial (Labcon, Alcon, Brasil).

Preparação dos extratos

Ao final do experimento, os peixes foram sacrificados por choque térmico. Em seguida, foram retirados por dissecação o estômago, ceco pilórico e intestino. Os tecidos foram estocados a -20ºC até preparação dos extratos.

Os extratos brutos do ceco pilórico e intestino foram obtidos de acordo com Alencar et al. (2003), homogeneizando os tecidos em 0,01M Tris-HCl (pH 8,0) contendo 0,15M de NaCl. O extrato bruto do estômago foi preparado homogeneizando o tecido em 0,1M Glicina-HCl (pH 2,0) contendo 0,15M de NaCl, usando um homogeneizador de tecidos (Bondine Electric Company, Chicago, IL). Os extratos foram centrifugados a 10.000 x g por 10 min a 4ºC (Sorvall RC 6 Plus Centrifuge, Thermo Scientific, EUA). As frações sobrenadantes (extrato bruto) foram estocadas a -20ºC para posteriores ensaios enzimáticos.

Ensaios enzimáticos

Os ensaios enzimáticos foram realizados em três repetições, por grupos de peixes de cada tanque, das cinco diferentes dietas testadas.

Atividade proteolítica inespecífica alcalina: Em microtubos, 50 µL de azocaseína a 1% (p/v), preparada em 0,1 M de Tris-HCl (pH 8,0) foi incubada por 60 min, com 30 µL dos extratos brutos do intestino e ceco pilórico. Em seguida, 240 µL de ácido tricloroacético (TCA) - 10% foram adicionados para parar a reação. Após 15 min, foi realizada a centrifugação em 8.000 rpm, por 5 min. Setenta microlitros do sobrenadante foram adicionados a 130 µL de NaOH 1 M em uma microplaca, sendo mensurados a 450 nm em um leitor de microplacas (Bio-rad 550). Uma unidade (U) de atividade enzimática foi considerada como sendo a quantidade de enzima necessária para produzir uma alteração de 0,001 na absorbância por minuto.

Atividade proteolítica inespecífica ácida (pepsina): Foram adicionados em microtubos, hemoglobina (100 µL, 2%, pH 2,5), o extrato bruto do estômago (50 µL) e 350 µL do tampão Glicina-HCl (pH 2,0). Após 60 min, foram adicionados 500 µL de ácido tricloroacético (TCA). Após 15 minutos os microtubos foram centrifugados a 8.000 rpm, por 10min. Em seguida, o sobrenadante foi coletado, e então realizada sua leitura a 280 nm em espectrofotômetro. Uma unidade (U) de atividade enzimática foi considerada como sendo a quantidade de enzima necessária para produzir uma alteração de 0,001 na absorbância por minuto.

Atividade de tripsina e quimiotripsina: As atividades de tripsina e quimiotripsina foram determinadas utilizando os substratos 8mM BApNA (N-α-benzoil-L-arginina-p-nitoanilida) e 8mM SApNA (N-succinyl-Ala-Ala-Pro-Phe-pNa), respectivamente. Os extratos brutos de intestino e ceco pilórico (30 µL) foram incubados, em microplaca, com os respectivos substratos (30 µL) e tampão Tris-HCl (140 µL, pH 8,0), durante 15 min. Em seguida, foi realizada a leitura em espectrofotômetro leitor de microplaca (Bio-rad) no comprimento de onda de 405 nm. Uma unidade de enzima foi definida como a quantidade de enzima necessária para hidrolisar 1 µg de substrato por $min.mg^{-1}$ proteína.

Atividade de leucino aminopeptidase: A atividade foi determinada utilizando o substrato leucina-β-naftalamida. Uma alíquota de 30 µL do extrato bruto, do intestino e ceco de cada tratamento foi incubada em microplacas, contendo 140 µL do tampão TRIS-HCl pH 8,0 e 30 µL do substrato. Após 15 min, foi realizada a leitura em um leitor de microplaca (Bio-rad), em um comprimento de onda de 405 nm. Uma unidade de enzima foi definida como a quantidade de enzima necessária para hidrolisar 1 µg de substrato por $min.mg^{-1}$ proteína.

Determinação da proteína total: A proteína total foi quantificada para determinar as atividades específicas das enzimas. A metodologia utilizada foi de acordo com Bradford (1976), utilizando albumina bovina como padrão.

Análise estatística

Os resultados expressos em media ± desvio padrão foram submetidos à análise de variância (ANOVA). Em seguida, foi aplicado o teste de Tukey ($P < 0,05$) com os dados obtidos dos peixes alimentados com dietas formuladas contendo diferentes níveis de hidrolisado de camarão. Foi realizado um Teste de Dunnett ($P < 0,05$) para comparar os resultados das atividades enzimáticas entre os peixes alimentados com as dietas contendo HPC e a dieta utilizada como controle externo.

Resultados

Os resultados dos ensaios enzimáticos dos peixes alimentados com as diferentes dietas estão dispostos na Tabela 2. Independente da dieta, a maior atividade proteolítica foi registrada no estômago dos beijupirás. De modo geral, foi detectada diferença nas atividades enzimáticas entre os peixes alimentados com as dietas contendo diferentes níveis de HPC. As maiores atividades proteolíticas ácidas (pepsina) foram registradas no estômago dos peixes alimentados com as dietas 24 e 36%, diferindo dos peixes alimentados com as demais dietas. Comparando a pepsina dos peixes alimentados com o controle externo entre as dietas experimentais contendo diferentes níveis de HPC, apenas os peixes alimentados com o nível de 12%, apresentaram uma atividade inferior.

Analisando as atividades enzimáticas do ceco pilórico, os peixes alimentados com o controle externo apresentaram atividade proteolítica inespecífica alcalina inferior aos peixes alimentados com HPC nas dietas, sendo a dieta 24%, superior às demais.

No intestino, avaliando os dados das atividades enzimáticas obtidas dos peixes alimentados com as dietas experimentais, com diferentes níveis de inclusão de HPC, com o controle externo, apenas o grupo que consumiu a dieta 36% demonstrou diferença na

atividade proteolítica alcalina total. E entre os resultados dos peixes alimentados com as dietas com o HPC, apenas os peixes alimentados sem HPC (0%), demonstraram atividade inferior.

No ceco pilórico, a maior atividade da tripsina foi observada para os peixes alimentados com a dieta 24%, e a menor atividade na dieta 0% (controle HPC). As dietas experimentais formuladas contendo HPC registraram maior atividade de tripsina que a dieta comercial utilizada como controle externo. Entretanto, no intestino, os peixes alimentados sem HPC e com a dieta contendo maior nível de HPC (36%) registraram maior atividade da tripsina. E apenas os peixes alimentados com a dieta 24% foi inferior ao controle externo.

As atividades de quimiotripsina tanto no ceco pilórico, como no intestino, não apresentaram diferença ($P<0,05$) entre os peixes alimentados com as diferentes dietas contendo HPC. No entanto, nos peixes alimentados com as dietas 0, 12 e 24% obtiveram maior atividade de quimiotripsina que o controle externo no ceco pilórico.

Leucino aminopeptidase no ceco pilórico apresentou maior atividade nos peixes alimentados com a dieta 24%, e menor para os peixes alimentados sem HPC (0%) e com menor nível de HPC na dieta (12%), sendo semelhante aos resultados da tripsina no mesmo órgão digestório. No intestino, a dieta com 24% de HPC promoveu uma maior atividade da leucino aminopeptidase nos peixes, quando comparado com as demais dietas experimentais (0, 12 e 36% de HPC). A atividade da leucino aminopeptidase tanto no ceco pilórico, como no intestino, registraram diferença entre os peixes alimentados com a dieta comercial e as dietas experimentais (0, 12, 24 e 36%). No intestino, peixes alimentados com o controle externo obtiveram inferior atividade desta enzima, e no ceco pilórico, os peixes alimentados com as dietas 0 e 12% de HPC registraram menor atividade.

Discussão

Os parâmetros de qualidade da água no presente estudo estiveram dentro das condições consideradas desejáveis para o desenvolvimento do beijupirá (Shaffer e Nakamura, 1989). Por serem pecilotérmicos, os peixes possuem dependência direta e indireta do ambiente em que vivem e as alterações nas condições ambientais afetam seu comportamento, além das suas funções fisiológicas (Cyrino et al., 2010). Em estudo realizado com *Seriola quinqueradiata* foi possível observar um aumento na síntese e no estoque de enzimas, além de um menor tempo de passagem da digesta no intestino, em temperaturas mais elevadas durante o cultivo, quando comparadas às atividades enzimáticas entre o período de inverno e verão (Kofuji et al., 2005; Miegel et al., 2010).

De modo geral, as atividades proteolíticas determinadas no presente estudo apresentaram diferença entre os peixes alimentados com as diferentes dietas experimentais, mesmo sendo formuladas para serem isoproteicas. De acordo com Kofuji et al. (2005), diferentes níveis proteicos na dieta podem afetar tanto o estoque como a atividade das enzimas proteolíticas, como é o caso da tripsina. As atividades enzimáticas digestivas dos beijupirás não se apresentaram proporcionais aos níveis crescentes de HPC das dietas. Entretanto, quando comparadas com as atividades enzimáticas dos peixes alimentados com a dieta comercial, houve diferença significativa com exceção à quimiotripsina determinada no intestino. Resultados similares foram encontrados por Santos (2008), que avaliou as atividades enzimáticas digestivas em tilápia do Nilo alimentadas com diferentes níveis de HPC, além de uma dieta comercial.

Alta atividade da pepsina foi registrada nos peixes avaliados no presente estudo, variando de 5.029,0 a 9.407,0 mU/mg proteína^{-1}. Pepsinas são endopeptidases, restritas ao estômago dos peixes, que hidrolisam as cadeias peptídicas e possuem maior afinidade para

ligações hidrofóbicas, como os aminoácidos tirosina e fenilalanina, além de preparar as dietas para a fase de quimo, durante a passagem pelo ceco pilórico e intestino (Almeida et al. 2006; Bakke et al., 2011). A atividade da pepsina nos peixes do presente estudo estão semelhantes aos resultados encontrados em truta (*Oncorhynchus mykiss*), "seabream" (*Sparus aurata*), tambaqui (*Colossoma macropomum*) e enguia (*Anguilla anguilla*) por Hidalgo et al. (1999) e Almeida et al. (2006). No estômago ocorre o início da digestão, e sob ação da pepsina e do ácido clorídrico, a maioria das proteínas são degradadas e desnaturadas, e parcialmente digerido, o alimento é encaminhado ao intestino (NRC, 2011). Entretanto, cada organismo possui sua capacidade intrínseca em digerir cada dieta, através das enzimas endógenas e/ ou exógenas (Garcia-Esquivel & Felbeck, 2006).

Apesar de estimar que 60% dos peixes possuam ceco pilórico, apenas algumas espécies apresentam este tecido bem desenvolvido. E além da mucosa ser similar a do intestino, o ceco pilórico auxilia na digestão e absorção dos nutrientes, por aumentar a superfície de contato, compensando assim o menor tamanho do intestino dos carnívoros, quando comparados com onívoros e herbívoros (Wilson & Castro, 2011). Esse fato pode explicar os maiores valores de atividades proteolíticas determinadas no ceco pilórico (tripsina, quimiotripsina e leucino aminopeptidase), assim como, Rungruangsak & Utne (1981) que detectaram uma maior atividade proteolítica no ceco pilórico, do que no intestino de trutas. Entretanto, Deguara et al. (2003), afirmam que há uma dificuldade em determinar o local específico da atividade de cada enzima, devido a habilidade dos peixes em se adaptar às dietas.

Os beijupirás alimentados com a dieta contendo 12% de HPC apresentaram uma maior atividade específica no ceco pilórico tanto para tripsina, como para leucino aminopeptidase, enquanto que a atividade de quimiotripsina não demonstrou diferença entre os peixes alimentados com as dietas com HPC, apenas quando comparados com a dieta comercial. A

atividade da tripsina está relacionada com a digestão proteica, absorção e transporte dos aminoácidos, afetando consequentemente na conversão alimentar e na taxa de crescimento. Além da qualidade da dieta, fatores ambientais e genéticos podem influenciar na atividade (Rungruangsak-Torrissen et al., 2006).

Em um estudo realizado com polvo, dietas contendo hidrolisado de peixe garantiram um aumento na atividade da tripsina e proteolítica total, indicando que o hidrolisado proteico de peixe pode induzir um aumento na atividade dessas enzimas, assim como uma deficiência na alimentação pode induzir a este mesmo efeito (Aguila et al., 2007). Esse fato indica uma relação inversa entre o crescimento e as atividades enzimáticas proteolíticas (Rungruangsak-Torrissen et al., 2006), o que pode explicar as diferenças significativas para a tripsina entre as diferentes dietas tanto no ceco pilórico, como no intestino dos beijupirás testados no presente trabalho.

O hidrolisado proteico de camarão produzido por autólise enzimática é um produto rico em aminoácidos livres e essenciais, e peptídeos de cadeia curta, o que garante uma alta digestibilidade da proteína bruta (Costa-Bomfim et al., in press). Peixes, incluindo o beijupirá, geralmente apresentam uma maior digestibilidade aparente com maiores níveis de inclusão de hidrolisados na dieta (Hevrøy et al., 2005; Costa-Bomfim et al., in press). Entretanto, uma relação inversa foi observada entre o crescimento e os níveis de inclusão de hidrolisados proteicos e silagens de pescado nas dietas, foram relatados em estudos com diversas espécies de peixe (Espe et al., 1999; Plascencia-Jatomea et al., 2002; Hevrøy et al., 2005), inclusive o beijupirá (Mach et al., 2010; Costa-Bomfim et al., Capítulo 3). Embora maiores níveis de hidrolisado proteico nas dietas, resultem em maior digestibilidade aparente, e consequentemente, é esperado um melhor desempenho produtivo nos peixes. Entretanto, o excesso de aminoácidos livres no trato digestório pode afetar a absorção destes nutrientes,

prejudicando o metabolismo (Geiger, 1947; Mach & Nortvelt, 2011). Esse desbalanceamento, portanto, pode desencadear uma resposta fisiológica que resulta no aumento da atividade das enzimas proteolíticas na tentativa de melhorar a digestão e absorção desses nutrientes. Além disso, alterações na estrutura da proteína e o fornecimento de enzimas exógenas também podem influenciar a produção de enzimas endógenas na digestão e absorção dos nutrientes, e finalmente no crescimento (Thongprajukaew et al., 2011). Esta possibilidade foi relatada por Debnath et al. (2007), que demonstraram que níveis de proteína na dieta acima de 25% resultaram em uma maior atividade proteolítica em alevinos de *Labeo rohita*. Apesar das dietas experimentais utilizadas neste estudo serem isoproteicas, o excesso de aminoácidos livres provavelmente acarretou em uma maior atividade da maioria das enzimas digestivas estudadas, quando o nível de hidrolisado proteico foi maior.

Conclusão

A inclusão de níveis crescentes de HPC nas dietas afetou nas atividades digestivas proteolíticas do beijupirá. As maiores atividades proteolíticas foram registradas no estômago, ceco pilórico e intestino dos beijupirás alimentados com a dieta contendo 12% de HPC, sugerindo que o excesso de aminoácidos livres pode ter induzido ao aumento das atividades enzimáticas proteolíticas.

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Tabela 1 - Formulação e composição centesimal das dietas experimentais em base seca.

Ingredientes (g $100g^{-1}$)	Dietas				
	0	12	24	36	Comercial[e]
Farinha de peixe [a]	60,70	52,90	45,10	37,30	Nd
Hidrolisado proteico de camarão	0	6,07	12,15	18,22	Nd
Farinha de trigo	20,60	21,20	21,80	22,40	Nd
Óleo de peixe [b]	6,50	7,00	11,13	11,76	Nd
Premix vitamínico e mineral [c]	2,00	2,00	2,00	2,00	Nd
Ácido ascórbico monofosfatado [d]	3.00	3,00	3,00	3,00	Nd
Celulose	9.88	10,51	12,15	18,22	Nd
BHT	0.02	0,02	0,02	0,02	Nd
Composição centesimal					
Proteína bruta	43,97	43,77	43,29	43,11	≥45,0
Lipídio total	13,40	14,05	13,93	14,37	≥10,0
Cinzas	12,66	12,22	11,44	10,56	≤20,0

nd = dados não disponíveis

[a] Corporación Pesquera Inca, Lima, Peru. [b] Pesquera Pacific Star S. A., Chile. [c] Composição por kg (DSM Produtos Nutricionais, Jaguaré, Brazil): Vitamina A 1.000.000 UI; Vitamina D3 312.500 UI; Vitamina E 18.750 UI; Vitamina K3 1.250 mg; Vitamina B1 2.500 mg; Vitamina B6 1.875 mg; Vitamina B12 3.75 mg; Ácido Nicotínico 12.500 mg; Ácido pantotênico 6.250 mg; Biotina 125 mg; Ácido Fólico 750mg; Vitamin C (Ascorbil monofosfatado) 31.250 mg; Colina 50.000mg; Inositol 12.500 mg; Cu 625mg; Zn 6.250mg; Mn 1.875mg; Se 12,5mg; I 62,5 mg; Co 12,5 mg; Fe 6,250 mg; BHA 1,520 mg; Etoxiquim 1,605 mg. [d] DSM Produtos Nutricionais, São Paulo, Brasil. [e] Dados fornecidos pelo fabricante.

Tabela 2 - Médias (± DP) das atividades enzimáticas digestivas (mU/mg. proteína^{-1}) no ceco, intestino e estômago de beijupirás alimentados com dietas contendo níveis crescentes de hidrolisado proteico de camarão (HPC) e uma dieta comercial como controle externo.

	Dietas experimentais					*P-value*	
	0	12	24	36	Dieta Comercial	HPC	HPC x controle externo
Ceco							
Proteolítica inespecífica	2094,2 [b*] (± 69,39)	2837,7 [b*] (± 354,1)	7310,2 [a*] (± 167,76)	2488,0 [b*] (± 236,8)	1391,1 (± 76,0)	<0,001	<0,001
Tripsina	316,23 [c*] (± 16,91)	485,96 [b*] (± 13,57)	793,87 [a*] (± 64,52)	470,41 [b*] (± 30,73)	230,41 (± 3,34)	<0,001	0,001
Quimiotripsina	79,01 [*] (± 11,83)	117,83 [*] (± 5,08)	99,18 [*] (± 27,12)	64,87 (± 3,60)	36,22 (± 2,68)	0,056	0,0012
Leucino aminopeptidase	397,47 [c*] (± 8,81)	379,30 [c*] (± 55,58)	881,14 [a*] (± 49,71)	597,83 [b*] (± 4,11)	505,54 (± 3,46)	<0,001	<0,001
Intestino							
Proteolítica inespecífica	49,77 [b] (± 18,11)	89,97 [ab] (± 3,02)	103,38 [ab] (± 61,09)	129,44 [a*] (± 3,97)	62,83 (± 8,59)	0,0305	0,016
Tripsina	134,73 [a] (± 67,25)	41,07 [b] (± 2,90)	18,54 [b*] (± 0,85)	138,38 [a] (± 10,07)	74,37 (± 1,36)	0,0044	0,0017
Quimiotripsina	71,82 (± 5,60)	74,44 (± 2,90)	65,92 (± 7,61)	64,10 (± 3,60)	58,92 (± 0,68)	0,3024	0,1034
Leucino aminopeptidase	129,18 [b*] (± 2,05)	379,11 [a*] (± 4,60)	41,27 [d*] (± 3,65)	104,46 [c*] (± 2,50)	64,75 (± 5,86)	<0,001	<0,001
Estômago							
Pepsina	6.179,0 [bc] (± 18,76)	5.029,0 [c*] (± 5,0)	9.407,0 [a] (± 18,3)	8.376,0 [ab] (± 5,16)	7.663,0 (± 11,59)	0,0044	0,0019

[1] Letras diferentes entre linhas indicam diferenças significativas (P<0,05).

[2] (*) indica diferença significativa entre as dietas formuladas (HPC) e o controle externo.

CONCLUSÃO GERAL

Com o crescimento da aquicultura, a busca por ingredientes alternativos à farinha de peixe tem sido intensa, uma vez que essa farinha é a fonte proteica mais utilizada na dieta de peixes, principalmente para espécies carnívoras que possuem uma maior exigência por proteína. Além do crescente aumento da demanda e consequentemente do custo da farinha de peixe, a sobrepesca das espécies utilizadas na sua fabricação afeta a sustentabilidade da atividade. Em vista disso, os resíduos do beneficiamento de pescado podem ser uma opção. Na presente Tese foi analisada a utilização de resíduos do processamento de camarão como ingrediente em dietas para o beijupirá *Rachycentron canadum*, espécie de peixe marinho nativo da costa brasileira. O estado atual sobre o uso de resíduos de crustáceos em dietas para peixes marinhos é inicialmente apresentado a fim de definir o contexto em que esta Tese foi desenvolvida.

No Capítulo 1, ao mesmo tempo em que a metodologia a ser aplicada em alguns dos estudos posteriores foi validada, são apresentados os resultados de um estudo sobre frequência alimentar em juvenis do beijupirá. Como conclusão desse Capítulo, foi observada a inexistência de alterações significativas no desempenho produtivo (ganho de peso, sobrevivência, taxa de crescimento específico, consumo alimentar, fator de condição e variação no tamanho), sob condições de laboratório, de beijupirás maiores que 110 g quando alimentados com mais de uma refeição diária.

Os Capítulos posteriores se basearam na produção, por meio de autólise enzimática, ou seja, sem uso de enzimas comerciais, de um hidrolisado bruto de camarão (*Litopenaeus vannamei*) (SPH), o qual, após centrifugação, resultou em um sobrenadante (hidrolisado de camarão centrifugado, CSPH) e um precipitado (carotenoproteína). No Capítulo 2, estes três produtos foram caracterizados quanto à composição centesimal e perfis de aminoácidos e de ácidos graxos, e os coeficientes de digestibilidade aparente (CDA) do CSPH foram estimados para o beijupirá. Os três produtos apresentaram alto conteúdo proteico, perfil de aminoácidos igual ou superior à farinha de peixe, embora o teor de umidade dos hidrolisados (SPH e CSPH) tenha sido significativamente superior à farinha de peixe. Além da composição nutricional, foi determinado o índice de aminoácidos indispensáveis (IAAI) dos três produtos, os quais apresentaram índice acima de 90%, confirmando o rico perfil de aminoácidos, e ainda sendo superior à farinha de peixe inteiro, considerada de boa qualidade. O CSPH, além de sua qualidade nutricional, apresentou CDA superior a 90% para proteína em juvenis de beijupirá, indicando este como um produto alternativo no fornecimento de fonte proteica para dietas de organismos aquáticos.

A partir dos diferentes níveis de inclusão de CSPH substituídos na proteína da dieta (0, 12, 24 e 36%), foi possível concluir que o uso do CSPH em 12% de substituição da proteína da dieta para o beijupirá, apresentou maior desempenho produtivo e uma redução nos custos com alimentação de 5,6%. Os resultados indicaram também que o excesso de aminoácidos livres do CSPH pode ter afetado a absorção destes e contribuído negativamente no crescimento dos peixes (Capítulo 3).

Ao avaliar o potencial de um novo ingrediente na dieta de uma espécie, além de sua composição nutricional, digestibilidade e utilização do nutriente para crescimento, a determinação da atividade enzimática deve ser realizada para complementar o estudo nutricional. Nesse sentido, a partir do uso do CSPH como fonte proteica na dieta do beijupirá, no Capítulo 4 foram determinadas as atividades enzimáticas proteolíticas dos peixes alimentados com os diferentes níveis de CSPH na dieta. Foi possível observar que, apesar das dietas terem sido isoproteicas, a inclusão de níveis crescentes de CSPH afetou as enzimas digestivas proteolíticas nos peixes e as maiores atividades proteolíticas foram registradas no ceco pilórico dos peixes alimentados com a dieta contendo 24% de CSPH na proteína da dieta em substituição à farinha de peixe, o que se pôde observar que a atividade enzimática pode não estar diretamente relacionada com o crescimento do peixe, como observados nos capítulos 3 e 4. Com base nesses resultados, recomenda-se que a inclusão de CSPH em dietas para o beijupirá não exceda 12% do conteúdo total de proteína bruta.

ANEXO I

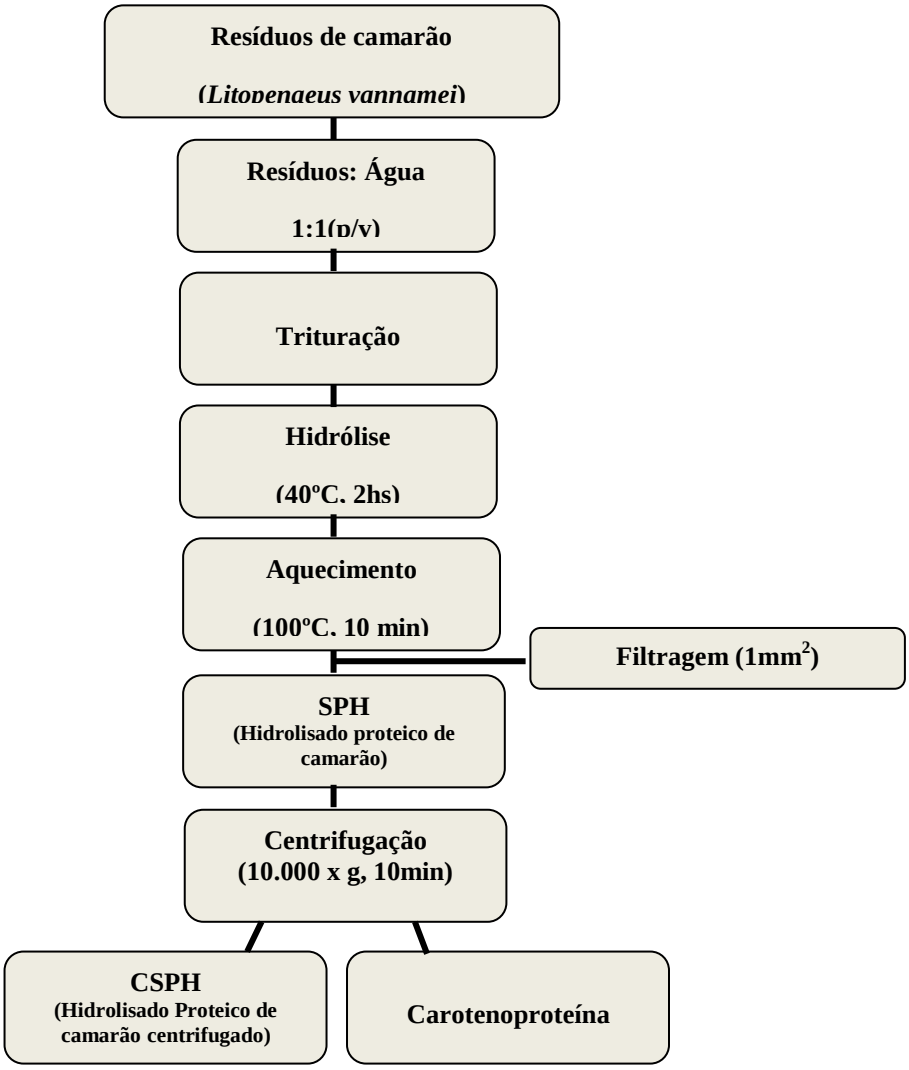

Figura 1. Fluxograma de obtenção dos produtos da autólise enzimática dos resíduos de camarão.