UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE PESCA E AQÜICULTURA
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQÜICULTURA

ALBINO LUCIANI GONÇALVES LEAL

UTILIZAÇÃO DE HIDROLISADO PROTÉICO DE CAMARÃO EM RAÇÕES PARA TILÁPIA DO NILO (*Oreochromis niloticus*, L.)

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Dissertação apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aqüicultura da Universidade Federal Rural de Pernambuco, para obtenção do título de Mestre em Recursos Pesqueiros e Aqüicultura.

Orientador: Dr. Eudes de Souza Correia.
Co-orientador: Dr. Ranilson de Souza Bezerra.

Recife, PE

Fevereiro, 2007

Universidade Federal Rural de Pernambuco
Programa de Pós-Graduação em Recursos Pesqueiros e Aqüicultura

Parecer da comissão examinadora da defesa de dissertação de mestrado de

ALBINO LUCIANI GONÇALVES LEAL

Utilização de hidrolisado protéico de camarão em rações para tilápia do Nilo (*Oreochromis niloticus*, L.)

Área de concentração: **Aqüicultura**

A comissão examinadora, composta pelos professores abaixo, sob a presidência do primeiro, considera o candidato **Albino Luciani Gonçalves Leal** como APROVADO COM DISTINÇÃO.

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DEDICATÓRIA

Às minhas famílias, presente e futura.

AGRADECIMENTOS

Ao Programa de Pós-Graduação em Recursos Pesqueiros e Aqüicultura da Universidade Federal Rural de Pernambuco, em nome de todos os Professores e Funcionários, que sempre contribuíram para o nosso crescimento.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela manutenção do Portal de Periódicos.

Ao Prof. Dr. Eudes de Souza Correia, pela amizade, confiança e orientações de trabalho e para a vida.

Aos membros da Banca Examinadora, titulares e suplentes, pelas críticas e sugestões que certamente melhoraram a qualidade deste trabalho.

À Empresa Pernambucana de Pesquisa Agropecuária (IPA), Diretoria de Extensão Rural, pelas concessões feitas à conclusão deste trabalho.

Aos pesquisadores Dr. Lourinaldo Barreto Cavalcanti (IPA) e M.Sc. Patrícia Fernandes de Castro (Embrapa Meio-Norte), e ao Dr. Ranilson de Souza Bezerra (UFPE), pelo apoio e sugestões a este trabalho.

Aos colegas do Laboratório de Sistemas de Produção Aqüícola e do Laboratório de Enzimologia, pelo sempre amistoso e solidário convívio.

Aos meus familiares, e em especial a Janaina Carvalho, que sempre me apoiaram e entenderam meus momentos de ausência.

RESUMO

A produção aqüícola requer rações de alta qualidade, com alto conteúdo protéico. Assim, a determinação de fontes protéicas de menor custo e que promovam bom crescimento é benéfica. Resíduos de camarão têm sido identificados como uma fonte de proteína animal de grande potencial. Hidrolisado protéico de camarão (HPC) foi considerado como uma excelente fonte alimentar e pode servir como uma adequada fonte de proteína e flavorizante em formulações alimentares. Este trabalho objetivou avaliar a qualidade nutricional do HPC através do desempenho em crescimento de juvenis da tilápia do Nilo e sua utilização protéica. SPH foi incluído em dietas isoprotéicas em níveis de 0, 5, 10 e 20% de substituição da proteína advinda da farinha de peixe (HPC0, HPC5, HPC10 e HPC20) e ofertadas aos peixes (1,7±0,4 g) estocados em aquários de 40 L, por um período experimental de 45 dias. A inclusão do HPC não produziu diferenças estatísticas (P≥0,05) no peso final (27,18, 29,46, 26,02 e 25,19 g), sobrevivência (100%), ganho de peso relativo (1.571, 1.624, 1.388 e 1.301%), ganho de peso diário, GPD (0,57, 0,62, 0,54 e 0,52 g dia^{-1}), taxa de crescimento específico, TCE (7,15, 7,38, 6,85 e 6,73 % dia^{-1}), conversão alimentar, CA (1,15, 1,09, 1,13 e 1,17) e eficiência protéica, EP (2,26, 2,33, 2,20 e 2,14), respectivamente. A inclusão do HPC nas dietas para a tilápia do Nilo afetou estatisticamente (P<0,05) a composição final dos peixes. Os teores de proteína e cinzas diminuíram e o teor de gordura aumentou com os níveis de inclusão do HPC. Este estudo claramente demonstra que o hidrolisado protéico de camarão pode ser incluído em dietas para a tilápia do Nilo sem efeitos adversos em crescimento e utilização protéica.

ABSTRACT

Aquaculture requires high-quality feeds with high protein content. So, the determination of less-expensive sources of protein which provides good growth is advantageous. Shrimp wastes have been identified as an animal protein source with great potential. Shrimp protein hydrolysate (SPH), a derived product obtained from shrimp wastes, was considered an excellent alimentary source and may serve as an useful source of protein and flavorants in food formulations. This work aimed to evaluate the nutritional quality of SPH through growth performance of juvenile Nile tilapia and its protein utilization. SPH was included in isonitrogenous diets at levels of 0, 5, 10 and 20% of fish meal protein replacement (SPH0, SPH5, SPH10 and SPH20) and offered to juvenile Nile tilapia (1.7±0.4 g) stocked in 40-L glass aquaria in a 45-day feeding trial. The inclusion of SPH did not produce statistical differences ($P \geq 0.05$) on final weight (27.18, 29.46, 26.02 and 25.19 g), survival (100%), relative weight gain (1,571, 1,624, 1,388 and 1,301%), average daily gain, ADG (0.57, 0.62, 0.54 and 0.52 g day^{-1}), specific growth rate, SGR (7.15, 7.38, 6.85 and 6.73 % day^{-1}), feed conversion ratio, FCR (1.15, 1.09, 1.13 and 1.17) and protein efficiency ratio, PER (2.26, 2.33, 2.20 and 2.14), respectively. The inclusion of SPH in diets for Nile tilapia statistically affected ($P<0.05$) the final fish body composition. Protein and ash contents decreased and fat content increased with SPH inclusion levels. This study clearly demonstrates that SPH could be included in diets for Nile tilapia without adverse effects on growth and protein utilization.

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1. INTRODUÇÃO

A crescente demanda por produtos pesqueiros para consumo humano tem resultado em um constante crescimento da produção aqüícola mundial. A aqüicultura é o setor da produção de alimentos de mais rápido crescimento, complementando e, em alguns casos, substituindo o pescado capturado na composição da oferta mundial (WATANABE, 2002).

A produção aqüícola requer rações de alta qualidade, com alto conteúdo protéico (LARA-FLORES et al., 2003). Geralmente, a proteína é o nutriente mais oneroso em rações aquáticas. Fontes protéicas marinhas (principalmente farinhas de peixe e de subprodutos) são utilizadas nestas rações por serem excelentes fontes de aminoácidos e ácidos graxos essenciais, vitaminas, minerais e, geralmente, aumentam a palatabilidade da ração (EL-SAYED, 1999; DAVIS & ARNOLD, 2000).

Atualmente, a farinha de peixe ainda permanece como a principal fonte protéica, compreendendo entre 20 e 60% das rações para peixes (WATANABE, op. cit.). Como a farinha de peixe é uma *commodity* de oferta limitada e demanda crescente (DAVIS & ARNOLD, op. cit.), a variabilidade da disponibilidade e constante flutuação nos preços deste insumo podem afetar seriamente a sustentabilidade e rentabilidade da aqüicultura (FARIA et al., 2001; OLVERA-NOVOA et al., 2002a).

A longo prazo, muitos países em desenvolvimento serão incapazes de manter a utilização da farinha de peixe como a principal fonte de proteína em rações aquáticas (EL-SAYED, op. cit.). A determinação de fontes protéicas de menor custo e que promovam bom crescimento é vantajoso para a indústria de rações como também para os aqüicultores (COYLE et al., 2004). Assim, pesquisas têm sido direcionadas a fontes protéicas alternativas que sejam, idealmente, de baixo custo e prontamente disponíveis como substitutas para a farinha de peixe (WEE & WANG, 1987; WATANABE, op. cit.).

A tilápia do Nilo *Oreochromis niloticus* é um dos mais importantes peixes cultivados nas regiões tropicais e subtropicais em todo o mundo. No Brasil, a produção de tilápia cultivada duplicou entre os anos de 2000 e 2004, passando de 32.459 para 69.078 toneladas, com crescimento médio de 22,4% ao ano no período (IBAMA, 2000; 2005). De toda a produção da aqüicultura continental brasileira em

2004, a tilápia respondeu por 38,4% do volume produzido, sendo a espécie com maior representatividade. Sua importância se torna maior quando analisada sua participação na produção da Região Nordeste, que correspondeu a 73% da produção da aqüicultura continental no mesmo período, totalizando 28,5 mil toneladas.

Apontada como a principal espécie da piscicultura brasileira devido a características biológicas e mercadológicas relevantes (FARIA et al., 2001), a tilápia é produzida em diferentes sistemas de cultivo e escalas, com maior ou menor dependência de insumos, principalmente ração. A intensificação do cultivo da tilápia requer o desenvolvimento de rações adequadas tanto para alimentação exclusiva em tanques como complementar em viveiros (KÖPRÜCÜ & ÖZDEMIR, 2005).

A aqüicultura é uma atividade geradora de renda e trabalho nas regiões em que se insere e o seu crescimento representa uma maior oferta de alimentos saudáveis e investimentos contínuos. Como atividade em rápido desenvolvimento tecnológico, requer a utilização de rações de alta qualidade, característica que implica elevado valor econômico deste insumo. Despesas com alimentação artificial podem representar mais de 50% dos custos de produção, com a proteína sendo o nutriente mais caro. O desenvolvimento de rações comerciais para aqüicultura tem sido tradicionalmente baseado na utilização de farinha de peixe como principal fonte de proteína, devido à qualidade nutricional deste ingrediente. Entretanto, o crescente preço internacional da farinha de peixe tem onerado as rações e, conseqüentemente, os custos de alimentação na aqüicultura.

Assim, a obtenção de ingredientes alternativos, como farinhas ou hidrolisados protéicos obtidos de resíduos do processamento industrial de alimentos, que atuem como fontes de proteína e que sejam mais baratos e acessíveis que a farinha de peixe, pode resultar em rações aqüícolas de menor custo, mantendo ou melhorando a qualidade nutricional das rações destinadas à alimentação animal.

2. OBJETIVOS

2.1 Objetivo Geral

Avaliar a utilização do hidrolisado protéico de camarão como fonte protéica em rações para juvenis da tilápia do Nilo *Oreochromis niloticus*, quando incluído como substituto à farinha de peixe.

2.2 Objetivos Específicos

- Avaliar o desempenho em crescimento de alevinos de tilápia alimentados com rações contendo diferentes concentrações de hidrolisado protéico de camarão;
- Comparar as relações comprimento-peso dos peixes;
- Mensurar o valor nutricional das rações através do seu aproveitamento;
- Examinar a composição corporal dos peixes.

3. REVISÃO BIBLIOGRÁFICA

A farinha de peixe é amplamente empregada na aqüicultura, sendo a principal fonte protéica nas rações para a maioria das espécies cultivadas, principalmente por apresentar bom perfil de aminoácidos e ácidos graxos essenciais, energia digestível, minerais e vitaminas, além de conferir melhor palatabilidade às rações (EL-SAYED, 1999), o que resulta em melhoras nos parâmetros de desempenho produtivo. Porém, seu custo geralmente elevado, baixa disponibilidade e qualidade às vezes duvidosa são limitantes ao uso da farinha de peixe em rações animais (FARIA et al., 2001), o que tem motivado a busca por potenciais substitutos à farinha de peixe.

Diversos produtos têm sido utilizados com o propósito de substituir total ou parcialmente a farinha de peixe em rações aquáticas, incluindo subprodutos de pescado ou de animais terrestres, sementes oleaginosas, plantas aquáticas, concentrados protéicos, proteína de organismos unicelulares (*single cell protein*) e subprodutos de leguminosas e cereais (EL-SAYED, op. cit.).

Farelo de soja tem sido preconizado como a principal fonte protéica de origem vegetal em rações para peixes, embora haja restrições (FURUYA et al., 2001a). O farelo de soja tem um alto teor de proteína com um bom perfil de aminoácidos, preço razoável, composição e disponibilidade constantes; entretanto, seu nível de metionina é baixo, contém aproximadamente 30% de carboidratos indigestíveis e vários compostos ou fatores antinutricionais que podem perturbar os processos digestivos (HERNÁNDEZ et al., 2006). Assim, a utilização do farelo de soja em rações animais exige um adequado processo de fabricação deste produto, através do aquecimento para inativação dos fatores antinutricionais (FURUYA et al., 2001b), ou a necessidade de suplementação de aminoácidos para reparar o seu perfil de aminoácidos (FURUYA et al., 2001c).

De forma geral, a viabilidade da substituição da farinha de peixe por ingredientes vegetais estará condicionada ao hábito alimentar do animal que se pretende alimentar. Em oposição aos peixes carnívoros, os peixes onívoros possuem adaptações morfológicas e fisiológicas que possibilitam a utilização de rações com elevadas porcentagens de ingredientes vegetais, pois utilizam melhor os carboidratos e a proteína (aminoácidos) dessas fontes (FURUYA op. cit.).

Kaushik et al. (2004) produziram dietas com redução de farinha de peixe de 100% a 2% e incremento de fontes protéicas vegetais (glúten de milho e trigo, farelos de soja e colza) para o robalo europeu *Dicentrarchus labrax*, resultando em desempenhos de crescimento idênticos e nenhuma conseqüência adversa, ressalvando-se a eficiência da suplementação de fósforo inorgânico nas dietas com maior inclusão de ingredientes vegetais.

A tilápia nilótica tem hábito alimentar onívoro, com sistema digestório que difere tanto de carnívoros como de herbívoros, o que permite utilizar um amplo espectro de alimentos (SKLAN et al., 2004a), digerir eficientemente os carboidratos da dieta (BOSCOLO et al., 2002) e maior capacidade de digerir proteínas vegetais. Assim, Olvera-Novoa et al. (2002a) testaram o uso de farinha de semente de girassol como fonte protéica substituta à farinha de peixe na alimentação de alevinos de tilápia (*Tilapia rendalli*), recomendando um nível de substituição de até 20%, o qual resultou em crescimento e eficiência alimentar similar ao proporcionado por uma ração com proteína derivada unicamente da farinha de peixe, porém com maior rendimento econômico. Contudo, os autores comentaram que níveis de inclusão superiores a este podem resultar em reduzido desempenho pelo agravamento da deficiência em fenilalanina e metionina e alto conteúdo de fibras não digeríveis da farinha de sementes de girassol.

Wee & Wang (1987) avaliaram a utilização de farinha de folhas de leucena (*Leucaena leucocephala*) como ingrediente substituto à farinha de peixe em dietas para alevinos de tilápia *O. niloticus*, não encontrando efeitos adversos no crescimento dos peixes com a inclusão de até 25% de farinha de folhas de leucena obtida a partir de folhas imersas em água por 48 horas. Segundo os autores, este procedimento eliminou a presença do aminoácido tóxico mimosina, o qual limita a utilização deste produto quando produzido por simples secagem ao sol.

A substituição da proteína da farinha de peixe por farinha de semente de algodão foi testada por Mbahinzireki et al. (2001), os quais conseguiram manter os mesmos índices de crescimento de alevinos de tilápia (*Oreochromis* sp.) com níveis de substituição de até 50%, sendo sua participação limitada pela presença de gossipol, o que reduz a aceitação e utilização de dietas baseadas em farinha de semente de algodão.

O grau de inclusão de fontes protéicas vegetais em rações aqüícolas parece ser limitado pela presença de fatores antinutricionais, em associação a deficiências em aminoácidos essenciais (FRANCIS et al., 2001) e à disponibilidade do fósforo, uma vez que, nos alimentos de origem vegetal, cerca de 70% deste mineral está complexado na forma de fitato, que não é utilizado pelos monogástricos e que promove também a redução na disponibilidade de outros elementos, como zinco, cálcio, ferro e manganês (FARIA et al., 2001). Com relação aos teores de fibras indigestíveis, Olvera-Novoa et al. (1997) recomendam o uso de concentrados protéicos como uma forma de eliminar as fibras indigestíveis do ingrediente integral, permitindo o uso de maiores níveis de materiais vegetais nas rações para peixes.

Vários subprodutos animais têm sido testados como principais provedores de proteína em rações para peixes. Coyle et al. (2004) avaliaram o uso de diferentes fontes protéicas (farinha de peixe, farinha de carne e osso e farelo de soja) em combinação com grãos e solúveis secos de destilaria para alimentar alevinos híbridos de tilápia (*Oreochromis niloticus* x *O. aureus*), não encontrando diferenças significativas em crescimento e aproveitamento do alimento proporcionados pela ração à base de farinha de peixe e aquelas em que este ingrediente foi substituído em até 30%.

Impulsionado por volumes crescentes de produção (cultivo e captura) de camarões, a farinha de resíduos de camarão tem sido identificada como uma fonte de proteína animal de grande potencial (FANINO et al., 2000), contribuindo ainda para a redução de problemas ambientais decorrentes da inadequada destinação de resíduos do processamento do camarão, como cabeças, carapaças e caudas (HEU et al., 2003). Uma das possíveis soluções para o problema do resíduo de cabeça de camarão é transformá-lo em materiais para uso na formulação de rações animais, inclusive peixes (CAVALHEIRO et al., 2007).

O resíduo do processamento do camarão é rico em proteína e quitina. A fermentação deste resíduo por ácido lático tem sido relatada como uma técnica eficaz e econômica de proteger esta biomassa da decomposição bacteriana, formando uma silagem que contém um líquido rico em proteínas, uma fração lipídica e quitina insolúvel; geralmente, o hidrolisado de resíduo de camarão tem alto teor de aminoácidos essenciais, indicando um alto valor nutricional (LÓPEZ-CERVANTES et al., 2006). Entretanto, o uso destes subprodutos pode ser restringido devido aos altos conteúdos de fibras e cinzas, resultando na formação de péletes fracos, com baixa hidroestabilidade (CAVALHEIRO et al., 2007). De fato, os altos conteúdos em cinzas e fibras reduziram a digestibilidade em tilápias das farinhas obtidas dos crustáceos *Gammarus kischineffensis* e *Astacus leptodactylus leptodactylus* (KÖPRÜCÜ & ÖZDEMIR, 2005). Elevados níveis de quitina também prejudicam peixes carnívoros. Olsen et al. (2006) relatam que a quitina presente nas dietas baseadas em farinha de *krill* (*Euphausia superba*) reduziu a absorção de lipídios e aumentou o teor de água (diarréia) nas fezes do salmão *Salmo salar*.

Fanino et al. (2000) determinaram a qualidade protéica de uma farinha de subprodutos de camarão (cabeça, apêndices e exoesqueleto) e demonstraram que o valor biológico da proteína é inferior ao da proteína da farinha de peixe; porém, quando aquela farinha foi suplementada com lisina e metionina houve melhora da qualidade protéica.

López-Cervantes et al. (2006) identificaram tirosina, treonina, leucina e glicina como os aminoácidos mais abundantes na fração protéica liofilizada do hidrolisado de resíduo de camarão. Estes autores afirmam, ainda, que aminoácidos livres são uma das mais importantes frações não-protéicas e alguns deles, como alanina, ácido glutâmico e glicina, são responsáveis pelo sabor e odor característicos destes produtos.

A inclusão de farinha de cabeça de camarão, produzida por silagem ácida, substituindo a farinha de peixe em até 100%, não reduziu os parâmetros de crescimento de alevinos de *O. niloticus* (CAVALHEIRO et al., 2007). A substituição total da farinha de peixe foi testada por El Sayed (1998) com o uso das farinhas de camarão, de carne e ossos, de subprodutos de frango e de sangue, sem diferença estatística no crescimento de alevinos de *O. niloticus* com os três primeiros produtos em comparação à farinha de peixe, embora tenham prejudicado os valores de conversão alimentar e eficiência protéica.

Silva (2004) elaborou um hidrolisado protéico a partir de cabeças do camarão marinho *Litopenaeus vannamei*, por meio da trituração deste resíduo e digestão por autólise (45°C), seguida por desativação enzimática (100°C), separação e centrifugação, obtendo-se o concentrado protéico sobrenadante, o qual foi considerado como uma excelente fonte alimentar, sobretudo de aminoácidos, com ácido glutâmico, ácido aspártico, leucina, lisina, tirosina e arginina como os mais abundantes. Heu et al. (2003) afirmam que produtos obtidos a partir do processamento de resíduos de camarão podem servir como uma adequada fonte de proteína e flavorizante em formulações alimentares, devido principalmente aos seus teores de aminoácidos livres.

4. ARTIGO CIENTÍFICO

Parte dos resultados obtidos do trabalho experimental dessa dissertação será apresentada em artigo intitulado **"Utilization of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus*, L.) feeds"** (manuscrito), que se encontra anexado a seguir.

MANUSCRITO

UTILIZATION OF SHRIMP PROTEIN HYDROLYSATE IN NILE TILAPIA (*Oreochromis niloticus*, L.) FEEDS

Manuscrito a ser submetido à revista *Aquaculture*, ISSN 0044-8486.

Utilization of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus*, L.) feeds

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Abstract

A 45-day feeding trial was carried out to evaluate the use of shrimp protein hydrolysate (SPH) in diets to *Oreochromis niloticus*, L. SPH was included in isonitrogenous diets at levels of 0, 5, 10 and 20% of fish meal protein replacement and offered to juvenile Nile tilapia (1.7±0.4g) stocked in 40-L glass aquaria. The inclusion of SPH did not produce significant differences ($P \geq 0.05$) on final weight, survival, weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU). The inclusion of SPH in diets for Nile tilapia significantly affected ($P < 0.05$) the final fish body composition. Protein and ash contents decreased and fat content increased slightly with SPH inclusion levels. This study clearly demonstrates that SPH could be included up to 6% in diets for Nile tilapia without adverse effects on growth and nutrient utilization.

Keywords: tilapia, shrimp protein hydrolysate, growth, protein utilization

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1 Introduction

Aquaculture requires high-quality feeds with high protein content (Lara-Flores et al., 2003). In general, protein is the most expensive nutrient in aquafeeds. Marine protein sources (mainly fish and by-products meals) generally enhance aquafeeds palatability and are excellent sources of essential amino acids and fatty acids, vitamins and minerals (El-Sayed, 1999; Davis and Arnold, 2000).

At present fish meal still remains as the major dietary protein source, comprising between 20 and 60% of fish feed (Watanabe, 2002). On the long-turn, many developing countries will be unable to depend on fish meal as a major protein source in aquafeeds (El-Sayed, 1999). The determination of less-expensive sources of protein which provides good growth is advantageous for diet manufactures and aquaculture producers alike (Coyle et al., 2004).

Nile tilapia, *Oreochromis niloticus*, Linnaeus (1758) is one of the most cultured fish in tropical and subtropical regions of the world. Tilapia is an omnivorous species that has a digestive system that differ both from those of carnivorous and from many herbivorous fish, utilize a wide spectrum of foods (Sklan et al., 2004a), use dietary carbohydrate efficiently (Boscolo et al., 2002) and has a great ability to digest plant protein.

Hence, many products has been tested as protein source to tilapia: soybean meal; *Leucaena* leaf meal (Wee and Wang, 1987); feather meal (Bishop et al., 1995); cowpea protein concentrate (Olvera-Novoa et al., 1997); shrimp, blood, meat and bone and poultry by-product meals (El-Sayed, 1998); cottonseed meal (Mbahinzireki et al., 2001); sunflower cakes, anchovy meal and wheat bran (Maina et al., 2002); sunflower meal (Olvera-Novoa et al., 2002a); a mix of soybean meal, cottonseed meal, sunflower meal and linseed meal (El-Saidy and Gaber, 2003); distillers dried grains with solubles (Coyle et al., 2004); corn gluten, rapeseed meal, sorghum and barley (Sklan et al., 2004b); soybean meal, maize gluten meal, dehulled flax, pea and canola protein concentrates (Borgeson et al., 2006).

Despite of this, the inclusion of plant protein sources in aquafeeds is limited by antinutritional factors, associated to amino acids imbalances (Francis et al., 2001), and fiber levels (Olvera-Novoa et al., 1997). Additionally, animal by-products demand attention because their potential risk to cause health-related problems (Olvera-Novoa et al., 2002b).

Stimulated by increasing shrimp production from catches and farming, the shrimp waste meal has been identified as an animal protein source with great potential (Fanino et al., 2000), and could reduce environmental problems as a result of improper deposition of inedible parts of shrimp, such as heads, shells and tails (Heu et al., 2003). Nevertheless, the use of shrimp waste meal could be restricted due to its high fiber and ash contents (Cavalheiro et al., 2007). Accordingly, ash and fiber contents reduced crustacean meal digestibility in tilapia (Köprücü and Özdemir, 2005) and decreased lipid absorption and increased water content in Atlantic salmon *Salmo salar* L. faeces (Olsen et al., 2006).

Silva (2004) produced a shrimp protein hydrolysate (SPH) from Pacific white shrimp, *Litopenaeus vannamei*, Boone (1931) heads, which was considered an excellent protein source (because of its amino acids profile) and low fiber content. Products obtained from shrimp processing wastes may serve as an useful source of protein and flavorants in food formulations, mainly due to its free amino acids levels (Heu et al., 2003; Ruttanapornvareesakul et al., 2005). This study aimed to evaluate the nutritional quality of SPH by assessing growth performance and protein utilization of juvenile Nile tilapia.

2 Materials and Methods

2.1 Diets

Four isonitrogenous (37% crude protein) and isocaloric (440 kcal 100 g^{-1}) experimental diets were formulated to feed *Oreochromis niloticus* juvenile (Tables 1 and 2). Shrimp protein hydrolysate was included in the diets at 0 (as control), 1.5, 3 and 6% inclusion levels, which corresponded to 0, 5, 10 and 20% of fish meal protein replacement. The SPH was incorporated to soybean meal and the dough was dried (65°C for 24h).

Table 1. Composition of the experimental diets.

Ingredients (%)	Diets			
	SPH0	SPH5	SPH10	SPH20
Fish meal (57% CP)	23.0	22.0	21.0	18.0
Shrimp protein hydrolysate (SPH) (43.6% CP)	0.0	1.5	3.0	6.0
Soybean meal (40.4% CP)	47.0	47.5	47.5	47.5
Wheat meal	16.0	13.5	13.5	15.5
Corn starch	10.5	12.0	11.5	9.5
Soybean oil	1.0	1.0	1.0	1.0
Dicalcium phosphate	1.0	1.0	1.0	1.0
Mineral and vitamin mix[1]	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5
Antioxidant BHT	0.02	0.02	0.02	0.02

CP = crude protein. BHT = butylated hydroxytoluene.

[1] Mineral and vitamin mix (quantity kg^{-1} premix): vitamin A (20,000 UI), vitamin D_3 (5,000UI), vitamin E (250 mg), vitamin K_3 (25 mg), vitamin B_1 (37.5 mg), vitamin B_2 (37.5 mg), vitamin B_6 (25 mg), vitamin B_{12} (0.053 mg), vitamin C (250 mg), niacin (200 mg), pantothenic acid (100 mg), biotin (1,25 mg), choline (1.000 mg), inositol (250 mg), Fe (100 mg), Cu (12 mg), Zn (125 mg), Mn (37,5 mg), Se (0,25 mg), I (1,25 mg), Co (0,25 mg).

The ingredients were mixed and the diets prepared by extrusion under industrial conditions to obtain 1-mm diameter pellets. A commercial diet (COM, 36% crude protein) was used as a reference.

2.2 Animals and Experimental Conditions

Juvenile sex-reversed Nile tilapia were obtained from Universidade Federal Rural de Pernambuco Aquaculture Station. Groups of eight fish were stocked in each of fifteen 40-L glass aquaria equipped with biological filter and continuous aeration. After a 7-day acclimatization period, diets were randomly assigned to three groups of fish. Fish were weighed (1.7±0.4 g) and measured (4.7±0.4 cm) before the onset of feeding trial. Diets were offered four times a day (800, 1100, 1400 and 1700 hrs), and feeding rates were gradually reduced from 15% to 6% of aquaria biomass, adjusted each nine days, during 45 days.

Aquaria were siphoned twice daily and submitted to a 66% water exchange. Water temperature, dissolved oxygen, pH, ammonia and nitrite were monitored and averaged 28.7±0.59ºC (mean±standard deviation), 3.5±0.92 mg L^{-1}, 8.1±0.19, 0.14±0.22 mg L^{-1} and 0.08±0.02 mg L^{-1}, respectively.

2.3 Growth and Nutrient Utilization

Fish performance was evaluated through weight gain rate (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU), according to the following formulae: WG = 100 ((BW_f - BW_i) / BW_i); ADG = WG (g) / time (days); SGR = 100 (Ln BW_i – Ln BW_f) / time (days); FCR = dry feed offered (g) / wet weight gain (g); PER = wet weight gain (g) / protein fed (g); and ANPU = 100 ((BW_f x BCP_f) – (BW_i x BCP_i)) / (TF x CP), where BW_i and BW_f = average initial and final body weight (g) of fish, respectively; BCP_i and BCP_f = initial and final body crude protein (g $100g^{-1}$) respectively; TF = total amount of diet fed (g), and CP = crude protein of diet (g $100g^{-1}$).

Table 2. Proximate analysis of the commercial and experimental diets.

Proximate analysis (as-fed basis, g kg^{-1})	Diets				
	COM	SPH0	SPH5	SPH10	SPH20

Dry matter	918.0	944.8	935.9	936.5	946.7
Crude protein	345.6	371.9	374.3	376.2	380.6
Ether extract	65.0	48.1	56.2	52.1	35.9
Crude fibre	34.9	39.7	38.8	41.1	46.6
Ash	67.5	105.7	102.9	101.6	101.9
Nitrogen-free extract	487.0	434.6	427.8	429.0	435.0
Calcium	14.3	22.2	21.7	20.0	17.2
Phosphorus	11.3	12.4	12.5	12.6	12.8
Gross energy (kcal 100 g^{-1})[1]	461.5	438.3	444.5	442.2	431.8
P/GE ratio (mg $kcal^{-1}$)	74.9	84.9	84.1	84.6	88.0

[1] Estimative based on 5.65, 4.2 and 9.5 kcal g^{-1} for protein, carbohydrate and lipid, respectively.

Fish length and weight data were plotted (*X* and *Y*, respectively) to allow analysis of length-weight relationship, using the mathematical model $Wt = \Phi Lt^{\theta}$ to adjust the tendency of these plots (Santos, 1978).

2.4 Analytical methods

At the end of trial, two fish from each aquarium were sampled and frozen for determination of body composition. Initial body composition analyses were performed on a pooled sample of eight fish which was frozen prior to the study. Moisture, lipid, protein and ash contents were determined using standard methods (AOAC, 1984).

2.5 Statistical Analysis

A one-way analysis of variance (ANOVA) was used to test the effects of SPH inclusion in the diets on fish performance. Tukey’s test was used at $\alpha = 0.05$ to test for differences among treatment means when *F*-values from the ANOVA were significant. The models of length-weight relationship were confronted using the statistic *W*, which was compared to qui-square distribution at $\alpha = 0.05$ (Mendes, 1999). Data obtained from commercial diet were not used in statistical analysis.

3 Results

The effects of SPH inclusion on tilapia performance and nutrient utilization are given on Table 3. The level of SPH incorporated on diets (0, 1.5, 3 or 6%) did not affect (*P*≥0.05) final fish weight (27.18, 29.46, 26.02 and 25.19 g), weight gain rate (1,571, 1,624, 1,388 and 1,301%), average daily gain (0.57, 0.62, 0.54 and 0.52 g day^{-1}) and specific growth rate (7.15, 7.38, 6.85 and 6.73% day^{-1}). No mortality was observed during the feeding trial. The indicators feed conversion ratio (1.15, 1.09, 1.13 and 1.17), protein efficiency ratio (2.26, 2.33, 2.20 and 2.14) and apparent net protein utilization (39.31, 40.39, 38.57 and 34.72) also were not affected by SPH inclusion.

The parameters of the mathematical models to evaluate length-weight relationships of fish fed different diets are shown in Table 4. Evaluation of these models revealed statistical differences (*P*<0.05) on fish growth. Fish fed SPH5 (1.5% inclusion rate) presented the best length-weight relationship. Higher SPH inclusion levels (3 and 6%) did not contribute for fish growth, resulting in similar or worse growth performance to that provided by the SPH0 diet.

Table 3. Growth performance and nutrient utilization in Nile tilapia fed diets with increasing substitution of fish meal by shrimp protein hydrolysate (SPH) and a commercial diet (COM).

Diets	SPH0	SPH5	SPH10	SPH20	COM
Initial weight (g)	1.68±0.45	1.72±0.38	1.75±0.41	1.81±0.49	1.55±0.18
Final weight (g)	27.18±9.38[a]	29.46±9.12[a]	26.02±9.20[a]	25.19±8.51[a]	16.40±6.46
Survival (%)	100.0	100.0	100.0	100.0	100.0
WG (%)[1]	1571±496[a]	1624±237[a]	1388±294[a]	1301±319[a]	961±158
ADG (g day^{-1})[2]	0.57±0.10[a]	0.62±0.04[a]	0.54±0.12[a]	0.52±0.10[a]	0.33±0.04
SGR (% day^{-1})[3]	7.15±0.58[a]	7.38±0.10[a]	6.85±0.68[a]	6.73±0.57[a]	5.29±0.38
FCR[4]	1.15±0.22[a]	1.09±0.16[a]	1.13±0.10[a]	1.17±0.12[a]	1.28±0.05
PER[5]	2.26±0.39[a]	2.33±0.36[a]	2.20±0.18[a]	2.14±0.22[a]	2.07±0.08
ANPU[6]	39.31±6.75[a]	40.39±6.31[a]	38.57±3.19[a]	34.72±3.61[a]	32.08±1.27

Values are mean ± standard deviation. Within a row, means with different superscript letters differ significantly (P<0.05) by Tukey test. Data obtained from commercial diet were not used in statistical analysis.

[1]Weight gain rate (WG) = 100 ((BW_f - BW_i) / BW_i); [2]Average daily gain (ADG) = WG (g) / time (days); [3]Specific growth rate (SGR) = 100 (Ln BW_i – Ln BW_f) / time (days); [4]Feed conversion ratio (FCR) = dry fed offered (g) / wet weight gain (g); [5]Protein efficiency ratio (PER) = wet weight gain (g) / protein fed (g); [6]Apparent net protein utilization (ANPU) = 100 ((BW_f x BCP_f) – (BW_i x BCP_i)) / (TF x CP), where BW_i and BW_f = average initial and final body weight (g) of fish, respectively; BCP_i and BCP_f = initial and final body crude protein (g $100g^{-1}$) respectively; TF = total amount of diet fed (g), and CP = crude protein of diet (g $100g^{-1}$).

The evolution of mean weight of Nile tilapia fed diets containing 0, 5, 10 and 20% of SPH protein as partial substitute for fish meal protein and commercial diet is presented in Figure 1. Mean weight of fish linearly enhanced throughout feeding trial. The experimental diets provided equal (P<0.05) growth performances among themselves.

Table 4. Parameters of the mathematical models ($Wt = \Phi Lt^{\theta}$) adjusted to length-weight data of fish fed diets with increasing shrimp protein hydrolysate (SPH) inclusion levels and commercial diet (COM) over a 45-days feeding trial.

Treatment	Φ	θ	R^2	n	C.S.[1]
SPH0	0.0134	3.1035	0.9919	129	b
SPH5	0.0132	3.1163	0.9950	126	a
SPH10	0.0142	3.0682	0.9924	128	b,c
SPH20	0.0149	3.0412	0.9933	125	c
COM	0.0163	2.9995	0.9889	127	-

[1]Comparative statistic using statistic W, compared to χ^2 distribution at $\alpha = 0.05$. Data obtained from commercial diet were not used in statistical analysis.

Initial and final body compositions of whole fish are shown in Table 5. The inclusion of SPH in diets for Nile tilapia significantly affected (P<0.05) final fish body composition. Protein and ash contents decreased and fat content increased slightly with SPH inclusion levels. Fish fed SPH 10 and SPH 20 presented higher fat contents (58.4 and 59.8 g kg^{-1}, respectively) than fish fed diets without (51.2 g kg^{-1}) or with the lowest SPH inclusion level (50.3 g kg^{-1}). The inclusion of SPH reduced ash contents; fish fed the diet with no SPH presented higher ash content (40.5 g kg^{-1}) than the others ones.

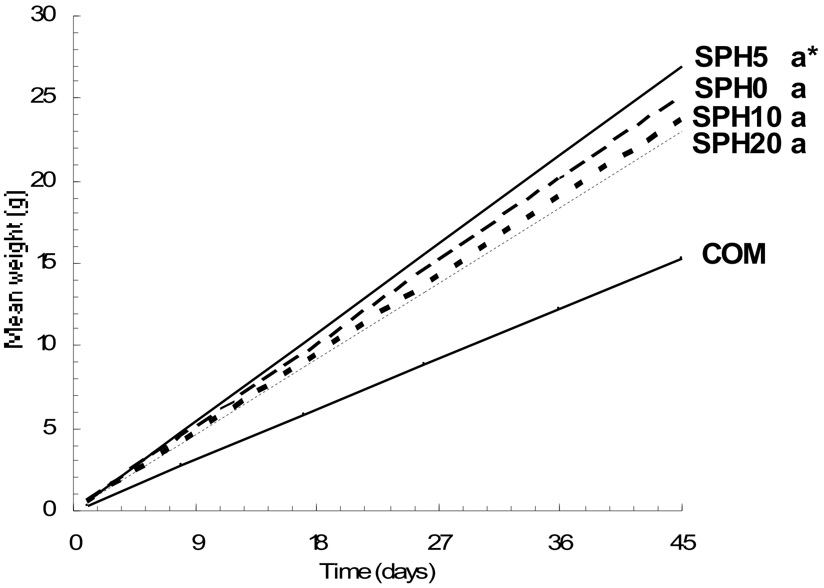

Figure 1. Mean weight evolution of Nile tilapia fed diets with increasing shrimp protein hydrolysate (SPH) inclusion levels and commercial diet (COM) over a 45-days feeding trial (*different superscript letters differ significantly, P<0.05). Data obtained from commercial diet were not used in statistical analysis.

4 Discussion

The present study clearly demonstrated that SPH could be included in diets for Nile tilapia without reducing growth rates. These findings revealed that the nutritional utilization values of the feed and protein (FCR and PER) in fish fed the test diets were not significantly different. This means that the four experimental diets tested have enough quality to assure good growth of the fish, corroborated by the maximum percentage of survival registered, which also reflect adequate handling and experimental conditions.

Table 5. Initial and final proximate composition (g kg^{-1} on as-fish basis) of whole body of Nile tilapia fed diets with increasing shrimp protein hydrolysate (SPH) inclusion levels and commercial diet (COM) over a 45-days feeding trial.

Fish	Dry matter	Protein[1]	Fat[1]	Ash[1]
Initial body composition	260.4	150.4±0.0[a]	45.3±0.0[a]	34.3±0.0[a]
Final body composition				
SPH0	270.3	163.2±0.2[b]	51.2±0.1[b]	40.5±0.1[c]
SPH5	266.2	161.9±0.0[b]	50.3±0.0[b]	37.4±0.0[b]
SPH10	274.0	163.0±0.5[b]	58.4±0.0[c]	35.9±0.0[a,b]
SPH20	272.3	153.7±0.0[a,b]	59.8±0.0[c]	36.2±0.0[a,b]
COM	282.5	142.8±0.1	89.0±0.2	30.5±0.1

[1]Each value is the mean (± standard deviation) of two replicates. Mean with common superscript in the same column are not significantly different ($P<0.05$). Data obtained from commercial diet were not used in statistical analysis.

Fish fed actively on all diets, but it was observed territorial behaviour related to feeding competition, which could conduce toward heterogeneous growth (Pereira-da-Silva et al., 2004), although not evaluated, in spite of adopting high feeding frequency (Sanches and Hayashi, 2001).

The inclusion of SPH as protein source did not affect feed conversion ratio. The utilization of dietary protein is dependent upon its essential amino acid profile. The more closely the dietary protein meets the needs of the animal for essential amino acids, the greater will be its assimilation and utilization for growth (Abdelghany, 2003).

Coyle et al. (2004) evaluated the use of fish, meat and bone, and soybean meals associated to distillers dried grains with solubles to feed hybrid tilapia fries, without significant differences in growth or feed utilization, except to diet without animal protein. According to these authors, it is necessary that a portion of the protein be of animal source. Shrimp meal was used as fish meal substitute by El-Sayed (1998), who did not find significant differences on Nile tilapia growth, even with feed conversion and protein efficiency ratios has been significantly damaged, maybe due to fiber and ash contents.

El-Saidy and Gaber (2003) proposed a plant protein mixture to totally replace fish meal on juvenile Nile tilapia diets because its use did not show negative effects on fish growth. Cavalheiro et al. (2007) also did not mention negative effects on growth of Nile tilapia when replacing 100% fish meal by shrimp head silage. Considering both studies, the better growth performance reached by fish in the present study should be due to superior nutritional quality of formulated diets. In fact, the production process of SPH reduces its fiber and ash contents by solid fraction removal. Accordingly, nutrients digestibility may be reduced due to high ash and chitin contents (Köprücü and Özdemir, 2005).

Taking account of the utilization of plant-protein meal as a sole protein source in fish diets request amino acid supplementation (Furuya et al., 2001; Zhou et al., 2005), and fish meal reduction did not produce any negative effect in growth in this study, it was concluded that SPH could be used as an amino acids source in plant protein-based diets, with a possible positive stimuli effect on fish feeding (Pereira-da-Silva and Pezzato, 2000). Shrimp by-products contain large amounts of extractive compounds as such free amino acids, which significantly influence the taste of feed, and they may potentially serve as a good source of seasoning (Heu et al., 2003).

On the other hand, if all essential amino acids are present in the wrong amount and proportion, the biological value will be low since protein being diverted for energy supply rather than for body tissues; in this case, the amino acids will be deaminated and consequently more excretion of ammonia (Fanino et al., 2000).

The nutritional quality of SPH10 and SPH20 diets could be inquired mainly with regard to fat whole-body content. The higher fat carcass content on fish fed these diets could be attributed to inadequate protein (amino acids) and energy balance. An excessive energy intake at moderate protein levels will lead to fat deposition or the excess protein will be deaminated and the carbon skeleton used as energy, leading to fat deposition (El-Sayed and Teshima, 1992).

The trend of whole-body composition reflects diet composition was observed regard to ash on fish fed experimental diets, where ash contents of diets and whole-body fish were directly related. Although the increasing SPH inclusion levels in experimental diets, the ash contents decreased. It is a result of combined effects of low ash content in SPH and gradual reductions on amounts of fish meal employed.

5 Conclusion

In conclusion, our findings suggest that shrimp protein hydrolysate could be included up to 6% in the diets for juvenile Nile tilapia without adverse effects on growth and nutrient utilization for up to 45 days. Further research will be required to evaluate higher shrimp protein hydrolysate inclusions and its influence on stimuli feeding, enzymatic alterations and economical values.

6 Acknowledgments

We express our thanks to *Poytara Indústria e Comércio de Rações Ltda.* for the manufacturing of the experimental diets and to *Laboratório de Análise de Plantas & Rações (LAPRA)* of the *Empresa Pernambucana de Pesquisa Agropecuária (IPA)* for proximate composition determinations. Recognition is given to all the technical staff of the *Laboratório de Enzimologia* for their assistance in conducting the feeding trial.

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5. CONCLUSÃO

O hidrolisado protéico de camarão pode ser incluído em até 6% em dietas para juvenis da tilápia do Nilo (*Oreochromis niloticus*, L.) sem efeitos adversos na utilização do alimento e no crescimento dos peixes por até 45 dias. Novas pesquisas são requeridas para avaliar maiores níveis de inclusão de hidrolisado protéico de camarão e sua influência como estimulante alimentar, alterações enzimáticas e aspectos econômicos.

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7. ANEXO

Guide for Authors – AQUACULTURE

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