BRUNA CÁRITAS SOUZA DO VALLE FERREIRA

USO DE HIDROLISADO PROTEICO DE PEIXE E FLOCO MICROBIANO EM SUBSTITUIÇÃO À FARINHA DE PEIXE NA ALIMENTAÇÃO DE PÓS-LARVAS DO CAMARÃO MARINHO *Litopenaeusvannamei* (BOONE,1931)

Recife

2013

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

USO DE HIDROLISADO PROTEICO DE PEIXE E FLOCO MICROBIANO EM SUBSTITUIÇÃO À FARINHA DE PEIXE NA ALIMENTAÇÃO DE PÓS-LARVAS DO CAMARÃO MARINHO *Litopenaeus vannamei* (BOONE,1931)

Bruna Cáritas Souza do Valle Ferreira

Orientadora: Profª. Drª. Roberta Borda Soares

Co-orientador: Prof. Dr.Sílvio Ricardo Maurano Peixoto

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

Recife

Março, 2013

Ficha catalográfica

V181u Valle, Bruna Cáritas Souza Ferreira
Uso de hidrolisado proteico de peixe e floco microbiano em substituição a farinha de peixe na alimentação de pós-larvas do camarão marinho *Litopenaeus vannamei* (Boone, 1931) / Bruna Cáritas Souza do Valle Ferreira – Recife, 2013.
62 f. : il.

Orientadora: **Roberta Borda Soares**
Dissertação (Mestrado em Recursos Pesqueiros e Aquicultura) – Universidade Federal Rural de Pernambuco, Departamento de Pesca e Aquicultura, Recife, 2013.
Inclui referências e anexo.

1. Carcinicultura 2. Fase berçário 3. Alimento proteico 4. Farinha de peixe 5. Floco microbiano 6. Hidrólise enzimática I. Soares, Roberta Borda, orientadora II. Título

CDD 639

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

USO DE HIDROLISADO PROTEICO DE PEIXE E FLOCO MICROBIANO EM SUBSTITUIÇÃO À FARINHA DE PEIXE NA ALIMENTAÇÃO DE PÓS-LARVAS DO CAMARÃO MARINHO *Litopenaeus vannamei* (BOONE,1931)

Bruna Cáritas Souza do Valle Ferreira

Esta dissertação foi julgada para a obtenção do título de **Mestre em Recursos Pesqueiros e Aquicultura** e aprovada em 18/02/2013 pelo Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura, em sua forma final.

Profª Drª Roberta Borda Soares

(Orientadora)
[Departamento de Pesca e Aquicultura]
[Universidade Federal Rural de Pernambuco]

Profª Drª Maria do Carmo Mohaupt Marques Ludke

[Departamento de Zootecnia]
[Universidade Federal Rural de Pernambuco]

Prof. Dr. Eudes de Souza Correia
[Departamento de Pesca e Aquicultura]
[Universidade Federal Rural de Pernambuco]

Prof. Dr. Ranilson de Souza Bezerra
[Departamento de Bioquímica]
[Universidade Federal de Pernambuco]

DEDICATÓRIA

Aos meus amados pais Alfredo e Sônia do Valle,

pelo amor e carinho dedicados.

Às minhas lindas sobrinhas Alice, Giovanna

e Brenda que enfeitam meus dias.

À Luciano Santos, meu esposo, pelo esteio, paciência, amor e cumplicidade em todos os momentos.

AGRADECIMENTOS

Mais uma vez agradeço a Deus por permitir que seja serena a minha caminhada e com grandes amigos à minha volta;

À Universidade Federal Rural de Pernambuco e aos professores do Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura pela contribuição na minha formação profissional;

Aos Orientadores Profª Roberta Soares e Profº Sílvio Peixoto pelos ensinamentos, disponibilidade, dedicação e atenção dadas, além da oportunidade de integrar sua equipe;

Aos órgão financiadores CNPQ e FACEPE;

Ao professor Eudes Correia pela eterna disponibilidade e atenção que dedica aos seus alunos, e pelo seu enorme coração que não consegue negar nada a ninguém;

Ao professor Ranilson pela disponibilização de espaço em seu laboratório para desenvolver minhas pesquisas;

Ao professor Ronaldo Cavalli pelo empréstimo dos livros pra consulta;

Á Camila Brito (Camilinha) e Nathalia Calazans (Nathi) por toda a força e amizade e por todos os grandes momentos de descontração dentro e fora do trabalho. Adoro vocês!

À Edmilson Dantas, companheiro nesta caminhada. Pensamos juntos, criamos juntos, nos aperreamos juntos, mas também comemoramos juntos os bons resultados;

À Emanuell Felipe, muito obrigada sempre pela disponibilidade em ajudar a qualquer hora e no que quer que seja. Você é verdadeiramente o amigo de todas as horas;

À Thaís Castelo Branco por toda ajuda com a tradução do artigo;

À toda equipe do Laboratório de Tecnologia em Aquicultura-LTA, Camila Brito, Nathalia Calazans, Edmilson Dantas, Vívian "Bora", Karin Barbosa, Camila Barros, Emanuell Felipe, Roberta Nery, Joana Vogeley, Juliana Interaminense, Bruna, Diego, Marcelo Soares e Jovêncio. Por todo empenho e dedicação aos nossos trabalhos, mesmo nos finais de semana e feriados. Obrigada gente!;

À Juliett Xavier pela disponibilidade, ajuda e presteza sempre que precisei;

À Fabiana Penalva e João Paulo pelos preciosos toques e ajuda no decorrer deste trabalho;

À Carolina Costa e Victor Andrade integrantes do LPM pela ajuda e disponibilidade;

Aos meus amados pais pela educação ofertada, pelos conselhos valiosos e acima de tudo, pelo amor dedicado;

Às minhas princesas, Alice, Giovanna e Brenda, crianças iluminadas cada uma com sua peculiaridade, são capazes de trazer alegria a qualquer um que tenha a oportunidade de conviver com elas;

Ao meu amado irmão Breno, um menino grande com um coração que não lhe cabe no peito e Bruno que apesar da distância sei que torce por mim;

Ao meu querido esposo Luciano, AMOR! Presente na minha vida em todos os momentos seja pra comemorarmos juntos ou me apoiar quando preciso. Certeza de poder contar em todos, todos os momentos. Obrigada!

Muito obrigada a todos vocês que fizeram e fazem parte da minha caminhada!

RESUMO

A presente dissertação teve como objetivo avaliar o efeito da substituição da farinha de peixe por hidrolisado proteico de peixe (HPP) e farinha de biofloco (FB) na alimentação de pós-larvas (2mg) do marinho camarão *Litopenaeus vannamei*. O HPP utilizado na elaboração das rações foi produzido a partir de resíduos de uma indústria de pescado. O biofloco para a produção da farinha foi obtido em tanques de cultivo de uma carcinicultura comercial. As rações para esta fase foram elaboradas para serem isoproteicas e isoenergéticas. Este experimento consistiu de seis tratamentos com três repetições cada, correspondendo ao tratamento controle o T0, utilizando a farinha de peixe como principal fonte proteica, e os demais tratamentos com substituição gradual da farinha de peixe por HPP e FB nas proporções de 10, 20, 30 e 40% correspondendo aos tratamentos T10, T20, T30 e T40 respectivamente. No experimento foi utilizado um tratamento com ração comercial que serviu como controle externo. Este ensaio teve duração de 42 dias. A sobrevivência dos camarões cultivados ficou acima de 99% em todos os tratamentos. As respostas estatísticas para os parâmetros de desempenho zootécnico (peso final, ganho de peso, taxa de crescimento específico) indicaram melhores resultados para os camarões alimentados com as rações formuladas quando comparados com os camarões alimentados com ração comercial. Os parâmetros testados tiveram seus pontos ideais indicados pela regressão, em níveis que variaram entre 15 e 16% de substituição da farinha de peixe por HPP e FB associados. Os resultados encontrados neste experimento indicaram o potencial dos ingredientes testados na substituição da farinha de peixe em rações para *L. vannamei*.

Palavras-chaves: carcinicultura, fase berçário, alimento proteico, farinha de peixe, floco microbiano, hidrólise enzimática

ABSTRACT

The present study aimed to evaluate the effect of replacing fishmeal for hydrolysate fish protein (HFP) and biofloc flour (BF) in diet of marine shrimp *Litopenaeus vannamei* postlarvae (2mg). The present study aimed to evaluate the effect of replacing fishmeal for hydrolyzed fish protein (HFP) and biofloc flour (BF) in Litopenaeus vannamei postlarvae. The HFP used in the diets formulation were produced from fish industry waste and the biofloc used for BF production was obtained in cultivation tanks of a commercial shrimp farm. Feed for this phase was designed to be isoenergetic and isoproteic. This experiment consisted of six treatments with three replicates each, corresponding to the control treatment T0, using fish meal as the main protein source. The other treatments (T10, T20, T30, T40) were a gradual replacement of fishmeal by HFP and BF in the proportions of 10, 20, 30 and 40% respectively. In the experiment a commercial feed was used as external control. It was a 42 days assay. Shrimp survival was above 99% in all treatments. After statistical analysis to zootechnical parameters (final weight, weight gain, specific growth rate) shrimps fed with formulated diets had better results than the ones fed with commercial feed. Tested parameters had ideal points indicated by regression in levels ranging between 15 and 16% of fishmeal replacement by HFP and associated BF. In the present study we inferred that BF and HFP are potential ingredients to replace fish meal in diets for *L. vannamei* postlarvae.

Keywords: shrimp, nursery phase, protein food, fish meal, microbial flake, enzymatic hydrolysis

LISTA DE TABELAS

Tabela 1. Proximal composition and amino acid of the main ingredients (g kg^{-1} of dry matter) which compose the feed..... 44

Tabela 2. Formulation of diets with partial replacement of fish meal by biofloc flour and fish protein hydrolyzate (g kg^{-1} dry matter)…….....45

Tabela 3. Profile of essential amino acids and non-essential and major polyunsaturated fatty acids (g kg^{-1} dry matter) in diets with replacement of fish meal by FPH and BF…..…….....46

Tabela 4.Mean values (±SD) performance of shrimps fed with rations containing different percentages of replacement of fishmeal by FPH and BF ranging from 10 to 40%.....47

LISTA DE FIGURAS

Figura 1. Regressions of final weight (A), weight gain (B), specific growth rate (C) and protein efficiency rate (D) of postlarvae of marine shrimp *Litopenaeus vannamei* fed diets with increasing substitution of flour fish by fish protein hydrolyzate (FPH) and biofloc flour (BF)…….....48

SUMÁRIO

Pág.

RESUMO VIII

ABSTRACT IX

LISTA DE TABELAS X

LISTA DE FIGURAS XI

1. INTRODUÇÃO 12

2. OBJETIVO.....13

3. REVISÃO DE LITERATURA 13

4. REFERÊNCIAS BIBLIOGRÁFICAS 19

5. ARTIGO CIENTÍFICO: Fishmeal replacement by fish protein hydrolysate and biofloc in diets for *litopenaeus vannamei* postlarvae.....20

6. REFERÊNCIAS BIBLIOGRÁFICAS.....36

7. ANEXOS 49

INTRODUÇÃO

O crescente déficit na quantidade de pescado capturado e a demanda de consumo tornaram a aquicultura uma das alternativas mais promissoras para o fornecimento de alimento de excelente valor nutritivo (CAMARGO e POUEY, 2005). A atividade da carcinicultura vem se mostrando um ramo da aquicultura extremamente promissor, com o cultivo, entre outras espécies, do camarão marinho *Litopenaeus vannamei*, sendo esta, a mais cultivada na América Latina e Ásia (ROJAS e ALFARO, 2007). No entanto, o déficit na pesca, além de incentivar o crescimento da aquicultura, trouxe consigo o problema da limitação na oferta de farinha de peixe e o aumento no seu valor.

Diversas pesquisas têm sido realizadas com o intuito de substituir total ou parcialmente a farinha de peixe na formulação de dietas para organismos aquáticos, mantendo os padrões nutricionais nos cultivos. Assim, ingredientes que confiram à ração níveis nutricionais adequados são de extrema importância. Proteínas vegetais e subprodutos de origem animal são algumas das fontes alternativas à farinha de peixe já investigadas (NAYLOR et al., 2009).

Dentre os subprodutos de origem animal com potencial para compor as dietas de organismos aquáticos estão os hidrolisados proteicos de peixes (HPP). O HPP está entre os produtos obtidos através da transformação de resíduos de pescado que possuem boa qualidade nutricional, sendo potenciais substitutos à farinha de peixe. Oetterer (2001), afirma que o HPP pode atingir uma concentração proteica de até 90%, além de apresentar propriedades funcionais úteis para a indústria alimentícia.

Outra potencial fonte proteica na alimentação para organismos aquáticos é o biofloco. Desenvolvido em sistemas de cultivo homônimo, oferece aos organismos cultivados em seu meio uma fonte a mais de nutrientes, complementando a proteína ofertada na ração. Estudos mostram que seu potencial nutritivo pode ser aproveitado como fonte proteica na formulação

de rações. Experimento desenvolvido por Soares et al. (2004), demonstrou que os bioflocos podem conter até 42% de proteína bruta, dependendo da sua composição.

Esses dois subprodutos já foram indicados como possíveis ingredientes na produção de rações para a aquicultura. No entanto, ainda não haviam sido reportados em conjunto. Neste contexto, o presente estudo visou avaliar o desempenho zootécnico do camarão marinho *L.vannamei* alimentados com rações contendo diferentes níveis de substituição da farinha de peixe por hidrolisado proteico de peixe (HPP) associado à farinha de biofloco (FB).

OBJETIVOS

Avaliar o desempenho zootécnico do camarão marinho *Litopenaeus vannamei* alimentado com rações formuladas com diferentes proporções de farinha de biofloco associado ao hidrolisado proteico de peixe adicionados à ração em substituição a farinha de peixe.

REVISÃO DE LITERATURA

Farinha de peixe

A pesca e a aquicultura são consideradas pela ONU como atividades estratégicas para a segurança alimentar sustentável do planeta, pois são capazes de fornecer alimento proteico de alta qualidade e gerar emprego tanto em países desenvolvidos, quanto em desenvolvimento (ARANA1999). No entanto, a atividade pesqueira tem se mostrado frágil, devido ao excessivo esforço de pesca, sofrido pelos estoques marinhos (ARANA, 1999). Esta fragilidade tem acarretado problemas com a oferta da farinha de peixe, item imprescindível à produção de ração na aquicultura. Segundo Naylor et al. (2000), o uso de farinha de peixe causa forte pressão de pesca sobre espécies forrageiras, ocasionando sobrepesca e até

depleção de alguns desses estoques, o que resulta na redução de alimento para as espécies em níveis tróficos superiores.

Grande parte da produção mundial de farinha de peixe tem sido direcionada a produção de ração para animais aquáticos. Em 2003 cerca de 53% da produção foi destinada à aquicultura (FAO, 2006). Os camarões cultivados geralmente são alimentados com dietas balanceadas constituídas aproximadamente por 25 a 35% de farinha de peixe (TACON E BARG, 1998). A exigência de proteína e lipídios na dieta de camarões peneídeos é considerada nutricionalmente importante, pois são fatores limitantes para o crescimento e componentes básicos na preparação de rações (CHO et al., 1994). Larvas de peixes e crustáceos necessitam de dietas com alto teor de energia para atender necessidades especiais de desenvolvimento. O fornecimento dessa energia é dado pelas proteínas e lipídios. As larvas destes animais requerem mais destes nutrientes do que juvenis da mesma espécie devido às maiores taxas de crescimento e metabolismo. Assim, uma dieta formulada para esta fase deve ter elevado nível de energia recomendando-se entre 50-60% de proteína e a presença dos ácidos graxos poli-insaturados eicosapentaenóico (20:5 n-3) – EPA e docosahexaenóico (22:6 n-3) -DHA numa proporção de 2:1 de respectivamente, para larvas marinhas (NRC, 2011). Animais cultivados em sistemas intensivos, alimentados com rações com deficiência de nutrientes essenciais, podem exibir um crescimento deficiente, deformidade ou serem susceptíveis a doenças (GUILLAUME, 1997).

As fontes proteicas de origem marinha principalmente a farinha de peixe, são as mais utilizadas para produção de rações por serem ótimas fontes de nutrientes (aminoácidos, ácidos graxos, vitaminas e minerais) e por aumentarem a palatabilidade das dietas (DAVIS e ARNOLD, 2000; EL-SAYED, 1999). Alguns estudos mostram que a concentração de proteína recomendada na alimentação de peneídeos varia entre 30 e 45% (AKIYAMA et al., 1991; ANDREWS e SICK, 1972; BALAZ, 1973; NEW, 1976; NEAL, 1980; PIEDAD-

PASCUAL, 1990). Porém, a estagnação da pesca e o elevado custo da farinha de peixe acabam encarecendo a produção de organismos aquícolas cultivados. Os custos com a alimentação na aquicultura chegam a responder por 70% dos gastos com o cultivo (KAUSHIK, 1989). Deste modo, a restrição ao uso da fariha de peixe será inevitável e produtos que possam substitui-la já são estudados.

A intensificação no desenvolvimento de pesquisas em busca ingredientes que possam suprir o potencial proteico da farinha de peixe e reduzir os custos com a produção é cada vez maior (NAYLOR et al., 2009). Alimentos que contenham pelo menos 20% de proteína bruta são considerados bons suplementos proteicos (DAVIS E ARNOLD, 2000) e dentre estes estão os ingredientes de origem vegetal e subprodutos de origem animal. No entanto, alguns autores reportam que fatores antinutricionais, deficiência em alguns aminoácidos, baixa palatabilidade e digestibilidade de alguns ingredientes de origem vegetal restringem o seu uso (DAVIS e ARNOLD, 2000; GATLIN et al., 2007; NUNES et al., 2006).

Já as fontes proteicas de origem marinha são frequentemente utilizadas na alimentação para organismos aquáticos por apresentarem excelente fonte de aminoácidos essenciais, ácidos graxos, vitaminas, minerais além de conferir aumento da palatabilidade das rações (DAVIS e ARNOLD, 2000). Dentre estes, estão as farinhas de peixe e lula e subprodutos de pescado, produzidos a partir do reaproveitamento dos resíduos gerados pelas indústrias de beneficiamento, sendo um deles o hidrolisado proteico de peixe.

Hidrolisado proteico de peixe

Desenvolvido inicialmente no Canadá na década de 40 (RUITER, 1999), o hidrolisado proteico de peixe (HPP) é obtido a partir do resultado da solubilização das proteínas do pescado através do processo de catalisação de enzimas proteolíticas que consiste na quebra de cadeias longas de moléculas proteicas resultando em partes solúveis e insolúveis. As partes

insolúveis contêm proteínas não hidrolisadas e outros materiais insolúveis, já a fração solúvel é rica em proteínas, peptídeos e aminoácidos livres (MARTONE, et al., 2005). O HPP teve sua primeira aplicação como fonte de nitrogênio amínico para a cultura de microrganismos, demonstrando que a carne de pescado hidrolisada por enzimas propicia um bom desenvolvimento bacteriano (FURLAN e OETTERER, 2002).

A hidrólise proteica pode acontecer de forma química ou enzimática. A hidrolise química está dividida em hidrólise ácida (ácidos orgânicos ou inorgânicos) ou alcalina (soluções de bases fortes). A hidrólise enzimática utiliza enzimas de origem animal, vegetal ou microbianas em sua produção (KRISTINSSON e RASCO, 2000; MARTONE et al., 2005). Dentro das enzimas microbianas, a alcalase destaca-se na produção industrial. Esta enzima, produzida a partir do *Bacillus licheniformis*, é amplamente utilizada nas indústrias alimentícias por ter gosto suave mesmo quando tem elevado grau de hidrólise (BENJAKUL e MORRISSEY, 1997; CENTENARO et al. 2009; KRISTINSSON e RASCO, 2000).

Segundo Hardy (1991), HPP's são ingredientes possíveis de serem utilizados na aquicultura principalmente como suplementos protéicos, atrativos e potenciadores da palatabilidade. Refstie et al. (2004) constataram que a substituição dietética de 10 a 15% de farinha de peixe por um HPP tratado com enzima comercial afetou positivamente o desempenho de crescimento de salmão do Atlântico. É relatado que ingredientes como os hidrolisados protéicos possuem propriedades nutracêuticas e têm sido utilizados como suplementos para melhorar as propriedades nutricionais dos alimentos (HAARD, 2001). Anggawati et al. (1990) ao utilizar hidrolisados de peixe na alimentação de *Penaeus monodon* observaram que a substituição de 3% da farinha de peixe por hidrolisado foi suficiente para aumentar o crescimento do camarão. Porém, eles não investigaram as razões deste incremento no crescimento do animal. No entanto, estudos sobre o crescimento de larvas de peixes confirmam que a alimentação com HPP melhora o crescimento e

desenvolvimento do sistema digestivo nesta fase (DAY et al, 1997; ZAMBONINO-INFANTE et al, 1997),conferindo a este produto alta capacidade de utilização. Hernandéz et al. (2011), encontraram um bom potencial proteico em hidrolisados de atum para a limentação de juvenis de *L.vannamei*. Cahu et al. (1999) observaram que a substituição de 25% de farinha de peixe por um HPP com enzima comercial melhorou a digestão no indivíduo adulto e o desenvolvimento de larvas de robalo enquanto taxas de substituição de 50 e 75% levou a uma redução do crescimento larval.

Outro produto que se apresenta como um potencial substituto ao uso da farinha de peixe é produzido em alguns ambientes de cultivo aquícola. O biofloco é utilizado em sistemas que trabalham com altas densidades, servindo de fonte extra de proteína para os organismos cultivados.

Flocos microbianos ou Bioflocos

O sistema BFT (Biofloc Technology) foi desenvolvido na década de 90 e possui como vantagens a reduzida ou zero troca de água e de descarga de efluentes, menor impacto ambiental devido à reutilização de nutrientes durante o ciclo, redução dos riscos de introdução de doenças, além do efeito benéfico da produção natural dentro dos tanques/viveiros sendo esta uma técnica sustentável no cultivo de camarões (AVNIMELECH, 2008; BOYD e CLAY, 2002; CRAB et al, 2007; WASIELESKY et al., 2006).

Em cultivos neste sistema, a comunidade autotrófica fitoplanctônica comumente presente nestes ambientes é parcialmente substituída por uma comunidade bacteriana heterotrófica mais estável que se junta às partículas inorgânicas, detritos orgânicos, microalgas, protozoários, entre outros, formando agregados microbianos (AVNIMELECH, 2007). Esta comunidade de microrganismos confere ao ambiente de cultivo uma maior variedade de alimento e aos organismos cultivados um melhor crescimento, conversão

alimentar e ganho de peso (VENERO et al., 2009). Os microrganismos tendem a formar agregados amorfos que podem ser utilizado por peixes e camarões, como uma fonte adicional de proteína (AZIM e LITTLE 2008; CRAB et al 2009; KUHN et al 2009). As comunidades bacterianas presentes no meio aquático, são capazes de processar a matéria orgânica acumulada, assimilar compostos nitrogenados e converte-los em proteína bacteriana (AVINIMELECH, 2007). No entanto, o excesso de biofloco no sistema de cultivo pode acarretar problemas na produção por conta da presença, em grande quantidade, de material em suspensão. Uma solução para o destino deste resíduo é sua utilização na alimentação dos organismos cultivados. Segundo Avnimelech (2006), o biofloco pode ser utilizado como nova fonte alternativa de alimento. Algumas poucas pesquisas já vêm avaliando a utilização deste produto como item substituto à farinha de peixe na formulação de rações. Bons níveis de proteína, aminoácidos, ácidos graxos e lipídios são parâmetros importantes que determinam a viabilidade dos bioflocos como alimento na aquicultura. Segundo alguns autores, os bioflocos podem alcançar níveis de proteína bruta que variam entre 24-50% (Azim e Little,2008; Avnimelech, 2009). Em estudo realizado por Kuhn et al. (2009) ao considerar dietas com o uso de diferentes concentrações de bofloco nas rações observaram que o crescimento do camarão melhorou em média de 49%. Segundo Izquierdo (2006) os lipídios presentes nos bioflocos podem contribuir para o crescimento dos animais. No entanto, Ju et al (2008) descreve que é possível que as melhores taxas de sobrevivência e crescimento estejam associadas não a nutrientes específicos presentes no floco, e sim aos efeitos sobre a taxa de ingestão, digestibilidade, absorção, assimilação e saúde dos animais. Contudo, ainda existem poucas pesquisas com a utilização de bioflocos como ingrediente em dietas pra camarões marinhos.

REFERÊNCIAS BIBLIOGRÁFICAS

AKIYAMA, D.M., DOMINY, W.G., LAWRENCE, A. Penaeid shrimp nutrition for the commercial feed industry: revised. In: Akiyama, D.M., Tan, R.K.H. (Eds.), Proceeding of the Aquaculture Feed Processing and Nutrition Workshop, September 19– 25, 1991. **American Soybean Association**, Singapore, pp. 80– 97, 1991.

ANDREWS, J.W., SICK, L.V. Studies on the nutritional requirements of penaeid shrimps. **Proc. World Maricult**. Soc. 3, 403– 414, 1972.

ANGGAWATI, A.M., MURTINI, J.T., HERUWATI, E.S. The use of hydrolyzedprotein concentrate in practical diets for Penaeus monodon juveniles. Research Report. Research Institute for Fish Technology. Palmerah Jakarta, 12 pp, 1990.

ARANA, L. A. V. **Aquicultura e desenvolvimento sustentável: subsídios para a formulação de políticas de desenvolvimento da aqüicultura brasileira**. Florianópolis: Editora da UFS, 1999.

AVNIMELECH Y. Sustainable land-based aquaculture- rational utilization of water, land and feed. **Mediterranean Aquaculture Journal** 1, 45–55, 2008.

AVNIMELECH, Y., KOCHBA, M. Effect of C:N ratio on the development, composition , structure and stability of microbial flocs. Abstracts of World aquaculture meeting, May 9–13, 2006, Florence, Italy, 2006.

AVNIMELECH, Y. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. **Aquaculture** 264, 140–147, 2007.

AZIM, M.E. AND LITTLE, D.C. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (Oreochromis niloticus). **Aquaculture** 283, 29–35, 2008.

BALAZ, G.H. Preliminary studies on the preparation and feeding of crustacean feeds. **Aquaculture** 2, 369–377, 1973.

BENJAKUL, B.; MORRISSEY, M.T. Protein hydrolysates from Pacific whiting solid wastes. **Journal of Agricultural and Food Chemistry** , v.45, p. 3424-3430, 1997.

BOYD C.E. e CLAY J.W.. Evaluation of Belize Aquaculture, Ltd: A Superintensive Shrimp Aquaculture System. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. Work in Progress for Public Discussion. Published by the Consortium. 17 pages, 2002..

CAHU, C.L., ZAMBONINO, J.L., QUAZUGUEL, M. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass Dicentrarchus labrax larvae. **Aquaculture** 171, 109–119,1999.

CAMARGO, S.G.O., POUEY J.L.O.F**.** Aqüicultura: um mercado em expansão**. Rev Bras Agrocienc**, v.11, p.393-396,2005.

CENTENARO, G. S.; PRENTICE-HERNÁNDEZ, C. E.; SALAS-MELLADO, M. Efeito da concentração de enzima e de substrato no grau de hidrólise e nas propriedades funcionais de hidrolisados protéicos de corvina (M*icropogonias furnieri*). **Quim. Nova**, v. 32 (7), p. 1792-1798, 2009.

CHO, C.Y., HYNES, J.D., WOOD, K.R., YOSHIDA, H.K. Development of highnutrient-dense, low-pollution diets and prediction of aquaculture wastes using biological approaches. **Aquaculture**, v.124, p.293-305, 1994.

CRAB, R., KOCHVA, M., VERSTRAETE, W. AND AVNIMELECH, Y. Bio-flocs technology application in over-wintering of tilapia. **Aquacult Eng** 40, 105–112, 2009.

DAVIS, D.A.; ARNOLD, C.R. Replacement of fish meal in pratical diets for the Pacific white shrimp, *Litopennaeus vannamei*. **Aquaculture**, v. 235, p. 291-298, 2000.

DAY, O.J., HOWELL, B.R., JONES, D.A. The effect of dietary hydrolyzed fish protein concentrate on the survival and growth of juvenile Dover sole, Solea solea (L.), during and after weaning**. Aquaculture Research**. 28,911– 921, 1997.

EL-SAYED, A.-F.M. Alternative dietary protein for farmed tilapia, *Oreochromis* spp. **Aquaculture**, v 179, p 149-168, 1999.

FAO. State of World Aquaculture. FAO Fisheries Technical Paper 500. IWAS/FRD, **FAO** Fisheries Departament, Rome, Italy. 134pp, 2006.

FURLAN,E.F. E OETTERER, M. Hidrolisado Proteico de Pescado. **Revista de Ciência e Tecnologia** V. 10, Nº 19 – pp. 79-89, 2002.

GATLIN, D.M., BARROWS, F.T., BROWN, P., DABROWSKI, K., GAYLORD, T.G., HARDY, R.W., HERMAN, E., HU, G., KROGDAHL, A., NELSON, R., OVERTURF, K., RUST, M., SEALEY, W., SKONBERG, D., SOUZA, E.J., STONE, D., WILSON, R., WURTELE, E. Expanding the utilization of sustainable plant products in aquafeeds: a review. **Aquaculture Research** 38, 551–579, 2007.

GUILLAUME, J. PROTEIN AND AMINO ACIDS. IN: D'ABRAMO, L.R., CONKLIN, D.E., AKIYAMA, D.M. Eds. **Crustacean Nutrition**. World Aquaculture Society, Baton Rouge, LA, pp. 26–50, 1997.

HAARD, N. F. **Enzymatic modification of proteins in food systems**. In Z. E. Sikorsky (Ed.), Chemical and functional properties of food proteins. New York: Technomic Publishing . pp. 155–190, 2001.

HARDY, R.W. **Fish hydrolysates: production and use in aquaculture feeds**. Aquaculture Feed Processing and Nutrition Workshop. American Soybean Association, Singapore, pp. 109–115, 1991.

HERNÁNDEZ, C., OLVERA-NOVOA M. A., SMITH D. M., HARDY R. W., GONZALEZ-RODRIGUEZ B. Enhancement of shrimp *Litopenaeus vannamei* diets based on terrestrial

protein sources via the inclusion of tuna by-product protein hydrolysates **Aquaculture** 317:117–123, 2011.

IZQUIERDO, M.I., FORSTER, S. ,DIVAKARAN, L., CONQUEST, O. DECAMP, A., TACON. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. **Aquaculture Nutrition**, 12: 192–202, 2006.

JU Z.Y., FORSTER, L., CONQUEST, W., DOMINY. Enhanced growth effects on shrimp(*Litopenaeusvannamei*) from inclusion of whole shrimp floc fractions to a formulated diet. **Aquaculture Nutrition**, 14: 533-543, 2008.

Kaushik, S.J. Use of alternative protein sources for intensive rearing of carnivorous fishes. In: Shiau, S.Y. (Ed). **Progress in Fish Nutrition. Proceedings of the Fish Nutrition** Symposium, Semptember 6-7, 1989, Keelung, Taiwan ROC, p: 181-208, 1989.

KRISTINSSON, H. G.; RASCO, B. A. Kinetics of the hydrolysis of atlantic salmon (*Salmo salar*) muscle proteins by alkaline proteases and a visceral serine protease mixture. **Process Biochemistry**, v. 36, n. 9, p.131-139, 2000.

KUHN, D.D., BOARDMAN, G.D., LAWRENCE, A.L., MARSH, L., FLICK, G.J. Microbial flocs generated in bioreactors is a superior replacement ingredient for fishmeal or soybean meal in shrimp feed. **Aquaculture** 296, 51–57, 2009.

MARTONE, C.B.; BORLA, O.P.; SÁNCHEZ, J.J. Fishery by-product as a nutrient source for bacteria and archaea growth media. **Bioresource Technology,** v. 96, p. 383-387, 2005.

NAYLOR, R.L., HARDY, R.W., BUREAU, D.P., CHIUA, A., ELLIOTT, M., FARRELL, A.P., FORSTER, I., GATLIN, D.M., GOLDBURG, R.J., HUA, K., NICHOLS, P.D. Feeding aquaculture in an era of finite resources. **PNAS** 106, 36, 2009.

NAYLOR R., GOLDBURG R.J., MOONEY H., BEVERIDGE M., CLAY J., FOLKE C., KAUTSKY N., LUBCHENCO J., PRIMAVERA J. & WILLIAMS M. Nature's subsidies to shrimp and salmon farming. **Science** 282, 883–884, 1998.

NAYLOR, R. L., GOLDBURG, R. J., PRIMAVERA, J. H., KAUTSKY, N., BEVERIDGE, M. C. M., CLAY, J., FOLKE, C., LUBCHENCO, J., MOONEY, H. AND TROELL, M. Effect of aquaculture on world fish supplies, **Nature**, vol. 405, pp. 1017-24, 2000.

NEAL, R.A. PENAEID SHRIMP CULTURE RESEARCH AT THE NATIONAL MARINE FISHERIES SERVICE GALVESTON LABORATORY.IN: PILLAY, T.V.R., DILL, W.A. (Eds.), Advances in Aquaculture. **FAO** Technical Conference on Aquaculture. 1980.

NEW, M.B. A review of dietary studies with shrimp and prawns. **Aquaculture** 9:101– 144, 1976.

NRC - National Research Council. **Nutrient requirements of fish and shrimp**. National Research Council of the National Academies, Washington, DC, USA, 2011.

NUNES, A.J.P., SA, M.V.C., FELIPE ANDRIOLA-NETO, F., LEMOS, D., Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, Litopenaeus vannamei. **Aquaculture** 260, 244–254, 2006.

OETTERER, M.**Produtos Obtidos por Interferência na Fração Protéica do Pescado**. Piracicaba: ESALQ, 2001

PIEDAD-PASCUAL, F., Feeds and feeding of cultured tiger prawns in Southeast Asia. SEAFDEC **Asian Aquaculture**. 12, 5 – 8, 1990.

REFSTIE, S., OLLI, J.J., STANDAL, H. Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon (Salmon salar) in response to graded levels of fish protein hydrolysate in the diet. **Aquaculture** 239, 331–349, 2004.

ROJAS, E.; ALFARO, J. In vitro manipulation of egg activation in the open thelycum shrimp *Litopenaeus*. **Aquaculture**, 264, 469-474, 2007.

RUITER, A.**Productos pesqueros**. In: El Pescado y los Productos Derivados de la Pesca. Composición, propriedades nutritivas y estabilidad. Zaragoza: Acribia, 1999.

SOARES, R., JACKSON, C., COMAN, F., PRESTON, N. Nutricional composition of flocculated material in experimental zero-exchange system for Penaeus monodon In: Australasian Aquaculture Sydney. **Australasian Aquaculture**– Profiting from Sustainability. p.98, 2004.

TACON, A.G.J., BARG, U.C. Major challenges to feed development for marine and diadromous finfish and crustacean species. In: De Silva, S.S. (Ed.), Tropical Mariculture. Academic Press, San Diego, CA, USA, p. 171–208, 1998.

WASIELESKY W., ATWOOD H., STOKES A. and BROWDY C.L.. Effect of natural production in a zero exchange suspended microbial floc super-intensive culture system for white shrimp *Litopenaeus vannamei*. **Aquaculture**, 258: 396-403, 2006.

ZAMBONINO-INFANTE, J.L., CAHU, C., PERES, A. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentratrachus labrax* larval development. **Journal Nutr**. 127, 608–614, 1997.

ARTIGO CIENTÍFICO

Artigo científico a ser submetido para publicação na revista *Aquaculture Nutrition*

Substituição da farinha de peixe por hidrolisado proteico de peixe e farinha de biofloco na alimentação de pós-larvas do camarão marinho *Litopenaeus vannamei*

Fishmeal replacement by fish protein hydrolysate and biofloc in diets for *litopenaeus vannamei* postlarvae

Bruna Cáritas Souza do Valle Ferreira*, Edmilson de Moura Dantas Jr., Juliett de Fátima Xavier da Silva, Ranilson de Souza Bezerra, Eudes de Souza Correia, Silvio Ricardo Maurano Peixoto, Roberta Borda Soares

[I] Universidade Federal Rural de Pernambuco (UFRPE), R. Dom Manoel de Medeiros, s/n, Departamento de Pesca e Aqüicultura – Laboratório de Tecnologia em Aquicultura. Dois Irmãos, 52171-900, Recife, PE, Brasil.
[II] Universidade Federal de Pernambuco (UFPE), Laboratório de Enzimologia.
*Autor para correspondência. E-mail: brunacaritas@hotmail.com
Telefone (81) 3320 6597/ 3320 6534

SUBSTITUIÇÃO DA FARINHA DE PEIXE POR HIDROLISADO PROTEICO DE PEIXE E FARINHA DE BIOFLOCO NA ALIMENTAÇÃO DE PÓS-LARVAS DO CAMARÃO MARINHO *Litopenaeus vannamei*

FISHMEAL REPLACEMENT BY FISH PROTEIN HYDROLYSATE AND BIOFLOC IN DIETS FOR *Litopenaeus vannamei* POSTLARVAE

Bruna do Valle[I*], Edmilson Dantas[I], Juliett Xavier[II], Ranilson Bezerra[II], Eudes Correia[I], Silvio Peixoto[I], Roberta Soares[I]

ABSTRACT

The present study aimed to evaluate the effect of gradual fishmeal replacement by fish protein hydrolysate (FPH) and biofloc flour (BF) in diets for white shrimp *Litopenaeus vannamei* postlarvae (2mg). Five diets (42% PB) were formulated replacing fishmeal in 0 (control), 10, 20, 30 and 40% (T0, T10, T20, T30 and T40). A commercial diet was used as a external control. The alternative ingredients FPH and BF were used in 1:1 proportion. After 42 days shrimp survival was above 99% in all treatments. A regression test indicated the ideal point to the other zootechnical parameters (final weight, weight gain, specific growth rate and protein efficiency) substitution levels between 15 and 16%. In the present study we inferred that BF and FPH are potential ingredients to replace fish meal in diets for *L. vannamei* postlarvae.

Keywords: shrimp, nursery, microbial flocs, enzymatic hydrolysis

INTRODUCTION

Feeding may correspond to 50% on total costs on intensive shrimp farming (Naylor, 1998) it is mainly due to the use of fishmeal as a main protein source in aquafeeds. Fishmeal has high palatability and is an excellent source of amino acids, fatty acids, vitamins and minerals (Cruz-Suarez et al., 2009). However, excessive pressure on marine fisheries stocks

and the increasing demand cause problems in fishmeal supply and the consequent increase in its value.

Aquaculture grows faster than any other animal food production sector (FAO 2010) and this growth must to be accompanied by efforts to promote sustainability. Fish and marine shrimp may consume more fish, as fishmeal, than it produces (Tacon and Metian, 2009). Thus, the partial or even complete replacement of fishmeal in aquafeeds is usually a target of many researches

Alternative ingredients have been studied like the plant products such as soybeans, canola, cotton and corn (Suárez 2009; Alvarez, 2007; Lim 1997). However, the presence of antinutritional factors, deficiency of some amino acids, low palatability and digestibility restrict its use (Davis and Arnold, 2000; Gatlin et al. 2007; Nunes et al. 2006).

Residues from fisheries industries are also an alternative to the use of fishmeal. The fish protein hydrolysate (FPH) produced from these residues present a high protein content, good balance of amino acids and fatty acids, and low ash volume in addition to high palatability and digestibility (Dabrowski, 1984, Goldhor and Regenstein, 1988; Oetterer, 2001; Sgarbieri, 1996). Thus FPH has essential features to aquafeed production and some authors considered it as a good substitute for fish meal. In a diet tested for *Penaeus monodon*, the replacement of only 3% of fishmeal by FPH improved animal growth (Anggawati et al., 1990).

More recently, the bioflocs produced in intensive farming systems known as BFT (Biofloc Technology), has been studied (Pan & Xu, 2013; Kuhn et al., 2010; Abreu et al., 2007). In this system, the shrimp consumes bioflocs actively and this can represent 20 to 30% of assimilated protein (Burford et al. 2003.2004). The bioflocs are formed by the junction of bacteria, fungi, invertebrates, small organic and inorganic particles (Avnimelech, 2007). Due to the composition variability, the biofloc protein content may vary from 24 to 40% and lipid

levels from 0.46 to 0.83% (Avnimelech, 2009). In BFT systems, when the concentration of bioflocs reaches maximum capacity, it is necessary to remove their surplus, which is normally discarded. Since it is a nutrients source, this residue has the potential to be used in the aquafeed production. Thus, the objective of this study was to evaluate the performance of *L.vannamei* postlarvae, fed diets replacing fishmeal with increasing levels of protein hydrolysate and biofloc flour.

MATERIALS AND METHODS

Production of fish protein hydrolysate (FPH)

The FPH was produced using residues from tilapia processing (carcass) acquired from fish industries. The methodology for FPH production was adapted from Bezerra (2000). After thawing, the carcasses were added to filtered water 1:1 followed by commercial enzyme (Alcalase) addition (0.5% of the total volume). This mixture was triturated in a blender for two minutes and submitted to a water bath (45ºC) for three hours under light and constant agitation for activating the process of hydrolysis by enzymes. Following this period, the hydrolysate product was subjected to a temperature of 100 ° C during 10 minutes for enzyme deactivation. The solid and liquid portions were separate by sieving through a mesh of $1mm^2$. The liquid part (FPH) was packed in a closed recipient and maintained at -20 ° C for further analysis of its composition.

Obtaining biofloc flour (BF)

The biofloc used in this study was collected from three *L.vannamei* BFT ponds. The development of biofloc was accompanied by daily samples of water with a graduated 1-L Imhoff cone. When a 20 mL/L volume was reached, the biofloc was collected by sedimentation tanks. To remove the water excess the material was filtered in a sieve sequence

of 250 and 50 µm nylon meshes, and a 10 µm cellulose filter. After the biofloc was distributed in fine layers and kept in a ventilated and sun protect area. To complete drying material was placed in a forced air circulating oven at 50 ºC for 48h. The dried biofloc was ground and sieved at 250µm. This material was packed in hermetically sealed containers and kept at -20 ºC for further analysis of its composition.

Analyses of composition of biofloc and FPH were according to Association of the Official Analytical Chemists (AOAC) (2005) methodology (Table 1).

Experimental diets

Five isoenergetic and isoproteic diets were formulated replacing fish meal in proportions of 0, 10, 20, 30 and 40% (Table 2). BF and FPH were utilized in proportion 1:1.

The dry ingredients were previously ground to 250µm. The diets were dried in a forced air circulating oven at 50 °C for 9 hours and pressed into 0.85 and 1.40 mm stainless steel sieves to obtain the required particle size. Diets were again displaced in the oven at the same temperature for 30 minutes to finish drying. Diets were stored in sealed plastic containers at -18 º C.

Experimental design

The experiment consisted of six treatments with three replicates each. T0 = control treatment without addition of BF and FPH; T10 = 10% replacement of fishmeal by combined BF and FPH; T20 = 20% replacement, T30 = 30% replacement, T40 = 40% replacement, and COM = commercial feed (Purina® CR1 and CR2, with a minimum of 40% crude protein) used as an external control.

The postlarvae of *L. vannamei* were acquired in PL8 (8 days post-larval stage) from a commercial hatchery. The animals were transferred to the laboratory and acclimated in a 310-L tank containing filtered seawater (1 µm) pre-treated with sodium hypochlorite (20 ppm).

The tank was kept under constant aeration and temperature controlled at 31-32°C by immersion heaters with thermostat. Animals were fed with commercial diet (Frippak Feeds® PL-INVE) and *Artemia* nauplii newly hatched.

Polyethylene tanks (50-L) were used as experimental units. The units were connected in a water recirculation system containing biological, mechanical and UV filters. One hundred and fifty postlarvae (PL10) with average initial wet weight of 0.00026±0.0023 g were stocked in each unit (3 PL/L). The experiment lasted 42 days. The animals were fed the diets described offered in excess, the feeding rate was 50% biomass for four weeks and reduced to 40% until the end of the experiment. The wet weight of the animals was checked weekly to adjust the feeding rate. The food was offered three times daily, at 08:00, 13:00 and 18:00. Bottom tanks were siphoned daily to remove feces, uneaten feed and exuviae. Water quality parameters (temperature, salinity, dissolved oxygen and pH) were measured daily using a multiparameter analyzer (YSI 556), ammonia and nitrite were measured by spectrophotometer (ALFAKIT-AT10P).

At the end of the experiment were evaluated the zootechnical performance parameters: final weight (FW), weight gain (WG = final weight - initial weight), feed conversion ratio (FCR = dry weight of feed offered (g) / gain weight (g)); specific growth rate (SGR = 100 (ln final weight - ln initial weight) / time); protein efficiency ratio (total weight gain/total protein intake); survival (Surv. = (final nº. of shrimps / nº initial) x 100).

Statistical analysis

The parameters of water quality and the zootechnical performance data were subjected to testing for normality and homogeneity of variances (Levene's test). Posteriorly, the data were subjected to analysis of variance ($p<0.05$) by the program Statistica 7.0. Performance data were later submitted to regression analysis by the program Sisvar 4.0 that indicated the

best point of replacement of fishmeal by the ingredients tested for each parameter. The results obtained with the commercial diet were not included in the regression analysis.

RESULTS

The water quality parameters did not differ significantly ($p<0.05$) among treatments. Mean (± SD) values of temperature, salinity, dissolved oxygen and pH were 27.67 ± 1.2^{o}C, 31.17 ± 0.56 g L^{-1}, 5.49 ± 0.49 mg L^{-1} and 7, 59 ± 0.11, respectively. Ammonia mean (± SD) concentrations were 0.106 ± 0.10 mg L^{-1} and nitrite 2.04 ± 0.61 mg L^{-1}. Mean (± SD) values for final weight (FW), weight gain (WG), specific growth rate (SGR) feed conversion ratio (FCR) and protein efficiency ratio (PER) and survival are described in Table 4. The animal survival was above 99% in all treatments ($p <0.05$).

Shrimps fed experimental diets showed no statistical differences between parameters of final weight, weight gain and specific growth rate. However, when compared with commercial feed, the animals fed experimental diets had better results. Although there is no statistical differences between treatments, animals fed with experimental diets had a decreasing trend to the parameters of final weight, weight gain and specific growth rate when fed diets containing higher levels of fish meal substitution. Feed conversion rate, protein efficiency rate and survival there were no statistical differences between treatments even when compared with commercial feed (Table 4).

Quadratic effect was observed for all performance parameters of shrimps fed experimental diets. The curves for weight gain and final weight indicated the optimal replacement level of 16.24% (Figure 1). The curves for the specific growth rate and protein efficiency rate indicated levels of 15.72% and 16.5%, respectively (Figure 1) as better replacement levels. The food in this experiment was supplied in excess and was not possible to collect feed remains in a efficient way. Thus, consumption data could not be evaluated

precisely impairing analysis of feed conversion. For this reason the regression was not applied to this parameter.

DISCUSSION

Water temperature and salinity in this experiment remained within the optimal environmental conditions for *L. vannamei* farming, recommended by Ponce-Palafox et al. (1997). Values of pH, dissolved oxygen, ammonia and nitrite were also in accordance with the recommendations for shrimp farming (Boyd, 1990).

The 1:1 ratio of FPH and biofloc established for the production of experimental diets was due to the high lipid content of fish protein hydrolysate. This fact has restricted the use of FPH in higher concentrations since diets with high fat levels inhibits shrimp growth at larval and juvenile stages (Gonzalez-Felix et al. 2002).

Whereas nutritional information for penaeid shrimp in the nursery phase are rather limited, in this experiment the diet balancing was based on the nutritional needs of shrimps in stages closer to the post-larval, whose crude protein is around 42%. Protein levels of 23 to 57% are indicated for penaeid shrimp larvae (Kanazawa, 1990) and for juvenile optimal protein level is 40% (NRC, 2011). Therefore, the ideal requirement of dietary protein for *L. vannamei* is between 423,7 and 441,2 g kg^{-1}(Li et al., 2001; Zhu et al., 2010), which are compatible with protein levels used in this study (Table 2).

The combination of tested ingredients (BF and FPH) had satisfactory nutritional levels in their composition, allowing their insertion in shrimp feed. Hydrolysate fish protein has essential characteristics in diet supplementation as the improvement in palatability, good digestibility and high solubility, and also high protein content and low ash content (Goldhor and Regenstein 1988). These last two points were also observed in our results, which are important factors in the preparation of products for aquaculture. The high protein content was

also observed for *Prionotus punctatus* hydrolysate, with protein level of 878,4 g/ kg^{-1} (Zavareze et al., 2009).

However, bioflocs have variable protein content with levels from 24 to 40.6% (Avnimelech, 2009). Accordingly, bioflocs in the present study presented crude protein level of 240 g kg^{-1} (Table 1). Bauer et al. (2012) found 230.9 g kg^{-1} of this nutrient in biofloc used in his experiment, while Soares et al. (2004) found 42% crude protein in the composition of biofloc. These results are linked to the composition of this product which may vary according to the microorganisms and inorganic particles that compose it.

Fishmeal is considered the main source of protein in the composition of aquafeed and has an excellent nutritional profile of essential amino acids (Pezzato et al., 2002). Its replacement by alternative ingredients can compromise the performance of the cultured organisms if there is nutritional deficiency. Partial replacement of fishmeal in the present study did not affect the essential amino acids (EAA) levels of the experimental diets that remained above those indicated by previous studies (Millamena et al., 1997,1998 and 1999; Teshima et al. 2002; Richard et al., 2010) (Table 3). However, as the levels of fishmeal replacement increased, there was a trend reduction in methionine levels (T30 = 8.6, T40 = 8.5), below of recommended by Richard et al. (2010) which is set at 9.0 g kg^{-1} for this species. This reduction is probably related to the low levels of EAA, including methionine (0.27%) in the biofloc used in this study. Avnimelech (2009) inferred that biofloc methionine levels may vary from 0.89 to 4.78%, differing from the results found in this study for this ingredient. This result may have been one of the factors that influenced the trend to reduced animal growth in treatments with increasing substitution of fish meal. When providing a diet with low methionine levels, as well as other essential nutrients, tends to have a depression in animal growth and low feed efficiency (NRC, 2011).

In contrast, FPH exhibited values of the EAA comparable to the fishmeal used. FPH improved the amino acids balance in the diets compensating the low levels in biofloc (Table 1). Previous study demonstrate that protein replacement of a basal diet for increasing values of FPH (4-20%) resulted in adequate concentrations of all essential amino acids for *L.vannamei* (Cordova-Murueta and Garcia-Caraño 2002). Hernandez et al. (2011) also observed increased levels of some amino acids in diets when substituted the meat meal by tuna hydrolysate protein in levels ranging from 2.5 to 10% substitution. Amino acids are responsible for the transport of minerals, formation and maintenance of tissues and formation of hormones and enzymes (Logato 2000). Thus, the feed quality is directly linked to good balance of amino acids.

In the present study main polyunsaturated fatty acids for the larval stage were within the ratio indicated by the NRC (2011) (Table 3). Penaeid shrimp have a specific diet requirement for unsaturated fatty acids. Highly unsaturated fatty acids are considered the most important nutrients to accomplish the needs of these animals, namely, eicosapentaenoic acids - EPA (20:5 n-3) and docosahexaenoic acid - DHA (22:6 n-3) (D'Abramo, 1997; Glencross & Smith, 2001; NRC, 2011). Formulated diets presented a 2:1 ratio of DHA and EPA, respectively, as indicated by the NRC (2011) for penaeid larvae nutrition. As previously mentioned, there was a decreasing trend of shrimp performance in the treatment T40. This result can also be associated with the total lipids present in greater levels in the T40 diet (101.9g kg^{-1}) (Table 2). Hu et al. (2008), using different levels of protein and lipid associated, observed that animals fed a diet containing 100 g kg^{-1} lipid showed reduced growth. These results were also observed by other authors (Andrews et al. 1972, Davis and Robinson, 1986; Glencross et al. 2001; Ward et al. 2003; Zhu et al., 2010) and some studies also suggest that lipid levels greater than 100g kg^{-1} can cause growth retardation of animals (Davis and Robinson, 1986; Kanazawa et al. 1977; Sheen and D'Abramo, 1991). This fact

may be related to the animal inability at this stage to metabolize high levels of lipids reducing the digestibility (NRC, 2011). The high lipid content of FPH (374.7 g kg^{-1}) (Table 1) observed differs from the results of FPH produced with other species such as *Prionotus puctatus* which showed levels of 40.4 g kg^{-1} (Zavareza et al., 2009) and *Micropogonias furnieri* with 210.9 g kg^{-1} (Martins et al., 2009).

The performance parameters evaluated in this experiment had satisfactory results regarding the fishmeal replacement by the ingredients tested. The indicated substitution level obtained in this study, between 15.72% and 16.5%, supports the indication of Hertrampf and Piedad-Pascual (2000) for inclusion of FPH in diets for crustaceans. These authors indicate inclusion rates ranging between 2 and 4% above this level there is no improvement in animal performance. Considering the proportion of FPH and BF (1:1) in this experiment, replacing 16% of fish meal, each ingredient tested (FPH and BF) represents 3.2%. *L. vannamei* fed commercial diets supplemented with fish protein hydrolysate (4.12 and 20%) or krill (5.15 and 25%) had an increase in weight gain, however, high amounts of FPH are not indicated to shrimps (Cordova-Murueta and García-Carreño, 2002). In tests with inclusion of tuna protein hydrolysate (0 to 100 g kg^{-1}) the higher level of hydrolysate in the diet reduced weight gain in *L.vannamei* (Suresh et al., 2011), which also supports with our results.

In diets with biofloc inclusion ranging from 0 to 15.8% shrimp growth has improved on average of 49% compared to the control diet (Kuhn et al.2009). Testing different percentages (0,25,50,75 and 100%) of biofloc combined with soy protein concentrate as fishmeal replacement Bauer et al (2012) did not observe difference in *L. vannamei* performance between the treatments and commercial diet. Thus authors indicate the possibility of total replacement of fishmeal using these products.

The results of this study indicate optimal levels of replacement of fish meal by fish protein hydrolysate and bioflocs between 15 and 16%. However, it must be held

supplementation of diets with methionine to correct the deficiency of this amino acid in biofloc flour.

ACKNOWLEDGMENTS

To funders Conselho Brasileiro de Pesquisa (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

REFERENCES

Abreu, P. C., Ballester, E.L.C. Odebrecht, C., Wasielesky, W. (Jr), Cavalli, R.O.,Graneli, W., Anesio, A.M. (2007) Importance of biofilm as a food source for shrimp (Farfantepenaeus Paulrnsis) evaluated by stable isotopes (δ^{13} C and δ^{15} N). Journal of Experimental Marine Biology and Ecology **347**,88-96

Alvarez J.S., Hernández-Llamas A., Galindo J., Fraga I., García T. & Villarreal H., (2007). Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp *Litopenaeus schmitti* (Pérez-Farfante & Kensley 1997). Aquaculture Research, **38**, 689-695

Andrews, J.W., Sick, L.V. & Baptist, G.J. (1972) The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. Aquaculture, **1**, 341–347.

Anggawati, A.M., Murtini, J.T., Heruwati, E.S., (1990). The use of hydrolyzed protein concentrate in practical diets for Penaeus monodon juveniles. Research Report. Research Institute Fish Technology, Palmerah, Jakarta. 12 pp.

Association of Official Analytical Chemists. (2007). Official Methods of Analysis. 18. ed. rev. 2. Gaithersburg, MD, USA. Crude protein in meat and meat products including pet foods, c. 39. Method 992, **15**. p. 6-7

Association of Oficial Analytical Chemists. (1995). Official methods of analysis of the Association of Analytical Chemists. Arlington: A.O.A.C., chapter 33. (method 920.39, C). p.10-1.

Avnimelech, Y. (2009). Biofloc Technology - A Practical Guide Book. Baton Rouge, Louisiana, United States. The World Aquaculture Society. 181p.

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture, **264**, 140-147.

Bauer,W., Prentice-Hernandez C., Tesser M.B., Wasielesky Jr W., Poersch L. H. S. (2012). Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacifc with shrimp *Litopenaeus vannamei* . Aquaculture **342–343,** 112–116.

Bezerra, R. S. (2000). Proteases digestivas no Tambaqui *Colossoma macropomum* (Cuvier, 1818). Tese Doutorado em Ciências Biológicas – Universidade Federal de Pernambuco, Recife.

Bligh, E.G. & Dyer,W.J. (1959). A rapid method for total lipid extraction and purification. *Can.J.Biochem.Physiol.* **37**,911-917.

Boyd, C.E., (1990). Water quality in ponds for aquaculture. Alabama Agricultural Experimental Station, Auburn University, Auburn, AL.

Burford M.A. Thompson,P.J., MCIntosh, R.P.,Bauman, R.H., Pearson D.C. (2004). The contribuition of flocculated material to shrimp (*Litopenaeus vannamei*)nutrition in a high-inensity, zero-exchange system. Aquaculture **232**,525-537.

Burford M.A. Thompson,P.J., MCIntosh, R.P.,Bauman, R.H., Pearson D.C. (2003). Nutrient and microbial dynamics in high-inensity, zero-exchange system shrimp ponds in Belize. Aquaculture **219**,393-411.

Córdova-Murueta, J.H., García-Carreño, F.L. (2002). Nutritive value of squid and hydrolysed protein supplement in shrimp feed. Aquaculture **210**, 371–384.

Cruz-Suárez, L.A., Tapia-Salazar, M., Villarreal-Cavazos, D., Beltran-Rocha, J., Nieto-López, M.G., Lemme, A., Ricque-Marie, D.(2009). Apparent dry matter, energy, protein and amino acid digestibility of four soybean ingredients in white shrimp Litopenaeus vannamei juveniles. Aquaculture **292**, 87–94.

Cunnif, P. Official Methods of Analysis.(2005). Arlington: Association of Official Analytical Chemists - AOAC, 18 ed.

D'Abrowski, K. (1984). The feeding of fish larvae: present state of art and perspectives. Reproduction, Nutrition and Development, **24 (6)**, 807-833.

Davis, D.A. & Robinson, E.H. (1986). Estimation of the dietary lipid requirement level of the white crayfish Procambarus acutus acutus. J. World Aquaculture. Soc. **17**, 37–43.

Davis D.A. & Arnold C.R . (2000). Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, **185**, 291-298.

FAO, (2010). The State of World Fisheries and Agriculture - 2010. Rome: FAO.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E.(2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research **38**, 551–579.

Glencross, B.D.; Smith, D.M. (2001). Optimizing the essential fatty acids, eicosapentaenoic and docosahexaenoic acid, in the diet of the prawn, *Penaeus monodon*. Aquaculture Nutrition, **7**, 101–112.

Glencross, B., Smith, M., Curnow, J., Smith, D.M. & Williams, K. (2001). The dietary protein and lipid requirements of post-puerulus western rock lobster, Panulirus cygnus. Aquaculture, **199**,119–129.

Goldhor, S.H. & Regenstein, J.M. U.S. (1988). Fisheries Products: a selective update and review. *Foodstuffs,* **60 (20)**,14-16.

González-Félix, M. L., Gatlin D. M., Lawrence A.L. III & Perez-Velazquez. (2002) Effect of various dietary lipid levels on quantitative essencial fatty acid requirements of juvenile Pacific with shrimp *Litopenaeus vannamei*. J. World Aquaculture. Soc. **33**,330-340.

Hagen S.R., Frost B., Augustin J. (1989) Precolumn Phenylisothiocyanate Derivatization And Liquid-Chromatography of Amino-Acids In Food. Journal of the Association of Official Analytical Chemists **72 (6)**, 912-916 .

Hernández, C., Olvera-Novoa M. A., Smith D. M., Hardy R. W., Gonzalez-Rodriguez B. (2011) Enhancement of shrimp *Litopenaeus vannamei* diets based on terrestrial protein sources via the inclusion of tuna by-product protein hydrolysates Aquaculture **317**,117–123.

Hertrampf, J.W., Piedad-Pascual, F.(2000). Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publisher, Dordrecht, The Netherlands. 573 pp.

Hu, Y., Tan, B., Mai, K., Zheng, S. & Cheng, K. (2008). Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. Aquaculture. Nutrition, **10**, 1–8.

Instituto Adolfo Lutz . (2005). Normas Analíticas do Instituto Adolfo Lutz. Métodos químicos e físicos para análise de alimentos, V.1. 4.ed. São Paulo: PROL. p. 118-119.

Instituto Adolfo Lutz. (1985). Normas Analíticas do Instituto Adolfo Lutz. Métodos químicos e físicos para análise de alimentos. 3 ed. São Paulo: IMESP. p. 21-22.

Kanazawa, A.; Teshima, S. (1977). Biosynthesis of fatty acids from acetate in prawn, *Penaeus japonicus.* Mem.Fat.Fish., Kagoshima Univ., **6**, 49-53.

Kanazawa, A. (1990). Microparticulate feeds for penaeid larvae. Adv..Trop. Aquacult. Actes de Colloque **9**,395-404 .

Kaushik, S.J. (1989). Use of alternative protein sources for intensive rearing of carnivorous fishes. In: Shiau, S.Y. (Ed). Progress in Fish Nutrition. Proceedings of the Fish Nutrition Symposium, Semptember 6-7, 1989, Keelung, Taiwan ROC, p: 181-208.

Kuhn D.D., Lawrence A.L., Boardman G.D., Patnaik S., Marsh L. & Flick G.J. (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei. Aquaculture*, **303**, 28-33.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick, G.J. (2009). Microbial flocs generated in bioreactors is a superior replacement ingredient for fishmeal or soybean meal in shrimp feed. Aquaculture **296**, 51–57.

Li, G.L., Zhou, C.H. & Zhou, Q.C. (2001) Effects of dietary protein level on the growth of *Litopenaeus vannamei*. Mar. Sci., **25**, 1–4.

Lim C, Beames R.M., Egles J.G., Predergast A.F., Mcleese J.M., Shearer K.D. & Higgs D.A., (1997). Nutritive value of low and high fibre canola meals for shrimp (*Penaeus vannamei*). *Aquaculture Nutrition*, **3**, 269–279.

Logato, P. V. R. (2000). Nutrição e alimentação de peixes de água doce. Ed. Aprenda Fácil. Viçosa/MG.

Martins, V. G., Costa, J. A. V., & Prentice-Hernández, C. (2009). Hidrolisado protéico de pescado obtido por vias química e enzimática a partir de corvina (*Micropogonias furnieri*). Química Nova, **32**, 61–66.

Naylor R., Goldburg R.J., Mooney H., Beveridge M., Clay J., Folke C., Kautsky N., Lubchenco J., Primavera J. & Williams M. (1998). Nature's subsidies to shrimp and salmon farming. Science **282**, 883–884.

NRC - National Research Council. (2011). Nutrient requirements of fish and shrimp. National Research Council of the National Academies, Washington, DC, USA.

Nunes, A.J.P., Sa, M.V.C., Felipe Andriola-Neto, F., Lemos, D., (2006). Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, Litopenaeus vannamei. Aquaculture **260**, 244–254.

Oetterer, M., Produtos Obtidos por Interferência na Fração Protéica do Pescado. Piracicaba: ESALQ, 2001.

Pezzato, L.E.; Miranda, E.C.; Barros, M.M.; Pinto, L.G.Q.; Furuya, W.M.; Pezzato, A.C. (2002). Digestibilidade aparente de ingredientes pela tilápia do Nilo (Oreochromis niloticus). Revista Brasileira de Zootecnia, Viçosa, **31(4)**, 1595-1604.

Ponce-Palafox, J. (1997). The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture, v. 157, p. 107-115.

Richard, L., Blanc, P.P., Rigolet, V., Kaushik, S.J., Geurden, I. (2010). Maintenance and growth requirements for nitrogen, lysine and methionine and their utilisation efficiencies in juvenile black tiger shrimp, Penaeus monodon, using a factorial approach. The British Journal of Nutrition **103**, 984–995.

Sgarbirei, V.C. (1996). Proteínas em alimentos protéicos: propriedades, degradações, modificações. São Paulo: Varela.

Soares, R., Jackson, C., Coman, F., Preston, N. (2004). Nutricional composition of flocculated material in experimental zero-exchange system for Penaeus monodon In: Australasian Aquaculture, 2004, Sydney. Australasian Aquaculture 2004 – Profiting from Sustainability. p.98.

Suárez J.A., Gaxiola G., Mendoza R., Cadavid S., Garcia G., Alanis G., Suárez A., Faillace J. e Cuzon G. (2009). Substitution of fish meal with plant protein sources and energy budget for white shrimp *Litopenaeus vannamei* (Boone, 1931) *Aquaculture*, **289**, 118–123.

Suresh A. V. Kumaraguru V. K.P., Nates Sergio. (2011).Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, Litopenaeus stylirostris fed diets formulated with high levels of poultry byproduct meal, Aquaculture **319**,132–140.

Tacon, A.G.J., Akiyama, D.M. (1997). Feed ingredients. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. World Aquaculture Society, Baton Rouge, USA, pp. 411–472.

Tacon, A.G.J. & Metian, M. (2009). Fishing for aquaculture: nonfood use of small pelagic forage fish – a global perspective. Rev. Fish. Sci., **17**, 305–317.

Ward, L.R., Carter, C.G., Crear, B.J. & Smith, D.M. (2003) Optimal dietary protein level for juvenile southern rock lobster, Jasus edwardsii, at two lipid levels. Aquaculture, **217**, 483–500.

White J.A., Hart R.J., Fry J.C. (1986). An Evaluation of the waters pico-tag system for the amino-acid-analysis of food materials. Journal of Automatic Chemistry **8 (4)**, 170-177.

Xu W.J. & Pan L.Q. (2013). Dietary protein level and C/N ratio manipulation in zero-exchange culture of Litopenaeus vannamei: Evaluation of inorganic nitrogen control, biofloc composition and shrimp performance Aquaculture Research, 1–10.

Zavareze, E. R., Silva, C.M., Mellado, M.S., & Prentice-Hernández, C. (2009). Funcionalidade de hidrolisados proteicos de cabrinha (*prionotus punctatus*) obtidos a partir de diferentes proteases microbianas. *Quim. Nova,* **32 (7)**, 1739-1743.

Table1. Proximal composition and amino acid of the main ingredients (g kg^{-1} of dry matter) which compose the feed

	Fishmeal	FPH	Biofloc flour	Soybean* meal	Wheat* bran	Wheat* flour
Essential amino acids						
Arginine	47	48.7	10.4	32.3	6.4	8.6
Histidine	20.6	11.8	3.3	11.7	3	3.9
Isoleucine	27.8	20.9	8.2	19.9	5.1	5.1
Leucine	48	35.7	15.6	34.2	8.9	9.2
Lysine	51	41.2	8.2	28.63	3.6	5.8
Methionine	27.5	17.1	2.7	6.1	2.1	1.9
Phenylalanine	27.6	22.5	10.7	21.8	6.3	5.5
Threonin	29.8	24.6	10	17.3	3.7	4.6
Valine	32	26.7	13.8	24	5.9	6.9
Análise proximal						
Crude protein	693.2	584.8	247.1	450	148	117
Crude fat	92.4	374.7	4	17.4	40	12
Ash	229.2	26.7	366	63	53	4
Moisture	71.1	813.2	86.3	117.8	110	120

*Data from NRC 2011

Table 2. Formulation of diets with partial replacement of fish meal by biofloc flour and fish protein hydrolyzate (g kg^{-1} dry matter)

Ingredients	T0	T10	T20	T30	T40	Commercial[a]
Fishmeal	400	360	320	280	240	
Biofloc flour	0	20	40	60	80	
FPH	0	20	40	60	80	
Soybean meal	130	130	150	190	225	
Wheat bran	150	150	150	150	150	
Wheat flour	230	230	230	190	140	
Yeast	25	25	30	30	40	
Gelatin	10	20	20	15	15	
Fish oil	30	20	10	15	20	
Mixture of vitamins and minerals[b]	10	10	10	10	10	
Bentonite	15	15	0	0	0	
Proximete analysis						
Crude protein	436	437.4	427.7	425.9	429.1	400
Crude fat	83	83.3	88.7	91.9	101.9	80
Ash	101.6	98.8	90.7	98.5	104.2	130
Moisture	173.7	158.4	168.5	185.4	170.5	130
NFE[c]	205.7	222.1	234.4	198.3	194.3	-
Gross Energy (Kj/g)	201.4	203.5	199.8	199.9	202.2	-

[a] Minimum levels of protein and lipids and maximum levels of ash and moisture in the feed established by the manufacturer
[b] Mineral and vitamin mix (Supremais, Campinas-SP): Composition per kg the product: Vit. A = 1.200.000 UI; vit. D3 = 200.000 UI; vit. E = 12.000 mg;vit. K3 = 2400 mg; vit. B1 = 4800 mg; vit. B2 = 4800 mg; vit. B6 = 4000 mg; vit. B12 = 4800 mg; folic acid = 1200 mg; Calcium pantothenate = 12.000 mg; vit. C = 48.000 mg; Biotin = 48 mg; Choline = 65.000 mg; Nicotinic acid = 24.000 mg; Fe = 10.000 g; Cu = 600 mg; Mn = 4000 mg; Zn = 6000 mg; I = 20 mg; Co = 2 mg e Se = 20 mg.
[c] NFE (carbohydrate digestion easier) NFE=100− (crude protein + crude fat + ash + moisture)

Table 3. Profile of essential amino acids and non-essential and major polyunsaturated fatty acids (g kg^{-1} dry matter) in diets with replacement of fish meal by FPH and BF.

	T0	T10	T20	T30	T40	Recommended levels
Essential amino acids						
Arginine	28.1	27.1	25.9	25.9	26.1	19.0 [a]
Histidine	11.5	10.8	10.4	10.2	10.2	8.0 [b]
Isoleucine	15.2	15.3	14.7	14.8	14.8	10.0 [b]
Leucine	29.1	28.5	27.7	27.9	28.1	17.0 [b]
Lysine	28	27.4	26.3	26	25.5	21.0 [a]
Methionine	10.3	9.5	9.2	8.6	8.5	9.0 [c]
Phenylalanine	17.3	17	16.7	16.6	16.9	14.0 [b]
Threonin	17	16.5	16.1	16	16.1	14.0 [d]
Valine	21.8	22	21.1	21.1	21	14.0 [e]
Nonessential amino acids						
Glutamic acid	66.7	66	65.8	64.5	65.7	
Aspártic acid	38.5	37.3	37.1	37.6	38.9	
Glycine	32.2	32.9	32.1	30.3	30.6	
Proline	24.5	24.9	24.8	23.8	24.3	
Alanine	25.9	23.8	22.9	24.7	24.7	
Tyrosine	12.7	12.3	11.9	11.8	11.9	
Cystine	4.3	4.7	4.6	4.6	4.2	
Serine	19.3	18.7	18.5	18.4	19	
Polyunsaturated fatty acids						
Eicosapentaenoic acid (20:5n-3)	76	68	55	54	52	
Docosahexaenoic acid (22:6n-3)	131	104	91	92	92	

[a] Millamena et al. (1998).
[b] Millamena et al. (1999).
[c] Richard et al. (2010).
[d] Millamena et al. (1997).
[e] Teshima et al. (2002).

Table 4. Mean values (±SD) performance of shrimps fed with rations containing different percentages of replacement of fishmeal by FPH and BF ranging from 10 to 40%.

Treatments	FW(g)	WG (g)	AFC	SGR (%/ dia)	PER	Survival (%)
T0	0.198 ± 0.022[a]	0.196±0.022[a]	2.847±0.289[a]	10.600±0.262[a]	0.0045±0.0005[a]	100±0[a]
T10	0.235 ± 0.026[a]	0.233±0.026[a]	3.571±0.306[a]	11.005±0.269[a]	0.0053±0.0006[a]	99.7±0.577[a]
T20	0.210 ± 0.033[a]	0.208±0.033[a]	3.186±0.436[a]	10.731±0.381[a]	0.0059±0.0008[a]	99.6±0.693[a]
T30	0.212 ± 0.057[a]	0.209±0.057[a]	4.054±0.613[a]	10.712±0.619[a]	0.0050±0.0013[a]	99.3±0.586[a]
T40	0.175 ± 0.007[a]	0.137±0.007[a]	3.815±1.247[a]	10.316±0.095[a]	0.0041±0.0002[a]	99.7±0.462[a]
Com	0.100 ± 0.006[b]	0.091±0.001[b]	2.952±0.077[a]	8.973±0.138[b]	0.0026±0[a]	99.7±0.462[a]

FW= final weight; GP= weight gain; AFC= apparent feed conversion; SGR= specific growth rate; TEP= protein efficiency rate
Different letters in the same column differ significantly ($p<0.05$)

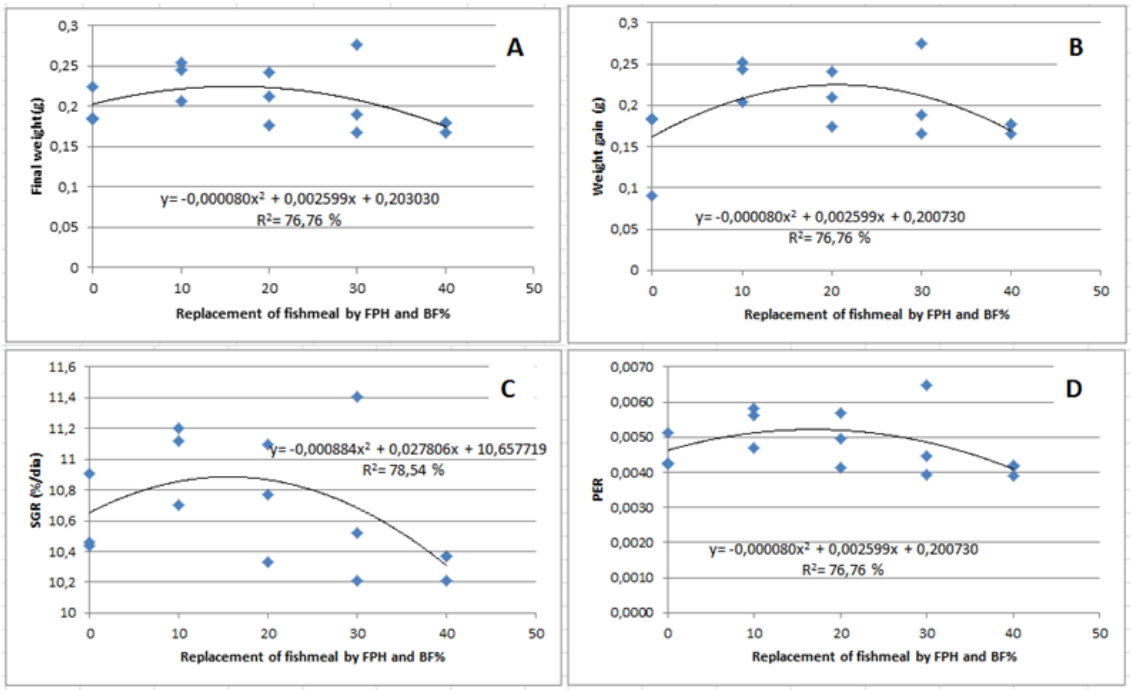

Figure 1. Regressions of final weight (A), weight gain (B), specific growth rate (C) and protein efficiency rate (D) of postlarvae of marine shrimp *Litopenaeus vannamei* fed diets with increasing substitution of flour fish by fish protein hydrolyzate (FPH) and biofloc flour (BF).

ANEXOS

Periódico : *Aquaculture Nutrition*

Site: http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2095

ISSN: 1365-2095 **Fator de impacto:** 2.179

Preparation of the Manuscript

All sections of the manuscript should be double-spaced and with 30mm margins. Articles are accepted for publication only at the discretion of the Editor(s). Authors will receive prompt acknowledgement of receipt of their paper and a decision will be reached within 3 months of receipt. A manuscript should consist of the following sections:

Title page

This should include: the full title of the paper; the full names of all the authors; the name(s) and address(es) of the institution(s) at which the work was carried out (the present addresses of the authors, if different from the above, should appear in a footnote); the name, address, and telephone and fax numbers of the author to whom all correspondence and proofs should be sent; a suggested running title of not more than fifty characters, including spaces; and six key words to aid indexing.

Main text

Generally, all papers should be divided into the following sections and appear in the order: (1) Abstract or Summary, not exceeding 150-200 words, (2) Introduction, (3) Materials and Methods, (4) Results, (5) Discussion, (6) Acknowledgements, (7) References, (8) Figure legends, (9) Tables, (10) Figures.

The Results and Discussion sections may be combined and may contain subheadings. The Materials and Methods section should be sufficiently detailed to enable the experiments to be reproduced. Trade names should be capitalized and the manufacturer's name and address given.

All pages must be numbered consecutively from the title page, and include the acknowledgements, references and figure legends, which should be submitted on separate sheets following the main text. The preferred position of tables and figures in the text should be indicated in the left-hand margin.

Units and spellings

Système International (SI) units should be used. The salinity of sea water should be given as g

L-1. Use the form g mL-1 not g/mL. Avoid the use of g per 100g, for example in food composition, use g kg-1. If other units are used, these should be defined on first appearance in terms of SI units, e.g. mmHg. Spelling should conform to that used in the Concise Oxford Dictionary published by Oxford University Press. Abbreviations of chemical and other names should be defined when first mentioned in the text unless they are commonly used and internationally known and accepted.

Scientific names and statistics

Complete scientific names should be given when organisms are first mentioned in the text and in tables, figures and key words. The generic name may subsequently be abbreviated to the initial, e.g. Gadus morhua L., otherwise G. morhua. Carry out and describe all appropriate statistical analyses.

References (Harvard style)

References should be cited in the text by author and date, e.g. Lie & Hemre (1990). Joint authors should be referred to by et al. if there are more than two, e.g. Hemre et al. (1990).

More than one paper from the same author(s) in the same year must be identified by the letters a, b, c, etc., placed after the year of publication. Listings of references in the text should be chronological. At the end of the paper, references should be listed alphabetically according to the first named author. The full titles of papers, chapters and books should be given, with the first and last page numbers; journal titles should be abbreviated according to World List of Scientific Periodicals.

Lie, O., Lied, E. & Lambertsen, G. (1988) Feed optimization in Atlantic cod (Gadus morhua): fat versus protein content in the feed. Aquaculture, 69, 333-341. Lall, S.P. (1989) The minerals. In: Fish Nutrition (Halver, J.E. ed.), 2nd edn, Vol. 1, pp. 219-257. Academic Press Inc., San Diego, CA, USA.

Work that has not been accepted for publication and personal communications should not appear in the reference list, but may be referred to in the text (e.g. A. Author, unpubl. observ.; A.N. Other, pers. comm.). It is the authors' responsibility to obtain permission from colleagues to include their work as a personal communication. A letter of permission should accompany the manuscript.

References in Articles

We recommend the use of a tool such as EndNote () or Reference Manager (http://www.refman.com/) for reference management and formatting. EndNote reference styles can be searched for

here:http://www.endnote.com/support/enstyles.asp

Reference Manager reference styles can be searched for here:http://www.refman.com/support/rmstyles.asp

Illustrations and tables

These should be referred to in the text as figures using Arabic numbers, e.g. Fig. 1, Fig. 2, etc., in order of appearance. Three copies of each figure should be submitted and each figure should be marked on the back with its appropriate number, together with the name(s) of the author(s) and the title of the paper. Where there is doubt as to the orientation of an illustration the top should be marked with an arrow.

Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Labelling should be clearly indicated on an overlay or photocopy. Colour illustrations are acceptable when found necessary by the Editor; however, the author may be asked to contribute towards the cost of printing.

Line drawings should be on separate sheets of white paper in black indelible ink (dot matrix illustrations are not permitted); lettering should be on an overlay or photocopy and should be no less than 4 mm high for a 50% reduction. Please note, each figure should have a separate legend; these should be grouped on a separate page at the end of the manuscript. All symbols and abbreviations should be clearly explained.

Tables should be self-explanatory and include only essential data. Each table must be typewritten on a separate sheet and should be numbered consecutively with Arabic numerals, e.g. Table 1, and given a short caption. No vertical rules should be used. Units should appear in parentheses in the column headings and not in the body of the table. All abbreviations should be defined in a footnote.

All tables and figures that are reproduced from a previously published source must be accompanied by a letter of permission from the Publisher or copyright owner.

Colour figures

It is the policy of *Aquaculture Nutrition* for authors to pay the full cost for the reproduction in print of their colour artwork. Therefore, please note that if there is colour artwork in your manuscript when it is accepted for publication, Wiley-Blackwell requires you to complete and return a colour work agreement form before your paper can be published. This form can be downloaded as a PDF*here. If you are unable to access the internet, or are unable to download the form, please contact the Production Editor at anu@wiley.com