UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
CENTRO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

YOLANDA DE MACEDO DANTAS

CONTRIBUIÇÃO DE FONTES NATURAIS E ARTIFICIAIS NO CRESCIMENTO DO CAMARÃO MARINHO *PENAEUS VANNAMEI* EM BAIXA SALINIDADE

Natal, 2021

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Natal, 2021

Universidade Federal do Rio Grande do Norte - UFRN
Sistema de Bibliotecas - SISBI
Catalogação de Publicação na Fonte. UFRN - Biblioteca Setorial Prof. Leopoldo Nelson - Centro de Biociências - CB

Dantas, Yolanda de Macedo.
Contribuição de fontes naturais e artificiais no crescimento do camarão marinho Penaeus vannamei em baixa salinidade / Yolanda de Macedo Dantas. - 2021.
35 f.: il.

Dissertação (mestrado) - Universidade Federal do Rio Grande do Norte, Centro de Biociências, Programa de Pós-graduação em Ecologia. Natal, RN, 2021.
Orientadora: Profa. Dra. Juliana Deo Dias.
Coorientador: Prof. Dr. Marcos Rogerio Camara.

1. Camarão branco do pacífico - Dissertação. 2. Nutrição - Dissertação. 3. MixSIAR - Dissertação. 4. Sustentabilidade - Dissertação. 5. Água oligohalina -Dissertação. I. Dias, Juliana Deo. II. Camara, Marcos Rogerio. III. Universidade Federal do Rio Grande do Norte. IV. Título.

RN/UF/BSCB CDU 639.512

Elaborado por KATIA REJANE DA SILVA - CRB-15/351

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia da Universidade Federal do Rio Grande do Norte, como parte das exigências para obtenção do título de Mestre em Ecologia.

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Dedico a Francisco Assis de Araújo Dantas e Maria Edinalva de Macedo Dantas, meus pais, por todo amor e dedicação.

AGRADECIMENTOS

Agradeço a Deus, minha fortaleza, por tudo, especialmente pela superação de cada obstáculo e por colocar anjos no meu caminho.

A minha orientadora, Dra. Juliana Deo Dias, por ter me ajudado a focar, pela dedicação, companheirismo, paciência e orientação ao longo dessa jornada.

A meu co-orientador, Dr. Marcos Rogério Câmara, por todas as contribuições e reflexões, pelo apoio, por ter me introduzido no meio dos produtores de camarão e por ter me guiado antes mesmo de ingressar no programa.

A minha família, pela qual sou muito grata pela união, pelo amor, suporte e incentivo.

A Luiz pelo amor, carinho, paciência, compreensão e por ser meu parceiro de vida.

A meus amigos e colegas do PPG Ecologia do Laboratório de Ecologia Aquática e do Departamento de Oceanografia e Limnologia (DOL) pela amizade, apoio, compartilhamento de aprendizado e auxílio na concretização deste trabalho.

Aos meus companheiros de coleta Leonardo e Fernanda, pela boa companhia, pela disposição, ajuda e colaboração.

Aos membros do Instituto de Química e do DOL, em especial a Bruno, Aline, Sany, Beethoven, Eduardo, Jonathas e Maurício, pelo suporte na execução das análises laboratoriais em meio a pandemia.

Aos professores Wallace Nascimento, Vanessa Becker e Renata Panosso por viabilizarem a execução de minhas análises fornecendo equipamentos e insumos de laboratório.

A Cyntia Moraes pela generosidade e atenção em compartilhar seu conhecimento e experiência com a análise de isótopos estáveis.

Aos proprietários e funcionários das fazendas de camarão pela receptividade e acolhimento.

Ao Programa de Pós-Graduação em Ecologia e à UFRN pela formação acadêmica.

Aos membros das bancas examinadoras pelo aprimoramento deste trabalho.

A todos que, de perto ou de longe, colaboraram com mentes brilhantes e braços fortes para a concretização deste trabalho.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Código de Financiamento 001.

O presente trabalho foi realizado com apoio do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

“Tem que perseverar!”

(Fábio Romano Sensei)

“Na natureza nada se cria, nada se perde, tudo se transforma.”

(Antoine Laurent Lavoisier)

RESUMO

A produtividade natural do viveiro cumpre um papel importante na nutrição do camarão e na manutenção da qualidade ambiental durante o cultivo. Organismos planctônicos e bentônicos, assim como detritos são apontados como as principais fontes nutricionais para o camarão. O presente estudo avaliou a contribuição relativa de fontes de alimento naturais e de rações comerciais para o crescimento do camarão *Penaeus vannamei* em fazendas de cultivo semi-intensivo com água de baixa salinidade. A análise de isótopos estáveis de carbono e nitrogênio foi empregada como método traçador das fontes no tecido muscular do animal. As contribuições relativas foram estimadas através de modelo de mistura utilizando a assinatura isotópica das amostras de camarão, ração comercial, matéria orgânica do sedimento e séston, além dos fatores discriminantes das fontes. Os resultados indicam que houve um padrão de incremento na contribuição das fontes de alimento naturais entre as fases de cultivo I (30 dias) e II (60 dias), no entretanto a principal fonte para o desenvolvimento do camarão foi a ração comercial. As fontes naturais tiveram sua maior contribuição (46,8%) na fase II sob baixas densidades de estocagem (Fazenda A) e a menor contribuição (26,9%) na fase I em alta densidade de estocagem (Fazenda B). A diferença nas contribuições relativas das fontes também está associada a características nutricionais da ração comercial, a disponibilidade de alimento natural e a condições ambientais. O incremento das fontes de alimentação natural contribui para práticas de cultivo de camarão semi-intensivos em baixa salinidade mais sustentáveis.

Palavras-chave: camarão branco do pacífico, sustentabilidade, ^{15}N, ^{15}C, MixSIAR, nutrição, água oligohalina.

INTRODUÇÃO GERAL

Ao longo dos últimos 60 anos o consumo anual médio de pescado tem avançado a uma taxa de quase o dobro do crescimento da população mundial. Do mesmo modo, a produção de camarão tem crescido mais rápido que a de outros animais aquáticos. Nesse sentido, o cultivo do camarão P*enaeus vannamei* se destaca como uma parcela significativa da carcinicultura global, correspondendo a mais da metade de todos os crustáceos cultivados. Entre 2010 e 2018, sua produção cresceu de 2649,5 milhares de toneladas para 4966,2 milhares de toneladas, representando 6% de toda produção de animais aquáticos (FAO, 2020). O Brasil, que ocupa a décima posição na produção de crustáceos (FAO, 2020), tem a região Nordeste como principal polo produtivo (Ximenes, 2021). Apesar do volume de camarão comercializado representar somente 9% da aquicultura nacional, o montante financeiro de R$ 1,18 bilhão corresponde a 23% do valor produzido na atividade em virtude do valor agregado do produto (Ximenes, 2021).

A maior parte dos cultivos nacionais opera dentro do sistema semi-intensivo (Nunes, 2019). Particularmente, a produção nesse sistema utiliza densidades de estocagem moderadas (10 a 30 camarões m^{-2}) associadas a estratégias de fertilização e fornecimento de ração em bandejas de alimentação em viveiros de terra. Quando necessário, também há trocas parciais de água e uso de aeração artificial (Tacon et al., 2004).

Tradicionalmente o cultivo de camarão marinho é realizado em zonas costeiras, no entanto também pode ocorrer longe da costa e em condições de baixa salinidade (Roy et al., 2010; Fierro et al., 2018). A nível mundial a produção de crustáceos longe da costa dobrou em 20 anos (FAO, 2018). Isso está ligado ao fato de os cultivos em águas interiores apresentarem vantagens econômicas e ambientais quando comparados aos de zonas costeiras, como: menor custo de terra; menos conflitos ligados ao uso de recursos comuns; e menor impacto em ecossistemas menos resilientes como o manguezal (Nunes e Lopez, 2001; Jory, 2017; Lacerda et al., 2021). Adicionalmente, cultivos em baixa salinidade estão associados a uma maior abundância e diversidade de microalgas, assim como a menor disseminação de doenças de camarão marinho (Nunes e Lopez, 2001; Vincent e Lotz, 2007; Aranguren Caro et al., 2021).

Por se tratar de uma espécie eurialina, *Penaeus vannamei* tem a capacidade de tolerar um amplo espectro de salinidade (0 a 50 g/L) (Pillay, 1990). Porém, seu ponto isosmótico, aquele no qual a pressão osmótica da água é igual à da hemolinfa, é indicado como sendo a salinidade de 20-25 g/L, podendo variar ao longo do ciclo de vida (Chong-Robles, 2014; Jaffer et al. 2019). O estresse salino gerado está associado a um custo energético extra para o camarão manter sua

osmorregulação e crescimento (Li et al., 2017). Em salinidades abaixo de 5 g/L, em geral, é observado menor performance de crescimento e sobrevivência (Diaz et al., 2001; Li et al., 2007). Porém, em um experimento laboratorial de 3 semanas com juvenis de *P. vannamei* em salinidade até 1g/L não foram encontradas diferenças significativas em taxas de sobrevivência, crescimento, ganho de peso e conversão alimentar (Jaffer et al. 2019).

Apesar da importância dos cultivos em baixa salinidade há uma lacuna de trabalhos abordando o tema não somente quanto a crustáceos, mas em aquicultura de maneira geral. Naylor et al. (2021) realizaram uma revisão na literatura aquícola dos últimos 20 anos e verificaram que apenas 25% dos artigos publicados tratam de cultivos em água doce. Num cenário onde mais de 75% do alimento aquático produzido para consumo humano é advindo da água doce essa estatística demonstra uma sub-representação do ambiente aquático continental (Naylor et al., 2021).

Quanto à nutrição do camarão, experimentos realizados em laboratório têm demonstrado que *P. vannamei* detecta o alimento através da visão, sendo capaz de distinguir cores e formas, de quimioreceptores, que propiciam olfato e paladar, e do tato, que revela a textura do objeto manipulado (Kawamura et al., 2017). No entanto, a visão não parece cumprir o papel mais importante, pois a atividade alimentar também ocorre na fase escura do dia (Pontes e Arruda, 2005). Na ausência de algum dos sentidos o comportamento alimentar apresenta plasticidade e o condicionamento através de dietas artificiais pode ser aprendido (Kawamura et al,. 2017).

Ao longo dos estágios do ciclo de vida, o camarão passa por mudanças no hábito alimentar. Nas fases iniciais, o séston, material particulado em suspensão na água como microorganismos e partículas inertes (Esteves, 1998), cumpre um importante papel na nutrição. Na fase de larva zoea alimenta-se de fitoplâncton, já na fase de mises e nas fases iniciais de pós-larva a dieta é a base de zooplâncton, principalmente rotíferos, copépodes e artêmias (Lavens e Sorgeloos, 1996). Já em estágios superiores o consumo de crustáceos, moluscos e detritos ocorre em quantidades significativas (Varadharajan e Pushparajan, 2013). Huang et al. (2020) destaca o meiobentos, principalmente nematóides, como potencial competidor por recursos por compartilharem dietas parecidas, mas também o aponta como um elo nutricional entre produtores primários e *P. vannamei* de tamanho maior. Os detritos presentes no sedimento também são uma importante fonte de proteína com alta digestibilidade devido a comunidade bacteriana associada que compõe de 5 a 10% da matéria no sedimento (Moriarty, 1997).

No viveiro, as fontes naturais cumprem um importante papel na nutrição da população cultivada bem como na ciclagem de nutrientes. Porém, a abundância limitada das fontes naturais não é suficiente para suprir nutricionalmente a população cultivada nas densidades de estocagem

utilizadas em sistemas semi-intensivos, portanto se faz necessário a suplementação com dietas artificiais (Burford et al., 2020).

A composição das rações comerciais tem como base farinha e óleo de peixe para atender a demanda nutricional e energética para o desenvolvimento do camarão em cativeiro. A farinha de peixe integra entre cerca de 30% da formulação das rações (Chatvijitkul et al., 2016) pela alta qualidade proteica e composição de ácidos graxos. Devido ao custo associado e ao fornecimento em declínio de ingredientes marinhos, subprodutos da pesca e da indústria do frango, bem como farelos e óleos vegetais vem sendo empregados (Barreto et al., 2020; Naylor et al., 2021). Buscando atender a demanda crescente por dietas suplementares e manter a sustentabilidade ecológica, econômica e social dos cultivos, outras fontes alternativas têm sido pesquisadas para a substituição da farinha de peixe, como leveduras (Gamboa-Delgado et al., 2016), algas (Perez-Velazquez et al., 2018), bactérias (Gamboa-Delgado et al., 2020) e vegetais (Gamboa-Delgado et al., 2013; Huang et al., 2017).

Uma das formas mais simples de quantificar a contribuição da ração comercial nos cultivos é o fator de conversão alimentar, que relaciona quantos quilos de ração foram necessários para gerar um quilo de animal cultivado (Chatvijitkul et al., 2016). De maneira complementar, alguns estudos também analisam o conteúdo estomacal para estimar a ingestão dessa fonte pelo animal (Porchas-Cornejo et al., 2012; Vinh, 2017) e/ou utilizam a análise de isótopos estáveis para medir a contribuição das fontes alimentares no tecido do camarão (Krummenauer et al., 2020). O carbono e nitrogênio são os principais elementos traçadores utilizados em estudos de ecologia alimentar aquática (Philippsen e Benedito, 2013).

Quando em equilíbrio, a composição isotópica do corpo do consumidor reflete a de sua fonte alimentar com uma pequena diferença (DeNiro e Epstein, 1978; Martinelli et al., 2019). Essa variação é atribuída ao acúmulo de isótopos raros no equilíbrio cinético de substrato e produto das reações bioquímicas e à memória isotópica causada pelo histórico alimentar (DeNiro e Epstein, 1978; Olive et al., 2003; Fry, 2006). A diferença encontrada entre a assinatura isotópica de um tecido do consumidor e sua dieta é chamada de fator discriminante (Philippsen e Benedito, 2013). Em ocasião de mudança na dieta, a alteração na assinatura isotópica do tecido vai depender de dois fatores: sua taxa de *turnover* metabólico, importante em animais grandes e maduros; e diluição da assinatura anterior pelo crescimento, dominante em animais pequenos e jovens (Fry e Arnold, 1982). O tempo estimado para alteração da assinatura isotópica de C em juvenis de *P. vannamei* alimentados somente com ração é de 15 dias (Sánchez et al., 2014).

A correta determinação do fator discriminante é importante para a estimativa da contribuição de uma fonte em relação a um consumidor através de modelos de mistura (Philippsen e Benedito, 2013; Phillips et al., 2014). Os modelos de mistura assumem os seguintes pressupostos: todas as fontes (presas) que contribuem para a mistura (consumidor) são conhecidas e quantificadas; os marcadores são conservados através do processo da mistura; os valores da mistura das fontes e do marcador são fixos; a soma das contribuições resulta em 1; e as fontes têm valores distintos do marcador (Stock et al. 2018).

A técnica de isótopos estáveis tem sido amplamente utilizada em pesquisas de alimentação de organismos aquáticos (Moraes e Henry-Silva, 2018), pois apresenta a vantagem de fornecer a informação do que o animal assimilou de alimento até a captura e não somente do que foi ingerido há pouco (Pereira e Benedito, 2007). Diante da importância socioeconômica e ambiental da carcinicultura e da lacuna de conhecimento quanto à contribuição da ração e das fontes naturais para o crescimento do camarão marinho em condições de cultivo comercial com água oligohalina, realizamos este trabalho, com emprego da análise de isótopos estáveis. Esta dissertação está estruturada em um capítulo, na forma de artigo, que objetivou avaliar a contribuição das fontes naturais e artificiais no desenvolvimento do camarão marinho *Penaeus vannamei* cultivado em água oligohalina, a ser submetido na revista Aquaculture.

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Contribution of natural and artificial sources to the growth of marine shrimp *Penaeus vannamei* in low salinity

Abstract: The natural productivity of a pond plays an important role in shrimp nutrition and in keeping the environmental quality during cultivation. Planktonic and benthic organisms as well as detritus are pointed out as the main nutritional sources for shrimp. This study evaluated the relative contribution of natural feed sources and commercial feeds to the growth of shrimp *Penaeus vannamei* on semi-intensive farms with low salinity water. The stable isotope analysis of carbon and nitrogen was used as a method to trace the sources in the animal's muscle tissue. The relative contributions were estimated through a mixing model using the isotopic signature of samples of shrimp, commercial feed, sediment organic matter, and seston, in addition to the discriminant factors of the sources. The results indicate that there was a pattern of increment in the contribution of the natural feed sources between the cultivation phases I (30 days) and II (60 days), but the main source for the development of the shrimp was the commercial feed. The natural sources had their greatest contribution (46.8%) in phase II under low stocking densities (Farm A) and the smallest contribution (26.9%) in phase I under high stocking density (Farm B). The difference in the relative contributions of the sources is also associated with nutritional characteristics of the commercial feed, the availability of natural food, and the environmental conditions. Increasing natural feed sources contributes to more sustainable low-salinity semi-intensive shrimp farming practices.

Key words: Pacific white shrimp, sustainability, ^{15}N, ^{13}C, MixSIAR, nutrition, oligohaline water.

1. Introduction

Shrimp farming has been a growing activity as an alternative to fishing to meet the global demand for animal protein. As an important economic activity, over the years it has considered issues such as biosafety, new technologies, and has a particular focus on sustainability (FAO, 2020; Naylor et al., 2021). Many authors have studied alternatives to replace or reduce the use of artificial diets with fish-based protein in shrimp nutrition (Kent et al. 2011; Gamboa-Delgado et al., 2013; Gamboa-Delgado et al., 2016; Magaña−Gallegos et al., 2018; Gamboa-Delgado et al., 2020), because the excess of artificial food decreases the water and soil quality, generating environmental impacts (Chaikaew et al., 2019). Furthermore, commercial feed is the largest productive cost in shrimp farming, which impacts economic sustainability (Maity and Saha, 2020; Nisar et al., 2021).

Therefore, the knowledge of ecological and nutritional aspects of shrimp farming is essential for decision-making in rearing management.

Some farms adopt the closed system to reduce the risk of disease spread and environmental impacts caused by effluents discharge. In that system, the water from a current cycle is kept being used in a future one. However, the accumulation of nutrients and organic matter increases over time and leads to intense eutrophication of the pond (Thakur and Lin, 2003; Chaikaew et al., 2019). In this "mature" water condition, smaller food conversation factors are found due to the greater availability of natural food (Thakur and Lin, 2003).

The natural productivity of a pond plays an important role in shrimp nutrition and in maintaining the environmental quality of semi-intensive shrimp cultures (Nunes et al., 1997; Bojórquez-Mascareño and Soto-Jiménez, 2013; Cardona et al., 2015). Periphytic, planktonic and benthic organisms have a high protein content, an adequate amino acid profile, in addition to suitable body and mobility characteristics to be preyed upon by shrimp, especially in the early life stages (Snell and Carrillo, 1984; Lavens and Sorgeloos, 1996; Helland et al., 2003; Abreu et al. 2007). As a benthic animal with an omnivorous habit, *Penaeus vannamei* also feeds on organic matter at the bottom of the pond (Huang et al., 2020).

Often, the contribution of natural sources exceeds that of commercial feed in the nutrition of cultivated shrimp (Gamboa-Delgado et al., 2003; Gamboa-Delgado, 2014; Magaña−Gallegos et al., 2018. Stomach content analysis of penaeid shrimps reports the presence of a wide variety of food items in addition to commercial feed, including protozoa, copepods, rotifers, diatoms, nematodes, amphipods, polychaetes, other microorganisms, and organic particles with intakes varying according to the availability of food and shrimp grow-out phase (Nunes et al., 1997; Kent et al., 2011; Vinh, 2017). Thus, the greatest contributions of the natural feed sources to the stomach content are found in a condition of high availability of natural foods (Porchas-Cornejo et al., 2012; Vinh, 2017), and the contribution of the natural diets decreases as the shrimp grows (Bojórquez-Mascareño and Soto-Jiménez, 2013).

Despite the multiple benefits of natural productivity to sustainability in shrimp farming, it is difficult to quantify its direct contribution to shrimp development through conventional analysis of ingested food. In this sense, the stable isotope analysis along with mixing models allows us to understand how consumers assimilate the elements consumed from their sources, enabling researchers to estimate the contributions of the diet (Peterson and Fry, 1987; DeNiro and Epstein, 1981; Fry, 2006; Stock and Semmens, 2016a; Moraes and Henry-Silva, 2018). The stable isotope technique is widely used in ecological studies to understand the trophic relationships and the flow

of energy and nutrients (Focken and Becker, 1998; Phillips, 2012; Phillips et al., 2014; Quinby et al., 2020). Looking at the pond as a small ecosystem with a limited number of sources (Gamboa-Delgado, 2014), its controlled conditions make it easier to track changes in the isotopic ratios of the animals (Manetta and Benedito-Cecilio, 2003; Phillips, 2012; Sacramento et al., 2016).

Although in recent years the number of studies using stable isotope analysis has increased (Quinby et al., 2020), most of those that analyze the contribution of different food sources for the shrimp diet are short term and/or short scale (Gamboa-Delgado and Le Vay, 2009; Bojórquez-Mascareño and Soto-Jiménez, 2013; Krummenauer et al., 2020). We know little about the effective contribution of each source in the field and there are even fewer studies under low salinity conditions (Naylor et al., 2021), where salt stress causes an extra energy cost for shrimp to maintain their osmoregulation and growth (Li et al., 2017). In a low-salinity cultivation condition, higher feed conversion ratios are observed (Moura, 2020). That shows a greater dependence on artificial feed.

In this study, we used the stable isotope technique to determine the relative contribution of natural food sources (seston and sediment organic matter) and commercial feed to shrimp *Penaeus vannamei* development reared in semi-intensive farms with oligohaline water. We predicted that (i) the contribution of natural sources is higher than artificial sources at the beginning of rearing phase (30 days), (ii) the contribution of natural sources exhibit a decreasing pattern between phases, and (iii) the contribution of natural sources may differ between farms management strategies.

2. Materials and methods

2.1. Study site and culture conditions

The study was conducted in two commercial shrimp farms located by the Ceará-Mirim River (5°37’46.8"S, 35°25'23.0"W), Rio Grande do Norte, Northeast of Brazil. The farms were chosen to represent distinct and commonly found semi-intensive production conditions. Farm A operates under a two-phase system, involving a nursery phase of about 20 days (initial stocking density of 2,000 post-larvae 10 m^{-3}), and a grow-out phase in earthen ponds (4-5 ha) at a 10 shrimp m^{-2} density. After each cycle, the water was recirculated in the system, and there was no water discharge. Replenishing was applied to compensate losses due to evaporation and infiltration. On other hand, Farm B adopts direct stocking of 30 post-larvae 10 m^{-2} in earthen ponds (1 ha) and does not recirculate water. A high-protein commercial feed (55% crude protein) was offered every 2

hours in the nursery phase (Farm A), whereas in the grow-out phase a 35% crude protein commercial feed was applied on both farms (Table S1).

2.2. Experimental design and field sampling

Between February and May 2020, we followed 4 independent productive ponds on each farm, taking samples at the 30th and 60th days of cultivation. Samples of shrimp, sediment organic matter, water, and zooplankton were collected at each time. Shrimp were captured by cast net. To represent the pond bottom, the sediment sample was a pool of three points collected following the slope of the pond, from the deepest point, next to the drain gate, to the shallowest point on the central plateau. The sediment was collected by a PVC tube of 34.8 mm inner diameter, and the top 0–3 cm sediment layer was considered (28.5 cm^{-3}). The water used to analyze the quality parameters and to obtain the seston was collected with a bucket near the drainage gate and transported in polyethylene bottles (4 L) in refrigerated isothermal boxes. To evaluate the zooplankton density, the sample was obtained by a plankton net with 68 µm mesh size and 30 cm in diameter pulled for 5 meters and preserved in 10% formol.

For stable isotope analysis, 3 shrimps 1 sediment sample, and 1 seston sample per pond were considered in each phase, totalizing 48 animals and 16 samples of each natural sources, respectively. A triplicate of each commercial feed was also analysed.

2.3. Environment quality parameters

In the field, the measurement of water temperature and dissolved oxygen, surface, and 1 m deep (bottom) (oximeter Instrutherm MO-900), were made in the same place as the water collection. In the laboratory, we measured salinity (ATC handheld optical refractometer), pH (pH meter luca-210p), alkalinity, total ammoniacal nitrogen (TAN), nitrite (NO_{2}^{-}), nitrate (NO_{3}^{-}) and total nitrogen (APHA, 2012). To determine total phosphorus (TP), we proceed with oxidation using alkaline potassium persulphate in an autoclave at 120 °C for 30 min followed by the spectrophotometric ascorbic acid method (Carmouze, 1994, Mackereth et al., 1978). Chlorophyll-a was extracted from 0.7 µm glass microfiber filter (GF-1, Macherey-Nagel) with 90% ethanol overnight (Jespersen and Christoffersen, 1987). The filtered water was used for orthophosphate (PO_{4}^{3-}) analysis following the total phosphorus methodology without persulfate digestion. The analysis of suspended particulate matter (SPM) was through filtration in a pre-combusted (500 °C, 4

hr) 0.7 µm glass microfiber filter (GF-1, Macherey-Nagel) and drying in an oven at 55-60 °C. The SPM was determined by comparing filter weights before and after filtering a known amount of water. Similarly, the sediment organic matter (SOM) was measured by comparing the weight of the sample dried at 105 °C and the weight after combustion in a muffle furnace (600 °C for 6 h) (Goldin, 1987). The environmental parameters are summarised in Table 1.

Table 1. Mean values (± standard deviation) of the environmental parameters during sampling phases in both farms. A1 - farm A, phase I; A2 - farm A, phase II; B1 - farm B, phase I; B2 - farm B, phase II.

Environmental variables	A1	A2	B1	B2
Temperature (°C)	31.93 ± 0.56	33.55 ± 0.87	32.68 ± 0.70	31.73 ± 1.25
Salinity (g/L)	1.83 ± 0.80	1.98 ± 0.76	1.00 ± 0.14	0.88 ± 0.25
Surface dissolved oxygen (mg/L)	8.20 ± 2.58	9.50 ± 1.48	7.43 ± 2.42	6.45 ± 0.71
Bottom dissolved oxygen (mg/L)	7.47 ± 2.73	7.08 ± 1.44	4.85 ± 2.87	4.53 ± 1.25
pH	8.55 ± 0.41	8.55 ± 0.25	8.40 ± 0.31	8.40 ± 0.52
Alkalinity (mg/L $CaCO_3$)	197.5 ± 32.8	175.0 ± 13.7	247.3 ± 137.7	163.3 ± 44.5
Suspended particulate matter (mg/L)	81.97 ± 9.62	72.73 ± 9.32	71.08 ± 39.22	82.01 ± 18.68
Total Nitrogen (mg/L)	1.47 ± 0.30	1.52 ± 0.40	0.94 ± 0.20	1.09 ± 0.15
Total ammoniacal nitrogen (mg/L)	0.39 ± 0.24	0.46 ± 0.20	0.07 ± 0.05	0.21 ± 0.08
Nitrite (mg/L)	<0.10	<0.10	<0.10	<0.10
Nitrate (mg/L)	0.58 ± 0.27	0.61 ± 0.34	0.66 ± 0.14	0.56 ± 0.03
Total phosphorus (mg/L)	0.09 ± 0.02	0.12 ± 0.02	0.25 ± 0.07	0.27 ± 0.03
Ortophosphate (mg/L)	0.03 ± 0.02	0.02 ± 0.00	0.05 ± 0.03	0.03 ± 0.04
Chlorophyll a (µg/L)	52.16 ± 19.64	112.7 ± 40.5	60.25 ± 34.21	175.1 ± 81.5
Sediment organic matter-SOM (%)	6.39 ± 1.13	5.54 ± 3.55	13.29 ± 3.75	8.00 ± 1.78
Zooplankton abundance (ind/L)	188.5 ± 104.2	124.5 ± 74.2	41.46 ± 10.72	23.51 ± 7.96
Rotifera (ind/L)	25.76 ± 35.74	42.83 ± 45.89	3.33 ± 5.95	6.12 ± 3.49
Cladocera (ind/L)	55.42 ± 47.90	0.20 ± 0.24	2.69 ± 2.70	0.63 ± 0.85
Copepoda (ind/L)	35.66 ± 41.75	26.83 ± 48.87	11.81 ± 15.32	5.53 ± 8.11
Others* (ind/L)	0.18 ± 0.28	0.49 ± 1.03	0.01 ± 0.03	0.09 ± 0.19

Others - Insecta and Ostracoda.

The counting and identification of the zooplankton groups (Rotifera, Cladocera, Copepoda, Insecta and Ostracoda) were carried out under an optical microscope by taking successive 2.5 ml homogenized aliquots. We applied the "Method 50 / Taxon" (Stahl and Stein 1994). If any group reached a count greater than or equal to 50 individuals, it would no longer be counted in the next aliquot. The counting was finished only when all groups reached at least 50 individuals counted or 10% of the concentrated sample was analyzed (approximately 20 aliquots). The zooplankton abundance was determinate in individuals per mL.

2.3. Shrimp performance

Each farm followed its own schedule according to production performance and market demand. To compare them in the study period, we considered linear mortality throughout the rearing phase and assumed a survival proportional to that found at the end of the cycle. The feed conversion ratio (FCR) was calculated by dividing the accumulated commercial feed input to that point by the estimated biomass gain. Survival, FCR and growth ratio (GR) equations are shown below.

$$\text{Survival (\%)} = (\text{estimated number of individuals at phase II} / \text{initial number of individuals}) * 100$$

$$\text{FCR} = \text{commercial feed supplied} / \text{biomass gain}$$

$$\text{GR (mg/day)} = [(\text{weight at phase II} - \text{initial weight}) / \text{number of days}]$$

2.5. Sample treatment for stable isotope analysis

Shrimp, water, and sediment samples were kept frozen until analysis. After thawing, the shrimp were peeled and had the intestine removed, and only the abdominal muscle tissue was analyzed. The water was filtered through a pre-combusted (500 °C, 4 hr) 0.7 µm glass microfiber filter (GF-1, Macherey-Nagel) with a low-pressure vacuum pump. The sediment was sieved in a 2 mm mesh to remove plant parts and macroinvertebrates. Subsamples were acidified to remove carbonate carbon, which is often more enriched in ^{13}C (Boutton, 1991). We applied as little as possible HCl 1N (drop-by-drop) until there is no formation of CO_2, then the sediment was recovered by centrifuging (Jacob et al., 2005, Kim et al., 2016). As acidification proportionally affects more the N content, the untreated subsample provided the N isotope signatures (Schlacher and Connelly, 2014). Shrimp tissues, filters, acidified and non-acidified sediment, and commercial

feeds were dried in an oven at a temperature of 55-60 °C for 24-48 h. Then, the filter with seston was scrapped, while other samples were ground to a fine powder using a mortar and pistil. All samples were weighed using an analytical balance (Sartorius) (aliquots of about 1.2 mg for shrimp tissue, 2.0 mg for commercial feed, 74.0 mg for non-acidified sediment, 11.0 mg for acidified sediment, and 5.2 mg for seston, all based on elemental content) into 5×3.5 mm tin capsules. Encapsulated samples were sent to the laboratory of the Stable Isotope Facility, Department of Plant Sciences, California University (Davis), USA, to perform stable isotope analysis of ^{13}C and ^{15}N based on natural abundance.

2.6. Stable Isotopes and mixing model

The results of the stable isotope analysis of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are expressed in delta notation (δ), which is per mill (‰) deviations from the standard (Peterson and Fry, 1987; Post, 2002; Philippsen and Benedito, 2013), according to the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(Rsample / Rstandard) – 1] * 1000 \quad (1)$$

Where 'Rsample' is the ratio between heavy and light isotopes, and the 'Rstandard' is derived from the standard. Vienna Pee Dee Belemnite and atmospheric air are the international standards for carbon and nitrogen, respectively.

The difference in isotopic ratio between the tissue samples and each feed item was calculated to find the discriminantion factor (Martínez del Rio et al., 2009; Philippsen and Benedito, 2013).

$$\Delta X = \delta X_{\text{muscle tissue}} – \delta X_{\text{feed item}} \quad (2)$$

Where Δ is the discrimination factor and X the chemical element represented by the heavier isotope (^{13}C or ^{15}N).

The Bayesian mixing model used to estimate the proportions of source contributions to the mixture (consumer) was run using the MixSIAR package (version 3.1.12) (Stock and Semmens, 2016a) on R software (version 3.6.3)(R Core Team, 2020). The mixing models for each farm phase included raw data of the isotopic signature of shrimps and sources, as well as discrimination factors for each source. We set an uninformative prior and assumed process error only for modeling the shrimp individuals (Stock and Semmens, 2016b). The Markov Chain Monte Carlo simulation method of the models had the following parameters: three chains of 1,000,000 iterations length, a burn-in of 500,000, and a thin of 500, which are default for the "very long" modeling (Stock and Semmens, 2016a). Convergence of posterior distributions for all variables in the model was verified

according to Gelman-Rubin (1992; Gelman et al., 2014) diagnose. The values of the mean contributions from the sources for each individual shrimp were used to calculate the mean contribution for each study period.

2.7. Statistical analysis

The relative contributions of feed sources at each farm and grow-out phase were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test. The Mann-Whitney test was performed to determine the difference in the relative contribution of each source between farm phases. All statistical analyses were performed in R 3.6.3 adopting the significance level of $p<0.05$.

3. Results

The results of isotopic composition showed mean values of -23.89 ± 1.41 ‰ ($\delta^{13}C$) and 5.47 ± 1.15 ‰ ($\delta^{15}N$) for the sediment organic matter (SOM). The means of carbon and nitrogen isotopic ratios for the commercial feeds were -21.68 ± 1.04 ‰ and 5.35 ± 1.50 ‰, respectively. The seston had a higher value of $\delta^{13}C$ (-10.30 ± 2.98 ‰) when compared with shrimp tissue -22.61 ± 1.01 ‰. As the discrimination factor is the difference between the isotopic ratio of the consumer's tissue and its source, those sources that presented the highest concentration of the ^{13}C isotope had a negative Δ. The δ13C and δ15N results obtained by the period in each farm, as well as the discrimination factors, are shown in Table 2.

Table 2

Mean values (± standard deviation) of the isotopic compositions and discrimination factors. Numbers I and II represent the rearing phases (Phase I - 30 days, Phase II - 60 days).

Samples	Isotopic composition		Discrimination factor	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\Delta^{13}C$ (‰)	$\Delta^{15}N$ (‰)
Farm A				
Shrimp I	-22.88 ± 1.21	8.77 ± 0.60	————	————
Seston I	-8.73 ± 1.23	6.03 ± 0.66	-14.14 ± 1.59	2.74 ± 0.82
SOM I	-23.24 ± 1.58	5.88 ± 0.33	0.38 ± 1.81	2.89 ± 0.65
Commercial feed I	-22.37 ± 0.09	4.01 ± 0.32	-0.49 ± 1.18	4.76 ± 0.64
Shrimp II	-22.82 ± 1.41	6.91 ± 1.03	————	————

Samples	Isotopic composition		Discrimination factor	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\Delta^{13}C$ (‰)	$\Delta^{15}N$ (‰)
Farm A				
Seston II	-9.06 ± 1.86	3.66 ± 0.47	-13.77 ± 2.13	3.25 ± 1.08
SOM II	-22.60 ± 0.76	6.61 ± 1.38	-0.22 ± 1.52	0.30 ± 1.57
Commercial feed II	-22.37 ± 0.09	4.01 ± 0.32	-0.45 ± 1.38	2.90 ± 1.03
Farm B				
Shrimp I	-22.49 ± 0.63	9.92 ± 1.19	———	———
Seston I	-8.73 ± 1.96	6.47 ± 0.52	-13.76 ± 1.82	3.45 ± 1.24
SOM I	-24.59 ± 0.96	4.46 ± 0.50	2.10 ± 1.03	5.46 ± 1.23
Commercial feed I	-22.37 ± 0.04	3.08 ± 0.26	-0.11 ± 0.61	6.84 ± 1.18
Shrimp II	-22.26 ± 0.48	7.34 ± 0.87	———	———
Seston II	-14.70 ± 0.99	4.19 ± 0.31	-7.56 ± 0.98	3.15 ± 0.89
SOM II	-25.13 ± 0.71	4.94 ± 0.83	2.87 ± 0.78	2.41 ± 1.11
Commercial feed II	-22.37 ± 0.04	3.08 ± 0.26	0.11 ± 0.46	4.26 ± 0.88

The mixing models converged (see Fig. S1). The Mann-Whitney test showed that the average contributions from feed sources and seston differed between periods at Farm A (commercial feed, W= 131, $p<0.05$; seston, W= 0, $p<0.05$), except for sediment organic matter. The same was observed at Farm B (commercial feed, W= 107, $p<0.05$; seston, W= 0.5, $p<0.05$). Farm A presented the higher contribution of autochthonous sources in the final rearing phase (46.78 ± 0.01%). However, the commercial feed proved to be the most important source to the growth of the reared animals, with contributions of 55.6 %, 53.2 %, 73.1 % and 71.0 % at A1 (Kruskal-Wallis X2(2) = 31, $p<0.05$), A2 (Kruskal-Wallis X2(2) = 25, $p<0.05$), B1 (Kruskal-Wallis X2(2) = 31, $p<0.05$) and B2 (Kruskal-Wallis X2(2) = 26, $p<0.05$), respectively (Fig.1). The mean values (± standard deviation) of contributions from SOM and seston were 24.0 % and 20.5 % at A1, 23.2 % and 23.6 % at A2, 17.5 % and 9.4 % at B1, and 15.0 % and 14.0 % at B2.

In terms of weight gain and feed conversion ratio, shrimps from Farm A (lower density) had better performance. At phase II, the average shrimp weight reared at farm A was 7.33 ± 1.40 g while at farm B 5.93 ± 1.50g. The estimated FCR indicated good efficiency in the use of the commercial feed, with average values of 0.65 ± 0.21 and 0.76 ± 0.32 for farms A and B, respectively. The

average survival of both farms remained above 80%. The results for zootechnical performance for *P. vannamei* shrimp farming during the study periods in each farm are shown in Table 3.

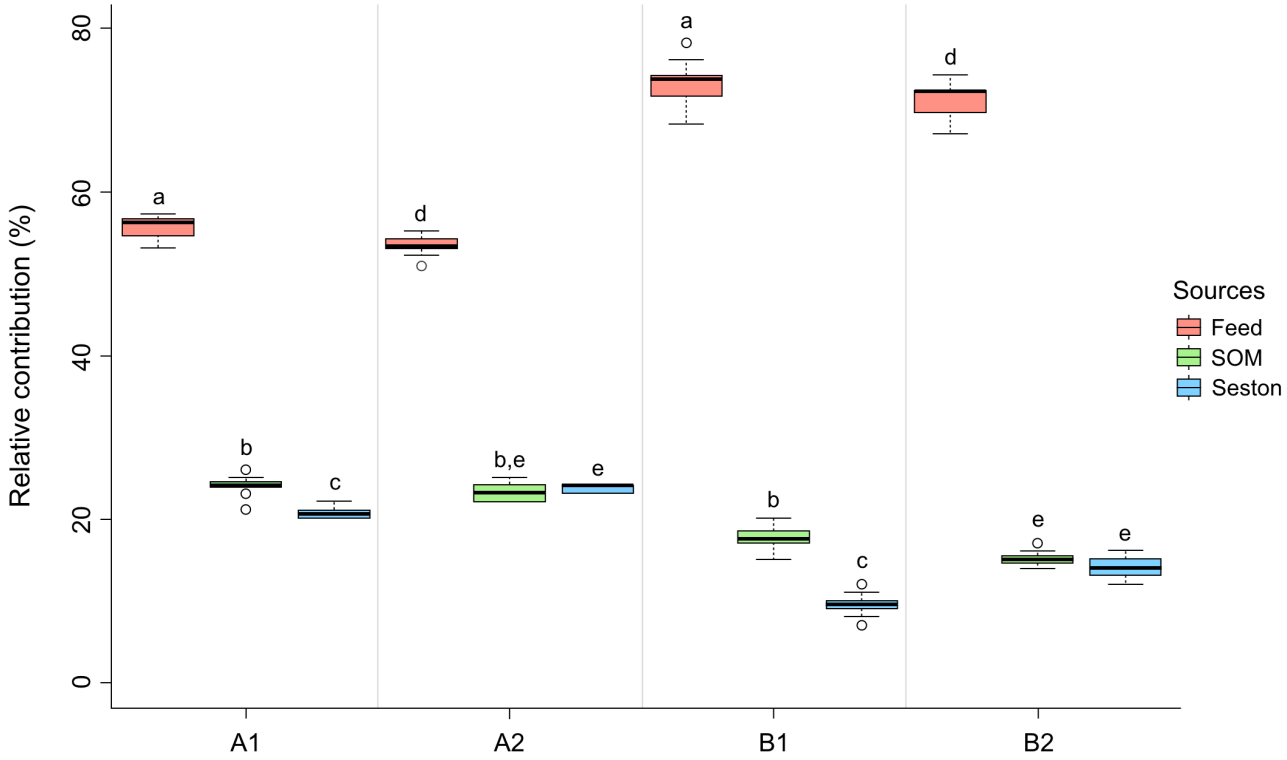

Fig.1 Relative contribution of sources (commercial feed, seston and sediment organic matter - SOM). A1 - Farm A at Phase I (30 days), A2 - Farm A at Phase II (60 days), B1 - Farm B at Phase I, B2 - Farm B at Phase II. Different letters indicate significant differences ($p<0.05$).

Table 3

Zootechnical performance of the shrimp (average ± standard deviation) in Farm A and Farm B. Feed conversion ratio (FCR); Growth rate (GR).

Parameter	Farm A	Farm B
Weight Phase I (g)	1.90 ± 0.87	2.17 ± 0.71
Weight Phase II (g)	7.33 ± 1.40	5.93 ± 1.50
Survival (estimated) (%)	82.42 ± 9.87	81.63 ± 7.41
FCR (estimated)	0.65 ± 0.21	0.76 ± 0.32
GR (mg/day)	122.06 ± 5.33	98.58 ± 15.71

4. Discussion

Our results suggest that natural food sources are important for the growth of shrimp reared in semi-intensive systems with low salinity water, with a temporal pattern of increasing its contribution, contradicting our prediction. Additionally, we observed that the commercial feed was the one that most contributed to the shrimp tissue during the analyzed period, and there was a difference in the relative contribution of the sources between the farms. The higher contribution of natural sources (46.8 %) was found on phase II under lower stocking density (Farm A - 10 ind/m^2). The lower contribution of these sources (26.9 %) occurred in rearing phase I at a higher stocking density (Farm B - 30 ind/m^2). These results can be attributed to a combination of factors such as the nutritional characteristics of the feed, the availability of natural food, and the environmental conditions.

The high contribution of commercial feed may be associated with its nutritional quality. Several studies have shown that commercial feed corresponds to a small amount, not exceeding 50%, of the stomach content of cultivated shrimp, which points to a high preference for natural food from the pond over formulated food (Nunes et al., 1997; Porchas-Cornejo et al., 2012; Gamboa-Delgado et al., 2013; Huang, 2020). However, the high protein content and digestibility of the components of commercial feed favor its assimilation in terms of nitrogen and carbon (Gamboa-Delgado, 2014). In this sense, Nunes et al. (1997) reported 16% of the stomach contents contributing 25% of the carbon growth in *Penaeus subtilis*. The large contribution of the artificial feed found here cannot be associated with commercial feed qualities only. Studies showed that there is an interaction between natural and artificial sources that contribute to the animal's performance. The presence of the natural diet, which is rich in digestive enzymes, also contributes to better absorption of artificial feed, in addition, it promotes better survival and energy conversion results when compared to diets restricted to artificial food (Xue et al., 2021).

In case of natural feed abundance, it can meet the demand for intermediate densities of small shrimps with minimal commercial feed participation (Huang et al., 2020). Bojórquez-Mascareño and Soto-Jiménez (2013) found 66% contribution from natural sources in ponds at a 60 shrimp (0.28g) m^{-2} density. The contribution decreased to 26 % to shrimps weighing 3.3 g. In the same study under treatment without commercial feed addition, zooplankton and benthic feed contributed 25% and 75% respectively in early stages, while larger shrimp depended exclusively on benthic. Similarly, Huang et al. (2020) found particulate organic matter (seston) as the major source in the early stages (66%) and nematodes (40%) contributed significantly to larger specimens, with the commercial feed not exceeding 12% contribution in semi-intensive cultivation. In rice fields, benthic organic matter is reported as the main source for shrimp nutrition (Burford, 2004a). In our

study, we found higher mean values of seston contribution (20.5 % and 23.6 %) in the closed system farm, which also showed higher zooplankton abundance (188.5 and 124.5 ind/L). Farm B had a high content of organic matter at the bottom of the pond, and this was the natural source that contributed the most (17.5 %) to the shrimp cultivated there.

Low contributions of natural sources are usually found in *P. vannamei* intensive systems, as observed by Burford et al. (2004b) in which the biofloc 120 animals m^{-2} contributed up to 29% of the nitrogen, and by Cardona et al. (2015), with 40% contribution to adult shrimps (25 animals $m^{-2)}$ coming from natural productivity. The low contribution of natural sources found in this study, specially at farm B (higher density) is comparable to those found in intensive systems with high densities in saline water. In cultivation conditions where higher stocking densities are employed, natural productivity is not sufficient for the satisfactory development of the population, resulting in the demand for food supplementation (Burford et al., 2020; Naylor, 2021).

The uneaten feed, feces, and metabolic wastes favor the accumulation of organic matter at the bottom of the pond. The high levels of organic matter found since the initial phases in Farm B indicate overfeeding or low feed consumption by the shrimp. The increase of chlorophyll-a levels in both farms also indicates that the input of commercial feed acts as a fertilizer. Despite the increase of chlorophyll-a, the excess organic matter favors anoxic conditions that interfere with the metabolism and survival of the benthic community (Boyd, 2015; Huang et al., 2020). Furthermore, the stress of low salinity affect the oxygen consumption by the *P. vannamei* (Bett and Vinatea, 2009; Zhang et al., 2009). Shrimps are constant feeders, but if oxygen levels are not sufficient to meet the costs associated with consuming and processing food, feeding is stopped (Rahmawati, 2021). Dissolved oxygen levels below 4 mg/L are associated with reduced bowel filling, indicating interruption of feeding mainly at night (Focken et al., 1998). Laboratory studies of the feeding behavior of P. *vannamei* juveniles show that it remains active also during the dark phase of the day (Santos et al., 2013) exploring the sediment in search of food, mainly at the beginning of the light phase (Pontes et al. 2006). A previous study points out that the offer of commercial feed reinforces the search and intake of food items actions (Pontes and Arruda, 2005). Low levels of oxygen at night may have influenced eating behavior and favor the consumption of commercial food available during the light phase of the day, mainly at Farm B.

Taking into account environmental and economic factors, the mixed diet brings good results for shrimp farming with oligohaline water. The benefit of favoring natural food sources to reduce the use of commercial food is linked to the greater sustainability of crops. The feed generally represents the highest farming cost and have many embedded resources. Energy, agricultural land,

water, and wild fish are economic and environmental resources associated with the production of commercial feed (Chatvijitkul et al., 2016). Shrimp cultivation has one of the largest FCR aquaculture activities, around 1.6 (Naylor et al., 2021). The high commercial feed contribution combined with FCR values below 1 found in the study farms indicate a good efficiency of crops. Additionally, more studies are needed to clarify the possible shrimp's dependence on the organic matter of commercial feed in low salinity conditions.

5. Conclusion

Our results showed natural food sources, such as seston and sediment organic matter, contribute to the growth performance of *P. vannamei* through semi-intensive systems under oligohaline farming conditions. Especially under conditions of higher density, the commercial feed proved to be the most important food source for shrimp growth. It indicates the importance of an integrated approach that optimizes natural food sources for semi-intensive marine shrimp farming more sustainably. The management of the food strategy, considering the feed ecology of the shrimp, and the maintenance of environmental parameters, in favor of the pond biota is the key to optimized production.

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Supplement Material

Table S1. Characteristics and components of commercial feeds used by Farms A and B. CP - Crude protein; min - minimum; max - maximum; DHA - Docosahexaenoic acid; UI - International unit.

Componente	Content	Farm A - 35% CP	Farm B - 35% CP
Moisture (g/Kg)	(max)	130	130
Protein (g/Kg)	(min)	350	350
Ether extract (g/Kg)	(min)	75	60
Crude fiber (g/Kg)	(max)	50	50
Mineral matter (g/Kg)	(max)	130	140
Calcium (g/Kg)	(min)	30	15
Calcium (g/Kg)	(max)	30	35
Phosphorus (g/Kg)	(min)	7	10
Sodium (g/Kg)	(min)	4	3.7
Mg (g/Kg)	(min)	-	1.5
DHA (mg/Kg)	(min)	-	140
Vit. A (IU/Kg)	(min)	7500	12000
Vit. D3 (IU/Kg)	(min)	2250	2000
Vit. E (IU/Kg)	(min)	150	160
Vit. C (mg/Kg)	(min)	300	225
Vit. K3 (mg/Kg)	(min)	15	40
Vit. B1 (mg/Kg)	(min)	22.5	15
Vit. B2 (mg/Kg)	(min)	15	30
Vit. B6 (mg/Kg)	(min)	15	40
Vit. B12 (mg/Kg)	(min)	75	20
Vit. B9 (mg/Kg)	(min)	5.2	5
Vit. B7 (mg/Kg)	(min)	0.3	0.2
Vit. B8 (mg/Kg)	(min)	525	-
Vit. B3 (mg/Kg)	(min)	74.5	60
Vit. B5 (mg/Kg)	(min)	45	70
Methionine (mg/Kg)	(min)	6000	-
Co (mg/Kg)	(min)	4	0.25
Cu (mg/Kg)	(min)	50	26
Fe (mg/Kg)	(min)	-	27
I (mg/Kg)	(min)	0.3	0.5
Mn (mg/Kg)	(min)	31.5	17
Se (mg/Kg)	(min)	0.2	0.2
Zn (mg/Kg)	(min)	90	66
Cr (mg/Kg)	(min)	0.01	0.05

Fig. S1 Convergence diagnoses for modeling shrimp individuals by phase and farm (76 variables), A1 (a), A2 (b), B1 (c), and B2 (d). The Potential Scale Reduction Factors of the Gelman-Rubin test indicated that the chains stabilized (converged) since the between-chain variance was not greater than within-chain variance (R <1.1), and the worst variables were included in the confident interval (C.I.).

a

```
############################################################
# Gelman-Rubin Diagnostic
############################################################

Generally the Gelman diagnostic should be < 1.05

Out of 76 variables: 0 > 1.01

0 > 1.05

0 > 1.1

The worst variables are:

             Point est. Upper C.I.
p.fac1[6,3]     1.007815   1.022809
ilr.fac1[6,2]   1.005769   1.019316
loglik[6]       1.005489   1.015088
p.fac1[5,1]     1.004948   1.019615
p.fac1[9,2]     1.004590   1.011465
p.fac1[5,2]     1.004046   1.013874
ilr.fac1[7,1]   1.003797   1.014018
p.fac1[12,1]    1.003769   1.016140
p.fac1[4,2]     1.003599   1.013578
ilr.fac1[4,1]   1.003155   1.008951
```

b

```
############################################################
# Gelman-Rubin Diagnostic
############################################################

Generally the Gelman diagnostic should be < 1.05

Out of 76 variables: 0 > 1.01

0 > 1.05

0 > 1.1

The worst variables are:

              Point est. Upper C.I.
p.fac1[12,2]     1.005692   1.015712
loglik[11]       1.004798   1.007647
ilr.fac1[12,1]   1.004320   1.017666
ilr.fac1[11,2]   1.003877   1.012823
ilr.fac1[9,2]    1.003764   1.015789
deviance         1.003653   1.007428
loglik[3]        1.003228   1.007430
ilr.fac1[8,2]    1.003136   1.009731
loglik[4]        1.003070   1.009071
ilr.fac1[2,1]    1.002808   1.011524
```

c

```
############################################################
# Gelman-Rubin Diagnostic
############################################################

Generally the Gelman diagnostic should be < 1.05

Out of 76 variables: 1 > 1.01

0 > 1.05

0 > 1.1

The worst variables are:

               Point est. Upper C.I.
p.fac1[6,3]      1.016148   1.032461
loglik[6]        1.008338   1.020266
p.fac1[3,3]      1.006206   1.007536
ilr.fac1[10,1]   1.004843   1.015364
p.fac1[10,2]     1.004252   1.012004
p.fac1[7,3]      1.003969   1.008931
loglik[9]        1.003860   1.008307
p.fac1[11,1]     1.003202   1.013195
ilr.fac1[11,1]   1.002954   1.011572
ilr.fac1[9,2]    1.002322   1.006206
```

d

```
############################################################
# Gelman-Rubin Diagnostic
############################################################

Generally the Gelman diagnostic should be < 1.05

Out of 76 variables: 1 > 1.01

0 > 1.05

0 > 1.1

The worst variables are:

              Point est. Upper C.I.
p.fac1[4,3]      1.016747   1.038160
p.fac1[12,3]     1.006923   1.017587
p.fac1[4,1]      1.006567   1.018100
p.fac1[11,3]     1.006550   1.017508
p.fac1[5,3]      1.005882   1.012751
p.fac1[9,3]      1.004365   1.011529
p.fac1[3,3]      1.004312   1.012073
ilr.fac1[2,2]    1.004040   1.014185
p.fac1[8,2]      1.003933   1.009290
ilr.fac1[8,1]    1.003864   1.009587
```