UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

GUILHERME MELGAÇO HELUY

UTILIZAÇÃO DE SUBPRODUTOS DO BENEFICIAMENTO DO CAMARÃO MARINHO *Penaeus vannamei* EM DIETAS PARA TILÁPIA DO NILO *Oreochromis niloticus*

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Resumo

O beneficiamento industrial do camarão gera grandes volumes de resíduos, sendo o cefalotórax um dos principais subprodutos, representando até 40% do peso total do animal. Apesar de frequentemente descartado, esse resíduo possui alto valor nutricional, sendo rico em proteínas, lipídios, minerais, quitina e carotenoides, como a astaxantina, com potencial para aplicações na nutrição aquícola. Portanto, este estudo avaliou os efeitos da inclusão de farinha e extrato lipídico oriundos do cefalotórax de camarão *Penaeus vannamei* como ingredientes funcionais na alimentação da tilápia do Nilo (*Oreochromis niloticus*). No primeiro estudo, dietas contendo níveis crescentes de extrato lipídico de cefalotórax de camarão (0,0%, 0,4%, 0,8%, 1,2% e 1,6%) foram testadas ao longo de 45 dias. Os resultados indicaram uma melhora significativa nos parâmetros de crescimento, conversão alimentar e eficiência proteica, além de alterações na composição corporal, com aumento dos níveis de proteína e redução do teor lipídico nos peixes que receberam as maiores concentrações do extrato. Além disso, constatou-se maior atividade de enzimas antioxidantes e melhora nos indicadores séricos sanguíneos, sugerindo efeitos positivos sobre o metabolismo e a saúde geral dos animais. No segundo estudo, foram analisadas a digestibilidade e os efeitos nutricionais da farinha de cefalotórax de camarão integral e da farinha com baixo teor lipídico, adicionadas a 20% e 30% nas dietas. Ambas apresentaram alta digestibilidade de nutrientes e energia e um perfil de aminoácidos adequado, sem a presença de aminoácidos limitantes e com score químico superior a outras fontes proteicas tradicionais. Além disso, observou-se um aumento significativo na atividade das enzimas digestivas e antioxidantes com a inclusão das farinhas, favorecendo assimilação dos nutrientes e a saúde hepática. A inclusão de ambas as farinhas em 20% foi considerada ideal para dietas destinadas à tilápia do Nilo. O terceiro estudo avaliou os efeitos imunomoduladores da farinha (0%, 5%, 10%, 15% e 20%) e do extrato lipídico (0,0%, 0,4%, 0,8%, 1,2% e 1,6%) de camarão inclusos separadamente em dietas para alevinos de tilápia, durante 45 dias. Verificou-se um aumento expressivo na contagem de leucócitos, trombócitos e hematócrito, especialmente em dietas contendo 1,6% de extrato lipídico e um aumento na contagem de leucócitos nas dietas contendo 20% de farinha. Esses achados demonstram o potencial desses ingredientes para fortalecer o sistema imunológico inato dos peixes, aumentando a sua resistência contra patógenos. Os resultados indicam que os bioprodutos oriundos do processamento de camarão são ingredientes eficientes e sustentáveis, podendo ser inclusos em rações aquícolas sem comprometer a qualidade nutricional ou a saúde dos peixes. Além disso, a adoção desses subprodutos na alimentação de tilápias contribui para minimizar os impactos ambientais associados ao descarte inadequado de resíduos da carcinicultura. Dessa forma, a incorporação desses ingredientes promove a economia circular e agrega valor à cadeia produtiva do camarão, ao mesmo tempo em que melhora a eficiência produtiva da piscicultura, consolidando-se como uma estratégia inovadora e promissora para o desenvolvimento sustentável do setor aquícola.

Palavras-chave: cefalotórax; farinha de camarão; extrato lipídico; digestibilidade; imunomodulador.

Abstract

The industrial processing of shrimp generates large volumes of waste, with the cephalothorax being one of the main by-products, representing up to 40% of the animal's total weight. Although often discarded, this waste has high nutritional value, being rich in proteins, lipids, minerals, chitin, and carotenoids such as astaxanthin, with potential applications in aquafeeds. Therefore, this study evaluated the effects of including *Penaeus vannamei* shrimp head meal and lipid extract as functional ingredients in the diets of Nile tilapia (*Oreochromis niloticus*). In the first study, diets containing increasing levels of shrimp head lipid extract (0.0%, 0.4%, 0.8%, 1.2%, and 1.6%) were tested over 45 days. The results indicated a significant improvement in growth parameters, feed conversion, and protein efficiency, as well as alterations in body composition, with increased protein levels and reduced lipid content in fish receiving the highest concentrations of the extract. Additionally, greater antioxidant enzyme activity and improved blood serum indicators were observed, suggesting positive effects on metabolism and overall health. In the second study, the digestibility and nutritional effects of whole shrimp head meal and low-lipid shrimp meal, included at 20% and 30% in the diets, were analyzed. Both meals exhibited high nutrient and energy digestibility and a suitable amino acid profile, with no limiting amino acids and a chemical score superior to traditional protein sources. Furthermore, significant increases in digestive and antioxidant enzyme activities were observed with the inclusion of these meals, promoting nutrient assimilation and liver health. The inclusion of both meals at 20% was considered optimal for Nile tilapia diets. The third study evaluated the immunomodulatory effects of shrimp meal (0%, 5%, 10%, 15% and 20%) and lipid extract (0.0%, 0.4%, 0.8%, 1.2% and 1.6%) included separately in diets for tilapia fingerlings over 45 days. A significant increase in leukocyte, thrombocyte, and hematocrit counts was observed, particularly in diets containing 1.6% lipid extract, and an increase in leukocyte counts was noted in diets containing 20% meal. These findings demonstrate the potential of these ingredients to enhance the innate immune system of fish, increasing their resistance to pathogens. The results indicate that shrimp processing by-products are efficient and sustainable ingredients, suitable for inclusion in aquafeeds without compromising fish nutritional quality or health. Moreover, the adoption of these by-products in tilapia diets contributes to reducing environmental impacts associated with improper disposal of shrimp farming waste. Thus, incorporating these ingredients promotes circular economy practices and adds value to the shrimp production chain, while improving the productivity and efficiency of aquaculture. This approach emerges as an innovative and promising strategy for the sustainable development of the aquaculture sector.

Keywords: cephalothorax; shrimp meal; lipid extract; digestibility; immunomodulator.

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Sumário

1. Introdução

1.1. Contextualização da Pesquisa

A produção mundial de crustáceos cresceu significativamente na última década, impulsionada pela elevada demanda do mercado consumidor. Em 2022, essa produção representou 8,7% do mercado global de espécies oriundas da aquicultura, com destaque para o camarão branco do Pacífico, *Penaeus vannamei* (Boone, 1931), que atingiu 6,8 milhões de toneladas, consolidando-se como a espécie aquícola mais cultivada (FAO, 2024). Nesse contexto, o Brasil ocupa a nona posição entre os principais produtores de crustáceos, com uma produção estimada de 113 mil toneladas de camarões cultivados, registrando um crescimento anual de 5,9%. A Região Nordeste responde por 99,6% do total nacional produzido (IBGE, 2022; FAO, 2024).

O processamento industrial do camarão gera grandes volumes de resíduos, que podem corresponder a até 70% do peso total do animal, sendo o cefalotórax a principal fração, representando até 50% desse total (MEZZOMO *et al*., 2013; GULZAR e BENJAKUL, 2018; CARMEN *et al*., 2023). No entanto, grande parte desses resíduos é descartada de forma inadequada, resultando em impactos ambientais significativos, como a contaminação do solo e da água (MEZZOMO *et al*., 2011; ZHOU *et al*., 2021).

Nesse cenário, a valorização dos resíduos do processamento do camarão, em especial do cefalotórax, surge como uma alternativa promissora para mitigar os impactos ambientais e promover a sustentabilidade na cadeia produtiva (ANEESH *et al*., 2020; SAINI *et al*., 2020). O aproveitamento desse subproduto não apenas reduz o desperdício e agrega valor ao setor aquícola, mas também fomenta a economia circular ao gerar insumos com aplicações diversas, como nas indústrias farmacêutica, médica, agrícola, cosmética e alimentar (KANDRA *et al*., 2012; MAO *et al*., 2017; NIRMAL *et al*., 2020; LIU *et al*., 2021).

Os subprodutos derivados do processamento de crustáceos apresentam grande potencial para a alimentação animal, especialmente na piscicultura. A farinha obtida a partir do cefalotórax de camarão destaca-se como um ingrediente nutricionalmente rico, contendo proteínas de alto valor biológico, um perfil balanceado de aminoácidos essenciais, além de lipídios, minerais e carotenoides (LIU *et al*., 2021; OSUNA-

SALAZAR *et al*., 2023). Como a proteína representa o componente mais oneroso das dietas (NRC, 2011) e desempenha um papel essencial na formação de tecidos, biossíntese de anticorpos, enzimas e hormônios (MAI *et al*., 2022) torna-se fundamental a busca por fontes proteicas alternativas que apresentem elevada digestibilidade, palatabilidade e baixos teores de fatores antinutricionais (HARDY e BREZAS, 2022).

O extrato lipídico do cefalotórax de camarão, por sua vez, é particularmente rico em ácidos graxos poli-insaturados, incluindo aqueles da série n-3, como o ácido eicosapentaenoico (EPA) e o ácido docosahexaenoico (DHA), além de conter carotenoides de alto valor biológico, como a astaxantina (GÓMEZ-GUILLÉN *et al*., 2018; GULZAR *et al*., 2020; YU *et al*., 2020). Peixes não possuem a capacidade de biossintetizar endogenamente os ácidos graxos poli-insaturados n-3 ou a astaxantina, tornando-se totalmente dependentes da alimentação para obter esses compostos essenciais (LIM *et al*., 2019; XIE *et al*., 2021). Esses nutrientes exercem um papel crucial no crescimento, reprodução e manutenção da saúde dos organismos aquáticos, influenciando a estrutura e o funcionamento das membranas celulares e atuando como precursores de eicosanoides, compostos que regulam processos inflamatórios e imunológicos (TOCHER, 2015; TURCHINI *et al*., 2022).

Além disso, observa-se um crescente interesse no desenvolvimento de rações funcionais, que não apenas atendam às exigências nutricionais, mas também promovam benefícios adicionais à saúde e ao desempenho dos peixes (BAI *et al*., 2015; MUELLER *et al*., 2023). Essas rações incorporam ingredientes funcionais contendo compostos bioativos, como a astaxantina, que apresentam efeitos comprovados na redução do estresse oxidativo, fortalecimento do sistema imunológico e ação anti-inflamatória (DAVE *et al*., 2020; NIRMAL *et al*., 2020; CABANILLAS-BOJÓRQUEZ *et al*., 2021; KHAN *et al*., 2023; TKACZEWSKA *et al*., 2024). Adicionalmente, esses ingredientes podem melhorar a absorção e o metabolismo dos nutrientes, impactando positivamente o crescimento e a eficiência alimentar dos peixes (WAAGBØ; REMØ, 2020). A inclusão de subprodutos do camarão também pode aumentar a palatabilidade das rações, estimulando um maior consumo e otimizando o desempenho produtivo das espécies cultivadas (HARDY e BREZAS, 2022; TKACZEWSKA *et al*., 2024). Embora alguns produtos à base de camarão já sejam utilizados como fontes proteicas e pigmentos em

dietas aquícolas (YI *et al*., 2015; LEDUC *et al*., 2018; SALAS-LEITON *et al*., 2020; HAQUE *et al*., 2021; LI *et al*., 2021; TRUNG *et al*., 2022; OSUNA-SALAZAR *et al*., 2023), ainda há escassez de estudos detalhando o potencial de aplicação de subprodutos do cefalotórax, como a farinha e o extrato lipídico, na alimentação de peixes, particularmente na tilápia do Nilo, *Oreochromis niloticus* (Linnaeus, 1758).

A tilápia do Nilo é um peixe dulcícola pertencente à família Cichlidae e representa a segunda espécie de peixe mais cultivada globalmente, com uma produção anual de 5 milhões de toneladas (FAO, 2024). Cultivada em regiões tropicais e subtropicais, sua introdução no Brasil ocorreu na década de 1960 (NOBREGA *et al*., 2020). Atualmente, a tilapicultura responde por 66,1% da produção de peixes de água doce no país, posicionando o Brasil como o quarto maior produtor mundial da espécie, com 408 mil toneladas anuais (IBGE, 2022; FAO, 2024). A tilápia do Nilo destaca-se por sua rusticidade, elevado desempenho produtivo, crescente aceitação no mercado consumidor, boa conversão alimentar, hábito alimentar onívoro e fácil adaptação às rações comerciais (FURUYA, 2010; NG e ROMANO, 2013; EL-SAYED, 2020; NOBREGA *et al*., 2020).

Diante desse panorama, a utilização de subprodutos do processamento do camarão na nutrição da tilápia apresenta-se como uma estratégia promissora e sustentável para a aquicultura. Essa abordagem não apenas agrega valor às cadeias produtivas do camarão e da tilápia, como também reduz a dependência de ingredientes tradicionais, melhora a eficiência nutricional das dietas e minimiza os impactos ambientais decorrentes do descarte inadequado de resíduos. Dessa forma, a incorporação desses ingredientes reforça a importância da economia circular e da inovação na formulação de rações para piscicultura, promovendo o desenvolvimento sustentável do setor.

1.2 Objetivos

1.2.1 Objetivo Geral

Avaliar os efeitos da inclusão da farinha e do extrato lipídico de cefalotórax de *Penaeus vannamei* em dietas sobre o crescimento, digestibilidade, eficiência de utilização de nutrientes e estado imunológico da tilápia do Nilo (*Oreochromis niloticus*).

1.2.2 Objetivos Específicos

- Caracterizar quimicamente a farinha e extrato lipídico de cefalotórax de *Penaeus vannamei*;
- Desenvolver e analisar as propriedades funcionais das dietas experimentais para tilápia do Nilo, incorporando os ingredientes experimentais;
- Determinar o coeficiente de digestibilidade aparente dos nutrientes presentes na farinha de camarão e o escore químico para a tilápia do Nilo;
- Analisar os efeitos da farinha de camarão sob a atividade enzimática digestiva e parâmetros antioxidantes de *Oreochromis niloticus*;
- Avaliar o desempenho das dietas experimentais contendo extrato lipídico de camarão, utilizando modelos zootécnicos e fisiológicos;
- Investigar os efeitos imunomoduladores das dietas experimentais em alevinos de *Oreochromis niloticus*.

1.3 Hipóteses

A inclusão da farinha e do extrato lipídico de cefalotórax de *Penaeus vannamei* como ingredientes alternativos na alimentação da tilápia do Nilo melhora os parâmetros zootécnicos e fisiológicos, promovendo o crescimento e fortalecendo a saúde dos animais.

2. Capítulo 1: Dietary supplementation of shrimp head lipid extract has significant effects on growth, body composition, antioxidant parameters and biochemical profile of Nile tilapia *Oreochromis niloticus*

Resumo

Este estudo explorou o potencial da incorporação do extrato lipídico de cabeça de camarão (ELCC) obtido do processamento de *Penaeus vannamei* na alimentação da tilápia do Nilo (*Oreochromis niloticus*). Foram testadas cinco dietas com níveis crescentes de ELCC: 0,0% (controle), 0,4%, 0,8%, 1,2% e 1,6%. Os peixes (4,38 ± 0,13 g e 6,36 ± 0,23 cm) foram distribuídos aleatoriamente em 20 tanques (50 L cada), com 10 peixes por tanque, e alimentados por 45 dias. O peso final, o ganho de peso, a conversão alimentar, a eficiência alimentar, a taxa de crescimento específico, a taxa de eficiência proteica, a taxa de retenção de proteína e o fator de condição melhoraram significativamente com o aumento dos níveis de ELCC na dieta. O teor de proteína corporal total também aumentou, enquanto o teor de lipídios diminuiu com a inclusão de ELCC. As atividades de catalase, superóxido dismutase e glutationa reduzida foram significativamente maiores no fígado e nos músculos da tilápia alimentada com níveis mais altos de ELCC na dieta. O aumento dos níveis dietéticos de ELCC também resultou em maiores concentrações séricas de proteína total, albumina e globulina, além da redução nos níveis séricos de glicose, colesterol, triglicerídeos, relação albumina/globulina, alanina aminotransferase, aspartato aminotransferase e amilase. Além disso, o ELCC provocou uma redução dose-dependente no teor de malondialdeído nas dietas após sete, trinta e sessenta dias de armazenamento. O ELCC é considerado um suplemento dietético adequado para *O. niloticus*, com um nível estimado de inclusão de 1,6%.

Palavras-chave: Astaxantina, ácidos graxos poli-insaturados, desempenho de crescimento, atividade antioxidante, imunomodulação.

Abstract

This study explored the potential of incorporating shrimp head lipid extract (SHLE) obtained from the processing of *Penaeus vannamei* in the feed of Nile tilapia (*Oreochromis niloticus*). Five diets containing increasing SHLE levels were tested: 0.0%

(control) 0.4%, 0.8%, 1.2% and 1.6%. Fish (4.38 ± 0.13 g and 6.36 ± 0.23 cm) were randomly distributed into 20 tanks (50 L each) at 10 fish/tank and fed for 45 days. The final body weight, weight gain, feed conversion ratio, feed efficiency, specific growth rate, protein efficiency ratio, protein retention rate, and condition factor improved significantly as dietary SHLE levels increased. Whole-body protein content also increased, while lipid content decreased with SHLE inclusion. The activities of catalase, superoxide dismutase, and reduced glutathione were significantly higher in the liver and muscles of tilapia fed higher dietary SHLE levels. Increasing dietary levels of SHLE also resulted in higher serum levels of total protein, albumin, and globulin, but reduced serum levels of glucose, cholesterol, triglycerides, albumin/globulin ratio, alanine aminotransferase, aspartate aminotransferase, and amylase. Additionally, SHLE caused a dose-dependent reduction in malondialdehyde content in the diets after seven, thirty, and sixty days of storage. SHLE is considered an adequate dietary supplement for *O. niloticus* at an estimated inclusion level of 1.6%.

Keywords: Astaxanthin, polyunsaturated fatty acids, growth performance, antioxidant activity, immunomodulation.

Introduction

Global crustacean production has grown in recent decades mainly due to high market demand. In 2022, aquaculture of the Pacific white shrimp, *Penaeus vannamei* (Boone, 1931), the main aquatic species farmed in the world, accounted for the production of 6.8 million tonnes (FAO, 2024). Residues from shrimp processing normally constitute up to 60% of the total weight, with the cephalothorax representing 35–50% (GULZAR and BENJAKUL, 2018; CARMEN *et al*., 2023). Although often regarded as having low commercial value, these residues contain bioactive compounds of high biological value, such as essential amino acids, minerals, chitin, polyunsaturated fatty acids, and carotenoids (NIRMAL *et al*., 2020; LIU *et al*., 2021). Moreover, in many cases the residues are disposed inappropriately, leading to environmental pollution problems (MEZZOMO *et al*., 2011; ZHOU *et al*., 2021). Therefore, the processing of shrimp residues represents an alternative for the full use of this material while generating gains in the production chain (ANEESH *et al*., 2020; SAINI *et al*., 2020). Several high value-

added products obtained from shrimp residues are currently used in the pharmaceutical, medical, agricultural, cosmetic and animal nutrition industries (MEZZOMO *et al*., 2013; YI *et al*., 2015; MAO *et al*., 2017; LEDUC *et al*., 2018; SALAS-LEITON *et al*., 2020; HAQUE *et al*., 2021; LI *et al*., 2021; LIU *et al*., 2021; TRUNG *et al*., 2022; OSUNA-SALAZAR *et al*., 2023).

Shrimp head lipid extract (SHLE) is rich in long-chain polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), as well as carotenoids such as astaxanthin, astaxanthin esters and β-carotenes (GÓMEZ-ESTACA *et al*., 2017; GULZAR *et al*., 2020; YU *et al*., 2020). Many of these biocompounds are known to have health-promoting effects, particularly PUFA (KHAN *et al*., 2023) and astaxanthin (RICCIO and LAURITANO, 2019). N-3 PUFA can help prevent cardiovascular and coronary events, and have anti-inflammatory, antithrombotic and antiaging properties (KHAN *et al*., 2023). Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4′-dione), the main carotenoid found in the shrimp cephalothorax (SAINI *et al*., 2018; DAVE *et al*., 2020), has high antioxidant properties, which reduce oxidative damage caused by reactive oxygen species (ROS), protecting hematopoietic tissue cells against damage and interrupting the formation of inflammatory processes (AMBATI *et al*., 2014; NIRMAL *et al*., 2020). Unlike its synthetic counterparts, astaxanthin obtained from natural sources is generally found in the esterified molecular form, thus having greater stability and, therefore, increased durability and bioavailability, in addition to being less prone to oxidation (BAI *et al*., 2015; ZHAO *et al*., 2023).

Fish are not able to biosynthesize either n-3 PUFA or astaxanthin *de-novo*, so they need to acquire them through feeding (NRC, 2011; LIM *et al*., 2019). Some bioproducts containing astaxanthin are already used as additives in fish feeds with significant gains in terms of growth, pigmentation and health status (LI *et al*., 2014; XIE *et al*., 2020; KHEIRABADI *et al*., 2022; ZHU *et al*., 2022; LONG *et al*., 2023). To date, however, only few studies have examined the effects of the dietary supplementation of astaxanthin sources in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), the second most farmed fish species in the world, with 5.3 million tonnes produced in 2022 (FAO, 2024). Although Aracati *et al*. (2021), Eldessouki *et al*. (2024), Harith *et al*. (2022) and Panase

et al. (2024) have provided valuable insights into the role of astaxanthin for Nile tilapia, there is still need to fully elucidate its impact on the physiological and immunological responses of this commercially important fish species. This study, therefore, evaluated the effects of increasing dietary concentrations of SHLE on growth performance, proximate composition, antioxidant activity in the liver and muscles, and immunological parameters of Nile tilapia.

Material and methods

Origin and composition of SHLE

SHLE was supplied by Bioingredientes®, Recife, Brazil, which has a pending patent application on the methodology for obtaining this product. The composition and fatty acid (FA) profile of SHLE was analytically verified (Table 1). Total lipids were gravimetrically quantified by Folch *et al*. (1957), while the FA profile was determined as methyl esters (FAME) using a Varian GC 430 gas chromatograph (Agilent, USA) equipped with a flame ionization detector (SECCI *et al*., 2019). The gross energy content was determined with a bomb calorimeter (AOAC, 2012). The astaxanthin concentration was determined by high-performance liquid chromatography (HPLC) (Shimadzu LC-10A, with UV-visible detector, 450 nm) following Kuhnen *et al*. (2009).

Preparation of experimental diets

Five isoproteic (27% digestible protein) and isoenergetic (3.200 kcal g^{-1} estimated digestible energy) diets were formulated (Table 2) to meet the nutritional requirements of Nile tilapia fingerlings (FURUYA, 2010; NRC, 2011; EL-SAYED, 2020). The experimental diets consisted of a basal diet with no inclusion of SHLE (control) and four diets with increasing levels of SHLE (0.4%, 0.8%, 1.2% and 1.6%) at the expense of soybean oil. The diets were estimated to contain 0, 2.75, 5.50, 8.25 and 11.0 mg g^{-1} of astaxanthin, respectively. Ingredients were mixed with warm water until forming a homogeneous blend that was passed through a 2-mm sieve. The pellets were dried in a forced-air oven at 55°C for 24 h. Dried diets were stored at −5°C until use. The proximate composition and pellet physical quality of the experimental feeds were determined following AOAC (2012) and Cai *et al*. (2022), respectively (Table 2).

Table 1. Contents of total lipids (g 100 g^{-1}), gross energy (kcal kg^{-1}), astaxanthin (µg g^{-1}), and selected (≥ 0.10 %) fatty acids (%) of the shrimp head lipid extract (SHLE) obtained from the processing of *Penaeus vannamei*.

Content	
Total lipids	63.0
Gross energy	8,367.8
Astaxanthin	687.0
Fatty acids	
14:0	0.60
15:0	1.53
iso-15:0	0.16
16:0	19.89
iso-16:0	0.16
16:1n-7	1.22
16:1n-9	0.23
16:4n-1	0.11
17:0	1.42
18:0	9.87
18:1n-7	2.79
18:1n-9 (cis + trans)	22.23
18:2n-6 (cis)	18.37
18:3n-3	0.83
20:0	0.53
20:1n-7	0.58
20:1n-9	0.89
20:1n-11	0.10
20:2n-6	1.95
20:3n-6	0.11
20:3n-3	0.14
20:4n-6	3.13
20:5n-3	5.37
22:0	0.84
22:4n-6	0.10
22:5n-6	0.39
22:5n-3	0.31
22:6n-3	4.59
24:0	0.36
24:1n-9	0.40
∑ Saturated	35.44
∑ Mono-unsaturated	28.60
∑ PUFA n-3	11.48
∑ PUFA n-6	24.11

Table 2. Ingredients, proximate composition and physical pellet quality of experimental diets (% dry matter) containing increasing levels of shrimp head lipid extract (SHLE) fed to Nile tilapia (*Oreochromis niloticus*) for 45 days.

	Dietary SHLE inclusion levels (%)				
	0	0.4	0.8	1.2	1.6
Ingredients (%)					
Soybean meal	60.78	60.79	60.80	60.82	60.83
Corn flour	33.78	33.71	33.65	33.58	33.50
Dicalcium phosphate	1.41	1.41	1.41	1.41	1.41
Soybean oil	1.38	1.04	0.69	0.34	0.00
Limestone	0.69	0.69	0.69	0.69	0.69
Carboxymethylcellulose	0.50	0.50	0.50	0.50	0.50
L-Threonine	0.43	0.43	0.43	0.43	0.43
Mineral-vitamin premix[a]	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.39	0.39	0.39	0.39	0.40
Salt	0.22	0.22	0.22	0.22	0.22
BHT	0.02	0.02	0.02	0.02	0.02
Shrimp head lipid extract	0.00	0.40	0.80	1.20	1.60
Total	100,00	100,00	100,00	100,00	100,00
Proximate composition (%)					
Dry matter	96.26	96.08	96.25	96.11	96.16
Crude protein	30.46	30.52	30.49	30.41	30.44
Digestible protein	27.00	27.00	27.00	27.00	27.00
Total lipids	3.27	3.23	3.34	3.25	3.31
Ash	5.32	5.21	5.30	5.25	5.18
Physical pellet quality parameters (%)					
Density	29.70	29.43	28.53	28.76	28.83
Durability index	99.48	99.32	99.48	99.65	99.69
Water solubility	71.66	71.53	71.61	71.58	71.41
Water resistance	71.64	71.13	71.89	71.17	72.22

[a] Vit. A (2,500,000 IU); Vit. D3 (60,000 IU); Vit. E (37,500 IU); Vit. K3 (3,750 mg); Vit. B1 (4,000 mg); Vit. B2 (4,000 mg); Vit. B5 (12 g); Vit. B6 (4,000 mg); Vit. B12 (4,000 mcg); Vit. C (50 g); folic acid (1,250 mg); niacin (22.5 g); biotin (15 mg); iron (15 g); zinc (12.5 g); manganese (12.5 g); copper (2,500 mg); iodine (375 mg); cobalt (125 mg); selenium (85.7 mg).

Experimental procedures

Forty five-day old, sex-reverted, genetically improved farmed tilapia - GIFT (*O. niloticus*) were acclimated to laboratory conditions for two weeks. Fish were then individually weighed and measured (4.38 ± 0.13 g; 6.36 ± 0.23 cm) and randomly distributed into twenty 50-L polyethylene tanks at a density of 10 fish $tank^{-1}$ (166 fish m^{-3}). Four replicated tanks were randomly assigned to each of the five experimental treatments. Feeding was carried out four times a day (9:00 am, 12:00 pm, 2:00 pm, and 5:00 pm) until apparent satiety (*ad libitum*). The amount of feed supplied daily was estimated by weighing the containers before the first and after the last feeding.

Experimental conditions and sampling

The tanks were connected to a recirculating aquaculture systems (RAS) equipped with mechanical, biological, and ultraviolet filters. Water temperature (27.6 ± 0.4 °C), dissolved oxygen (5.80 ± 0.30 mg L^{-1}), and pH (7.74 ± 0.30) were monitored daily using a digital multiparametric probe (YSI™ Model 550A Dissolved Oxygen Instrument, USA). Salinity was maintained at 3. Non-ionized ammonia (NH_3, 0.035 ± 0.01 mg L^{-1}), nitrite-nitrogen ($N\text{-}NO_2$, 0.25 ± 0.3 mg L^{-1}), and nitrate-nitrogen ($N\text{-}NO_3$, 4.18 ± 0.31 mg L^{-1}) were measured every five days (BAIRD *et al*., 2017). A natural photoperiod of 12L:12D was established.

At the end of the experimental period (45 days), 24 h fasted fish were anesthetized with 150 mg L^{-1} of Tricaine methane sulfonate (MS-222, Sigma-Aldrich) (ARAÚJO *et al*., 2018), counted, weighed and measured. Blood samples were also collected from four fish per tank by caudal vein puncture. Subsequently, all the anesthetized fish were euthanized by sectioning the bone marrow and bleeding the gills. The liver and viscera of four fish per tank were weighed to determine the hepatosomatic (HSI) and viscerosomatic (VSI) index, respectively. Additionally, the liver and muscle of four fish per tank were collected, pooled, and stored at −20 °C for analysis of antioxidant activity. The remaining fish were euthanized and stored at −20 °C for whole-body composition analysis.

Growth performance

The performance parameters were obtained as:

Initial body weight (IBW; g) = individually weighed at the beginning of the experimental period;

Final body weight (FBW; g) = individually weighed at the end of the experimental period;

Survival rate (%) = 100 x (final fish number / initial fish number);

Weight gain (WG; %) = 100 × (FBW (g) − IBW (g)) / IBW (g);

Feed intake (g day^{-1}) = total dry feed fed per fish (g) / days of experiment;

Feed conversion ratio = dry feed fed (g) / WG (g);

Feed efficiency = wet weight gain (g) / dry feed fed (g);

Specific growth rate (% day^{-1}) = 100 x [(ln FBW – ln IBW) / days of experiment];

Protein efficiency ratio (g) = WG (g) / protein intake (g);

Protein retention rate (%) = 100 x [(FBW (g) x final body protein (g)) – (IBW (g) x initial body protein (g))] / protein intake (g);

Condition factor (g cm^{-3}) = 100 x (FBW (g) / body length $(cm)^3$);

Eviscerated weight (%) = 100 x (eviscerated fish weight / FBW);

Hepatosomatic index (%) = 100 x (liver weight (g) / FBW (g));

Viscerosomatic index (%) = 100 x (viscera weight (g) / FBW).

Whole-body composition

Approximately 80 g of whole fish (four fish per tank) were sampled for proximal composition analysis (AOAC, 2012). Samples were dried in a forced ventilation oven at 55 °C for 24 h, followed by drying at 105 °C for 16 h, to determine the moisture content. Crude protein was determined using the Kjeldahl method. Total lipids were evaluated using a Goldfisch extractor, using petroleum ether as solvent, for 16 h. Ash was obtained after burning in a muffle furnace at 600 °C for 4 h. Nitrogen-free extract was quantified by subtracting 100% of the dry matter from the contents of crude protein, total lipids and ash. All whole-body composition analyses were performed in triplicates per tank (n = 12 per treatment).

Antioxidant parameters in liver, muscle, and experimental diets

Samples of the experimental diets, liver and muscle tissue were pooled, homogenized (40 mg mL^{-1} 0.1 M Tris-HCl buffer and 0.15 mM NaCl pH 8), and centrifuged at 8,000 *g* for 15 min at 4 °C. The total protein concentration was determined

by the method of Bradford (1976) using bovine serum albumin as standard. The activity of catalase (CAT) in the liver and muscle was determined by measuring the decrease in hydrogen peroxide concentration by absorbance at 240 nm, at 25 °C, in a UV spectrophotometer using a quartz cuvette (AEBI, 1984). Liver and muscle superoxide dismutase (SOD) activity was calculated by measuring the inhibition of absorbance of adrenaline auto-oxidation in a spectrophotometer at 480 nm (BANNISTER and CALABRESE, 1987). Reduced glutathione (GSH) levels in the liver and muscle were estimated using the method of Sedlak and Lindsay (1968), which is based on the reaction between Ellman's reagent (DTNB) with the free thiol, which gives rise to a more acidic mixed disulfide (2-nitro-5-thiobenzoic acid). The reaction product then formed was measured by spectrophotometric reading at 412 nm. The lipid peroxidation of the liver, muscle, and experimental diets was measured by the content of Malondialdehyde (MDA), in which the samples were mixed with 25 mL of TCA (7.5%) and filtered; subsequently, 5 mL of TBA (Thiobarbituric acid, 0.01M) (thiobarbituric acid) were added to 5 mL of extracts. The samples were then subjected to a water bath at 90 °C for 45 min, and a spectrophotometry reading was performed at 532 nm (WALLIN *et al*., 1993). To evaluate lipid peroxidation in the experimental diets, a sample from each treatment was analyzed after 7, 30 and 60 days of storage, with the diets stored in containers wrapped in aluminum foil, protected from light and humidity, and kept at room temperature (25 °C). All analyses of antioxidant activity were performed in triplicate per tank (n = 12 per treatment).

Biochemical profile

Blood samples were collected from four fish in each tank with no EDTA, pooled, and then centrifuged at 2,000 *g* for 25 min at 4 °C. The collected serum was stored at −20 °C (JAGRUTHI *et al*., 2014). Glucose, cholesterol, triglycerides, serum protein, albumin, globulin, albumin/globulin ratio, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and amylase were determined in triplicate per tank (n=12 per treatment) according to Young (1997) with commercial diagnostic kits (Laborlab Ltd., Guarulhos, Brazil). Reading was carried out on the spectrophotometer following the manufacturer's protocol.

Statistical analyzes

Experimental data were initially subjected to the Shapiro-Wilk and Levene tests to verify normality and homoscedasticity, respectively. The values of antioxidant activity were transformed into a log (x + 1) to reduce the effect of extreme values. Data were subjected to a one-way analysis of variance (ANOVA) and, when differences between the means were detected, the Tukey post-hoc test was applied at a 5% probability level. The results are presented as means ± standard deviation (SD). Data were also subjected to Pearson correlation analysis to measure the intensity of the relationship between the variables and inclusion levels of SHLE. For feed lipid peroxidation data, a two-way ANOVA with no replicates was applied. The analyses were conducted using the free software Jamovi version 2.3.28.

Results

Composition of SHLE

The shrimp head lipid extract (SHLE) obtained from the processing of *Penaeus vannamei* exhibited a total lipid content of 63.0 g 100 g^{-1} and a gross energy value of 8,367.8 kcal kg^{-1}. Additionally, the extract contained 687.0 μg g^{-1} of astaxanthin (Table 1).

Fatty acid analysis revealed that monounsaturated fatty acids (MUFAs) accounted for 28.60% of the total fatty acids, while saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) represented 35.44% and 35.59%, respectively. The PUFA composition was primarily represented by n-6 fatty acids (24.11%), with a lower proportion of n-3 PUFAs (11.48%). Among the individual fatty acids, oleic acid (C18:1n-9) was the most abundant (22.23%), followed by palmitic acid (C16:0, 19.89%) and linoleic acid (C18:2n-6, 18.37%). Stearic acid (C18:0) was present at 9.87%, whereas eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) were detected at 5.37% and 4.59%, respectively. Arachidonic acid (ARA, C20:4n-6) contributed 3.13% of the total fatty acid profile.

Fish performance

In general, final body weight, feed conversion ratio, feed efficiency, protein efficiency ratio, protein retention rate, and condition factor of fish fed diets containing 1.2% and 1.6% SHLE were significantly higher than those fed the control diet (Table 3).

Fish fed the diet containing 1.6% SHLE also showed the highest weight gain and specific growth rates ($P < 0.05$) with a high correlation. No significant effects of increasing dietary levels of SHLE were observed for survival rate, feed intake, eviscerated weight, and hepatosomatic and viscerosomatic indexes ($P > 0.05$).

Whole-body composition

The results of the whole-body composition analysis of Nile tilapia are summarized in Table 4. The total moisture and lipid content of tilapia fed with 0.4% to 1.6% SHLE were lower than in fish from the control group, while the opposite occurred for the body crude protein content of fish fed with 0.8% to 1.6% SHLE ($P < 0.05$), both with a high correlation. There were no significant differences in the levels of ash and nitrogen-free extract.

Antioxidant parameters in liver, muscle, and experimental feeds

The results of the antioxidant activity in the liver and muscles of Nile tilapia are described in Table 5. The liver and muscle of fish fed with the inclusion of 1.6% SHLE in the diet showed the highest activities of catalase, superoxide dismutase, and glutathione reduced ($P < 0.05$), and the data indicate a high correlation between enzymatic activity and the dietary inclusion levels of SHLE. However, no significant differences were found in MDA content between the control and the other treatments ($P > 0.05$). The MDA content in the experimental diets decreased at the three times evaluated, as the levels of SHLE included in the diet increased ($P < 0.001$) (Fig. 1).

Biochemical profile

The effects of SHLE on tilapia serum parameters are shown in Table 6. Fish fed with 1.2% and 1.6% SHLE had the lowest levels of glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, and amylase regarding the control treatment ($P < 0.05$), with high negative correlations between these variables and SHLE inclusion levels. The dietary inclusion of 1.6% SHLE also resulted in the lowest albumin/globulin ratio ($P < 0.05$). Fish fed with the inclusion of 1.6% SHLE had the highest levels of serum protein, albumin and globulin, with a highly positive correlation ($P < 0.05$).

Table 3. Mean (± SD) initial and final body weight (IBW and FBW, respectively), survival rate (SR), weight gain (WG), feed intake (FI), apparent feed conversion ratio (FCR), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), protein retention rate (PRR), condition factor (CF), eviscerated weight (EW), hepatosomatic index (HSI) and viscerosomatic index (VSI) of Nile tilapia (*Oreochromis niloticus*) fed diets containing increasing concentrations of shrimp head lipid extract (SHLE) for 45 days.

	Dietary SHLE inclusion levels (%)					*P*	R
	0	0.4	0.8	1.2	1.6		
IBW (g)	4.36 ± 0.16	4.36 ± 0.16	4.44 ± 0.14	4.46 ± 0.09	4.32 ± 0.09	0.543	0.032
FBW (g)	22.31 ± 1.56^{c}	22.33 ± 0.27^{c}	23.66 ± 0.57^{bc}	24.68 ± 0.77^{ab}	26.38 ± 1.05^{a}	<0.001	0.851
SR (%)	82.5 ± 8.1	95.0 ± 5.8	95.0 ± 5.8	92.5 ± 6.6	95.0 ± 7.2	0.406	0.309
WG (%)	412.3 ± 32.4^{b}	412.7 ± 16.2^{b}	432.8 ± 20.1^{b}	453.9 ± 28.2^{b}	510.8 ± 20.4^{a}	<0,001	0.800
FI (g/day)	0.50 ± 0.02	0.48 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.48 ± 0.04	0.427	-0.319
FCR	1.29 ± 0.09^{c}	1.20 ± 0.03^{bc}	1.12 ± 0.03^{b}	1.08 ± 0.02^{ab}	0.97 ± 0.06^{a}	<0.001	-0.907
FE	0.78 ± 0.04^{d}	0.83 ± 0.02^{cd}	0.89 ± 0.02^{bc}	0.92 ± 0.02^{b}	1.03 ± 0.06^{a}	<0.001	0.904
SGR (% day^{-1})	3.62 ± 0.14^{b}	3.63 ± 0.07^{b}	3.71 ± 0.08^{b}	3.80 ± 0.11^{ab}	4.02 ± 0.07^{a}	<0.001	0.798
PER (g)	2.61 ± 0.16^{d}	2.76 ± 0.07^{cd}	2.97 ± 0.08^{bc}	3.07 ± 0.08^{b}	3.43 ± 0.22^{a}	<0.001	0.904
PRR (%)	36.24 ± 2.11^{d}	39.03 ± 0.96^{cd}	43.30 ± 0.99^{bc}	45.94 ± 1.15^{b}	52.15 ± 3.50^{a}	<0.001	0.944
CF (g/cm^{3})	1.43 ± 0.06^{c}	1.51 ± 0.04^{c}	1.63 ± 0.04^{b}	1.67 ± 0.03^{ab}	1.76 ± 0.04^{a}	<0.001	0.929
EW (%)	90.56 ± 0.33	90.76 ± 0.52	90.72 ± 0.30	90.78 ± 0.58	90.79 ± 0.48	0.739	0.152
HSI (%)	0.59 ± 0.11	0.55 ± 0.08	0.56 ± 0.05	0.61 ± 0.09	0.58 ± 0.13	0.542	0.055
VSI (%)	9.43 ± 0.33	9.23 ± 0.52	9.27 ± 0.30	9.21 ± 0.58	9.20 ± 0.48	0.739	-0.152

Different superscript letters on the same row indicate significant differences ($P < 0.05$). Pearson correlation coefficient (R): values above 0.7 indicate a high correlation between the variable and SHLE inclusion levels.

Table 4. Whole-body composition (%) on dry matter basis of Nile tilapia (*Oreochromis niloticus*) fed diets containing increasing levels of shrimp head lipid extract (SHLE) for 45 days.

	SHLE inclusion levels					*P*	R
	0%	0.4%	0.8%	1.2%	1.6%		
Moisture	81.11 ± 0.01^{e}	80.87 ± 0.01^{d}	80.48 ± 0.01^{c}	80.06 ± 0.01^{b}	79.77 ± 0.01^{a}	<0.001	-0.996
Crude protein	68.97 ± 0.02^{c}	69.16 ± 0.13^{c}	69.81 ± 0.16^{b}	70.04 ± 0.13^{b}	70.47 ± 0.12^{a}	<0.001	0.971
Total lipids	13.03 ± 0.03^{e}	12.87 ± 0.09^{d}	12.68 ± 0.02^{c}	12.20 ± 0.02^{b}	11.96 ± 0.07^{a}	<0.001	-0.974
Ash	16.14 ± 0.04	16.25 ± 0.05	16.15 ± 0.02	16.15 ± 0.06	16.13 ± 0.04	0.090	-0.263
Nitrogen free extract	1.86 ± 0.01	1.72 ± 0.08	1.36 ± 0.18	1.61 ± 0.18	1.44 ± 0.21	0.140	-0.614

Different superscript letters on the same row indicate significant ($P < 0.05$). Pearson correlation coefficient (R): values above 0.7 indicate a high correlation between the variable and inclusion levels of SHLE.

Table 5. Mean (± SD) activity of catalase (CAT; U mg^{-1} protein), superoxide dismutase (SOD; U mg^{-1} protein), reduced glutathione (GSH; µM mg^{-1} protein) and malondialdehyde (MDA; µM mg^{-1} protein) in the liver and muscle tissues of Nile tilapia (*Oreochromis niloticus*) fed diets containing increasing levels of shrimp head lipid extract (SHLE) during 45 days.

	SHLE inclusion levels					*P*	R
	0%	0.4%	0.8%	1.2%	1.6%		
Liver							
CAT	35.68 ± 6.84^{e}	52.33 ± 5.99^{d}	69.36 ± 6.39^{c}	91.97 ± 5.03^{b}	109.02 ± 6.53^{a}	<0.001	0.974
SOD	18.73 ± 5.03^{c}	22.34 ± 5.55^{c}	30.11 ± 3.47^{bc}	38.48 ± 6.33^{ab}	43.99 ± 5.45^{a}	<0.001	0.896
GSH	4.21 ± 0.03^{d}	5.52 ± 0.11^{c}	5.99 ± 0.15^{bc}	6.77 ± 0.05^{b}	9.13 ± 0.78^{a}	<0.001	0.944
MDA	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.445	0.358
Muscle							
CAT	20.42 ± 6.38^{e}	36.13 ± 5.05^{d}	54.58 ± 6.86^{c}	67.63 ± 4.41^{b}	95.40 ± 6.81^{a}	<0.001	0.969
SOD	15.35 ± 2.43^{c}	15.48 ± 2.59^{c}	25.94 ± 5.33^{b}	32.35 ± 5.58^{ab}	37.82 ± 3.44^{a}	<0.001	0.906
GSH	8.35 ± 0.16^{d}	9.79 ± 0.28^{c}	13.86 ± 0.17^{b}	14.04 ± 0.05^{b}	15.41 ± 0.42^{a}	<0.001	0.952
MDA	0.09 ± 0.01	0.09 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.139	0.557

Different superscript letters on the same row indicate significant differences ($P < 0.05$). Values of the Pearson correlation coefficient (R) above 0.7 indicate a high correlation between the variable and SHLE inclusion levels.

Table 6. Mean (± SD) levels of glucose (GL; mg dL^{-1}), total cholesterol (TC; mg dL^{-1}), triglycerides (TG, mg dL^{-1}), total protein (TP; g dL^{-1}); albumin (AB; g dL^{-1}), globulin (GB; g dL^{-1}); albumin/globulin ratio (AB/GB), alanine aminotransferase (ALT; U L^{-1}), aspartate aminotransferase (AST; U L^{-1}), and amylase (AM; U L^{-1}) in the serum of Nile tilapia (Oreochromis niloticus) fed diets containing increasing levels of shrimp head lipid extract (SHLE) for 45 days.

	SHLE inclusion levels					*P*	R
	0%	0.4%	0.8%	1.2%	1.6%		
GL	52.40 ± 4.90^{b}	49.37 ± 5.61^{b}	42.17 ± 5.34^{ab}	37.72 ± 4.72^{a}	33.32 ± 2.22^{a}	<0.001	-0.861
TC	195.05 ± 6.9^{c}	191.83 ± 4.8^{c}	158.45 ± 6.2^{b}	132.37 ± 5.0^{a}	128.36 ± 5.7^{a}	<0.001	-0.949
TG	158.14 ± 4.9^{c}	145.25 ± 4.3^{b}	142.52 ± 2.7^{b}	137.89 ± 3.8^{ab}	132.28 ± 4.9^{a}	<0.001	-0.889
TP	4.01 ± 0.22^{c}	4.29 ± 0.13^{bc}	4.33 ± 0.25^{bc}	4.67 ± 0.08^{b}	5.30 ± 0.19^{a}	<0.001	0.888
AB	1.90 ± 0.01^{d}	1.98 ± 0.02^{c}	1.99 ± 0.03^{c}	2.10 ± 0.02^{b}	2.18 ± 0.03^{a}	<0.001	0.952
GB	2.11 ± 0.22^{c}	2.30 ± 0.11^{bc}	2.34 ± 0.23^{bc}	2.56 ± 0.08^{b}	3.12 ± 0.18^{a}	<0.001	0.848
AB/GB	0.91 ± 0.09^{b}	0.86 ± 0.03^{b}	0.85 ± 0.08^{b}	0.82 ± 0.02^{ab}	0.70 ± 0.04^{a}	0.005	-0.721
ALT	13.87 ± 0.46^{c}	12.07 ± 0.80^{b}	12.12 ± 0.25^{b}	10.91 ± 0.33^{a}	10.23 ± 0.58^{a}	<0.001	-0.904
AST	105.24 ± 2.0^{c}	89.48 ± 1.5^{b}	87.25 ± 2.2^{b}	77.74 ± 1.7^{a}	77.26 ± 2.3^{a}	<0.001	-0.927
AM	113.32 ± 3.2^{d}	102.77 ± 1.8^{c}	96.19 ± 4.4^{bc}	92.56 ± 3.4^{ab}	86.63 ± 3.9^{a}	<0.001	-0.933

Different superscript letters on the same row indicate significant differences ($P < 0.05$). Values of the Pearson correlation coefficient (R) above 0.7 indicate a high correlation between the variable and SHLE inclusion levels.

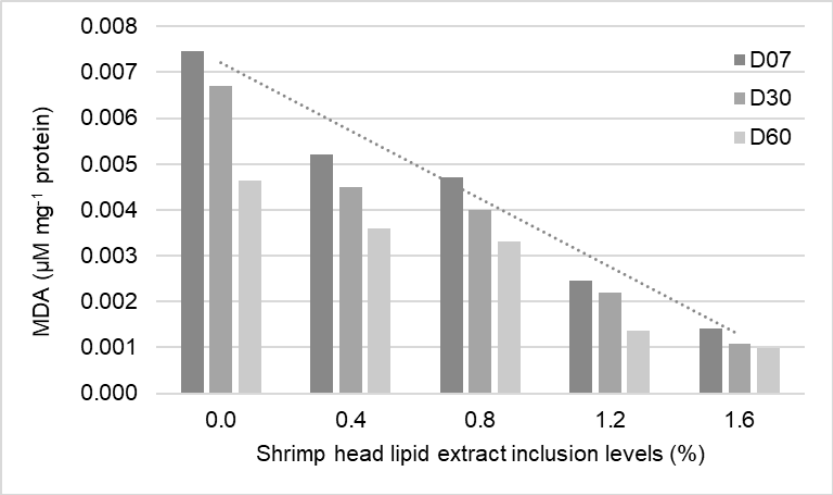

Fig 1. Malondialdehyde content (MDA; µM mg^{-1} protein) in experimental diets containing increasing levels of shrimp head lipid extract (SHLE) after 7 days (D07), 30 days (D30) and 60 days (D60) of storage ($P < 0.001$). The dotted line represents the decreasing trend in MDA levels as SHLE inclusion increases, indicating a potential reduction in lipid peroxidation.

Discussion

A positive response to the dietary supplementation of SHLE to Nile tilapia was clearly observed. In general, performance parameters were superior in tilapia fed with higher dietary levels of SHLE. Nile tilapia is considered less dependent on n-3 PUFA, particularly EPA and DHA, than other commercially important fish species (TRUSHENSKI and ROMBENSO, 2020). Although n-3-PUFA are considered essential, they can be synthesized from C18 PUFA through desaturation (ENGDAW and GEREMEW, 2024) hence its n-3 PUFA requirement can be met with linolenic acid (18:3n-3). Nevertheless, the higher weight gain in tilapia fed increasing dietary levels of SHLE may be related to increased dietary n-3 PUFA levels. This is in line with the findings of Sarker *et al*. (2016) and Trevi *et al*. (2024) as they also observed significantly higher growth of *O. niloticus* fed diets enriched with the n-3 PUFA-rich microalgae *Schizochytrium* sp. Other studies, however, found no significant changes in the growth performance of *O. niloticus* when fed n-3 PUFA-enriched diets (AL-SOUTI *et al*., 2012; LIU *et al*., 2019; LI *et al*., 2020b).

Dietary supplementation of n-3 PUFA may not only affect the growth performance of Nile tilapia, but also plays an important role in their overall health status, as well as

positively affecting the n-3-PUFA content in the whole body of the fish. Higher levels of n-3 PUFA in both diet and fish tissues generally imply greater instability due to increased risk of oxidation. Therefore, requirements for antioxidants are usually higher with increasing degree of fatty acid unsaturation and increasing levels of n-3 PUFA in the diet (WATANABE *et al*., 1981; NRC, 2011). Antioxidant compounds are vital in removing excess reactive oxygen species (ROS) and reduce cell damage, strengthening the antioxidant defense system of animals (SILA *et al*., 2015; NIRMAL *et al*., 2020). Under regular environmental and physiological conditions, ROS are neutralized by endogenous enzymatic antioxidants such as SOD and CAT, by non-enzymatic antioxidants such as GSH, and by exogenous antioxidants, such as carotenoids (KHEIRABADI *et al*., 2022). Astaxanthin has the highest antioxidant capacity among carotenoids, being superior to vitamin E as its structure contains long conjugated double bonds and α-hydroxyketones, which allows the acceptance of electrons from free radicals for subsequent neutralization, completing the chain reactions (COSTA and MIRANDA-FILHO, 2020; ZHU *et al*., 2022).

Besides its direct antioxidant action, astaxanthin also has indirect antioxidant action. In the present study, the activity of CAT, SOD, and GSH in the liver and muscle of tilapia fed diets containing increasing levels of SHLE was progressively higher than the control. Previous studies also showed that the dietary supplementation of astaxanthin increased the antioxidant capacity of fish (LIU *et al*., 2016; LI *et al*., 2019; ZHU *et al*., 2020; WU and XU, 2021; KHEIRABADI *et al*., 2022; XU *et al*., 2022; ZHU *et al*., 2022; LONG *et al*., 2023; ZHAO *et al*., 2023). Notably, Panase *et al*. (2024) and Eldessouki *et al*. (2024) reported elevated levels of SOD and CAT in the liver, and SOD and GSH in the serum of different tilapia species when diets included *Paracoccus carotinifaciens* and *Haematococcus pluvialis* enriched with astaxanthin, respectively. These findings further corroborate the capacity of astaxanthin to enhance antioxidant responses across different tilapia species.

According to Xie *et al*. (2020), Mu *et al*. (2020), and Zhao *et al*. (2023), astaxanthin and n-3 PUFA have a hepatoprotective role by activating the mRNA expression of the Nrf2 cytoprotective signaling pathway, which positively regulates the transcription of genes encoding antioxidant defense proteins, such as CAT, SOD, and GSH, enhancing

their activity and reducing oxidative stress. The inclusion of n-3 PUFA-rich fish oil as the primary lipid source in the diet has been shown to significantly upregulate hepatic Nrf2 expression in Japanese sea bass (*Lateolabrax japonicus*) and large yellow croaker (*Larimichthys crocea*) compared to diets containing soybean oil and linseed oil or rapeseed oil (TAN *et al*., 2017; MU *et al*., 2020). This upregulation of Nrf2 led to increased transcription of antioxidant enzyme genes (CAT, SOD1, SOD2, and GPx), resulting in an enhanced hepatic antioxidant capacity. Additionally, pufferfish (*Takifugu obscurus*) fed with astaxanthin can even significantly increase the expression of CAT and SOD mRNA, in response to the increased production of reactive oxygen species before and, mainly, after exposure to a thermal stress situation, in relation to the control group (CHENG *et al*., 2018).

In certain cases, carotenoids may exhibit pro-oxidant properties (RIBEIRO *et al*., 2018). According to the authors, indications of carotenoids exhibiting pro-oxidant activity include a decrease in GSH and SOD activity, in addition to an increase in lipid peroxidation. However, these effects were not observed in the current study. Therefore, the carotenoids present in SHLE demonstrated no pro-oxidant activity in Nile tilapia when included at dietary levels of up to 1.6%.

MDA is a biomarker of oxidative stress, a secondary product of lipid peroxidation, formed by the rupture and degradation of PUFA present in fish feed and also in cell membranes (HALLIWELL and GUTTERIDGE, 2015). Due to its high antioxidant capacity, astaxanthin can reduce excess tissue MDA, especially when the animal is exposed to environmental stressors or infections, in addition to also reducing MDA in fish feed, especially in incorrect storage circumstances, both situations that cause an increase in in the production of ROS and lipid peroxides (GRASSI *et al*., 2016; ELBAHNASWY and ELSHOPAKEY, 2023).

Liu *et al*. (2016) mentioned that *Pelteobagrus fulvidraco* fed a diet containing 80 mg kg^{-1} astaxanthin presented no reduction in MDA concentrations under regular conditions when compared to the control group. A significant reduction in MDA was only observed after a challenge with the bacterium *Proteus mirabilis*. In our study, MDA levels remained stable in all treatments, indicating that the experimental conditions were within acceptable levels for Nile tilapia, which concurs with Kheirabadi *et al*. (2022) and

Panase *et al*. (2024). Grassi *et al*. (2016) observed a reduction in rancidity, expressed as lower MDA content, in Nile tilapia feeds stored for six and twelve months. The inclusion of 350 mg kg^{-1} of Carophyll pink® (astaxanthin 10%) apparently increased the oxidative stability of the feeds.

The levels of biochemical parameters evaluated here are similar to reference values for Nile tilapia (CHEN *et al*., 2003; MAUEL *et al*., 2007). The assessment of blood biochemical parameters is an indicator of nutritional conditions and general and specific physiological disorders that may compromise fish health (CHENG *et al*., 2018).

Elevated glucose, cholesterol, and triglycerides are considered secondary indicators of physiological response to a specific stressor or liver dysfunction (LIM *et al*., 2019). In the present study, glucose, cholesterol, and triglyceride levels decreased as dietary SHLE levels increased. The decline in glucose is associated with the antioxidant and immunomodulatory properties of astaxanthin, which reduced the stress of tilapia, as glucose is synthesized and released into the bloodstream in stressful situations to meet the high energy demand (SOPINKA *et al*., 2016). The reduction in cholesterol and triglycerides may be related to the better use of lipids through the inclusion of astaxanthin in the diet, by positively regulating the expression of genes related to lipid catabolism and negatively regulating the expression of genes related to lipogenesis and transport of lipids (XU *et al*., 2022). Similar results have been previously described for different fish species (SHEIKHZADEH *et al*., 2012; LI *et al*., 2014; LIU *et al*., 2016; LIM *et al*., 2019; ARACATI *et al*., 2021; HAQUE *et al*., 2021).

The inclusion of n-3 PUFA, particularly omega-3 fatty acids such as EPA and DHA, has been associated with enhanced lipid metabolism and reduced triglyceride levels. Notably, n-3 PUFA have been reported to reduce the secretion of very low-density lipoprotein, a key transporter of triglycerides, while simultaneously promoting mitochondrial β-oxidation, thereby decreasing the availability of fatty acids for triglyceride synthesis (HARRIS *et al*., 1990; HOUSTON *et al*., 2017; MU *et al*., 2020; ZHAO *et al*., 2020). Additionally, these fatty acids stimulate lipid catabolism and modulate the expression of lipogenic genes, ultimately limiting fat deposition, a finding consistent with previous studies investigating the effects of n-3 PUFA on lipid metabolism in Nile tilapia (Ribeiro *et al*., 2008; Ferreira *et al*., 2011; Ayisi *et al*., 2018;

Liu *et al*., 2019; Nassef *et al*., 2019; Li *et al*., 2020b).

Total serum protein content, albumin, globulin, and albumin/globulin ratio are parameters that reveal the physiological and nutritional conditions of fish. The function of albumin is related to homeostasis, through the maintenance of blood osmotic pressure, in addition to transporting hormones, fatty acids, ions, and carotenoids, while globulin acts as a precursor for the synthesis of immunoglobulins (ANDREEVA, 2010; RATHORE and YUSUFZAI, 2018). The increase in serum protein, albumin, and globulin through feeding with SHLE is in line with previous studies that showed that fish fed with different sources and concentrations of astaxanthin were superior to the control, which signals a better innate immunity status in the animals (JAGRUTHI *et al*., 2014; LIU *et al*., 2016; LIM *et al*., 2019; ZHU *et al*., 2020; HAQUE *et al*., 2021). Astaxanthin, due to its high antioxidant capacity in the liver, the site of albumin and globulin synthesis and secretion, may have amplified the metabolic mechanisms for protein synthesis in fish liver tissue (LIM *et al*., 2019).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and amylase are enzymes that act as biochemical markers used to evaluate the nutritional and health conditions of fish. ALT and AST are endocellular transaminases, present mainly in liver tissue, and are released excessively into the blood in situations of cellular injury, mainly in hepatocytes, therefore they are enzymes that indicate tissue damage caused by stress or infections (TANG *et al*., 2019). Amylase is an endocarbohydrase that acts in the intestine, in the hydrolysis of polysaccharides (starch and glycogen), which originates oligosaccharides (ROBYT, 1984). This enzyme is produced by the pancreas and its high concentration in plasma is an indicator of inflammatory processes in pancreatic cells, a situation where intense intracellular enzyme activation occurs, resulting in damage to acinar cells (ZHANG *et al*., 2018). Due to its capacity as an immunomodulator and antioxidant, astaxanthin increases the immune response to infectious processes (WU and XU, 2021) and significantly reduces oxidative stress, interrupting the process of inflammation (OZBEYLI *et al*., 2020). Such results can be observed by the decrease in the concentration of ALT, AST, and amylase in the blood, as seen in the present study. Similar findings were described in studies that evaluated different sources and concentrations of astaxanthin (LIU *et al*., 2016; CHENG *et al*., 2018; ZHANG *et al*.,

2018; LIM *et al*., 2019; OZBEYLI *et al*., 2020; ZHU *et al*., 2020; HAQUE *et al*., 2021; WU and XU, 2021; ELDESSOUKI *et al*., 2024).

In *Oreochromis niloticus*, higher dietary n-3 PUFA levels have been reported to reduce serum ALT and AST activity, an effect associated with enhanced hepatic antioxidant capacity and decreased triglyceride accumulation (AYISI, ZHAO, and WU, 2018; LIU *et al*., 2019; NASSEF *et al*., 2019). These metabolic adaptations contribute to improved lipid homeostasis, attenuation of lipotoxicity, and overall hepatic protection, as reported by Liu *et al*. (2019). Moreover, higher dietary n-3 PUFA levels have been associated with reduced serum TNFα concentrations, a key mediator of inflammation and immune function regulation, which may consequently contribute to decreased ALT and AST levels (MA *et al*., 2018; LI *et al*., 2020a; MU *et al*., 2020), further reinforcing the hepatoprotective effects of n-3 PUFA through anti-inflammatory and metabolic regulatory mechanisms.

Previous studies have suggested that different dietary sources of astaxanthin can improve the productive performance of several fish species (LI *et al*., 2014; LIU *et al*., 2016; XIE *et al*., 2020; HAQUE *et al*., 2021; KHEIRABADI *et al*., 2022; TRUNG *et al*., 2022; XU *et al*., 2022; ZHU *et al*., 2022; LONG *et al*., 2023; ZHAO *et al*., 2023; ELDESSOUKI *et al*., 2024). The effect of dietary astaxanthin on fish performance seems to be directly related to the species, stage of development, feeding habit, and astaxanthin source used (CHENG *et al*., 2018; LIM *et al*., 2018).

As feed intake did not differ between treatments, the action of SHLE may be related to a higher efficiency in the use of dietary nutrients as astaxanthin has been shown to improve the structure of the liver of *O. niloticus*, a central organ of intermediary metabolism (SEGNER *et al*., 1989), which may be related to the higher performance of tilapia in our study. The supplementation of increasing concentrations of SHLE had no effect on survival, thus showing that it has no negative effect on the health status of tilapia.

According to Zhao *et al*. (2023), the best performance indices of fish supplemented with dietary astaxanthin, both from a synthetic source and through *Haematococcus pluvialis*, are directly related to different beneficial effects of the carotenoid on metabolism, including antioxidant capacity, innate immune response, and

hepatomorphology. The authors observed that the inclusion of 0.33% *H. pluvialis* powder or 0.1% of the synthetic pigment Lucantin Pink® (93.70 and 94.10 mg kg^{-1} of astaxanthin, respectively) in the diet increased weight gain, specific growth rate, and decreased feed conversion ratio in rainbow trout (*Oncorhynchus mykiss*).

In the present study, an increase in weight gain was observed after feeding diets supplemented with SHLE, which may be related to the body crude protein content that was significantly higher than that of the control diet. These results corroborate those found by Kheirabadi *et al*. (2022), after providing diets with different levels of the red yeast *Phaffia rhodozyma* (1.55%, 3.73%, and 4.7%), rich in astaxanthin (1.6 mg g^{-1} of yeast) in the growth of *O. mykiss*. Our results showed that the whole-body lipid content of Nile tilapia was significantly reduced in the SHLE-supplemented diets. According to Kalinowski *et al*. (2011), this decrease might be due to astaxanthin stimulating the use of lipids as an energy source. Eldessouki *et al*. (2024) also reported significant increase in the whole-body protein along with a decrease in body lipids in the hybrid red tilapia (*O. niloticus* x *Oreochromis mossambicus*) fed astaxanthin-rich *Haematococcus pluvialis*.

The increase in protein content and reduction in body lipid content observed here may also be related to the increased dietary levels of n-3 PUFA fed to Nile tilapia, which is consistent with previous studies using different n-3 PUFA sources (RIBEIRO *et al*., 2008; FERREIRA *et al*., 2011; LIU *et al*., 2019; NASSEF *et al*., 2019; EL ASELY *et al*., 2020). These studies reported enhancements in growth performance and lipid metabolism, indicating that the improved fat utilization and reduced body lipid accumulation in Nile tilapia may result from the capacity of n-3 PUFA-rich diets to modulate lipid metabolism by downregulating lipogenic activity in hepatic and adipose tissues, ultimately leading to a more favorable lipid profile.

Conclusion

The dietary supplementation of SHLE improved the productive parameters and whole-body composition of *O. niloticus*, mainly by increasing weight gain, feed efficiency, and body protein content, in addition to reducing feed conversion ratio and body lipid content. Additionally, SHLE improved the antioxidant capacity thus reducing oxidative stress. Besides, with the inclusion of SHLE, there was a dose-dependent

reduction in MDA content in fish feed sampled after 7, 30, and 60 days of storage. Furthermore, SHLE also reduced serum parameters indicating infections and inflammatory processes, and increased parameters indicating fish health. At an optimal dietary inclusion level estimated at 1.6%, shrimp head lipid extract demonstrated to be an adequate dietary supplement for Nile tilapia.

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Ethical approval

All experimental work was approved by the Ethics Committee on Animal Use of the Federal Rural University of Pernambuco - CEUA/UFRPE (protocol nº. 8148060922).

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3. Capítulo 2: Comparative evaluation of two shrimp head meals as dietary ingredients for Nile tilapia *Oreochromis niloticus*: effects on nutrient digestibility, amino acid chemical score, digestive enzyme activity and antioxidant parameters

Resumo

A digestibilidade e os efeitos enzimáticos e antioxidantes da farinha de cabeça de camarão (*Penaeus vannamei*) como fonte proteica na dieta da tilápia do Nilo (*Oreochromis niloticus*) foram avaliados. Duas farinhas de camarão foram testadas: farinha de camarão integral (FCI) e farinha de camarão com baixo teor lipídico (FCBL). Níveis dietéticos de 20% e 30% das farinhas substituíram, respectivamente, 80% e 70% da dieta de referência. O experimento, com duração de 60 dias, foi conduzido em um sistema Guelph modificado, com nove tanques de alimentação e nove unidades de coleta de fezes, cada uma contendo 10 peixes (61,85 ± 10,06 g). Tanto a FCI quanto a FCBL apresentaram alta digestibilidade de nutrientes e energia, sendo que a inclusão de 20% gerou os resultados mais eficientes. Os aminoácidos essenciais e não essenciais foram altamente digestíveis, sem aminoácidos limitantes, e o escore químico e o índice de aminoácidos indispensáveis de ambas as farinhas superaram os das fontes proteicas convencionalmente usadas em rações para aquicultura. A inclusão de 20% de FCI e FCBL aumentou significativamente a atividade das enzimas digestivas (protease alcalina, tripsina, quimotripsina, lipase e amilase). A inclusão de FCI e FCBL melhorou a capacidade antioxidante hepática por meio da atividade da catalase e da glutationa reduzida, sem alterar os níveis de malondialdeído. Embora ambas as farinhas sejam consideradas fontes proteicas de alta qualidade e possam ser incluídas na dieta de *O. niloticus* em até 30%, a inclusão de 20% foi considerada ideal. A FCBL, obtida após extração parcial dos lipídios, representa uma opção eficiente para o aproveitamento integral dos resíduos de camarão, agregando valor à cadeia produtiva. Assim, a incorporação de FCI e FCBL na dieta dos peixes promove a aquicultura sustentável e os princípios da economia circular, reutilizando subprodutos da indústria do camarão e aumentando a rentabilidade da aquicultura de peixes e camarões.

Palavras-chave: digestibilidade, escore químico, enzimas digestivas, capacidade antioxidante, economia circular.

Abstract

The digestibility and enzymatic and antioxidant effects of shrimp head meal (*Penaeus vannamei*) as a protein source in the diet of Nile tilapia (*Oreochromis niloticus*) were evaluated. Two shrimp meals were tested: whole shrimp meal (WSM) and low-lipid shrimp meal (LLSM). Dietary levels of 20% and 30% of shrimp meal replaced 80% and 70% of the reference diet, respectively. The 60-day experiment was conducted in a modified Guelph system, with nine feeding tanks and nine fecal collection units, each containing 10 fish (61.85 ± 10.06 g). Both WSM and LLSM produced high nutrient and energy digestibilities, with 20% inclusion yielding the most efficient results. Essential and non-essential amino acids were highly digestible, with no limiting amino acids, and the chemical score and indispensable amino acid index of both WSM and LLSM exceeded those of protein sources conventionally used in aquafeeds. Inclusion of 20% WSM and LLSM significantly increased the activity of digestive enzymes (alkaline protease, trypsin, chymotrypsin, lipase, and amylase). Inclusion of WSM and LLSM enhanced liver antioxidant capacity via catalase and reduced glutathione activities, without altering malondialdehyde levels. Although both WSM and LLSM are considered high-quality protein sources and up to 30% can be effectively included in the diet for *O. niloticus*, inclusion of 20% was found to be optimal. LLSM, which was obtained after partial lipid extraction, represents an efficient option for fully utilizing shrimp waste and adding value to the production chain. Thus, incorporating WSM and LLSM into fish diets promotes sustainable aquaculture and circular economy principles by reusing shrimp industry by-products, enhancing profitability of fish and shrimp aquaculture.

Keywords: digestibility, chemical score, digestive enzymes, antioxidant capacity, circular economy.

Introduction

Aquaculture has expanded significantly in recent decades, propelled by the increasing demand for high-quality protein sources in aquafeeds. Traditionally, fish meal is the primary animal-based protein source in diets for various aquatic species (NRC, 2011). However, overfishing and rising production costs have led to efforts to find

sustainable alternatives to reduce the use of or replace fish meal entirely (ARAGÃO *et al*., 2022). In this context, utilizing waste products, such as shrimp industry by-products, offers an economically and environmentally interesting option.

Global crustacean production reached approximately 12.75 million tons in 2022, with penaeid shrimp farming accounting for 62.2% of this total, and projections indicate continued growth in the coming decades (FAO, 2024). The disposal of by-products like shrimp cephalothorax and shells poses environmental challenges, contributing significantly to solid waste, representing between 40% and 60% of the processed biomass (MAO *et al*., 2017; DENG *et al*., 2020). When poorly managed, these wastes can lead to environmental liabilities, such as soil and water pollution due to organic matter decomposition (FERRARO *et al*., 2010). This highlights the importance of sustainable solutions, such as reusing by-products within circular economy principles (CHARY *et al*., 2024).

Repurposing fishery waste has shown promise in food, pharmaceutical, and cosmetic industries, as well as in the feeding of various aquaculture species (NIRMAL *et al*., 2020; ABUZAR *et al*., 2023). Fish processing by-products are rich in high-quality proteins and essential amino acids, such as methionine and lysine, which are often limiting in plant-based diets (MONTOYA-CAMACHO *et al*., 2019; LIU *et al*., 2021). Furthermore, bioactive compounds found in cephalothorax, such as polyunsaturated fatty acids, carotenoids, chitin, and minerals, exhibit antioxidant, antimicrobial, and anti-inflammatory properties, contributing to both fish nutrition and health (NIRMAL *et al*., 2020; CABANILLAS-BOJÓRQUEZ *et al*., 2021; TKACZEWSKA *et al*., 2024). Incorporating cephalothorax meal into feeds may also improve palatability, promoting feed intake and growth performance in cultured species (TKACZEWSKA *et al*., 2024).

Digestibility trials are essential for evaluating the potential of new feed ingredients by measuring the proportion of energy and nutrients that animals can extract post-digestion and absorption (GLENCROSS *et al*., 2007). Ingredients are considered to have good digestibility when their coefficient exceeds 70% (OLIVEIRA FILHO and FRACALOSSI, 2006). With aquaculture advancements, diets have increasingly been formulated based on digestible rather than crude nutrients thus demanding accurate digestibility data for each ingredient (BAI *et al*., 2022). Such an approach is crucial to

efficiently meet specific nutritional needs, maximize performance and promote sustainability (FRACALOSSI *et al*., 2016; BAI *et al*., 2022).

Though an ingredient's digestibility remains consistent regardless of inclusion levels, this relationship may vary, particularly in chitin- and mineral-rich ingredients like shrimp cephalothorax meal. High dietary inclusion levels of these compounds can reduce digestibility, affecting fish nutritional efficiency (GLENCROSS *et al*., 2007). Therefore, it is essential to evaluate the inclusion of shrimp head meal at different levels, such as 20% and 30%, which are commonly used in digestibility trials for novel ingredients (NRC, 2011; FRACALOSSI *et al*., 2016).

Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) ranks as the second most cultivated fish globally, with 5.3 million tons produced in 2022 (FAO, 2024). Originating from Africa and the Middle East, Nile tilapia has been successfully introduced to various tropical and subtropical regions, including parts of Asia and Latin America, hence demonstrating adaptability to diverse environmental conditions and efficient feed conversion (NOBREGA *et al*., 2020). Known for its resilience, rapid growth, robust reproduction in captivity, disease resistance, and omnivorous feeding habits, this species readily accepts commercial feeds (NG and ROMANO, 2013; EL-SAYED, 2020a).

Utilizing shrimp head meal as a protein source represents a promising alternative. Therefore, this study evaluated the digestibility, intestinal enzymatic activity, and liver antioxidant capacity of Nile tilapia (*O. niloticus*) fed two forms of marine shrimp (*Penaeus vannamei*) head meal at dietary inclusion levels of 20% and 30%.

Materials and methods

Origin and evaluation of the ingredients

The shrimp (*P. vannamei*) head meals used in this study were obtained from Bioingredientes®, Recife, Brazil. Two types of meals were tested: whole shrimp head meal (WSM), processed to retain the full nutrient profile, and low-lipid shrimp head meal (LLSM), which was produced after partial lipid extraction to yield a lipid extract. Proximate composition of these meals (Table 1) was analyzed according to AOAC (2012). Samples were oven-dried to assess dry matter content (Method 934.01), crude protein was determined by the Kjeldahl method (Method 990.03), and ether extract was

measured using a Goldfisch apparatus with petroleum ether as the solvent (Method 920.39). Ash content was determined by incineration in a muffle furnace at 600°C (Method 942.05), while calcium and phosphorus levels were analyzed via atomic absorption spectrophotometry (Method 983.19) and phosphomolybdate reaction, respectively (Method 984.08). Gross energy was determined using a Parr adiabatic bomb calorimeter, while determination of chitin content followed Clark *et al*. (1993) and Hornung and Stevenson (1971), including NaOH digestion and quantification by micro-Kjeldahl with a conversion factor of 6.89.

Table 1. Proximal composition (% dry matter) and gross energy (kcal kg^{-1}) of whole shrimp head meal (WSM) and low-lipid shrimp head meal (LLSM).

	WSM	LLSM
Dry matter	95.86	97.31
Crude protein	49.97	54.18
Ether extract	8.72	2.22
Ash	17.59	21.15
Calcium	5.83	8.12
Phosphorus	1.23	1.69
Chitin	9.32	12.56
Gross energy	3,898.02	3,907.61

Experimental diets

A completely randomized experimental design with five treatments and three replicates was used. For the standard ingredient digestibility assay, the treatments consisted of a reference diet (RD) formulated (Table 2) to meet the nutritional requirements of Nile tilapia (FURUYA, 2010; NRC, 2011; EL-SAYED, 2020b) and four test diets in which 20% and 30% of the RD was replaced by each test ingredient (WSM_{20}, WSM_{30}, $LLSM_{20}$, and $LLSM_{30}$). Apparent digestibility was determined using the indirect method with 1% acid-insoluble ash (Celite®) as inert indicator (GODDARD & MCLEAN, 2001). The proximal composition and physical quality of the diets were analyzed according to AOAC (2012), and Cai et al. (2022), respectively (Table 3).

Table 2. Composition (%) of the reference diet fed to Nile tilapia (*Oreochromis niloticus*).

Soybean meal	64.30
Corn flour	27.79
Starch	4.00
Dicalcium phosphate	1.39
Soybean oil	0.81
Mineral-vitamin premix[a]	0.40
Limestone	0.36
Salt	0.34
DL-Methionine	0.30
L-Threonine	0.30
BHT	0.01
Total	100.00

[a] Vit. A (2,500,000 IU); Vit. D3 (60,000 IU); Vit. E (37,500 IU); Vit. K3 (3,750 mg); Vit. B1 (4,000 mg); Vit. B2 (4,000 mg); Vit. B5 (12 g); Vit. B6 (4,000 mg); Vit. B12 (4,000 mcg); Vit. C (50 g); folic acid (1,250 mg); niacin (22.5 g); biotin (15 mg); iron (15 g); zinc (12.5 g); manganese (12.5 g); copper (2,500 mg); iodine (375 mg); cobalt (125 mg); selenium (85.7 mg).

Table 3. Proximal composition (% dry matter), gross energy (kcal kg^{-1}) and physical pellet quality parameters (%) of the reference diet (RD), and experimental diets containing 20 or 30% of whole shrimp head meal ($WSM_{20\%}$ and $WSM_{30\%}$, respectively) or low-lipid shrimp head meal ($LLSM_{20\%}$ and $LLSM_{30\%}$, respectively) fed to Nile tilapia (*Oreochromis niloticus*).

	RD	$WSM_{20\%}$	$WSM_{30\%}$	$LLSM_{20\%}$	$LLSM_{30\%}$
Dry matter	96.22	96.67	96.9	96.29	97.21
Crude protein	32.15	36.69	39.14	37.10	39.72
Ether extract	3.25	4.34	4.89	3.04	2.94
Ash	8.65	10.14	11.19	11.39	12.92
Calcium	0.73	1.72	2.24	2.16	2.89
Phosphorus	0.51	0.57	0.65	0.68	0.79
Chitin	0.06	2.62	3.45	3.66	5.06
Gross energy	3,899	3,971	3,940	3,955	3,956
Density	74.80	72.57	73.43	76.10	72.40
Durability index	99.06	98.95	99.33	99.04	99.27
Water solubility	72.99	72.21	72.69	73.23	73.52
Water resistance	79.52	78.59	79.80	78.26	80.17

Experimental procedures and sampling

A modified Guelph system (FRACALOSSI *et al*., 2016), comprising nine 250-L polyethylene feeding tanks and nine 150-L fiberglass tanks for fecal collection, was used. Ninety genetically improved Nile tilapia (GIFT) males (61.85 ± 10.06 g) were randomly distributed in the feeding tanks at a density of 10 fish per tank (40 fish m^{-3}). After a 10-

day acclimation period, fish were fed until apparent satiety (*ad libitum*) three times daily (10:00, 13:00, 16:00). One hour after the last feeding, fish were transferred to the fecal collection tanks (density of 67 fish m^{-3}) and returned to feeding tanks two hours before the first feeding the following day (BORGHESI *et al*., 2008; GUIMARÃES *et al*., 2008). Feces were collected during the retention period in the fecal collection tanks and stored at -20 °C. The 60-day trial was divided into two phases: on the initial 30 days, fish were fed solely the diet containing the 20% test ingredient, while in the last 30 days the 30% inclusion diet was offered. The RD was provided continuously, with feces from the first five days of each phase discarded to clear the digestive tract.

The experiment was conducted under natural photoperiod (12 h light: 12 h dark), with continuous aeration in all tanks. Salinity was maintained at 3, with a daily water renewal rate of 20% in the feeding tanks and 100% in the collection tanks. Mean water temperature (27.44 ± 0.5 °C), dissolved oxygen (6.12 ± 0.3 mg L^{-1}), and pH (7.43 ± 0.3) were monitored daily using a digital multiparametric probe (YSI™ model 550A). Non-ionized ammonia (0.042 ± 0.06 mg L^{-1}), nitrite-nitrogen (0.32 ± 0.5 mg L^{-1}), and nitrate-nitrogen (4.38 ± 0.45 mg L^{-1}) were measured every five days (BAIRD *et al*., 2017).

At the end of each experimental phase, three fish per tank were fasted for 24 h and euthanized using 150 mg L^{-1} tricaine methanesulfonate (MS-222, Sigma-Aldrich) (ARAÚJO *et al*., 2018), followed by sectioning the bone marrow and bleeding the gills. Intestine and liver samples were collected for analysis of digestive enzyme activity and antioxidant activity.

Apparent digestibility and amino acid scoring

Fecal samples were analyzed following AOAC (2012). Acid-insoluble ash was determined based on Atkinson *et al*. (1984), where dried feces and diet samples were incinerated at 600°C for 16 h, treated with HCl, and re-incinerated to quantify ash. The apparent digestibility coefficients (ADC) for dry matter, protein, energy, ash, ether extract, calcium, phosphorus, and chitin were calculated as per Cho *et al*. (1985); Fracalossi *et al*. (2016); Fricke *et al*. (2022) and Li *et al*. (2021).

The shrimp head meal with the highest digestibility was analyzed for essential and non-essential amino acid digestibility using liquid chromatography (HAGEN *et al*., 1989; WHITE *et al*., 1986) with tryptophan concentrations determined colorimetrically at 590

nm (LUCAS and SOTELO, 1980). The amino acid chemical scores (CS) were calculated according to Sgarbieri (1996) using the amino acid requirements of Nile tilapia (FURUYA, 2010; NRC, 2011) and egg protein (BAI *et al*., 2022) as reference. Amino acids with a CS above 100 indicate high nutritional value, while a CS below 100 denotes limiting amino acids (PIRES *et al*., 2006). Indispensable amino acid index (IAAI) was calculated in comparison to egg protein (BAI *et al*., 2022).

Digestive enzyme activities

For digestive and antioxidant enzyme analyses, intestine and liver samples were homogenized (40 mg mL^{-1} in 0.1 M Tris-HCl and 0.15 mM NaCl, pH 8) and centrifuged at 8000 *g* for 15 min at 4 °C. Total protein was determined using bovine serum albumin as a standard (BRADFORD, 1976).

Total alkaline protease activity was measured following Silva *et al*. (2020) with 1% azocasein as a substrate in 0.1 M Tris-HCl buffer, pH 8, with spectrophotometric readings at 450 nm. One unit of enzyme activity was defined as the amount hydrolyzing azocasein with an absorbance change of 0.001 per min per mg of protein.

Trypsin, chymotrypsin, and leucine aminopeptidase activities were measured using Nα-benzoil-DL-arginine-p-nitroanilide (BApNA), Suc-Ala-Ala-Pro-Phe p-nitroanilide (SApNA), and leucine-p-nitroanilide (Leu-p-Nan) as substrates, respectively, with absorbance at 405 nm for trypsin and chymotrypsin, and 525 nm for leucine aminopeptidase. One unit of enzymatic activity was defined as the amount of enzyme required to release 1 μmol of p-nitroaniline per minute, with an extinction coefficient of 9100 M^{-1} cm^{-1} (SILVA *et al*., 2020).

Lipase and amylase activities were measured using p-nitrophenyl palmitate (p-NPP) and soluble starch as substrates, respectively. Lipase activity was assessed by reading absorbance at 405 nm, while amylase activity was quantified by the DNSA method, with maltose as the standard curve reference. One unit of lipase activity was defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmol of p-nitrophenol (p-NP) per minute per mg of protein, using an extinction coefficient of 17,500 M^{-1} cm^{-1}. For amylase, one unit of activity was defined as the amount of enzyme that releases 1 μg of maltose per minute per mg of protein.

Liver antioxidant capacity

Catalase (CAT) activity was measured by H_2O_2 reduction at 240 nm at 25 °C (AEBI, 1984). Reduced glutathione (GSH) was quantified following Sedlak and Lindsay (1968), using Ellman's reagent (DTNB) at 412 nm. Lipid peroxidation was determined as malondialdehyde (MDA) using thiobarbituric acid reactive substances (TBARS), with absorbance measured at 532 nm (WALLIN *et al*., 1993).

Statistical analysis

Data were tested for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. Two-way ANOVA was applied, followed by Tukey's post-hoc test for significant differences ($P < 0.05$) in apparent digestibility results. One-way ANOVA was applied, followed by Tukey's post-hoc test for significant differences ($P < 0.05$) in digestive enzyme activities and liver antioxidant capacity results. Analyses were conducted using Jamovi software version 2.3.28.

Results

Apparent digestibility of nutrients

Overall, the apparent digestibility coefficients (ADC) for all nutrients in both shrimp head meals exceeded 70%, except for chitin at the 30% inclusion level of both WSM and LLSM (Table 4). Dry matter, crude protein, gross energy, and calcium digestibility were significantly higher in diets containing 20% inclusion of both meals ($P < 0.05$). Ash, phosphorus, and chitin showed the highest ADC in diets containing 20% LLSM ($P < 0.05$), whereas ether extract digestibility was significantly higher in diets with 20% WSM inclusion ($P < 0.05$).

Table 5 presents the raw values and apparent digestibility coefficients of amino acids in WSM and LLSM. As the highest ADM were achieved with the inclusion of 20% of the meals (Table 4), amino acid digestibility analyses were conducted at this inclusion level. Both WSM and LLSM contained high levels of amino acids, particularly lysine, arginine, leucine, aspartic acid, glutamic acid, glycine, and alanine, with concentrations exceeding 3%. In terms of digestibility, both meals exhibited high digestibility of essential and non-essential amino acids, with ADC values above 89% for all evaluated amino acids.

Table 4. Mean (± SD) apparent digestibility coefficients for dry matter, crude protein, gross energy, ether extract, ash, calcium, phosphorus and chitin in diets with 20% and 30% inclusion of whole shrimp head meal (WSM) and low-lipid shrimp head meal (LLSM) fed to Nile tilapia (*Oreochromis niloticus*).

SM	IL (%)	Apparent digestibility coefficients (%)							
		Dry matter	Crude protein	Gross energy	Ether extract	Ash	Calcium	Phosphorus	Chitin
WSM	20	90.87±0.19[a]	93.61±0.16[a]	91.45±0.06[a]	96.72±0.08[a]	90.73±0.14[b]	88.80±0.18[a]	89.98±0.14[b]	73.83±0.13[b]
	30	82.51±0.18[b]	86.40±0.10[b]	85.04±0.15[b]	92.43±0.08[c]	83.69±0.23[c]	77.25±0.12[b]	82.84±0.19[c]	58.51±0.14[c]
LLSM	20	90.63±0.24[a]	93.74±0.10[a]	91.50±0.09[a]	95.35±0.11[b]	91.55±0.19[a]	88.71±0.12[a]	91.20±0.11[a]	76.21±0.09[a]
	30	80.24±0.17[c]	84.45±0.06[c]	82.83±0.13[c]	86.35±0.08[d]	83.48±0.19[c]	74.47±0.16[c]	82.53±0.15[c]	53.97±0.12[d]
P Shrimp meal (SM)		< .001	< .001	< .001	< .001	0.271	< .001	< .001	< .001
P Inclusion level (IL)		< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
P SM * IL		< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001

Different letters on the same column indicate significant differences ($P < 0.05$).

Table 5. Raw values (mean ± 0.02 SD) and apparent digestibility coefficients (ADC) of amino acids in diets with 20% inclusion of whole shrimp head meal (WSM) and low-lipid shrimp head meal (LLSM) fed to Nile tilapia (*Oreochromis niloticus*).

	$WSM_{20\%}$		$LLSM_{20\%}$	
	Raw value (%)	ADC (%)	Raw value (%)	ADC (%)
Essential Amino acids				
Lysine	3.42	94.69	3.66	95.44
Methionine	1.45	95.67	1.59	95.99
Threonine	2.46	94.49	2.66	94.61
Histidine	1.47	94.97	1.56	95.35
Arginine	3.67	95.36	3.97	95.85
Tryptophan	0.58	95.74	0.68	95.74
Valine	2.46	94.60	2.69	94.96
Isoleucine	2.26	93.50	2.42	95.06
Leucine	3.34	94.04	3.67	95.04
Phenylalanine	2.63	94.32	2.89	95.12
Non-essential Amino acids				
Cystine	0.48	95.34	0.52	95.28
Aspartic Acid	4.46	94.69	4.83	95.39
Glutamic Acid	6.6	95.28	7.15	95.36
Serine	2.1	93.91	2.27	94.99
Glycine	4.71	91.34	5.10	90.98
Taurine	0.2	92.54	0.21	95.95
Alanine	3.03	93.65	3.28	94.63
Proline	2.31	93.93	2.50	94.58
Tyrosine	2.32	94.98	2.51	95.44
Hydroxyproline	0.02	92.90	0.02	89.07

Amino acid chemical scores

The limiting amino acids for the different meals, evaluated based on their chemical scores, are detailed in Tables 6 and 7. No essential amino acids were identified as limiting for WSM and LLSM when compared to the amino acid requirements of Nile tilapia. However, when compared to egg protein, isoleucine was identified as a limiting amino acid in both meals. In comparison with traditional protein sources, both shrimp meals exhibited higher chemical scores and indispensable amino acid indices.

Table 6. Chemical score (CS) of amino acids (AA) for whole shrimp head meal (WSM), low-lipid shrimp head meal (LLSM), fish meal (FM), soybean meal (SBM), and bovine meat and bone meal (MBM), relative to the amino acid requirements for Nile tilapia.

Amino acids	%AA tilapia[1]	$\%CS_{WSM}$	$\%CS_{LLSM}$	$\%CS_{FM}$[2]	$\%CS_{SBM}$[2]	$\%CS_{MBM}$[2]
Lysine	1.6	213.75	228.75	208.13	185.63	170.63
Methionine	0.7	207.14	227.14	184.29	90.00	100.00
Methionine + Cystine	1.0	193.00	211.00	225.00	135.00	121.00
Threonine	1.1	223.64	241.82	200.00	170.00	155.45
Histidine	1.0	147.00	156.00	103.00	125.00	89.00
Arginine	1.2	305.83	330.83	270.83	290.83	318.33
Tryptophan	0.3	193.33	226.67	146.67	223.33	93.33
Valine	1.5	164.00	179.33	172.67	152.67	153.33
Isoleucine	1.0	226.00	242.00	208.00	223.00	151.00
Leucine	1.9	175.79	193.16	185.26	192.11	154.74
Phenylalanine	1.1	239.09	262.73	186.36	225.45	139.09
Phenylalanine + Tyrosine	1.6	309.38	337.50	223.75	261.25	161.25
Crude protein	-	49.97	54.18	54.90	48.20	51.90

[1]NRC (2011); [2]Rostagno (2024).

Table 7. Chemical score (CS) of amino acids (AA) for whole shrimp head meal (WSM), low-lipid shrimp head meal (LLSM), fish meal (FM), soybean meal (SBM), and bovine meat and bone meal (MBM), relative to egg protein, and the indispensable amino acid index (IAAI).

	%AA egg[1]	$\%CS_{WSM}$	$\%CS_{LLSM}$	$\%CS_{FM}$[2]	$\%CS_{SBM}$[2]	$\%CS_{MBM}$[2]
Lysine	7.2	47.50	50.83	46.25	41.25	37.92
Methionine	4.1	35.37	38.78	31.46	15.37	17.07
Methionine + Cystine	6.3	30.63	33.49	35.71	21.43	19.21
Threonine	4.9	50.20	54.29	44.90	38.16	34.90
Histidine	2.1	70.00	74.29	49.05	59.52	42.38
Arginine	6.4	57.34	62.03	50.78	54.53	59.69
Tryptophan	1.5	38.67	45.33	29.33	44.67	18.67
Valine	7.3	33.70	36.85	35.48	31.37	31.51
Isoleucine	8.0	28.25	30.25	26.00	27.88	18.88
Leucine	9.2	36.30	39.89	38.26	39.67	31.96
Phenylalanine	6.3	41.75	45.87	32.54	39.37	24.29
Phenylalanine + Tyrosine	10.0	49.50	54.00	35.80	41.80	25.80
Crude protein	-	49.97	54.18	54.90	48.20	51.90
IAAI (%)	-	439.08	478.41	384.05	391.78	317.25

[1]Bai *et al*. (2022); [2]Rostagno (2024).

Digestive enzyme activities

The digestive enzyme activities in Nile tilapia juveniles are presented in Table 8. The inclusion of 20% of each shrimp meal resulted in higher enzyme activities compared to the inclusion of 30% of each shrimp meal and the reference diet ($P < 0.05$). The exception was leucine aminopeptidase, which showed lower activity in treatments with 20% and 30% inclusion of WSM compared to treatments with 20% and 30% inclusion of LLSM ($P < 0.05$).

Table 8. Mean (± SD) levels of alkaline protease (U.mg^{-1} protein), trypsin (mU.mg^{-1} protein), chymotrypsin (mU.mg^{-1} protein), leucine aminopeptidase (mU.mg^{-1} protein), lipase (U.mg^{-1} protein), and amylase (mg maltose min^{-1} mg protein^{-1}) in the intestine of Nile tilapia (*Oreochromis niloticus*) fed a reference diet (RD), and diets containing 20% and 30% whole shrimp head meal ($WSM_{20\%}$ and $WSM_{30\%}$, respectively) or low-lipid shrimp head meal ($LLSM_{20\%}$ and $LLSM_{30\%}$, respectively).

	RD	$WSM_{20\%}$	$WSM_{30\%}$	$LLSM_{20\%}$	$LLSM_{30\%}$	*P*
Alkaline protease	5.44 ± 0.60^{c}	10.75 ± 1.76^{a}	7.76 ± 0.25^{b}	9.99 ± 0.41^{a}	7.20 ± 0.43^{bc}	< 0.001
Trypsin	3.09 ± 0.74^{b}	6.29 ± 0.90^{a}	3.83 ± 0.43^{b}	6.13 ± 0.82^{a}	4.04 ± 0.80^{b}	< 0.001
Chymotrypsin	14.32 ± 2.26^{c}	32.27 ± 1.50^{a}	18.76 ± 1.69^{b}	33.53 ± 1.07^{a}	21.18 ± 1.28^{b}	< 0.001
Leucine aminopeptidase	0.42 ± 0.04^{c}	0.54 ± 0.08^{b}	0.52 ± 0.05^{bc}	0.73 ± 0.07^{a}	0.70 ± 0.02^{a}	< 0.001
Lipase	0.09 ± 0.01^{c}	0.20 ± 0.01^{a}	0.13 ± 0.03^{bc}	0.21 ± 0.03^{a}	0.14 ± 0.01^{b}	< 0.001
Amylase	153.18 ± 8.27^{c}	240.39 ± 15.89^{a}	97.65 ± 7.22^{d}	183.49 ± 7.01^{b}	107.72 ± 16.09^{d}	< 0.001

Different letters on the same row indicate significant differences ($P < 0.05$).

Liver antioxidant capacity

The antioxidant activity results in the liver of Nile tilapia are shown in Table 9. Catalase (CAT) activity was significantly higher in all test diets compared to the reference diet ($P < 0.05$). Reduced glutathione (GSH) activity was highest in treatments with 20% inclusion of both meals ($P < 0.05$). Malondialdehyde (MDA) levels remained unchanged regardless of dietary WSM or LLSM inclusion level ($P > 0.05$).

Table 9. Mean (± SD) activity of catalase (CAT; U mg^{-1} protein), reduced glutathione (GSH; µM mg^{-1} protein) and malondialdehyde (MDA; µM mg^{-1} protein) in the liver of Nile tilapia (*Oreochromis niloticus*) fed a reference diet (RD), and diets containing 20% and 30% whole shrimp head meal ($WSM_{20\%}$ and $WSM_{30\%}$, respectively) or low-lipid shrimp head meal ($LLSM_{20\%}$ and $LLSM_{30\%}$, respectively).

	RD	$WSM_{20\%}$	$WSM_{30\%}$	$LLSM_{20\%}$	$LLSM_{30\%}$	*P*
CAT	33.82 ± 5.32^{b}	65.24 ± 8.87^{a}	67.29 ± 8.93^{a}	60.14 ± 9.81^{a}	65.63 ± 9.68^{a}	< 0.001
GSH	12.45 ± 0.68^{c}	36.82 ± 2.59^{a}	20.60 ± 3.69^{b}	32.41 ± 2.55^{a}	18.39 ± 1.21^{b}	< 0.001
MDA	0.023 ± 0.006^{a}	0.028 ± 0.001^{a}	0.029 ± 0.007^{a}	0.028 ± 0.007^{a}	0.022 ± 0.001^{a}	< 0.001

Different letters on the same row indicate significant differences ($P < 0.05$).

Discussion

Both whole shrimp head meal and low-lipid shrimp head meal exhibited high nutrient digestibility for Nile tilapia. This is consistent with previous research by Chi *et al*. (2017) and Fines and Holt (2010), who reported ADCs above 70% for protein, energy, lipids, and amino acids in *Rachycentron canadum* fed with whole shrimp and crab meals at 30% dietary inclusion. In that study, ADCs of some nutrients also exceeded 90%. Additionally, shrimp-derived byproducts, such as protein hydrolysates and fermented meals, have demonstrated high ADC values for protein, amino acids, energy, and lipids (NWANNA, 2003; KHOSRAVI *et al*., 2015; LI *et al*., 2021), underscoring the versatility of shrimp-based ingredients in optimizing aquafeeds.

In contrast, Boscolo *et al*. (2004) reported low energy digestibility (68.38%) but high protein digestibility (88.79%) when evaluating 20% cinnamon shrimp meal

inclusion for *O. niloticus*. Similar patterns of lower digestibility values (58–79%) have also been observed in other species such as *Sciaenops ocellatus*, *Cromileptes altivelis*, and *Oncorhynchus mykiss* (HARDY *et al*., 2007; LAINING *et al*., 2003; LI *et al*., 2004). These variations highlight possible differences among species and the potential impact of shrimp meal processing on final feed digestibility (FONTES *et al*., 2019).

The high digestibility observed in diets with shrimp head meal in this study may be attributed to the presence of attractant amino acids like glycine, alanine, proline, and arginine, which are generally more concentrated in shrimp meal than in fish meal (CARR *et al*., 1996). These amino acids are known to enhance palatability, promoting feed acceptance (KITAGIMA and FRACALOSSI, 2011).

While Glencross *et al*. (2007) suggested that ingredient interactions do not alter diet digestibility, our findings revealed a significant reduction in digestibility with 30% shrimp meal inclusion. This discrepancy could be related to higher levels of minerals and chitin in shrimp meal, which, at elevated inclusion levels, may form a physical barrier, hindering the digestion and absorption of other nutrients (BOSCOLO *et al*., 2004; FONTES *et al*., 2019). Kitagima and Fracalossi (2011) also noted lower dry matter and protein digestibility (59.81% and 81.41%, respectively) in *Ictalurus punctatus* fed shrimp and fish viscera meal compared to other protein sources, attributing this to the high mineral content (42.0%) of the test ingredients.

On the other hand, omnivorous species such as tilapia, whose diets may include crustaceans and insects, can partially digest chitin due to the release of chitinase, an enzyme produced primarily in the stomach and pancreas (ROTTA, 2003; LU and KU, 2013; TIPPAYADARA *et al*., 2021). Chitinase degrades chitin into N-acetylglucosamine, facilitating absorption (GUTOWSKA *et al*., 2004; KROGDAHL *et al*., 2005). In the present study, chitin digestibility ranged from 58.51% to 76.21%, similar to results by Köprücü and Özdemir (2005), who reported 69.3% and 71.5% chitin digestibility for crawfish and gammarid meals for Nile tilapia. Fontes *et al*. (2019) also observed chitin ADCs between 59.8% and 81.3% in various insect meals, though without direct correlation between chitin content and digestibility in tilapia.

Overall, the digestibility of dry matter, energy, protein, and amino acids in both shrimp head meals at 20% inclusion was comparable to widely used protein sources in

tilapia feed. This includes plant-based sources such as soybean meal (GUIMARÃES *et al*., 2008) and corn gluten meal (MEURER *et al*., 2003; GUIMARÃES *et al*., 2008), as well as animal-based sources like fish meal, meat and bone meal, and poultry by-product meal (MEURER *et al*., 2003; GUIMARÃES *et al*., 2008; XAVIER *et al*., 2014). Additionally, ingredients like fish meal, meat and bone meal, and soybean meal are highlighted in aquaculture diets due to their balanced essential amino acid profiles (NRC, 2011; EL-SAYED, 2020b; MAI *et al*., 2022). In this study, both WSM and LLSM showed superior essential amino acid scores and IAAI compared to these traditional ingredients, indicating they represent high-quality protein sources for Nile tilapia.

Analysis of the chemical score of WSM and LLSM indicated that, compared to the nutritional requirements of Nile tilapia, no essential amino acid was limiting. However, when compared to the amino acid profile of egg protein, (BAI *et al*., 2022), all essential amino acids were limiting in shrimp meal, especially isoleucine. This suggests that while shrimp meals are valuable protein sources, amino acid supplementation might be required in higher-demand diets.

The ability of fish to utilize nutrients is linked to the presence and activity of digestive enzymes, which vary by species, age, and feeding habits (HANI *et al*., 2018). Omnivorous fish exhibit greater enzymatic plasticity compared to species with more selective feeding habits (MORAES and DE ALMEIDA, 2020). Furthermore, high-quality feed increases digestive capacity, which was observed in the present study, as enzymatic activity in diets with 20% and 30% WSM and LLSM was comparable to or higher than the reference diet. Moreover, according to Amer *et al*. (2021), substrates with low bioavailability, like chitin, and excess minerals may reduce enzymatic activity when exceeding tilapia's digestive capacity, which helps explain the lower digestibility at 30% inclusion compared to 20%.

Shrimp head meal is rich in carotenoid pigments, mainly astaxanthin (KANDRA *et al*., 2012). These bioactive compounds can enhance antioxidant capacity by stimulating catalase and reduced glutathione synthesis, protecting cells and tissues from oxidative damage (SOWMYA and SACHINDRA, 2012; COSTA and MIRANDA-FILHO, 2020). Recent studies by Eldessouki *et al*. (2024) and Panase *et al*. (2024) support the antioxidant potential of astaxanthin-enriched diets for tilapia, as observed in this study. Additionally,

stable malondialdehyde levels, a common biomarker for oxidative stress and lipid peroxidation (HALLIWELL and GUTTERIDGE, 2015), further confirm the antioxidant potential of shrimp meals.

Conclusion

The inclusion of shrimp head meals (WSM and LLSM) in diets for *Oreochromis niloticus* resulted in significant nutritional benefits, such as high nutrient and energy digestibility, enhanced intestinal enzyme activities, and increased hepatic antioxidant capacity. Incorporating a 20% inclusion level of both WSM and LLSM resulted in optimal apparent digestibility coefficients and ensured efficient digestibility of essential and non-essential amino acids, with no limiting amino acids for Nile tilapia.

These findings highlight WSM and LLSM as high-quality, bioavailable protein sources with amino acid profiles suitable for tilapia and chemical scores exceeding those of conventional protein sources. The use of LLSM, derived from partial lipid extraction, proved advantageous by adding value to shrimp industry by-products and promoting full resource utilization. Incorporating WSM and LLSM enhances the profitability of fish and shrimp production chains, aligning with circular economy principles and environmental sustainability in aquaculture by repurposing nutrient-rich waste.

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Ethical approval

All experimental work was approved by the Ethics Committee on Animal Use of the Federal Rural University of Pernambuco - CEUA/UFRPE (protocol nº. 8148060922).

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4. Capítulo 3: Immunomodulatory effect of shrimp by-products in diets for Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Aquaculture plays a critical role in food security, with Nile tilapia (*Oreochromis niloticus*) being one of the most widely farmed species due to its resilience and adaptability. However, the reliance on traditional feed ingredients poses sustainability challenges. This study aimed to investigate the immunomodulatory effects of incorporating shrimp cephalothorax lipid extract (SCLE) and shrimp cephalothorax meal (SCM) in Nile tilapia diets as sustainable feed alternatives. Two experiments were conducted using diets with varying levels of SCLE (0.4%, 0.8%, 1.2%, and 1.6%) and SCM (5%, 10%, 15%, and 20%). A total of 200 healthy, uniformly sized Nile tilapia fingerlings were selected for two trials with average weights of 4.38±0.13 g and 3.24±0.07 g, respectively. Fish were distributed across 20 tanks and fed *ad libitum* for 45 days. Hematological parameters were analyzed, revealing that diets with SCLE significantly increased leukocyte, thrombocyte, and hematocrit levels, particularly at 1.6% inclusion. SCM inclusion also led to higher leukocyte counts, especially at 20%. These findings suggest that SCLE and SCM positively impact the innate immune system of Nile tilapia, enhancing their ability to resist pathogens. Additionally, the use of shrimp by-products in aquafeeds presents a viable strategy for reducing environmental waste and promoting more sustainable practices in aquaculture.

Keywords: Sustainable ingredients, Hematological parameters, Leukocyte count, Innate immune system, Shrimp waste.

RESUMO

A aquicultura desempenha um papel fundamental na segurança alimentar, com a tilápia do Nilo (*Oreochromis niloticus*) sendo uma das espécies mais cultivadas devido à sua resiliência e adaptabilidade. No entanto, a dependência de ingredientes tradicionais nas rações apresenta desafios de sustentabilidade. Este estudo teve como objetivo investigar os efeitos imunomoduladores da inclusão de extrato lipídico de cefalotórax de camarão (SCLE) e farinha de cefalotórax de camarão (SCM) nas dietas de tilápia do Nilo como alternativas sustentáveis de alimentação. Foram conduzidos dois experimentos utilizando dietas com níveis variados de SCLE (0,4%, 0,8%, 1,2% e 1,6%) e SCM (5%, 10%, 15% e 20%). Um total de 200 alevinos de tilápia do Nilo peixes saudáveis e de tamanho uniforme foram selecionados para dois ensaios, com pesos médios de 4,38±0,13 g e

3,24±0,07 g, respectivamente. Os peixes foram distribuídos em 20 tanques e alimentados *ad libitum* por 45 dias. Os parâmetros hematológicos foram analisados, revelando que dietas com SCLE aumentaram significativamente os níveis de leucócitos, trombócitos e hematócrito, especialmente com 1,6% de inclusão. A inclusão de SCM também levou a contagens mais altas de leucócitos, especialmente com 20%. Esses resultados sugerem que SCLE e SCM têm um impacto positivo no sistema imunológico inato da tilápia do Nilo, melhorando sua capacidade de resistir a patógenos. Além disso, o uso de subprodutos do camarão em rações aquícolas apresenta uma estratégia viável para reduzir o desperdício ambiental e promover práticas mais sustentáveis na aquicultura.

Palavras-chave: Ingredientes sustentáveis, Parâmetros hematológicos, Contagem de leucócitos, Sistema imunológico inato, Resíduos de camarão.

RESUMEN

La acuicultura desempeña un papel crucial en la seguridad alimentaria, siendo la tilapia del Nilo (*Oreochromis niloticus*) una de las especies más cultivadas debido a su resistencia y adaptabilidad. Sin embargo, la dependencia de ingredientes tradicionales en las dietas plantea desafíos de sostenibilidad. Este estudio tuvo como objetivo investigar los efectos inmunomoduladores de la inclusión de extracto lipídico de cefalotórax de camarón (SCLE) y harina de cefalotórax de camarón (SCM) en las dietas de tilapia del Nilo como alternativas sostenibles de alimentación. Se realizaron dos experimentos utilizando dietas con diferentes niveles de SCLE (0,4%, 0,8%, 1,2% y 1,6%) y SCM (5%, 10%, 15% y 20%). Un total de 200 alevines de tilapia del Nilo sanos y de tamaño uniforme fueron seleccionados para dos ensayos, con pesos promedio de 4,38±0,13 g y 3,24±0,07 g, respectivamente. Los peces se distribuyeron en 20 tanques y se alimentaron *ad libitum* durante 45 días. Se analizaron los parámetros hematológicos, revelando que las dietas con SCLE aumentaron significativamente los niveles de leucocitos, trombocitos y hematocrito, especialmente con un 1,6% de inclusión. La inclusión de SCM también condujo a un aumento en los recuentos de leucocitos, especialmente con un 20%. Estos hallazgos sugieren que SCLE y SCM tienen un impacto positivo en el sistema inmunológico innato de la tilapia del Nilo, mejorando su capacidad para resistir patógenos. Además, el uso de subproductos del camarón en las dietas acuícolas presenta

una estrategia viable para reducir los desechos ambientales y promover prácticas más sostenibles en la acuicultura.

Palabras clave: Ingredientes sostenibles, Parámetros hematológicos, Conteo de leucocitos, Sistema inmunológico innato, Residuos de camarón.

1 INTRODUCTION

Aquaculture stands out as one of the fastest-growing global sectors, playing a fundamental role in food security and the provision of animal protein, particularly in developing countries and coastal regions. Fish, the primary output of this industry, accounts for 16% of global animal protein consumption and serves as the main food source for approximately 950 million people (Pradeepkiran, 2019).

In this context, Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) has become the third most raised species worldwide, with a production of 5 million tons in 2022 (FAO, 2024). Originally native to Africa and the Middle East, this species is now farmed in about 135 countries (Prabu *et al.*, 2019). Its popularity in aquaculture is attributed to its resilience to adverse environmental conditions, disease resistance, high productivity, and ease of adaptation to formulated feed (Arumugam *et al.*, 2023).

As global fish consumption continues to rise, the demand for sustainable production solutions also increases, particularly in terms of nutrition, which remains largely dependent on fishmeal and fish oil—ingredients with limited availability that can contribute to overfishing and the depletion of marine ecosystems (Hua *et al.*, 2019). In this context, agro-industrial by-products, derived from non-commercial parts of agricultural resources, have gained attention as potential nutritional ingredients and sources of bioactive compounds in aquaculture, promoting physiological responses and enhancing the health of farmed organisms (Leyva-López *et al.*, 2020).

Shrimp is another highly significant aquaculture commodity. After processing, non-commercial parts such as the cephalothorax and exoskeleton account for approximately 70% of the shrimp's weight, with residue global production reaching 4 million tons in 2022 (FAO, 2024). These waste, often discarded improperly, contribute to substantial environmental damage (Mezzomo *et al.*, 2013). However, due to their

composition—rich in proteins, minerals, lipids, chitin, and carotenoids—these raw materials have considerable potential for utilization in the pharmaceutical and food industries (Kandra; Challa; Jyothi, 2012; Mezzomo *et al.*, 2013; Parjikolaei *et al.*, 2015).

Shrimp meal and lipid extract obtained from the shrimp cephalothorax are two by-products with environmental and socioeconomic relevance. Shrimp meal is a protein-rich feed ingredient that contains essential amino acids, minerals, carotenoids, and chitin (Liu *et al.*, 2021). El-Sayed (2020) demonstrated that shrimp meal could completely replace fishmeal in Nile tilapia diets without negatively affecting growth performance. Additionally, protein sources derived from shrimp have shown satisfactory digestibility in Nile tilapia, with values ranging from 74% to 87% (NRC, 2011). Shrimp lipid extract is another potential ingredient, containing polyunsaturated fatty acids, including DHA and EPA, along with α-tocopherol and astaxanthin (Gómez-Guillén *et al.*, 2018).

Both shrimp meal and lipid extract are rich sources of carotenoids, particularly astaxanthin, the primary pigment found in shrimp (Gómez-Estaca *et al.*, 2017). This carotenoid has been shown to possess antioxidant, pigmenting, anti-inflammatory, and immunomodulatory properties in various aquaculture species (Bai; Katya; Yun, 2015; Gómez-Guillén *et al.*, 2018; Costa; Miranda-Filho, 2020; Kheirabadi *et al.*, 2022).

The provision of functional feeds that act as positive immunomodulators, enhancing the immune response of fish, plays a crucial role in aquaculture (Devi *et al.*, 2019). Bioactive components help mitigate the impact of environmental stressors and diseases, providing a natural preventive alternative (Waagbø; Remø, 2020; Mueller *et al.*, 2023). Despite these benefits, the specific immunomodulatory effects of astaxanthin in species such as Nile tilapia remain underexplored and require further investigation.

Since fish lack the physiological mechanisms to biosynthesize astaxanthin, they must acquire it through their diet in natural environments (Lim; Bachok; Hii, 2017). In aquaculture systems where natural sources of astaxanthin are absent, supplementation must be provided through formulated feeds (Parjikolaei *et al.*, 2015). Therefore, the present study aims to evaluate the immunomodulatory effects of including shrimp cephalothorax meal and lipid extract in the diets of Nile tilapia fingerlings.

2 METHODOLOGY

2.1 PREPARATION OF EXPERIMENTAL DIETS

Two experiments were developed, the first evaluating the inclusion of shrimp cephalothorax lipid extract (SCLE) and the second one with the inclusion of shrimp cephalothorax meal (SCM) in the diets, both supplied by Bioingredientes®, Recife, Brazil. Five isoproteic and isoenergetic diets were produced in both experiments (Table 1 and Table 2), meeting the nutritional requirements of Nile tilapia fingerlings (Furuya, 2010; NRC, 2011; El-Sayed, 2020). The experimental diets consisted of a basal diet with 0% inclusion of the test ingredient (control group) and four diets with increasing levels of SCLE (0.4%, 0.8%, 1.2%, and 1.6%) and SCM (5%, 10%, 15%, and 20%).

Feed ingredients were mixed with warm water for a few minutes until exhibiting an adequate texture. Subsequently, the resulting homogeneous blend was passed through a 2-mm sieve in a meat-mincing machine. The formed pellets were dried in a forced-air oven at 55°C for 24 h. All dried diets were stored at −5°C. The proximate composition analyses of the experimental feeds were carried out following (AOAC, 2012).

Table 1. Ingredients and proximate composition of diets with SCLE inclusion (% dry matter)

Ingredients (%)	Shrimp lipid extract inclusion levels				
	0%	0.40%	0.80%	1.20%	1.60%
Soybean meal	60.78	60.79	60.80	60.82	60.83
Ground corn grain	33.78	33.71	33.65	33.58	33.50
Dicalcium phosphate	1.41	1.41	1.41	1.41	1.41
Soybean oil	1.38	1.04	0.69	0.34	0.00
Limestone	0.69	0.69	0.69	0.69	0.69
Carboxymethylcellulose	0.50	0.50	0.50	0.50	0.50
L-Threonine	0.43	0.43	0.43	0.43	0.43
Mineral-vitamin premix [a]	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.39	0.39	0.39	0.39	0.40
Salt	0.22	0.22	0.22	0.22	0.22
Antioxidant BHT	0.02	0.02	0.02	0.02	0.02
Shrimp cephalothorax lipid extract [b]	0.00	0.40	0.80	1.20	1.60
Total	100	100	100	100	100
Proximate composition					
Dry matter (%)	96.26	96.08	96.25	96.11	96.16
Crude protein (%)	30.46	30.52	30.49	30.41	30.44
Digestible protein (%)	27.00	27.00	27.00	27.00	27.00
Crude lipid (%)	3.27	3.23	3.34	3.25	3.31
Ash (%)	5.32	5.21	5.30	5.25	5.18
Digestible energy (kcal/g)	3.200	3.200	3.200	3.200	3.200

[a] Vit. A (2,500,000 IU); Vit. D3 (60,000 IU); Vit. E (37,500 IU); Vit. K3 (3,750 mg); Vit. B1 (4,000 mg); Vit. B2 (4,000 mg); Vit. B5 (12 g); Vit. B6 (4,000 mg); Vit. B12 (4,000 mcg); Vit. C (50 g); folic acid (1,250 mg); niacin (22.5 g); biotin (15 mg); iron (15 g); zinc (12.5 g); manganese (12.5 g); copper (2,500 mg); iodine (375 mg); cobalt (125 mg); selenium (85.7 mg).

[b] Astaxanthin (687 µg/g); total lipids (63.0 g/100 g); gross energy (8,367.77 kcal/kg); digestible energy (8,200.41 kcal/kg); ∑MUFA (28.60%); ∑PUFAω-1 (0.20%); ∑PUFAω-3 (11.48%); ∑PUFAω-4 (0.17%); ∑PUFAω-6 (24.11%); ∑SFA (36.01%). Source: Prepared by the authors.

Table 2. Ingredients and proximate composition of diets with SCM inclusion (% dry matter)

Ingredients (%)	Shrimp meal inclusion levels				
	0%	5%	10%	15%	20%
Soybean meal	63.85	57.70	51.56	45.42	39.28
Ground corn grain	28.79	30.99	33.18	35.38	37.57
Shrimp cephalothorax meal [a]	0.00	5.00	10.00	15.00	20.00
Limestone	2.42	1.89	1.35	0.81	0.27
Soybean oil	1.81	1.64	1.47	1.30	1.13
Dicalcium phosphate	1.39	1.11	0.83	0.56	0.28
Carboxymethylcellulose	0.50	0.50	0.50	0.50	0.50
Mineral-vitamin premix [b]	0.40	0.40	0.40	0.40	0.40
L-Threonine	0.28	0.25	0.22	0.19	0.17
Salt	0.21	0.19	0.17	0.15	0.12
DL-Methionine	0.19	0.18	0.17	0.17	0.16
L-lysine	0.09	0.08	0.07	0.06	0.05
Antioxidant BHT	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100
Proximate composition					
Dry matter (%)	96.05	96.15	96.18	96.22	96.31
Crude protein (%)	30.67	30.62	30.56	30.51	30.46
Digestible protein (%)	28.00	28.00	28.00	28.00	28.00
Crude lipid (%)	4.15	4.38	4.61	4.84	5.07
Ash (%)	5.55	5.79	6.03	6.28	6.52
Digestible energy (kcal/g)	3.200	3.200	3.200	3.200	3.200

[a] Dry matter (87.86%); crude protein (55.19%); digestible protein (51.70%); gross energy (3,572.71 kcal/kg); digestible energy (3,267.56 kcal/kg); crude lipids (8.72%); ash (17.59).

[b] Vit. A (2,500,000 IU); Vit. D3 (60,000 IU); Vit. E (37,500 IU); Vit. K3 (3,750 mg); Vit. B1 (4,000 mg); Vit. B2 (4,000 mg); Vit. B5 (12 g); Vit. B6 (4,000 mg); Vit. B12 (4,000 mcg); Vit. C (50 g); folic acid (1,250 mg); niacin (22.5 g); biotin (15 mg); iron (15 g); zinc (12.5 g); manganese (12.5 g); copper (2,500 mg); iodine (375 mg); cobalt (125 mg); selenium (85.7 mg). Source: Prepared by the authors

2.2 EXPERIMENTAL PROCEDURE

The rearing phases were conducted at the Professor Johei Koike Aquaculture Station, at the Federal Rural University of Pernambuco (UFRPE), Recife-PE, Brazil. The experimental procedures adopted involving animals were approved by the Ethics Committee on Animal Use of the Federal Rural University of Pernambuco – CEUA/UFRPE (protocol nº. 8148060922).

The Nile tilapia (*Oreochromis niloticus*) fingerlings of the GIFT (Genetically Improved Farmed Tilapia) strain, reverted males at 45 days old, were obtained in a commercial hatchery and acclimated to the laboratory conditions for fourteen days. After this period, fish were individually weighed and measured. A total of 200 healthy and uniformly sized fish were selected, with an average weight of 4.38±0.13 g and length of 6.36±0.23 cm in the first trial (with SCLE), and 3.24±0.07 g and 5.42±0.45 cm in the second trial (With SCM). These fish were then randomly distributed in twenty polyethylene tanks, each with a volume of 40 L. The stocking density was 10 fish per tank, equivalent to 166 fish m^{-3}. Four tanks were randomly assigned to each of the five experimental treatments. The experimental diets were offered four times daily (9:00 am, 12:00 pm, 2:00 pm, and 5:00 pm) until apparent satiety (*ad libitum*).

Salinity was maintained at 3‰ and each tank was provided with continuous aeration. The tanks were connected to Recirculating Aquaculture Systems (A, B, C, and D) and were equipped with mechanical, biological, and ultraviolet filters. Daily pH, water temperature, and dissolved oxygen were monitored using a digital multiparametric probe (YSI™ Model 550A Dissolved Oxygen Instrument, USA). Additionally, non-ionized ammonia, nitrite-nitrogen, and nitrate-nitrogen were measured every five days based on Baird; Eaton; Rice (2017) (Table 3).

Table 3. Average water quality parameters in the Recirculating Aquaculture Systems in both experiments

Evaluated parameters	Recirculating Aquaculture Systems				*P*-value
	A	B	C	D	
First experiment (with SCLE)					
pH	7.6±0.52	7.8±0.29	7.6±0.49	7.7±0.35	0.32
WT (°C)	27.9±0.49	27.6±0.34	27.8±0.47	27.5±0.51	0.43
O_2 (mg L^{-1})	5.7±0.44	5.6±0.47	5.8±0.39	5.8±0.31	0.28
NH_3 (mg L^{-1})	0.03±0.01	0.04±0.02	0.03±0.02	0.02±0.01	0.56
N-NO_2 (mg L^{-1})	0.31±0.19	0.42±0.18	0.29±0.11	0.26±0.12	0.39
N-NO_3 (mg L^{-1})	4.43±0.39	4.28±0.42	4.50±0.58	4.56±0.46	0.44
Second experiment (with SCM)					
pH	7.8±0.28	7.9±0.32	7.8±0.15	7.7±0.22	0.64
WT (°C)	29.6±0.42	29.2±0.35	29.5±0.22	29.3±0.53	0.56
O_2 (mg L^{-1})	5.6±0.5	5.5±0.42	5.6±0.38	5.7±0.48	0.58
NH_3 (mg L^{-1})	0.04±0.02	0.03±0.01	0.02±0.01	0.03±0.01	0.46
N-NO_2 (mg L^{-1})	0.62±0.32	0.55±0.41	0.66±0.32	0.54±0.38	0.36
N-NO_3 (mg L^{-1})	4.56±0.49	4.61±0.29	4.42±0.46	4.33±0.31	0.39

WT (water temperature); O_2 (dissolved oxygen); NH_3 (non-ionized ammonia); N-NO_2 (nitrite-nitrogen); N-NO_3 (nitrate-nitrogen). Source: Prepared by the authors.

2.3 HEMATOLOGICAL ANALYZES

At the end of the experimental test (45 days in both experiments), four fish per tank were anesthetized with 150 mg L^{-1} of Tricaine methane sulfonate (MS-222, Sigma-Aldrich) (Popovic *et al.*, 2012; Araújo *et al.*, 2018). Blood samples were collected by caudal vein puncture, using disposable syringes containing 1 µL of 10% EDTA. For total and differential leukocyte counts, blood smears were prepared in triplicate per individual (36 blood smears per treatment), with the slides stained panchromatically (Rosenfeld, 1947), using a drop of blood. Leukocytes were measured using the indirect method, which considered the number of neutrophils, eosinophils, monocytes, heterophils, basophils, and lymphocytes for every 300 erythrocytes counted in each blood smear, to determine the percentage of each cell, using a light microscope at 40× magnification. The number

of thrombocytes was also counted for every 300 erythrocytes. Blood was also collected to measure hematocrit, using the centrifuge for microhematocrit at 5,000 *g* for 5 minutes (Goldenfarb *et al.*, 1971).

2.4 STATISTICAL ANALYZES

Before analysis, the data were subjected to the Shapiro-Wilk and Levene tests to verify normality and homoscedasticity, respectively. Data were subjected to a one-way analysis of variance and, when differences between the means were detected, the Tukey post-hoc test was applied at a 5% probability level. The results were presented as means ± standard deviation. The analyses were conducted using the Jamovi software version 2.3.28.

3 RESULTS AND DISCUSSIONS

The hematological parameters of Nile tilapia fed diets with SCLE are detailed in Table 4 and Fig. 1. Total leukocyte counts, as well as lymphocyte, monocyte, and basophil counts, were higher ($P < 0.05$) in fish fed with from 1.6% SCLE. Moreover, neutrophil, eosinophil, thrombocyte, and hematocrit levels increased significantly ($P < 0.05$), including from 1.2% SCLE. No significant effect ($P > 0.05$) was observed on heterophil counts.

Regarding the inclusion of SCM, the hematological data (Table 5 and Fig. 2) revealed that total leukocyte counts were significantly higher ($P < 0.05$) in fish fed diets containing 20% SCM. Lymphocyte levels increased significantly ($P < 0.05$) with the inclusion of from 15% SCM, while monocyte counts were elevated ($P < 0.05$) with from 5% SCM. No significant changes ($P > 0.05$) were detected in neutrophil, thrombocyte, or hematocrit levels. No basophil, eosinophil, or heterophil were observed.

Table 4. Total and differential leukocyte count (%), thrombocyte (%), and hematocrit (%) of Nile tilapia fingerlings fed with different concentrations of shrimp cephalothorax lipid extract

Items	Shrimp lipid extract inclusion levels (%)					*P*-value
	0	0.4	0.8	1.2	1.6	
Lpc	14.50±4.34^{c}	15.37±4.96^{c}	15.67±4.89^{c}	19.48±3.71^{b}	24.19±4.70^{a}	<0.001
Mc	0.36±0.37^{c}	0.46±0.35^{c}	0.95±0.34^{b}	1.02±0.53^{b}	1.35±0.58^{a}	<0.001
Bp	0.42±0.35^{c}	0.85±0.54^{b}	0.92±0.40^{b}	1.02±0.34^{b}	1.89±0.59^{a}	<0.001
Np	0.05±0.12^{b}	0.06±0.13^{b}	0.07±0.14^{b}	0.15±0.20^{ab}	0.20±0.26^{a}	0.014
Ep	0.03±0.09^{b}	0.04±0.10^{b}	0.05±0.12^{b}	0.13±0.20^{ab}	0.18±0.23^{a}	0.001
Hp	0.03±0.10	0.02±0.09	0.04±0.10	0.04±0.11	0.06±0.13	0.741
Lkc	15.41±4.45^{c}	16.81±4.92^{c}	17.72±4.91^{c}	21.84±3.79^{b}	27.89±4.63^{a}	<0.001
Tc	0.74±0.55^{c}	0.83±0.59^{c}	1.22±0.39^{b}	1.80±0.35^{a}	1.77±0.50^{a}	<0.001
Ht	26.25±1.14^{c}	26.75±1.36^{c}	28.00±2.01^{bc}	29.16±1.95^{ab}	30.50±2.07^{a}	0.001

Lpc (lymphocytes); Mc (monocytes); Bp (basophils); Np (neutrophils); Ep (eosinophils); Hp (heterophils); Lkc (total leukocytes); Tc (thrombocytes); Ht (hematocrit). Values are presented as mean ± SD. Different letters on the same row indicate significant differences by one-way ANOVA and Tukey tests ($P < 0.05$). Source: Prepared by the authors.

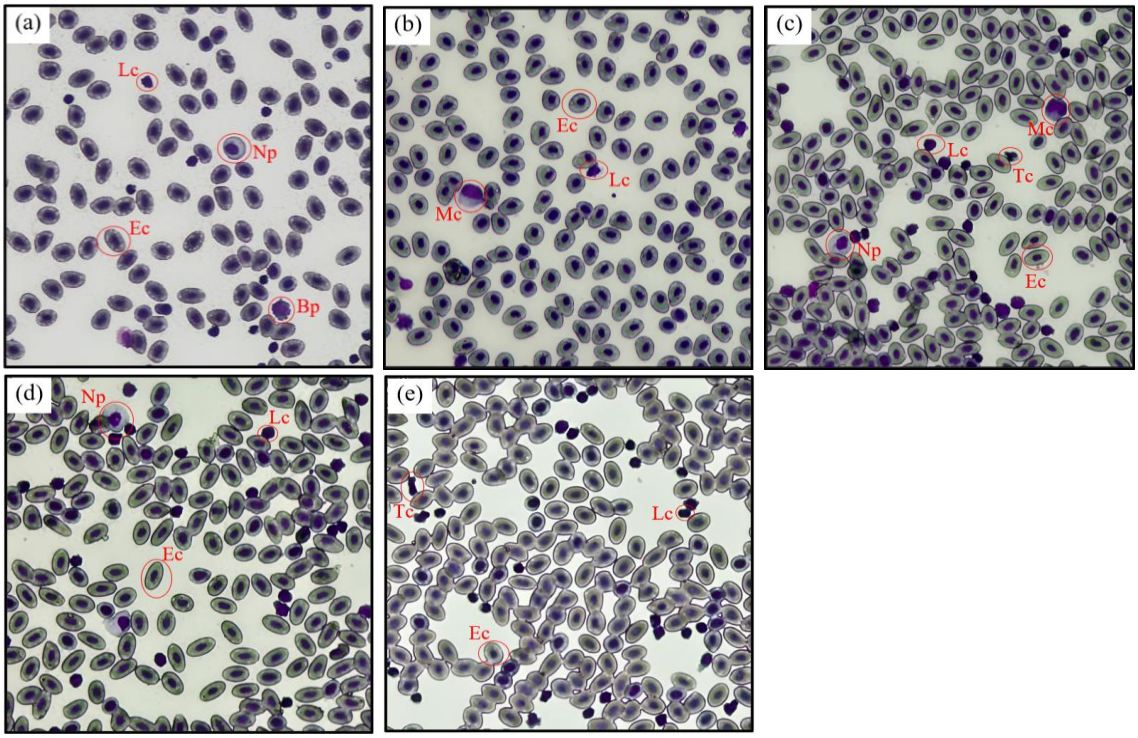

Fig. 1 Visual comparison between erythrocytes and categories of leukocytes present in the peripheral blood of *Oreochromis niloticus* fed with diets containing 0.0% (a), 0.4% (b), 0.8% (c), 1.2% (d), and 1.6% (e) shrimp cephalothorax lipid extract. Scale: 40×. Bp (basophils); Ec (erythrocytes); Lc (lymphocytes); Mc (monocytes); Np (neutrophils); Tc (thrombocytes). Source: Prepared by the authors.

Table 5. Total and differential leukocyte count (%), thrombocyte (%), and hematocrit (%) of Nile tilapia fingerlings fed with different concentrations of shrimp cephalothorax meal

Items	Shrimp meal inclusion levels (%)					*P*-value
	0	5	10	15	20	
Lpc	22.25±0.72^{c}	25.38±1.48^{b}	25.39±1.27^{b}	26.25±1.04^{a}	27.06±1.61^{a}	<0.001
Mc	0.70±0.26^{b}	0.76±0.28^{ab}	0.88±0.31^{ab}	0.91±0.36^{a}	0.92±0.36^{a}	0.010
Np	1.46±0.40^{a}	1.54±0.45^{a}	1.50±0.46^{a}	1.47±0.48^{a}	1.57±0.43^{a}	0.801
Lkc	24.41±0.77^{c}	27.70±1.41^{b}	27.57±1.34^{b}	28.31±1.09^{b}	29.22±1.67^{a}	<0.001
Tc	4.84±1.34^{a}	4.48±1.27^{a}	5.06±1.20^{a}	4.75±1.03^{a}	4.69±1.35^{a}	0.382
Ht	31.32±6.77^{a}	29.50±6.25^{a}	30.25±5.01^{a}	31.16±7.52^{a}	29.56±4.91^{a}	0.959

Lpc (lymphocytes); Mc (monocytes); Np (neutrophils); Lkc (total leukocytes); Tc (thrombocytes); Ht (hematocrit). Values are presented as mean ± SD. Different letters on the same row indicate significant differences by one-way ANOVA and Tukey tests ($P < 0.05$). Source: Prepared by the authors.

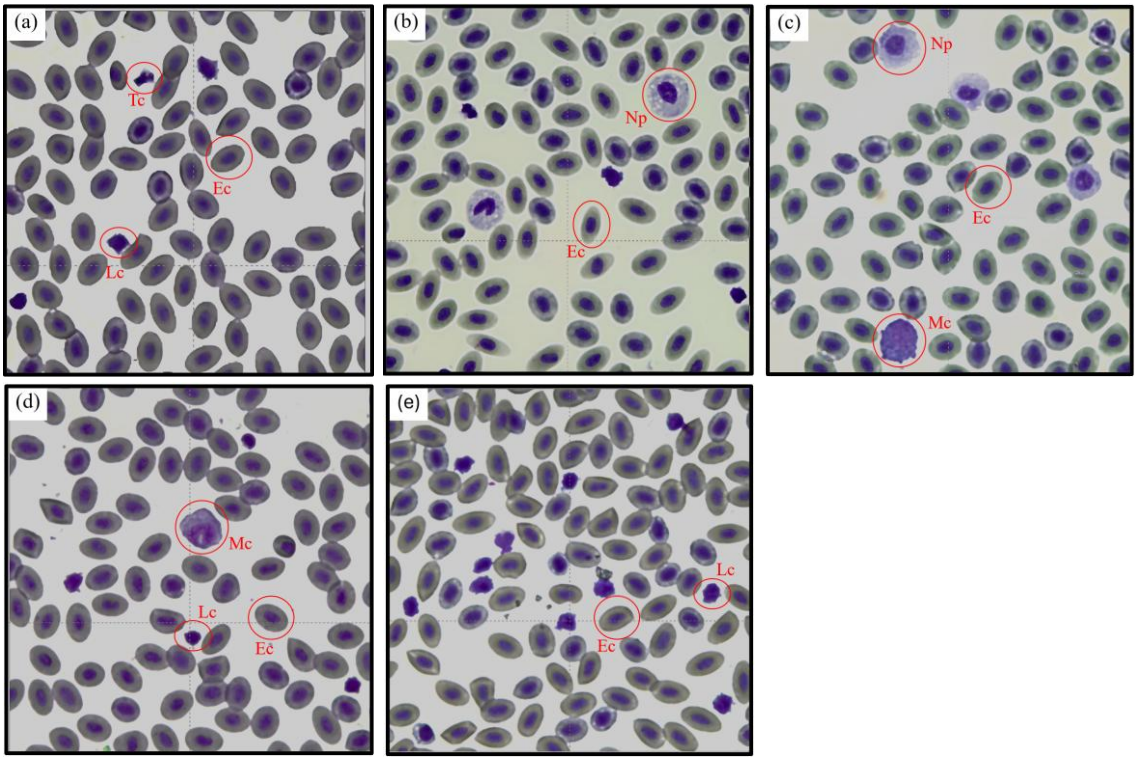

Fig. 2 Visual comparison between erythrocytes, categories of leukocytes, and thrombocytes present in the peripheral blood of *Oreochromis niloticus* fed with diets containing 0% (a), 5% (b), 10% (c), 15% (d), and 20% (e) shrimp cephalothorax meal. Scale: 40×. Ec (erythrocytes); Lc (lymphocytes); Mc (monocytes); Np (neutrophils); Tc (thrombocytes). Source: Prepared by the authors.

The innate immune system of fish serves as the primary line of defense against pathogens and harmful substances, acting as a rapid response mechanism (Fazio, 2019; Alves *et al.,* 2021). Immunomodulation regulates this response by activating various leukocyte types. The differential leukocyte count, a hematological tool, helps identify and quantify these cells, facilitating the assessment of health and the body's resilience in combating infections or environmental stress (Devi *et al.,* 2019; Riccio; Lauritano, 2019).

Leukocytes are classified based on functions. Lymphocytes are crucial for the adaptive immune system, specifically in antibody production, while monocytes and neutrophils are essential for phagocytosis and defense against pathogens. Eosinophils help combat parasitic infections and manage allergic responses and basophils are involved in inflammatory processes (Tavares-Dias *et al.,* 2009; Witeska *et al.,* 2022).

Thrombocytes are fragments derived from megakaryocytes and are important for hemostasis and blood clotting (Ortiz; Esteban, 2024). Thrombocytes and lymphocytes are the most frequent cellular defense elements observed in fish blood smears, consistent with the findings of this study (Tavares-Dias; Oliveira, 2009). Hematocrit, a key indicator of fish health and oxygen transport efficiency, represents the proportion of erythrocytes in blood volume (Seibel; Bassmann; Rebl, 2021). Fish typically exhibit hematocrit values ranging from 20% to 45%, which can fluctuate due to water quality, nutrition, and overall health (Tavares-Dias *et al.,* 2009).

Alterations in these cellular metrics can indicate stress from environmental or physiological factors (Shahjahan *et al.,* 2022). However, since water quality parameters remained consistent and within optimal ranges for Nile tilapia, these factors likely did not affect the fish’s health or contribute to the observed treatment differences.

The dietary inclusion of immunomodulatory compounds, such as astaxanthin, can enhance resistance to pathogens, especially during stressful periods, offering protective benefits (Alves *et al.,* 2021). Thus, improving immune responses represents a viable strategy for disease prevention in aquaculture (Ahmed; Reshi; Fazio, 2020). In this study, the inclusion of SCLE in the diet led to increases in differential leukocyte counts, thrombocytes, and hematocrit levels, with a similar rise in leukocyte count observed with SCM inclusion ($P < 0.05$).

Our findings are consistent with those reported by Jagruthi *et al*. (2014), where higher levels of astaxanthin inclusion in the diet of *Cyprinus carpio* significantly increased the number of blood cells before and after challenge against *Aeromonas hydrophila*, which reduced mortality in these groups compared to other treatments. Similar findings were also observed by other authors (Li *et al.,* 2019; Lim *et al.,* 2019; Haque *et al.,* 2021). The increase in lymphocytes within the normal range may mean greater defense of the body against future stressful and pathological factors (Clauss; Dove; Arnold, 2008). Therefore, the higher leukocyte levels observed in this study appear to indicate immunological stimulation in Nile tilapia fed SCLE and SCM.

According to Li *et al*. (2014), astaxanthin indirectly influences the hematological parameters because of its antioxidant properties. In this case, astaxanthin can inhibit the production of mitochondrial ROS in the hematopoietic tissue, where leukocytes, thrombocytes, and erythrocytes are formed (Ghoneum *et al.,* 2013), which favors cellular stability by minimizing the deleterious effects of ROS on fatty acids present in the plasma membrane (Li *et al.,* 2014; Costa; Miranda-Filho, 2020), contributing to the production of blood cells. Thus, the higher concentrations of leukocytes, thrombocytes, and hematocrit in the present study are indicators of a positive effect of SCLE and SCM in tilapia feed.

4 CONCLUSION

The present study demonstrated that the inclusion of shrimp cephalothorax lipid extract (SCLE) in Nile tilapia diets significantly increased differential leukocyte counts, thrombocytes, and hematocrit levels, while shrimp cephalothorax meal (SCM) also led to elevated leukocyte counts. These hematological improvements indicate that both SCLE and SCM have a positive immunomodulatory effect, enhancing the innate immune response in Nile tilapia. This highlights the potential of shrimp by-products as sustainable alternatives to conventional feed ingredients, thereby contributing to enhanced fish health and resilience in aquaculture systems. Moreover, the environmental benefits of utilizing shrimp processing residues are significant, offering a strategy for reducing waste while promoting sustainable aquaculture practices.

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5. Considerações finais

Os resultados desta tese demonstraram, de maneira geral, que os subprodutos do beneficiamento industrial do camarão, especificamente a farinha e o extrato lipídico de cefalotórax, são ingredientes promissores para a alimentação da tilápia do Nilo, agregando valor a resíduos que, normalmente, são descartados.

No primeiro estudo, a inclusão do extrato lipídico em níveis crescentes resultou em melhorias significativas no crescimento, na conversão alimentar e na eficiência proteica, além de alterações benéficas na composição corporal dos peixes. O aumento da proteína corporal e a redução do teor lipídico indicam um aproveitamento nutricional eficiente, enquanto a elevação da atividade antioxidante e as melhorias nos indicadores séricos sanguíneos sugerem um efeito positivo na saúde geral dos animais. Além disso, a menor peroxidação lipídica das rações contendo extrato lipídico pode aumentar a validade desses produtos.

O segundo estudo confirmou que tanto a farinha de cabeça de camarão integral quanto a de baixo teor lipídico apresentam alta digestibilidade e um perfil equilibrado de aminoácidos, sem fatores antinutricionais que comprometam a nutrição dos peixes, além de exibirem elevado escore químico e índice de aminoácidos essenciais. A inclusão de 20% dessas farinhas na dieta da tilápia foi considerada ideal, promovendo maior eficiência alimentar. Além disso, os peixes alimentados com essas dietas apresentaram aumento na atividade das enzimas digestivas e nas propriedades antioxidantes, favorecendo a assimilação dos nutrientes e a saúde hepática.

O terceiro estudo evidenciou o potencial imunomodulador da farinha e do extrato lipídico de cefalotórax de camarão. Foi observada uma resposta imune aprimorada nos peixes que receberam dietas contendo 1,6% de extrato lipídico e 20% de farinha, com aumento expressivo na contagem de leucócitos, trombócitos e hematócrito. Esses achados indicam que a incorporação desses ingredientes pode fortalecer o sistema imunológico inato da tilápia, tornando os peixes mais resistentes a infecções.

Dessa forma, os bioprodutos derivados do processamento do camarão se mostraram alternativas viáveis e sustentáveis para a nutrição aquícola, permitindo sua inclusão sem comprometer a qualidade nutricional das rações ou o desempenho produtivo dos peixes.

Além dos benefícios zootécnicos e imunológicos, a utilização desses ingredientes pode contribuir significativamente para a economia circular e a sustentabilidade da cadeia produtiva aquícola, reduzindo o impacto ambiental associado ao descarte inadequado de resíduos da carcinicultura. Do ponto de vista econômico, a adoção desses subprodutos pode gerar ganhos tanto para a cadeia produtiva da piscicultura quanto para a da carcinicultura, promovendo maior eficiência no uso dos recursos e agregando valor aos resíduos da indústria do camarão.

Os achados desta pesquisa reforçam a viabilidade do uso de subprodutos da indústria pesqueira como ingredientes funcionais na alimentação de peixes, promovendo maior eficiência produtiva e menor dependência de fontes convencionais. Assim, a inclusão da farinha e do extrato lipídico de cefalotórax de camarão representa uma estratégia inovadora para a piscicultura, unindo eficiência nutricional, sustentabilidade ambiental e viabilidade econômica.

Para estudos futuros, recomenda-se investigar os efeitos a longo prazo da inclusão desses ingredientes na dieta de tilápias, avaliar a aplicabilidade desses bioprodutos em outras espécies aquícolas e analisar os impactos econômicos da implementação dessas dietas em larga escala. Estudos adicionais podem também explorar novas técnicas de processamento e otimização da extração dos compostos bioativos presentes nesses subprodutos, ampliando suas aplicações na nutrição animal e potencializando seus benefícios funcionais.

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