Stefan Willem Bert Claessens

Mussel meal as a potential ingredient in diets for the whiteleg shrimp (*Litopenaeus vannamei)*

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Master's degree in Aquaculture and Fisheries

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Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam na listagem de referências incluída

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Stefan Willem Bert Claessens

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Abstract

The global aquaculture production is growing immensely in all aspects and has already surpassed the output from wild caught fish and shellfish industries. The farming of *Litopenaeus vannamei* is one of the biggest contributors to this market. Originally, *L. vannamei* is native to the tropical marine habitats, but due to its high value, farming of these species expanded to the subtropical areas. Therefore, low temperatures have become one of the major constraining factors to the *L. vannamei* culture. Besides this, concerns about the sustainability of this industry lead to the search for new, healthy and sustainable ingredients for aquafeeds, like bivalves, due to their nutritional value and low trophic level. In this experiment, mussel meal (species *Perna perna*) was evaluated as a potential ingredient in *L. vannamei* diets to improve growth and cold resistance of the shrimp. Five experimental diets (0%, 1%, 2%, 3% and 4% of mussel meal inclusion) were evaluated for 8 weeks in twenty polyethylene tanks of 400 liter (n = 4). Each tank was stocked with 40 shrimps (3.5 ± 0.5 g), filled with sea water and kept under constant aeration and temperature of 28.4 ± 0.4 °C. Every day 100% of the water was exchanged to maintain the water quality. After 8 weeks of experiment a thermal shock treatment was performed to analyse the cold resistance of the shrimp. Shrimps that were fed with the 1% and 2% mussel meal diets had a significantly higher final weight, weekly weight gain and lower FCR than the control, 3% and 4% mussel meal treatments. The shrimps fed with the 2% mussel meal diet had the best growth results. Further, no differences were observed in thermal shock resistance and survival among the treatments. In conclusion, mussel meal can be used as a potential ingredient in whiteleg shrimp diets.

Keywords: *Litopenaeus vannamei,* Mussel meal, thermal shock resistance, *Perna perna*

Resumo

A produção global em aquacultura tem tido um crescimento exponencial, tendo já superado o total de peixes e mariscos capturados através da pesca. Um dos segmentos produtivos mais importantes da aquacultura é o grupo dos crustáceos marinhos, com uma contribuição de quase 10% para o mercado global. Dentro deste grupo, a espécie *Litopenaeus vannamei*, vulgarmente conhecida como camarão de perna branca ou camarão branco do Pacífico, representa 84% da produção total de camarões marinhos produzidos em todo o mundo. Ao considerar todas as espécies produzidas na indústria da aquacultura mundial, *L. vannamei* foi a 3ª espécie mais produzida em 2018 com um volume total de mais de 6,5 milhões de toneladas, tendo em 2020 apresentado um crescimento na produção de 8,78%, com um valor total de 40 mil milhões de dólares. *L. vannamei* é uma espécie nativa dos habitats marinhos tropicais, mas devido ao alto valor da produção desta espécie, a sua introdução foi expandida para as áreas subtropicais. Deste modo, as baixas temperaturas tornaram-se um dos principais fatores limitantes na produção de *L. vannamei*. De forma a manter um crescimento da indústria assente em princípios de sustentabilidade, a aquacultura tem tentado identificar novos ingredientes, saudáveis e sustentáveis do ponto de vista ambiental, tal, como os bivalves. Os bivalves têm um grande potencial como forma de diminuir a pressão sobre a captura de peixes utilizados para a produção de farinha de peixe, pois são ricos em proteínas, lípidos e minerais e minimizam as perdas de energia nas transferências tróficas para a produção de proteína animal, uma vez que são espécies de baixo nível trófico. Nesta experiência, foi testada a utilização da farinha de mexilhão (espécie *Perna perna*) como ingrediente potencial em dietas de *L.vannamei* e avaliou-se o seu impacto no crescimento e na resistência térmica dos camarões. No total, cinco dietas experimentais (0%, 1%, 2%, 3% e 4% de inclusão de farinha de mexilhão) foram testadas durante 8 semanas em vinte tanques de polietileno de 400 litros (n = 4). Em cada tanque foram colocados 40 camarões (3,5 ± 0,5 g). Foi utilizado um regime de 12 horas de luz e 12 horas de escuro e cada tanque continha água do mar (30,6 ± 0,4 mg/L) mantida sob aeração constante e a uma temperatura de 28,4 ± 0,4 °C. Todos os dias, 100% da água foi trocada, de forma a manter a sua qualidade. A temperatura e o oxigénio foram medidos duas vezes ao dia (manhã e tarde) usando um medidor YSI Pro 20. Amostras de água foram retiradas semanalmente de cada tanque para medir a concentração de amónia total (TAN) e de nitritos, a alcalinidade, o pH e a salinidade. Os camarões foram alimentados quatro vezes ao dia (8:00, 11:00, 14:00 e 17:00h) com as respetivas dietas, seguindo a tabela de alimentação de Van-Wyk e Scarpa. Uma vez por semana foi determinada a biomassa total de cada tanque e contado o número de camarões. Após as 8 semanas de experiência foi realizado um teste de choque frio potencialmente letal para quantificar a resistência ao frio dos camarões. Dez camarões de cada tanque contendo água do mar a 28,4 ± 0,4°C foram transferidos simultaneamente para aquários de 60 L contendo ± 25 litros de água do mar a 10,9 ± 0,1°C, onde permaneceram por 1 hora, sob aeração constante. Após esse período, foram transferidos para baldes brancos com ± 30 litros com água do mar a 28,5 ± 1,0 °C e a mortalidade foi monitorizada por 24 horas. Após 8 semanas, os camarões que foram alimentados com as dietas com 1% e 2% de farinha de mexilhão tiveram um peso final e um ganho de peso semanal significativamente maior, assim como uma menor taxa de conversão de alimento do que os camarões alimentados com as dietas controlo (com 0% de farinha de mexilhão), 3% e 4% de farinha de mexilhão. Os camarões alimentados com a dieta com 2% de farinha de mexilhão tiveram os melhores resultados de crescimento e atingiram um peso 10% maior que os do tratamento controlo. Além disso, não foram observadas diferenças na resistência ao choque frio e na sobrevivência entre os tratamentos. Com isto, conclui-se que a farinha de mexilhão pode ser utilizada como potencial ingrediente em dietas para camarões.

Palavras-chave: *Litopenaeus vannamei*, farinha de mexilhão, resistência ao choque térmico, *Perna perna*

Abbreviations

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
BFT	Biofloc technology
BW	Body weight
°C	Degree Celsius
$CaCO_3$	Calcium carbonate
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DW	Dry weight
E.g.	*Exempli gratia*
EPA	Eicosapentaenoic acid
EU	European Union
ER	Endoplasmic reticulum
FAO	Food and Agriculture Organization
g	Gram
h	Hour
IU	International unit
kg	Kilogram
kJ	Kilojoule
L	Liter
m	Meter
m^2	Square metre
m^3	Cubic metre
mg	Milligram
n	Numbers, Sample Size
p	p-value, Probability Value
PL	Post Larvae
r	Correlation coefficient
RAS	Recirculating aquaculture system
SD	Standard deviation
TAN	Total ammonia nitrogen
TEP	Tetraethoxypropane
UFSC	Universidade Federal de Santa Catarina
U.S.	United States of America
WWF	World Wildlife Fund
YSI	Yellow Springs Instruments

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1. Introduction

1.1. World aquaculture

In the last decades, the global aquaculture production experienced immense growth in terms of the total quantity, the variety of farmed species, and applied production systems (Bostock et al., 2010; Engle et al., 2017). Aquaculture is now the fastest growing food-producing sector in the world. In the year 2014, the total volume of farmed aquatic animals matched the total wild caught fish and shellfish for the first time in history (FAO, 2016). The World Bank, the United Nations and the Food and Agriculture Organization concluded that by 2030 over 60 per cent of the consumed fish and shellfish will be provided by aquaculture (World Bank, 2013). This fast growth is largely driven by the ever-increasing human population of already 7,5 billion people to date, estimated to reach close to 9,5 billion in the year 2050 (United Nations, 2017). Also, the consumption per person is increasing. On a global average the annual consumption of fish and shellfish is now more than 20 kg per capita per year (FAO, 2016).

This shift toward more contribution from aquaculture to human nutrition will most likely continue hence after. This means that the problems of food security and nutrition will persist and increase. Nonetheless, satisfying the world's need for food cannot be done in a way that generates further toll on already fragilized ecosystems, increases greenhouse emissions or promotes further environmental degradation (FAO, 2017). Furthermore, socioeconomic changes such as rising incomes, increased urbanization, and ageing populations are generating a shift in food consumption patterns, resulting in an increased demand for animal derived protein in developing countries (Henchion et al., 2017).

One of the long-lasting goals for the aquaculture industry, and consequently for fish nutritionists, has been the reduction of feeding costs since in this sector more than half of the production costs are related to feeds (Arru et al., 2019; Henry et al., 2015; Rana et al., 2009). Nonetheless, this cost reduction must be done while advocating for animal welfare and sustainability, and logically for final product quality. The high demand, especially for aquatic protein, which has led to enormous pressure on many natural resources and fish stocks, has increased the prices. Increasing prices and decline of supplies have led to the search for less expensive sources such as animal and fisheries by-products, and bacterial and plant proteins (Ayadi et al., 2012).

One of the most important production segments of aquaculture is the group of marine crustaceans, with a contribution of almost 10% to the global market. Within this group, the species *Litopenaeus vannamei*, commonly known as whiteleg shrimp or Pacific white shrimp comprise nearly 84% of the total farmed marine shrimp production worldwide (FAO, 2020). When considering all produced species in the world aquaculture industry *L. vannamei* was ranked 3rd in 2018 with a total volume of more than 6.5 million tons and had a growth in production of 8.78% in 2020 with a total value of 40 billion U.S. dollars (FAO, 2020).

1.2. *Litopenaeus vannamei*

The whiteleg shrimp or Pacific white shrimp (*Litopenaeus vannamei)* was officially published in 1931 by Mr. Boone (Boone, 1931). In the 1970s *L. vannamei* were caught from the wild on the coast of Panama and were successfully spawned by French scientists in Tahiti and the species life-cycle was closed for the first time (FAO, 2022). After this the species has been introduced widely around the world and grow out as one of the most important aquaculture species due to fast growth, tolerance to high (stocking) densities, wide range of salinity, low dietary protein requirement and high survival rates (Argue et al. 2002; Moss et al. 2001, 2007, 2011; Rocha et al. 2010; Briggs et al. 2004).

Originally, *L. vannamei* is native to the tropical marine habitats of the Eastern Pacific coast, ranging from northern Mexico through Central America until the north of Peru, where the water temperatures are normally above 20 °C throughout the year. Adults live near the coast or offshore in depths reaching up to 70 m and mate and spawn in the open ocean. Post-larvae migrate inshore to spend their juvenile, adolescent and sub-adult stages in coastal estuaries, lagoons or mangrove areas. The life-cycle of *L. vannamei* is rather complex and passes through 3 several morphologically differing stages. Maturity is attained at approximately 6-7 months of age when males have reached over 20 grams and females over 28 grams of body weight. A full-grown female can spawn up to a quarter million eggs at once, which are fertilized by male sperm in the external environment. About 16 hours after spawning and successful fertilization, the first larval stages, called nauplii, hatch from the eggs. The nauplii can swim intermittently and do not require feed but rely on internal yolk sac reserves as nutrition. After a few days, through metamorphic processes, the first larval stage is developed into the protozoa, which actively feed on phytoplankton and unicellular algae. Further development phases lead through the mysis and early post-larvae (PL) stage. The PL change their planktonic habit about 5 days

after molting, move inshore and begin feeding on benthic detritus, worms, bivalves and crustaceans (FAO, 2022).

L. vannamei is farmed widely in tropical areas and expanded to subtropical areas. The main producers of *L. vannamei* are China, India, Thailand, Vietnam, Ecuador and Mexico. *L. vannamei* can be cultivated in different ways. The broodstock are stocked in maturation tanks in a dark room supplied with clean, filtered seawater. The feeds consist of a mixture of fresh and formulated broodstock diets and the optimal husbandry conditions like temperature, photoperiod, aeration and water parameters are continuously controlled by trained personnel. For reproduction the one eyestalk ablation technique is applied to each female which leads to repeated maturation and spawning. After successful spawning and fertilization by the males, the hatched healthy nauplii are attracted by light and collected and transferred to holding tanks or directly to larval rearing tanks. Hatchery systems range from specialized, small, unsophisticated, often inland, backyard hatcheries to large, sophisticated and environmentally controlled installations, together with maturation units. Nauplii are stocked into flat, or preferably 'V' or 'U' shaped tanks with a volume of 4–100 m^3, made from concrete, fiberglass or other plastic lined material. The larvae are fed with live food (microalgae and *Artemia*), supplemented by micro-encapsulated, liquid or dry formulated diets. In these tanks the shrimp larvae go through 3 developmental stages: nauplii (1-2 days), zoea (3-5 days) and mysis (3-5 days) until they reach the PL stage. At 10-12 days after reaching the PL stage, the post-larvae are transferred directly to the grow-out tanks/ponds or to an intermediate nursery tank system (FAO, 2022).

The final grow-out systems can be classified as extensive, semi-intensive, intensive, and super-intensive, mainly depending on the stock density of the PLs. The extensive technique is commonly found in developing countries, where the *L. vannamei* are cultivated in earthen ponds with minimal or no water pumping or aeration. The shrimps are fed mainly with natural feeds enhanced by fertilization of the ponds, with a stocking density of 4-10 PL/m^2. In semi-intensive systems, higher densities are stocked (around 10-30 PL/m^2), water is regularly exchanged by pumping and ponds are provided with aeration. The shrimps feed on natural feeds like in extensive systems, supplemented with formulated diets. In intensive systems around 60-300 PL/m^2 are stocked. These systems can be ponds that can be completely drained, concrete raceways or circular tanks of various dimensions. Water can be exchanged and strong aeration is necessary. The shrimps are fed with artificial diets around 4-5 times per day (FAO, 2022).

Recently, researchers developed a super-intensive systems in the United States of America. These super-intensive raceways systems are enclosed in greenhouses, using no water exchange (only the replacement of evaporation losses) and stock densities over 300 PL/m^2. These systems have high biosecurity and a small ecological footprint and can produce cost-efficient, high quality shrimps. Alternative systems are biofloc systems (BFT) and recirculating aquaculture systems (RAS). These systems are widely applied to farm shrimp and have the same principles with a low water exchange and controlled environment (Martins et al., 2010).

1.3. Current issues in shrimp farming

1.3.1. Temperature stress in whiteleg shrimp

L. vannamei is a tropical species, originally farmed in tropical areas and known to be sensitive to low temperatures. Due to its high economic value, the farming areas of the *L. vannamei* have been expanded to the sub-tropical areas. Therefore, low temperatures have become one of the major constraining factors in the *L. vannamei* culture. In poikilothermic organisms, like the *L. vannamei*, cold stress imposes multiple physiological impairments, including disruption of protein and membrane integrity, mitochondrial respiration, malfunction, oxidative stress and loss of ion homeostasis and neuromuscular coordination (Hayward et al., 2014). In shrimp farming, this cold stress could lead to dramatic productivity losses, owing to growth inhibition, immune response suppression and higher susceptibility to diseases (Kautsky et al., 2000). For example, shrimp farming in southern China has been adversely affected by winter mortality for several decades, especially in 2008 (Li et al., 2013). Also in Brazil, especially in Southern Brazil where the weather is quite unstable, cold stress plays an important role as a natural trigger for disease outbreaks.

To overcome these temperature related environmental stress factors, the robustness of the *L. vannamei* must be improved. Other poikilothermic organisms from temperate regions (cold-tolerant animals) developed biochemical and physiological mechanisms to overcome the stress caused by cold. For example, in insects, a few mechanisms have been identified as being involved in cold resistance capacity, such as up regulation of aquaporins, detoxification enzymes and heat shock proteins, which are responsible, for the facilitation of water movement between inner and outer compartments of cells, reduction of oxidative stress and inhibition of protein denaturation. Reduced membrane fluidity during chilling is very harmful to cell

functioning since it immobilizes membrane proteins and impairs cell signaling and nutrient/ion transport. Several other biochemical mechanisms, like increment of unsaturated fatty acids and cholesterol proportion, insertion of unsaturated fatty acids in the sn-2 position of glycerophospholipids, enhancement of short and long chain fatty acids ratio (16:0/18:0), and restructuration of polar head groups of glycerophospholipids can also help to achieve higher membrane fluidity (Teets & Denlinger, 2013). On the other hand, cold-intolerant animals may achieve cold resistance by feed supplementation. Gilthead seabream (*Sparus aurata*) fed with a diet supplemented with taurine, betaine, vitamin C and unsaturated phospholipids showed a significantly higher resistance to winter thermal stress, likely associated with an increase in fish oxidative stress defenses, amino acid and energy metabolism modulation, and endoplasmic reticulum (ER) stress mitigation (Richard et al., 2016). *L. vannamei* fed with highly unsaturated fatty acids diets showed an increase in immune response after exposure to handling stress (Mercier et al., 2009). Furthermore, Schleder et al. (2017) found an increase in thermal shock resistance of this species after feeding a diet supplemented with *Sargassum filipendula*.

1.3.2. Use of low-trophic species

The pressure of aquafeeds on capture fisheries calls for new, healthy and sustainable ingredients. Some marine suspension feeding organisms foraging low in the food web, like bivalves, have a large potential to lower the pressure on capture fish used for fishmeal. One of the reasons is that they minimize energy losses in trophic transfers to build animal protein. In natural food webs, the vast majority (~90% on average; range 80-95%) of the energy captured by primary producers is lost through energy expenditure (such as growth, reproduction, foraging, predation, avoidance and other mechanisms) and only a small fraction passes to the trophic level above (Bonhommeau *et al*., 2013; Tucker & Rogers, 2014; Watson *et al*., 2013; Sanders *et al*., 2016). The inherent inefficiency of trophic transfers through food webs means that the higher the trophic level of an animal eaten by humans; the more ecosystem energy is embodied in its production. Recent reports from the World Resources Institute, World Wildlife Fund, Asia Pacific Fisheries Commission, and High-Level Group of Scientific Advisors to the European Union recognize this inefficiency, and advocate for farming and consuming 'fish low in the food-chain' to help achieve production and sustainability objectives for aquaculture (Waite et al., 2014; WWF, 2016; SAPEA, 2017). Another reason for the use of low-trophic species is their nutritional value. For example, mussels have been proposed as a candidate for

incorporation into aquafeeds due to their high protein content (~50% of the whole animal ash-free dry weight). Besides this, low-trophic species can efficiently exploit the largest trophic resource in the marine environment., detrital matter and phytoplankton, are possible to cultivate in high-density and three-dimensional environment (*e.g*. Cubillo et al., 2012) and have well-tested culture technologies available and existing worldwide market and facilities.

Focusing on the trophic level as a metric of sustainability omits important aspects of resource efficiency. Through a combination of feed technologies, nutrition, selective breeding, feed and on-farm management practices, feed conversion ratios have, on average, improved (decreased) for all species globally. Emphasis on the trophic levels of farmed species also biases our understanding of the impacts of feeds in general. While there has been considerable attention paid to the sustainability implications of using relatively high trophic level ingredients derived from forage fish, these now comprise a relatively small proportion of modern feeds. Crops (trophic level = 1) now dominate feed composition across all aquaculture species (Pahlow et al., 2015; Tacon & Metian, 2015). But according to sustainability, the overstressed terrestrial agroecosystems, water and land use and transport should be under consideration.

1.4. *Perna perna*

The *Perna perna* is a brown mussel widely distributed and native in the tropical and subtropical regions of the Atlantic Ocean and Western Indian Ocean (Siddall, 1980). It is found in the waters of the West and East Coast of Africa, the coast of South America up to the Caribbean and on the coast of India and Sri Lanka. In the 1990s this species was also found on the coast of Mexico and the United States, where the *P. Perna* was accidentally introduced via boat hulls and water ballasts of ships from Venezuela (Holland et al., 1997).

P. perna is cultivated in Africa and South America and is the most abundant Mytilidae on the Brazilian coast, with Santa Catarina as the most important region for shellfish mariculture (Santos and Della-Giustina 2017). The bivalve can grow quickly to the commercial size of 60 to 80 mm in just 6 or 7 months, which makes this species of huge commercial interest. Also, mussels of warm waters tend to grow larger than cold water species (Schurink and Griffiths, 1993).

The mussel is easily recognized by its brown color and has a smooth elongate shell (Figure 1.1), but its best identifying characteristic is an internal divided posterior adductor and retractor

muscle scar. In *Perna* spp., these two muscles attach separately to the shell, resulting in a discontinuous scar (Figure 1.2). In nature, the *P. perna* is normally found on rocky substrates, from the inter-tidal zone to several meters deep in the infralittoral zone. The mussel matures early in natural populations, with maturation occurring at sizes as small as 35 mm, well before the animal is a year old (Souza et al., 2019). The *P. perna* exhibits gonadal activity throughout all periods of the year and has a prolonged spawning season. In some tropical regions, they spawn the year round (Hicks and McMahon, 2002). In sub-tropic regions two great spawning events were monitored, one in early spring and one in mid-summer (Stakowian et al., 2020). Adult males and females release the eggs and sperm into the water column and external fertilization occur. After fertilization, a free swimming trochophore is formed that develops in a veliger. After several days the veliger goes through a metamorphose and settles on a stable, hard substrate surface.

The *P. perna* is a filter feeder that feeds mainly on phytoplankton. These phytoplankton contain essential fatty acids, linoleic (18:2n−6) and linolenic (18:3n−3) acids, and long chain polyunsaturated fatty acids (Jónasdóttir, 2019). In filter-feeding bivalves, the type of phytoplankton food is closely linked with the levels of polyunsaturated fatty acids in the animal, in particular those with fairly long chains (20 and 22 carbons) (Fernández-Reiriz et al., 1996; Caers et al., 2000). The levels of polyunsaturated fatty acids in phytoplankton varies in quality and quantity according to natural cycles of plankton growth and environmental parameters, like temperature, salinity and photoperiod. Besides this the lipid quality and quantity also varies in the filter-feeding bivalves with the physiological status of the organisms, in particular with the reproductive status, as gametes contain high levels of lipid reserves (Grkovic et al., 2019). Besides high levels of polyunsaturated fatty acids, mussels also contain a range of vitamins and minerals such as A and C-vitamins and trace minerals (Saritha et al., 2015). The unsaturated fatty acids, vitamins and minerals provide good health benefits for animals and humans.

Figure 1.1: *Perna perna* (photo by Felipe Vieira)

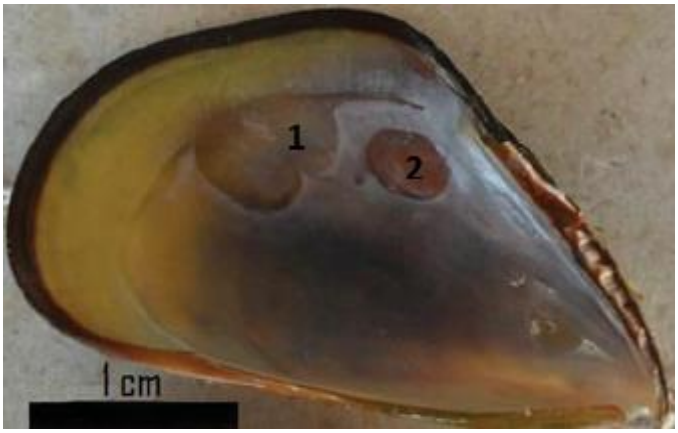

Figure 1.2: Divided posterior adductor (1) and retractor (2) muscle scar (photo by Carla R. Lourenço)

1.5. Mussel meal in aquaculture feeds

Mussel meal is characterized by a high lipid and protein content with similar amino acid patterns as fishmeal, thus numerous studies were done by partly replacing fishmeal by mussel meal in diets of aquatic species. Many studies with different mussel species and aquatic species showed good results. For example, Wagner et al., (2019) investigated the effects of replacing 40% of the dietary fishmeal, with either mussel meal, zygomycete fungi, extracted baker's yeast or non-extracted baker's yeast on the lipid and metabolic profile of the Arctic char (*Salvelinus alpinus*). The fish metabolism was least affected by mussel meal, which suggests that it may be suitable to replace fishmeal in Arctic charr diets by this ingredient. Wang et al., (2019) evaluated the effects of replacing fishmeal with mussel meal or meat and bone meal in low-fishmeal diets on growth performance, body composition, digestibility, antioxidant capacity and IGF-I-gene expression of juvenile Ussuri catfish (*Pseudobagrus ussuriensis*). Good results were found with mussel meal diets and the findings demonstrated that mussel meal could substitute 400 g/kg of fishmeal in low-fishmeal diets, without influencing the health and growth of the Ussuri catfish.

In shrimps, very few studies concerning the use of mussel meal were performed. Cavalli et al. (2004) measured the growth and feed utilization of the shrimp *Farfantepenaeus paulensis* fed with different marine protein sources (fishmeal, squid meal and mussel meal). The inclusion of mussel meal in diets led to similar shrimp growth and feed utilization as the inclusion of fishmeal. At the moment, Green Blue Health Pty Ltd is running a study that evaluate the use of mussel meal, at different inclusion levels, as a replacement of fishmeal in black tiger prawn diets. The research is being conducted at the Brible Island research facility in Queensland,

Australia, were they mostly focused to improve the palatability of the prawn feeds. Green Blue Health Pty Ltd is particularly interested to see if prawn diets with the inclusion of mussel meal will increase the black tiger prawn acceptance of locally available and sustainable ingredients such as poultry and arable by-products (Byrne, 2021).

2. Objectives

The main goal of this work is to identify if the ingredient mussel meal (species *Perna perna*) can be used in whiteleg shrimp (*Litopenaeus vannamei*) diets to improve growth and resistance to thermal shock. Five different inclusion levels of mussel meal (0%, 1%, 2%, 3% and 4%) in the diet will be tested and mortality and growth will be evaluated for 8 weeks. Shrimp robustness will be further assessed through a potential lethal thermal shock.

3. Material and Methods

3.1. Mussel meal preparation

To prepare the mussel meal (*P. perna*) 20 kilograms of mussels were bought in the south of Florianópolis (Brazil) at Paraíso das Ostras (27°49'00.5"S 48°33'49.6"W). After that the mussels were transported to the Marine Shrimp Laboratory (Barra da Lagoa, Brazil) and cooked for 10 minutes. The shells were removed and the males (pale color) and females (reddish color) were separated in boxes (Figure 3.1A). The cooked mussels were placed in a ventilated oven at 70 °C for 48 hours (Figure 3.1B). Under these conditions the proximate composition of the mussel (e.g., amino acid profile) will not alter significantly and the final water content is kept at <10%. After 48 hours of drying, 25% of the initial biomass was left and the dried mussels were transferred in sealed bags to LABNUTRI in Armação (Brazil). The dried mussel meat was then minced with a commercial mincer in portions of 250 grams at a medium speed of 5 minutes (Figure 3.1C). After this, the ground mussel meat was put in a mixer in portions of ±100 grams for 30 seconds to change the mussel meat crumbles into powder. A sieve of 600 µm was used to sieve the mussel meal. The mixer process was repeated till all the mussel meat was turned into mussel meal (<600 µm) (Figure 3.1D). After this the mussel meal (male and female

separated) were analyzed for protein, lipid, moisture and ash content by LABNUTRI (Table 3.1).

The analysis of the proximate composition of the mussel meal followed procedures standardized by the "Association of Official Analytical Chemists" (AOAC, 2006) and was run in triplicates. Moisture content was determined by drying the samples at 105 °C for 24 h and ash content by incineration in a muffle furnace at 500 °C for 12 h. The crude protein was analyzed by using the combustion/Dumas method and total lipid by petroleum ether extraction using a Soxtherm Multistat/ SX PC (Gerhardt).

Figure 3.1: Pictures of the different steps of the mussel meal preparation

Table 3.1: Dry weight, crude protein, lipids and mineral composition of *P. perna* (males and females)

P. perna	**Dry weight (DW, %)**	**Crude protein (% DW)**	**Lipids (% DW)**	**Minerals (% DW)**
Female	95.75	57.43	11.83	11.99
Male	95.89	56.35	13.64	11.16

3.2. Diets

In this Thesis, five diets were formulated by using Optimal Fórmula 2000® version 19.102.009 based on the formulation for commercial diets and with different levels of mussel meal (*P. perna*) inclusion: 0% (Control), 1%, 2%, 3% or 4% (Table 3.2) All five diets were formulated to be diets isoproteic, isolipidic and isoenergetic. The selected ingredients were previously

collected, weighed and sieved with a 600 μm sieve and brought to the Nutrição de Espécies Aquícolas (LABNUTRI, Armação, Brazil), where the experimental diets were produced. First the dry ingredients were mixed for 15 minutes in a concrete mixer. After 15 minutes the mixture of dry ingredients was put back in a bucket were the oils (soy oil, lecithin and fish oil) were added. These oils were first mixed with the dry mixture by hand and afterwards putted back again for 10 minutes in the concrete mixer. The resulting mixture was pelletized with an extruder at 70 °C and a 2.5 mm sieve. Then the pellets were put in an oven (50 °C) and dried for around 24 hours till the moisture content was < 10%. Diets were analysed for proximate composition following the procedures standardized by the "Association of Official Analytical Chemists" (AOAC, 2006), as described in Section 3.1. and were run in duplicates.

Table 3.2: Composition of the experimental diets, containing 0% (control), 1%, 2%, 3%, and 4% of mussel meal (*P. perna*).

		Diets			
Ingredients (g/kg)	**Control**	**1%**	**2%**	**3%**	**4%**
Soybean meal	324.3	320.3	316.3	312.3	310.3
Wheat flour	150.0	150.0	150.0	150.0	150.0
Fish meal	126.0	125.0	122.0	119.0	115.0
Poultry meal	150.0	145.0	142.0	139.0	135.0
Mussel meal (*P. perna*)	**0.0**	**10.0**	**20.0**	**30.0**	**40.0**
Carboxymethylcellulose	5.0	5.0	5.0	5.0	5.0
Soy lecithin	25.0	25.0	25.0	25.0	25.0
Monocalcium phosphate	25.0	25.0	25.0	25.0	25.0
Fish oil	20.0	20.0	20.0	20.0	20.0
Soy oil	10.0	10.0	10.0	10.0	10.0
Vitamin-C[a]	0.7	0.7	0.7	0.7	0.7
Vitamin premix[b]	5.0	5.0	5.0	5.0	5.0
Mineral premix[c]	17.0	17.0	17.0	17.0	17.0
Magnesium sulphate	15.0	15.0	15.0	15.0	15.0
Kaolin	100.0	100.0	100.0	100.0	100.0
Sodium chloride	12.0	12.0	12.0	12.0	12.0
Potassium chloride	10.0	10.0	10.0	10.0	10.0
Methionine	5.0	5.0	5.0	5.0	5.0
TOTAL	1000.0	1000.0	1000.0	1000.0	1000.0

Proximate composition (% DW)					
Moisture	8.86	6.83	9.74	10.83	8.19
Crude protein	39.76	39.71	39.79	39.51	39.57
Lipids	7.90	7.68	8.59	8.46	8.64
Ash	24.31	24.06	24.20	24.11	24.14
Gross energy (kJ/g)	17.69	16.81	16.89	16.90	16.85

[a]L-ascorbic acid-2-monophosphate 35%. DSM Produtos Nutricionais Brasil (São Paulo, SP, Brazil).

[b]Vitamin premix: InVivo mix (Paulínia, Brasil) - vitamin A 3,000,000 IU; vitamin D3 1,000,000 IU; vitamin E 70,000 IU; vitamin K3, 14 g; vitamin B1 30 g; vitamin B2 20 g; vitamin B6 33 g; vitamin B12 50,000 µg; pantothenic acid 40 g; biotin 750 mg; nicotinic acid 70 g; folic acid 3,000 mg; excipient for 1000 g.

[c]Mineral Premix: InVivo mix (Paulínia, SP, Brasil) - Potassium 6,100 mg; copper 23,330 mg; zinc 10,000 mg; manganese 20,000 mg; selenium 125 mg; iodine 1000 mg; cobalt 50 mg; excipient for 1000 g.

DW: dry weight

3.3. Experimental set up

The experiment was carried out at the Marine Shrimp Laboratory (Laboratório de Camarões Marinhos - LCM/UFSC) at Barra da Lagoa, Brazil. Twenty polyethylene tanks of 400 liter were used and each tank was stocked with 40 shrimps (3.5 ± 0.5 g). A 12 hours light and 12 hours dark regime was used and each tank was filled with sea water (30.6 ± 0.4 ‰) and kept under constant aeration and temperature of 28.4 ± 0.4 °C. Every day 100% of the water was exchanged to keep the water quality parameters within ideal values, and after the water exchange the tanks were cleaned with a hose to remove the remaining feces and carapaces from the shrimps. All the twenty tanks had the same design where the inlet was put close to the wall of the tank and the outlet and aeration in the middle of the tank (Figure 3.2). The tail-end of the inlet was put clockwise to improve the waterflow while changing the water. The aeration was set on a way that there was enough oxygen in the water (>5 mg/l) and keeping the water quiescent so the shrimps are swimming peaceful and do not have to swim against the current provided by the bubbles. The five experimental diets (0%, 1%, 2%, 3% and 4% of mussel meal inclusion) were evaluated for 8 weeks. Each diet was randomly assigned to four replicate (n = 4) tanks in the experimental room (Figure 3.3).

Figure 3.2: Individual tank design used in the experiment. All 20 tanks had the same design with the inlet next to the wall and outlet and aeration in the middle of the tank.

Figure 3.3: Experimental set up with 20 tanks of 400 liter (n = 4 for each experimental diet).

3.4. Feeding

The shrimps were fed four times a day (8:00, 11:00, 14:00 and 17:00h) with their respective diets following the feeding table by Van-Wyk and Scarpa (1999); Table 3.3. The sinking pellets were put in a feeding basket and after 1.5 hour the feeding baskets were checked on spillages.

Table 3.3: Feeding table for high-intensity tank production of *Litopenaeus vannamei (Van-Wyk and Scarpa, 1999)*.

Average Shrimp Wt. (g)	Feed Rate (% BW/day)
<.1	35 – 25
0.1 - 0.24	25 – 20
0.25 – 0.49	20 – 15
0.5 – 0.9	15 – 11
1.0 – 1.9	11 - 8
2.0 – 2.9	8 – 7
3.0 – 3.9	7 – 6
4.0 – 4.9	6 – 5.5
5.0 – 5.9	5.5 – 5.0
6.0 – 6.9	5.0 – 4.5
7.0 – 7.9	4.5 – 4.25
8.0 – 8.9	4.25 – 4.0
9.0 – 9.9	4.0 – 3.75
10.0 – 10.9	3.75 – 3.5
11.0 – 11.9	3.5 – 3.0
12.0 – 12.9	3.25 – 3.0
13.0 – 13.9	3.0 – 2.75
14.0 – 14.9	2.75 – 2.5
15.0 – 15.9	2.5 – 2.3
16.0 – 16.9	2.3 – 2.1
17.0 – 17.9	2.1 – 2.
18.0 – 18.9	2.0 – 1.9
19.0 – 19.9	1.9 – 1.8
20.0 – 20.9	1.8 – 1.7

3.5. Biometry

Once a week the total biomass for each tank was determined and the number of shrimps was counted. The weekly biometry was done 1.5 hour after the first or second feeding round. The lid, inlet and aeration pipe of each tank were removed and the water level was reduced to ± 15 cm. In this way the shrimps were easier and faster to catch and reduced the stress during the biometric analysis. All shrimps were first placed in a white bucket with system water and transported to the scale. The shrimps were put in a net and the adhering water was removed for 5 seconds. The shrimps were weighed with a scale (Marte AD2000) with accuracy of 0.01 g. After weighing, the shrimps were put back in the tank where they were counted.

3.5. Water quality

Oxygen and temperature were measured twice a day (in the morning and afternoon) using a YSI Pro 20 meter that was calibrated once a month. The temperature was kept at 28.4 °C ± 0.4 °C and dissolved oxygen were kept at levels between 6.0 and 6.4 mg $L^{-1.}$. Weekly, water samples were taken to measure the total ammonia nitrogen (TAN), nitrite, alkalinity, pH and salinity. Total ammonia nitrogen (TAN) was measured by the indophenol with trione method, according to Grasshoff et al. (1983). Nitrite was measured by the Griess reaction method (Strickland and Parsons, 1972). Alkalinity was measured by titration using APHA method 2320-B (APHA, 2005). pH was measured with a Tecnal pH-meter and the salinity was measured by using a YSI EcoSense® probe, model EC300A.

3.6. Thermal shock

At day 57 (one day after finishing the experiment), 10 shrimps from each tank were subjected to an abrupt, potential lethal thermal stress (cold shock). The 10 shrimps (18.0 ± 1,8 g) were simultaneously transferred from tanks with seawater at 28.4 ± 0.4 °C to 60 L aquarium filled with ± 25 liter of seawater from 10,9 ± 0.1 °C under constant aeration, where they remained for 1 hour (Figures 3.4 and 3.5). After this period, they were transferred to white buckets with ± 30 liter with seawater at 28.5 ± 1.0 °C, and mortality was monitored for 24 hours. The seawater used in the thermal shock trial was from the same reservoir as the experiment, which had the same salinity of 30.50 mg L^{-1}.

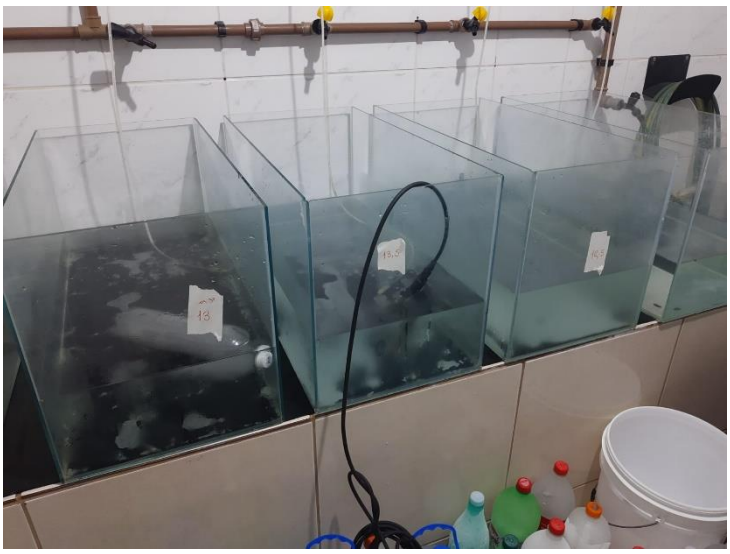

Figure 3.4: Thermal shock setup.

Figure 3.5: YSI Pro 20 probe was used to keep the temperature at 10.9 ± 0.1 °C.

3.7. Shrimp growth performance

On the final day of the experiment (56th day), the total number of shrimps and total final biomass were measured during the final biometry. The survival rate, feed conversion rate (FCR) and weekly week gain (g/week) were calculated with the following formulas:

$$\text{Survival rate (\%)} = \frac{Final\ number\ of\ individuals}{Initial\ number\ of\ individuals} \times 100$$

$$\text{FCR} = \frac{Total\ feed\ intake\ (g)}{Total\ final\ biomass\ (g) - Total\ initial\ biomass\ (g)}$$

$$\text{Weekly weight gain (g/week)} = \frac{final\ weight\ (g) - initial\ weight(g)}{number\ of\ weeks}$$

$$\text{Weight gain (\% initial weight)} = \frac{final\ weight\ (g) - initial\ weight(g)}{initial\ weight\ (g)} \times 100$$

3.8. Statistical analysis

All data was analyzed using the statistical software program IBM® SPSS® Statistics Version 28.0.1.0(142). Data are presented as means ± standard deviation and the data expressed as a percentage were arcsine square root transformed previously to the statistical analysis. The Shapiro-Wilk test was used to test the obtained data for normal distribution and the Levene's test determined the homogeneity of variance. A one-way analysis of variance (ANOVA) was applied for the growth performance (feed conversion ratio, survival and final weight) and water quality data (temperature, dissolved oxygen, TAN and nitrite), followed by Dunnett to compare the different treatments with the control and Turkey's to compare the different treatments between each other. The mortality data after the thermal shock treatment was analyzed by the Kaplan-Meier test. All tests used a significance level of 5% ($p < 0.05$).

4. Results

4.1. Water quality parameters

4.1.1. Temperature and dissolved oxygen

The temperature and dissolved oxygen did not show any significant differences among the treatments during the eight weeks (Figure 4.1 and Figure 4.2). The highest recorded temperature was 29.2 ± 0.2 °C on day 23 and the lowest temperature on day 13 (27.9 ± 0.3 °C) with an average temperature of 28.5 ± 0.4 °C during the experiment. The dissolved oxygen (DO) slightly decreased along the experiment as the biomass inside the tanks increased, but no significant differences were found among the treatments. The highest DO was recorded on day 2, with 6.85 ± 0.06 mg/l, while the lowest DO level was recorded on day 55 (6.05 ± 0.1 mg/l) with an average DO level of 6.33 ± 0.23 mg/l during the experiment.

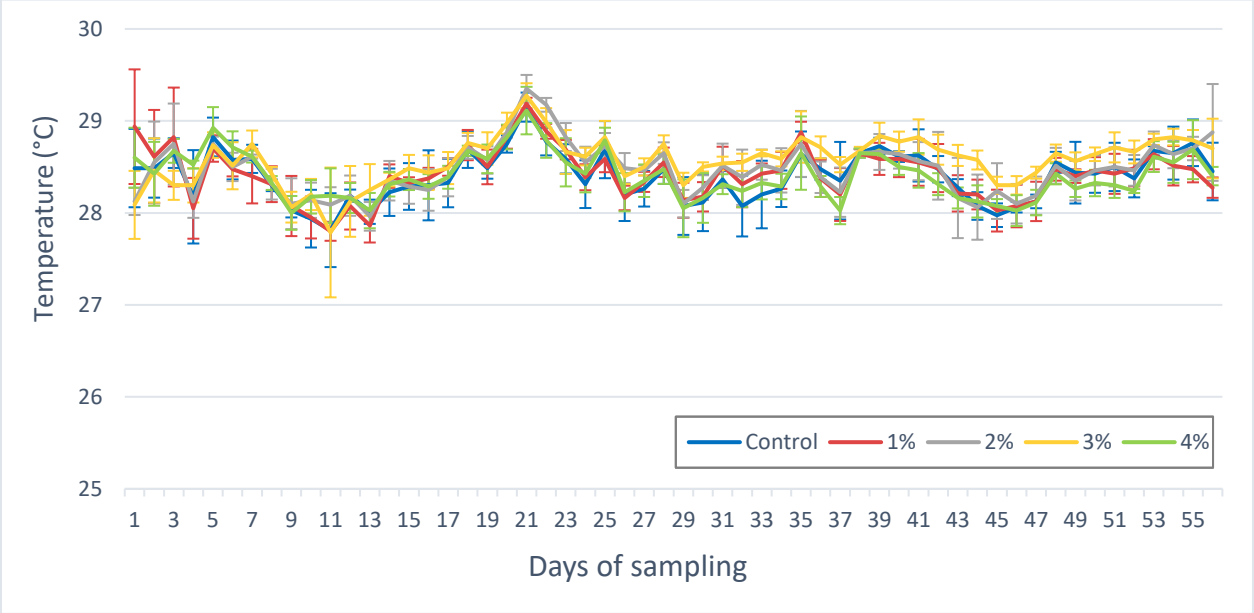

Figure 4.1: The temperature in each treatment along the experiment (56 days). The values are means ± SD (n = 4). No statistically significant differences were found among the five treatments.

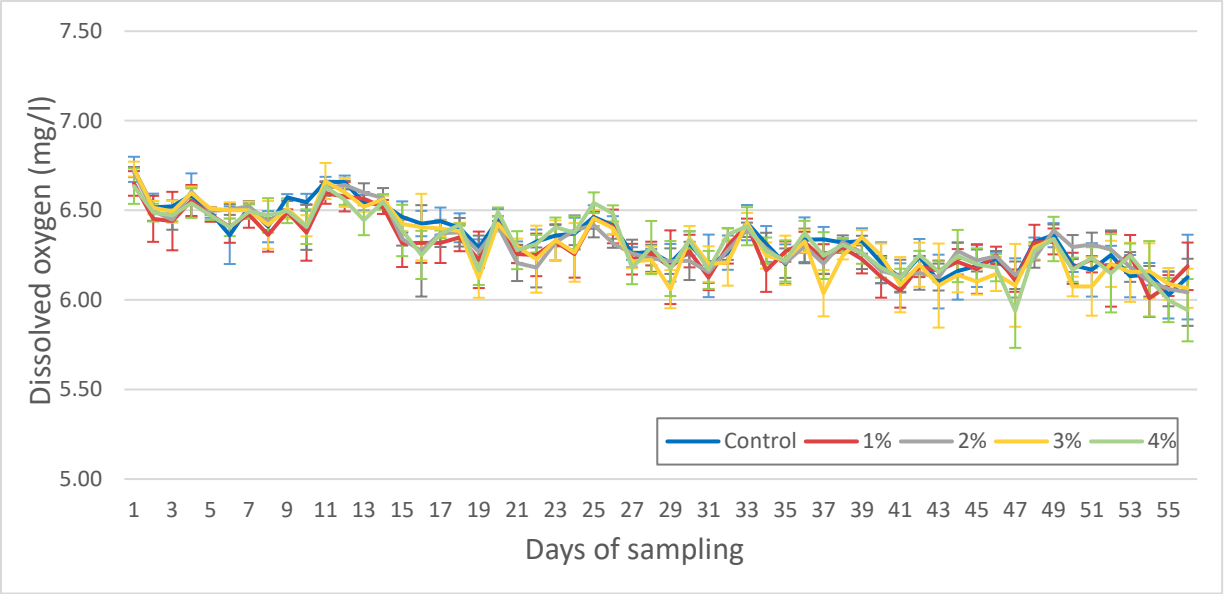

Figure 4.2: The dissolved oxygen (DO) in each treatment along the experiment (56 days). The values are means ± SD (n = 4). No statistically significant differences were found among the five treatments.

4.1.2. Total ammonia nitrogen and nitrite

The total ammonia nitrogen (TAN) and nitrite concentrations did not show any significant differences among the treatments during the experiment (Figure 4.3 and Figure 4.4). The lowest TAN concentrations were measured in the first week of the experiment (0.38 ± 0.06 mg/l) and increased slightly during the experiment. The highest TAN concentrations were monitored in week 6 and 7 with an average of 1.44 ± 0.22 mg/l. The nitrite concentrations were also increasing during the experiment, with the lowest levels of nitrite in the first week (0.00 mg/l) and the highest levels in the last week (0.25 ± 0.14 mg/l).

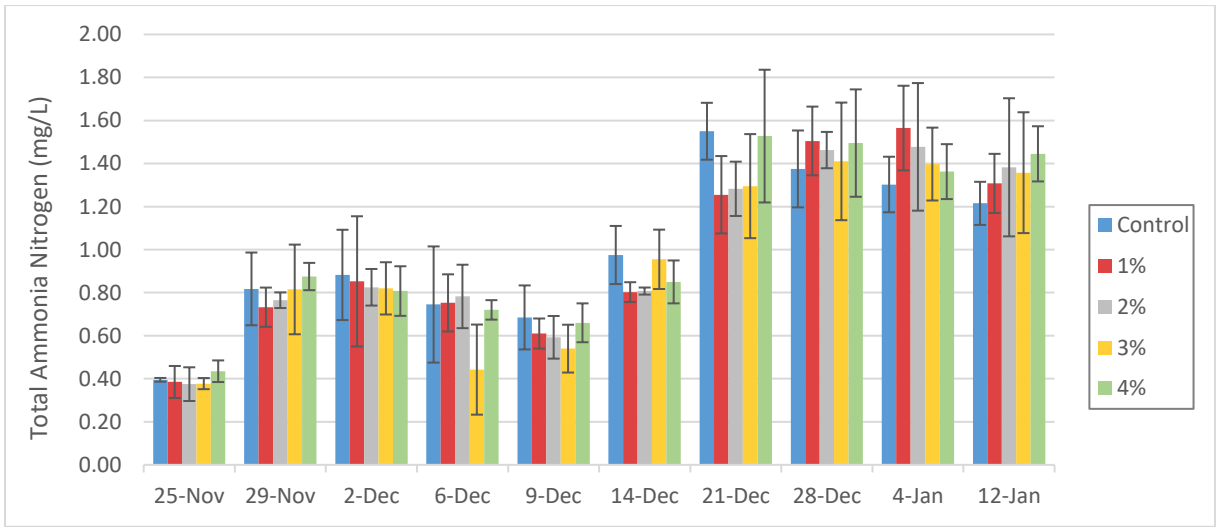

Figure 4.3: Total Ammonia Nitrogen (TAN) concentrations along the experiment. The values are means ± SD (n = 4). No statistically significant differences in TAN levels were found among the treatments.

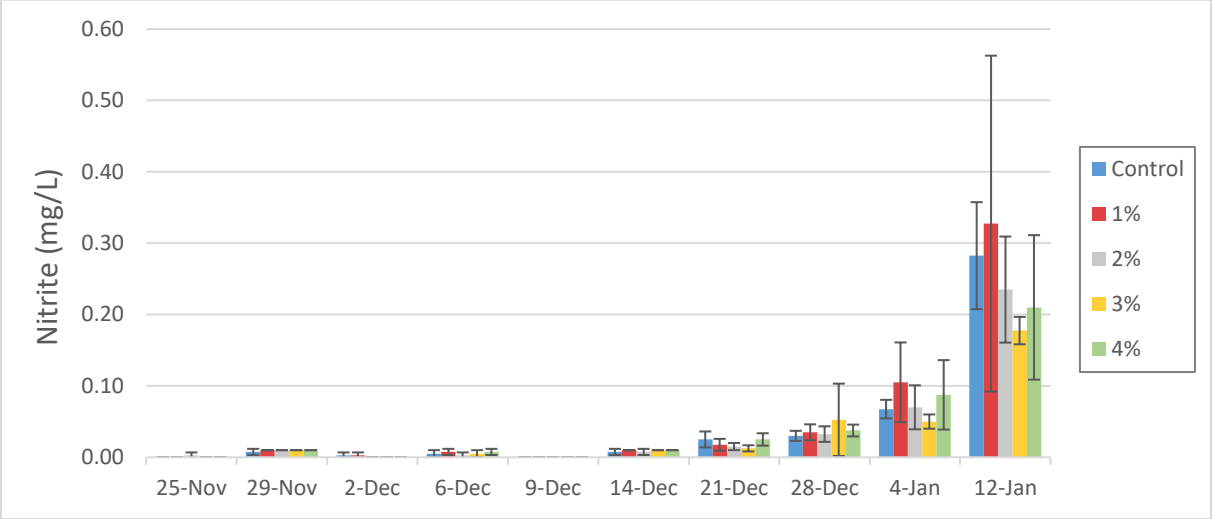

Figure 4.4: Nitrite concentrations along the experiment. The values are means ± SD (n = 4). No statistically significant differences in nitrite concentrations were found among the treatments.

4.1.3. Alkalinity, salinity and pH

Alkalinity, salinity and pH did not change much along the experiment. The alkalinity was between 118 and 128 mg/l for all the treatments (Figure 4.5), the salinity was between 30 and 31 mg/L (Figure 4.7) and the pH was between 7.98 and 8.10 (Figure 4.6).

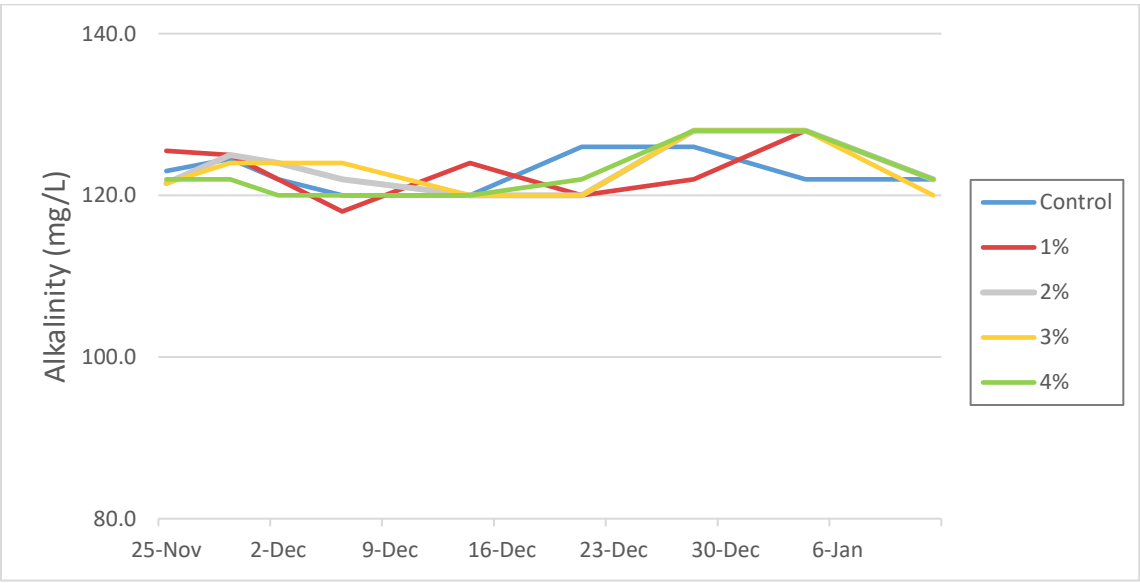

Figure 4.5: Alkalinity values of the five treatments during the experiment.

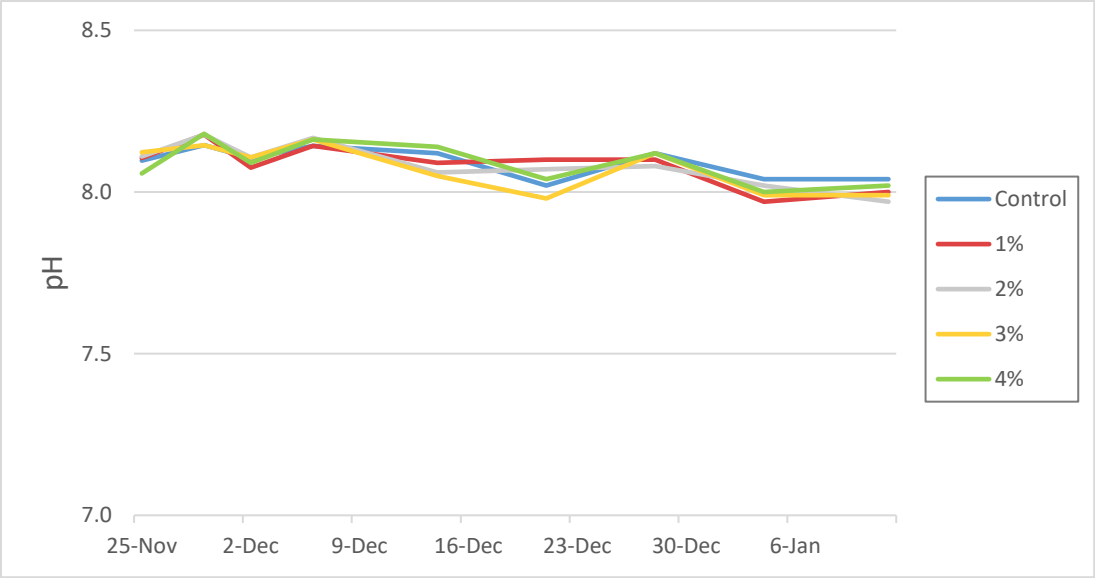

Figure 4.6: pH values of the five treatments during the experiment.

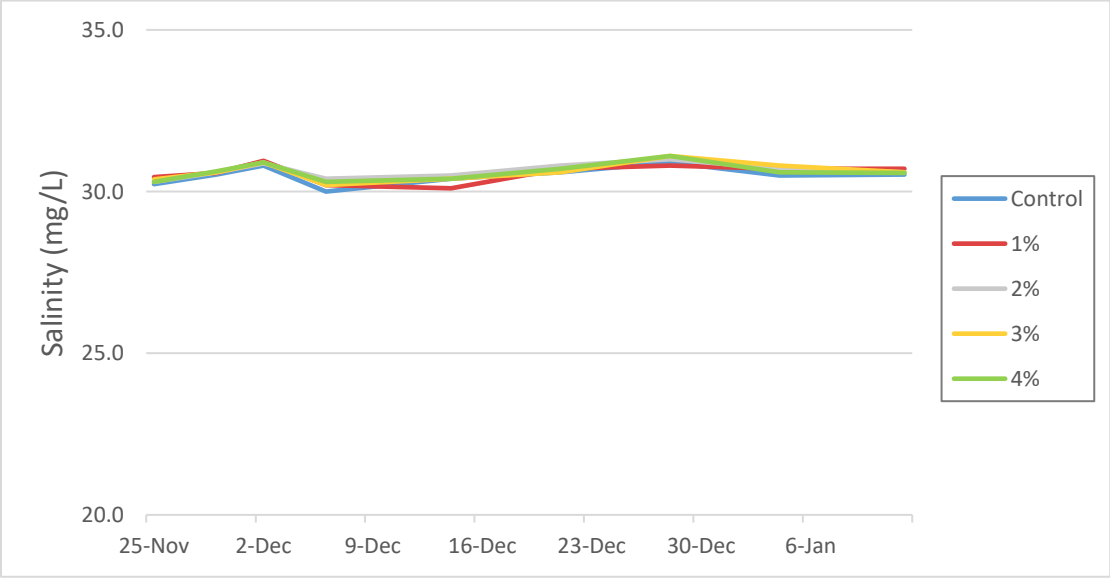

Figure 4.7: Salinity values of the five treatments during the experiment.

4.2. Growth

After 56 days of experiment, final weight, average weekly weight gain and feed conversion ratio were measured for the five treatments (Table 4.1). The shrimps that were fed with the 1% and the 2% of mussel meal diets had a significantly higher final weight and weekly weight gain and lower FCR than the shrimps fed with the Control, 3% and 4% diets.

Table 4.1: Growth performance of *L. vannamei* from the different treatments after 56 days of experiment. Results are means ± SD (n = 4). Absence of letters indicate no significant differences among the treatments. Different letters indicate significant differences ($p < 0.05$) among the treatments.

Treatment	Initial weight (g)	Final weight (g)	Weight gain (% initial weight)	Weekly weight gain (g/week)	FCR
Control	3.50 ± 0.00	18.50 ± 0.30 ^{b}	429.1 ± 9.0 ^{b}	1.88 ± 0.04 ^{b}	1.25 ± 0.03 ^{b}
1%	3.51 ± 0.01	19.63 ± 0.18 ^{a}	459.8 ± 5.0 ^{a}	2.02 ± 0.02 ^{a}	1.21 ± 0.03 ^{a}
2%	3.51 ± 0.03	20.34 ± 0.54 ^{a}	479.8 ± 18.5 ^{a}	2.10 ± 0.07 ^{a}	1.16 ± 0.02 ^{a}
3%	3.50 ± 0.00	18.11 ± 0.31 ^{b}	418.0 ± 9.2 ^{b}	1.83 ± 0.04 ^{b}	1.24 ± 0.02 ^{b}
4%	3.50 ± 0.01	18.45 ± 0.38 ^{b}	426.7 ± 10.7 ^{b}	1.87 ± 0.05 ^{b}	1.25 ± 0.02 ^{b}

FCR: Feed conversion ratio

4.3. Survival

The results of the final survival showed no significant differences among the treatments. After 56 days of experiment, the average survival was >94% in all treatments (Figure 4.8).

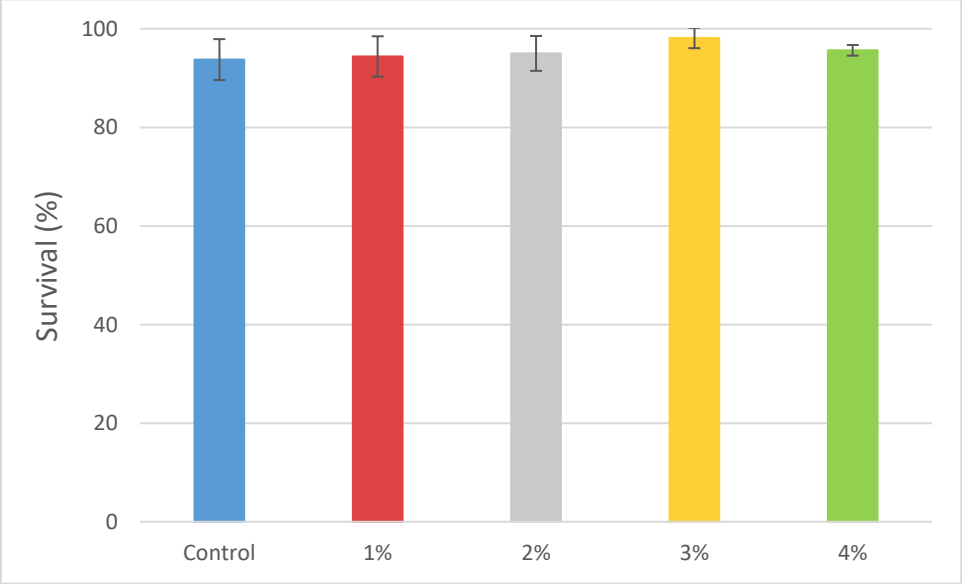

Figure 4.8: Survival of *L. vannamei* fed diets with different inclusion levels of *P. perna* for 56 days. Results are means ± SD (n = 4). Absence of letters indicate no significant differences among the treatments.

No correlation was found between the shrimp final weight and survival (Figure 4.9).

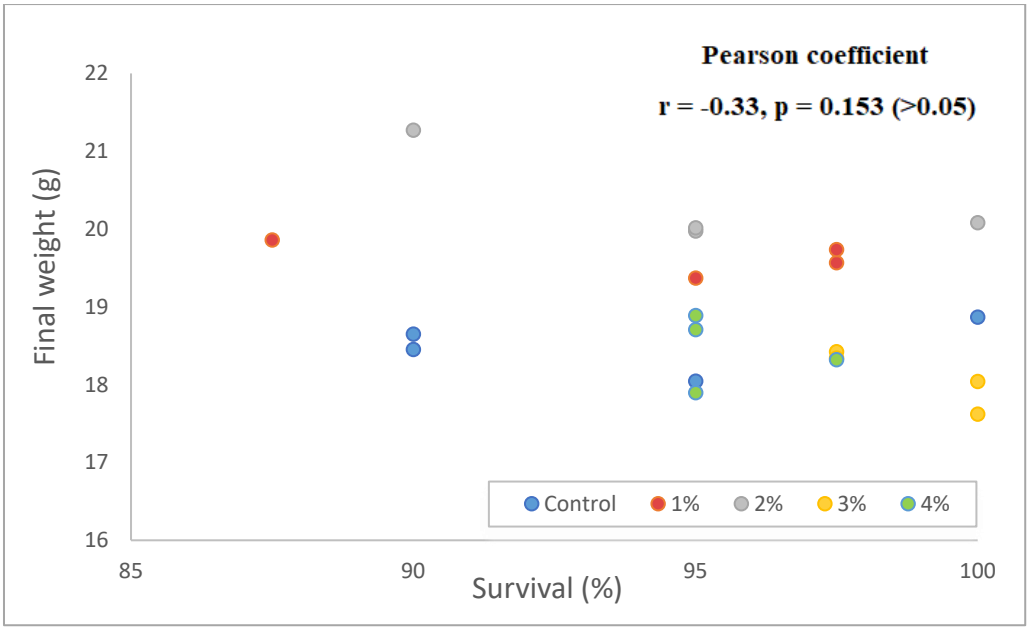

Figure 4.9: Correlation between final weight and survival of *L. vannamei* fed diets with different inclusion levels of *P. perna* for 56 days.

4.4. Thermal shock

The survival 24 hours after the potential lethal thermal shock had no significant differences among the treatments (Figure 4.10). The survival after 24 hours was between 20 and 70% in all treatments.

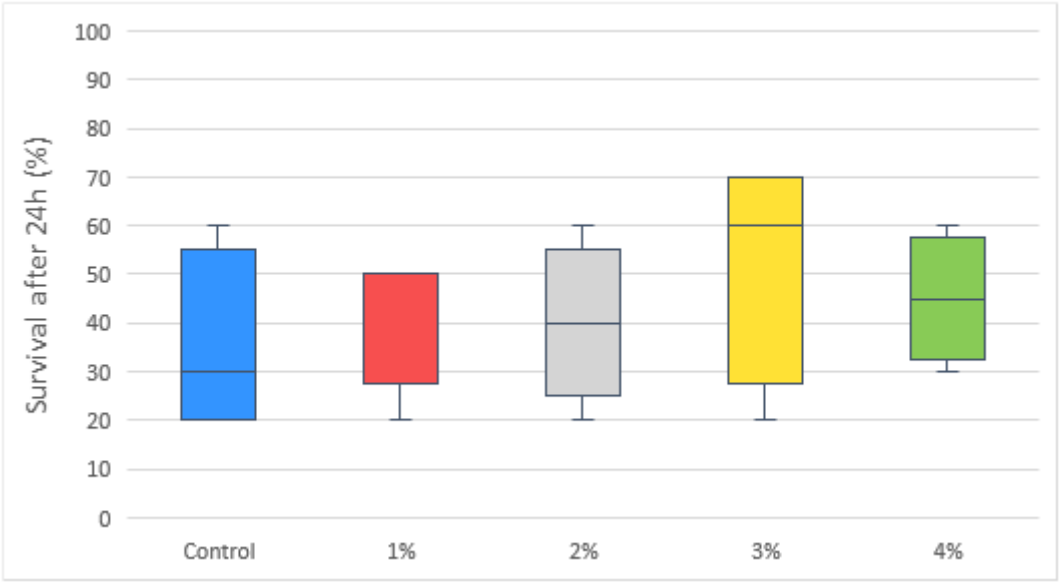

Figure 4.10: Survival of *L. vannamei* 24 hours after the thermal shock (n = 4). Box = 25th and 75th percentile, bars = min and max values, thick line = median value. The absence of letters indicates no significant differences were found among the treatments.

5. Discussion

During the eight weeks of experiment no significant differences were observed for temperature, dissolved oxygen, total ammonia nitrogen and nitrite among the five different treatments. The temperature and dissolved oxygen were kept relatively constant around 28.5 ± 0.4 ℃ and the dissolved oxygen around 6.33 ± 0.22 mg/L. The species *L. vannamei* naturally occurs in oceanic habitats with all year temperature above 20 °C (FAO, 2022), with optimum values for growth and survival around 28 to 30°C for *L. vannamei* juveniles (Palafox et al., 1997). The DO concentrations were maintained well above the limits considered adequate for aquaculture (> 5 mg/L) (Boyd & Gautier, 2000). The total ammonia nitrogen and nitrite slightly increased during the experiment. The TAN concentration in the beginning of the experiment was around 0.38 ± 0.06 mg/l and the highest in week 7 with an average of 1.44 ± 0.23 mg/L with an outlier of 1.87 mg/l in tank 11 (2% mussel meal diet) in week 7. Toxicity levels of TAN are highly dependent on temperature, salinity and pH, being TAN levels more toxic (because of the higher concentration of the un-ionized fraction NH_3) at lower temperatures, lower salinities and higher pH levels (Bower & Bidwell, 1978). Also, the age and size of the shrimp has influence on the resilience to toxic ammonia (Waikhom et al., 2018). In this study, the water temperature and salinity 'safety' levels for rearing *L. vannamei* juveniles was estimated to be 3.75 mg/L TAN (Lin et al., 2001). Therefore, the levels of TAN in all tanks of this experiment can be considered within safe levels. Also, the nitrite increased during the experiment as the biomass in the tanks increased. The nitrite in the beginning of the experiment was 0 mg/L and increased until an average of 0.25 ± 0.14 mg/L with an outlier of 0.63 mg/L in tank 2 (2% mussel meal diet). This is probably due to the growth of the nitrifying bacteria during the experiment, which convert ammonia into nitrite. Toxicity levels of nitrite are dependent on salinity and pH levels in the water. The toxicity levels for *L. vannamei* decrease with an increase in the salinity levels (Ramírez-Rochín at al., 2016). Studies have reported an antagonistic effect between the concentration of chloride ions and nitrite uptake in the hemolymph of aquatic organisms. This can be explained by possible competition for the same transport site in the HCO_3/Cl exchanger protein, which is located on the apical side of gill cells (Jensen, 2003; Tomasso, 2012). The 'safety' level for rearing *L. vannamei* in the conditions of this study was estimated to be ± 20 mg/l (Lin & Chen, 2003). Therefore, even in the tanks with the highest nitrite levels, were still way below toxic levels. The temperature, DO, TAN and nitrite that were maintained along the experiment can be considered good and did not affect the shrimps.

Since the experiment was performed in static water with 100 per cent daily water exchange the alkalinity, salinity and pH levels did not change much along the experiment. *L. vannamei* are capable of inhabiting waters with salinities ranging from 0.5 to 40 ppt (Saoud et al., 2003). Marine shrimps develop best with a pH ranging from 7 to 9 (Van Wyk and Scarpa, 1999). Concerning alkalinity, Poersch et al., (2014) performed an experiment testing alkalinity levels between 70 and 300 mg $CaCO_3$/L in a biofloc system and no differences were detected in shrimp survival. The level of alkalinity is more important for the growth of heterotrophic and nitrifying bacteria, due to the consumption of inorganic carbon by these bacteria (Ebeling et al., 2006). Therefore, in our experiment the parameters alkalinity, salinity and pH were in a suitable level for rearing *L. vannamei.*

When the water parameters were suitable for rearing *L. vannamei* and did not show any significant differences among the five treatments, significant differences were found in the growth performance between the five different diets. An addition of 1% and 2% mussel meal in the diets resulted in significantly higher shrimp final weight, weekly weight gain and lower FCR, while the 3% and 4% mussel meal diets shown similar results as the control. The exact cause for this remains unclear. It could be possible that the amino acid profile from the 1% and 2% are better matching with the amino acid requirements of the shrimps. Shrimp growth and nitrogen loading is influenced by the quantity and quality of protein supplied to the diet (Swanepoel, 2018). Even when the experimental diets had similar protein levels, the amino acid profile could be different. During the formulation of the experimental diets the levels of soybean meal, fishmeal and poultry meal vary a few percent to comprise the mussel meal (rich in protein) to keep the total protein levels in the diets the same. This can cause differences in amino acid profiles between the experimental diets. A unbalanced amino acid profile in the diets can reduce growth performance and cause unavoidable amino acid losses (Aragão et al., 2004)

Another reason for the differences in growth could be the oxidation of the lipids, and specially the oxidation of the highly unsaturated fatty acids. Bivalves in coastal areas are an excellent sources of n-3 polyunsaturated fatty acids (PUFAs), including the long-chained eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) (Chakraborty et al., 2014; Astorga Espana et al., 2007). But these highly unsaturated fatty acids are also known to be readily susceptible to oxidation due to the high number of unsaturated carbon-carbon bonds in the fatty acids (Ackman and Gunnlaugsdottir, 1992). The mechanism of lipid oxidation begins with auto-oxidation involving the direct reaction of unsaturated fatty acids with molecular oxygen to form hydroperoxides. This is followed by secondary reactions

yielding diperoxides if further oxidation takes place, or ketoacylglycerols if the hydroperoxides are dehydrated. Fission of hydroperoxides yield products containing carbonyl and hydroxy groups which will react further to form other products. These products of secondary oxidation of unsaturated fatty acids contribute to off flavor and include toxic compounds frequently associated with rancidity (Halliwell and Chirico, 1993). Furthermore, carbonyl groups produced by the fission of aldehydic hydroperoxides can react with the ε amino group of lysine, thereby reducing the nutritive value of the protein. The negative effects of oxidative rancidity mainly in diets containing oxidized fish oils on growth, feed intake and health have been demonstrated in several fish species. In comparison with fish, only a few studies on effects of lipid oxidation have been carried out in shrimp. Bautista and Subosa (1997) examined the effect of feeding diets containing increasing levels of tetraethoxypropane (TEP, an oxidation product) to black tiger shrimp. The unsaturated fatty acid content of the diets decreased as the TEP content increased. Yang et al. (2014) found a negative effect on growth performance and an increase in oxidative stress in the shrimps fed with oxidized fish oil. Also, during storage of feedstuffs, oxidation of the lipids can take place. Specially for sub-tropical and tropical regions with high humidity and temperature throughout the year, lipids in either feedstuffs or diets are readily susceptible to oxidation if storage is prolonged. Laohabanjong et al. (2009) tested different ways of fish meal storage and found a decrease in growth performance and diet utilization of the shrimp, according of the oxidation of the diets. There were no differences in survival of the shrimp. Even when marine invertebrates, particularly bivalve mollusks are a rich source of antioxidants (Chakraborty et al., 2016) and with experimental diets supplemented with antioxidants (like vitamin C), oxidation can occur because of the susceptibility to oxidation of the highly unsaturated fatty acids. This could be a possible explanation for the decrease in growth performance of the diets supplemented with 3% and 4% of mussel meal compared with the 1% and 2% mussel meal diets.

When some shrimps died in the tank, small density differences occur. Several research showed significantly differences in growth and final weight related with different densities (Kotiya & Vadher, 2021). In this experiment no relation was found between survival and final weight ($r = 0.32$, $p = 0.15$). So the small differences in the tank density did not cause the differences in growth.

Mussel meal is rich in different compounds like minerals and unsaturated fatty acids that could improve the robustness of the shrimp, as shown in previous studies. For instance, Mercier et al. (2009) reported a beneficial role of diet enriched with HUFA on tolerance to handling stress

and immune response of *L. vannamei* juveniles. Also, an improvement of osmoregulation capacity was monitored in *L. vannamei* juveniles, when diets were supplemented with unsaturated fatty acids (Chen et al., 2019). In the current experiment, no differences were measured in survival among the treatments after the thermal shock. Due to the huge differences in survival (20% up to 70%) within the same treatment, a critical note has to be given on the procedure used for the thermal shock treatment. The size of the shrimp influences the cold resistance, where bigger shrimps are more resistance than smaller shrimps (Pontinha, 2017). Small size differences in the sampled shrimps could have influenced the survival results. Also, the sample size of 10 shrimps per tank could be too small to have statistical power. Due to the shortage of the shrimps, the sample size could not be larger.

6. Conclusion

Shrimps fed with the 1% and 2% mussel meal diets had a significantly higher final weight, weekly weight gain and low FCR then the shrimps fed with the control, 3% and 4% mussel meal diets. The shrimps fed with the 2% mussel meal diets showed the best growth results. After 8 weeks of experiment, shrimps fed with the 2% mussel meal diets were significantly 10% heavier than the control. Further, no differences were observed in cold resistance and survival among the treatments. In conclusion, mussel meal can be used as a potential ingredient in whiteleg shrimp diets. In addition, further experiments investigating the amino acid profile, lipid profile and oxidation of the experimental diets should be designed to better understand the underlying processes why the 2% mussel meal diets improved growth performance.

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