Algae Meals as a substitute for fish meal and fish oil in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*)

by

Lorena Garcia

B.Sc., Jorge Tadeo Lozano University, 2009

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Appplied Animal Biology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2013

© Lorena Garcia, 2013
Abstract

Traditionally, marine fish meal (FM) and fish oil (FO) have been the major feed ingredients used as protein and lipid sources in the formulation of aquaculture feeds. However, these commodities are limited by their availability, high cost as well as negatively impacting the sustainability of wild fish stocks. Thus, finding alternatives sources to replace or reduce the use of FM and FO in aquafeeds is critical. The primary goal of this research was to determine the effects incorporating algal meals into diets fed to the Pacific white shrimp (Litopenaeus vannamei) on production for and organoleptic qualities. Twenty circular tanks composed an outdoor closed system with a volume capacity of 530-L. Specific Pathogen-Resistant (SPR) Pacific white shrimp (mean weight ± SD, 1.31± 0.029) were stocked at a density of 28 shrimp/m² in a green water system. Shrimp were assigned five dietary treatments. The experimental diets, fed twice daily, were isolipidic (8%), and contained three protein levels: 34%, 37% and 48%. Spirulina and Schizochytrium were used to substitute FM and FO in the experimental diets. Two FM based feeds were used as controls. The production parameters: final weight (9.4 to 14.9 g), percent weight gain (479-680%), growth rate (0.7-1.3 g/week), feed conversion ratio (1.6-2.5) and survival (85-95%) were estimated. Treatment effects for production parameters were evaluated using one-way analyses of variance (ANOVA). Differences (P ≤ 0.05) among the treatments existed for all production parameters except survival. Organoleptic qualities of shrimp were analyzed by analysis of variance (ANOVA). Differences existed for the desirable attributes of aroma, color and sweetness, but not for the attributes of firmness, moistness, and fattiness. Differences were not detected for the undesirable attributes off-odor, fish flavor, earthiness, off-flavor, and overall unpleasantness. The diet containing Spirulina at a 37% protein level with the inclusion of DHA from Schizochytrium, was
perceived as not containing undesirable attributes and thus shows promise. Results from this study show that Pacific white shrimp can accept algal meals in their feeds and that the inclusion of certain algae ingredients in shrimp feeds could be commercially viable when considering consumer acceptance.
Preface

Chapter 2 will be submitted for publication [Chapter 2: Garcia-Hoyos, L.M., Quintero, H., Forster, I., Cliff, M., and McKinley, S. 2013. Algae meals as a substitute to fish meal and fish oil in practical diets for the Pacific white shrimp (*Litopenaus vannamei*)].

Dr. Herbert Quintero and Ian Forster formulated the diets of this experimental study. Diets were manufactured at the Center for Aquaculture and Environmental Research (CAER).

Dr. Herbert Quintero and I created the husbandry protocol of the feeding trial and the experimental design. I conducted the experimental trial for 3 months in Colombia where I was responsible for experiment set up, general husbandry, sampling sessions, collection and analysis of data. Co-authors Dr. Herbert Quintero, Ian Forster and Margaret Cliff provided guidance and commentary in the statistical analysis of data and preparation of the final manuscript. Dr. Margaret Cliff contributed with assistance in the analysis and interpretation of sensory data.
Table of Contents

Abstract ..................................................................................................................................... ii
Preface....................................................................................................................................... iv
Table of Contents ........................................................................................................................ v
List of Tables ....................................................................................................................................vii
List of Figures ...................................................................................................................................x
List of Abbreviations ...................................................................................................................xii
Acknowledgements .................................................................................................................. xiv
Dedication..................................................................................................................................... xv

1 Introduction ........................................................................................................................... 1
  1.1 Shrimp aquaculture .........................................................................................................2
  1.2 The use of fish meal and fish oil in aquaculture feeds .....................................................3
  1.3 Plant protein and oil sources for aquaculture feeds as alternatives ...............................7
  1.4 Microalgae as an alternative ingredient for aquaculture feeds .......................................11
  1.5 Thesis objectives .......................................................................................................... 15
    1.5.1.1 Research questions and hypothesis ......................................................................16

2 Algae meals as a substitute for fish meal and fish oil in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) ...................................................................................18
  2.1 Introduction .....................................................................................................................18
  2.2 Materials and methods .................................................................................................21
    2.2.1 Source of shrimp and experimental design ...............................................................21
    2.2.2 Feed formulation and management ........................................................................22
2.2.3 Chemical analyses................................................................. 23
2.2.4 Sensory evaluation................................................................. 24
2.2.5 Statistical analyses ............................................................... 25

2.3 Results and discussion ............................................................. 26
2.3.1 Water quality ................................................................. 26
2.3.2 Growth performance and production parameters .............. 26
2.3.3 Chemical composition of shrimp body .................................. 31
2.3.4 Sensory evaluation ............................................................... 32

2.4 Tables .................................................................................. 35

2.5 Figures ................................................................................ 51

3 General discussion, limitations and future directions ................ 54
3.1 Research contributions .......................................................... 54
3.2 Approaches for the evaluation of sensory characteristics in aquaculture products using a three factor Analysis of Variance ................................................................. 55
3.3 Research limitations and future directions ............................ 56
3.4 Analysis of thesis work in future research directions .......... 58

Bibliography .................................................................................. 60
List of Tables

Table 1.1. General composition of different algae (% of dry matter)………………………………………36

Table 2.1. Nutritional composition of practical diets for *Litopenaus vannamei* KJ: Kilojoules/100g. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%)………………………………………………………………………………………..……37

Table 2.2. Average of water quality parameters during the 10-week culture period for *Litopenaeus vannamei*. Values are means ± SD. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%)………………………………………………………………………………………………………38

Table 2.3. Average production parameters at the end of a 10-week culture period for *Litopenaus Vannamei* fed practical diets with varying levels of algae meal and fish meal diets 1. Values are means ± SD.FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%)…………………………………………………………………………………………39
Table 2.4. Amino acid composition of practical diets for *Litopenaus vannamei* (%)\(^1\). FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%)…………………………………………………………………………..40

Table 2.5. Whole body chemical composition of *Litopenaeus Vannamei* fed various diets\(^1\). KJ: Kilojoules/100g. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%)…………………………………………………………………………………………42

Table 2.6. Fatty acid composition (% of FAME) of diets\(^1\). FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%)…………………………………….43
Table 2.7. Fatty acid composition (% FAME) in shrimp muscle tissue: FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%).

Table 2.8. Mean sensory characteristics (n = 20; 5 judge ×4 replications) of shrimp at the end of the feeding trial. Values are means ± SE mean. R represents the standard expected value for each attribute. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%).

Table 2.9. Analysis of variance of sensory characteristics ratings (5 judges): degrees of freedom (df), F-ratios, and error mean squares (MSE). J*S: Judge and sample interaction. J*R: Judge and replicate interaction. S*R: Sample and replicate interaction.

Table 2.10. Correlation matrix among the sensory attributes (df = 3).
List of Figures

Figure 2.1. Final body weight of Pacific white shrimp fed two control (FM-37; COM-36) and, three experimental (AS-37, ASS-48, ASS-34) diets reared under a green water system. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%) Error bars represent SD……………………………………………………………………………………………….51

Figure 2.2. Mean scores for the sensory attributes aroma, color and sweetness. Bars represent aroma, color and sweetness of the various treatments. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%). Treatments that do not share the same letter are significantly different at $P \leq 0.05$. Error bars represent SE (standard error).…………………………………………………………………………………………………52

Figure 2.3. Cobweb with descriptive profiles of undesirable attributes for *Litopenaeus vannamei* indicating the results of sensory tests where judges evaluated shrimp that had been reared on experimental and control diets. FM-37 (Fish meal based feed, main protein source: fish meal at
37%); AS-37 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%). The center of the figure designates low intensity. Intensity of each attribute increases with distance ..................
List of Abbreviations

Abbreviations in text

ANOVA Analysis of variance
AOAC Association of Official Analytical Chemists
ArA Arachinodic acid
AS-37 Algae based diet (main protein source: *Spirulina*-37% with inclusion of algal oil DHA)
ASS-34 Algae based diet (main protein source: *Schizochitrium, spirulina*-34%)
ASS-48 Algae based diet (main protein source: *Schizochitrium, spirulina*-48%)
BHT Butylated hydroxytoluene
CAER Center for Aquaculture and Environmental Research
COM-36 Commercial fish meal feed (36% protein)
DHA Docosahexaenoic acid
DPA Decosapentaenoic acid
EPA Eicosapentaenoic acid
FA Fatty acid
FAME Fatty acid methyl ester
FAO Food and Agriculture Organization of the United Nations
FCR Feed conversion ratio
FM Fish meal
FM-37 Fish meal based diet (main protein source: fish meal-37%)
FO Fish oil
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUFA</td>
<td>Highly unsaturated fatty acids</td>
</tr>
<tr>
<td>IAA</td>
<td>Indispensable amino acids</td>
</tr>
<tr>
<td>LC</td>
<td>Long-chain fatty acids</td>
</tr>
<tr>
<td>LSD</td>
<td>Least-significance difference</td>
</tr>
<tr>
<td>LNA</td>
<td>Linolenic acid</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyls</td>
</tr>
<tr>
<td>PLS</td>
<td>Post-larval shrimp</td>
</tr>
<tr>
<td>POP</td>
<td>Persistent organic pollutants</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>SBO</td>
<td>Soybean oil</td>
</tr>
<tr>
<td>SDA</td>
<td>Specific dynamic action</td>
</tr>
<tr>
<td>SPR</td>
<td>Specific-pathogen resistant</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
</tbody>
</table>

**Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>Ammonium</td>
</tr>
</tbody>
</table>
Acknowledgements

I would like to express my deepest gratitude to Dr. Scott McKinley for the opportunity he has offered, and for his intellectual contribution in the completion of this research. I am especially grateful to Dr. Herbert Quintero for his support during this research, time, advice and some very stimulating scientific discussions. I would like to extend my gratitude to Ian Forster for his willingness to help whenever needed and for following the progress of this research.

Also, I would like to thank Dr. Margaret Cliff for providing advice on statistical analysis of the sensory evaluation component of this research. I am also very grateful to my committee members Drs. Zhaoming Xu, James Thompson and Marina von Keyserlingk for their promptness and assistance throughout the preparation of this thesis.

I would like to acknowledge my gratitude to Andres Suarez, Jaime Faillache and all personnel from CENIACUA, Colombia, for their technical assistance during fieldwork. I would like to further thank Mahmoud Rowshandeli from CAER and Bianca Arney for their patience and help during laboratory analysis.

Special thanks must be given to my parents Alba and Raul, who have supported me in the direction of following my dreams. Thanks to my sister Ana Maria, who is always willing to help and cheer me up. Finally, thanks to my husband Michael Rosaine, for his incredible support, patience, constant encouragements and sweet love.
Dedication

To my husband, Michael and my family: Ana, Raul and Alba.
1 Introduction

Fish meal (FM) and fish oil (FO) have traditionally been considered essential ingredients in aquaculture feeds for carnivorous (e.g. salmon, trout, sea bass, eel) and omnivorous (e.g. tilapia, catfish, common carp, shrimp) species. Numerous aquaculture feed formulations continue to include FM and FO at levels that surpass 50% and 30%, respectively (Tacon and Metian, 2008a). Generally, these commodities are the primary and most costly ingredients in aquaculture feeds. Other challenges encountered when using FM and FO are associated with environmental and safety issues linked to the use of potentially contaminated by-products and the negative effect of decreasing wild fish stocks from FM production from natural fish stocks. Food safety risks in commercial aquafeeds may include salmonellae, mycotoxins, veterinary drug residues, persistent organic pollutants (POP), heavy metals, and excess mineral salts. These contaminants can have a potential effect on the health of cultured species, and may be passed along the food chain via contaminated aquaculture produce to humans (Tacon and Metian, 2008b).

Recently, an average of 25 million metric tons of raw material, approximately 25% of the world’s capture fisheries, has been used to produce FM and FO (De Silva, 2010). The utilization of this volume of raw material has been the subject of continued controversy as the wild capture fisheries reaches its maximum sustainable levels. In an attempt to address these issues, the aquaculture industry has endeavored to find alternative protein and oil sources that can partially or entirely substitute FM and FO in feeds of farmed aquatic animals. Hundreds of studies have tested alternative protein and lipid sources in aquaculture feeds without compromising the production and economic performance of farmed fish and crustaceans; therefore, there is great promise and need for incorporating alternative and sustainable protein and lipid sources in aquafeeds. FM and FO replacements are of paramount importance to the aquaculture sector.
1.1 Shrimp aquaculture

The commercial production of farmed shrimp has been expanding rapidly over the past two decades and this trend is expected to continue as global population increases and the demand for quality seafood continues to rise (Tacon and Forster, 2001). In 2001, marine shrimp were the second highest farmed world aquaculture species with an estimated value of US$8.4 billion (FAO, 2003). Measured by production, *Litopenaeus vannamei* is the most successful internationally introduced marine crustacean species for aquaculture. In 2010, it accounted for 71.8% of world production of all farmed marine shrimp species of which 77.9% was produced in Asia, with the rest in North and South America (FAO, 2012).

Several factors have led to the growth of shrimp farming. Growing demand for shrimp from countries in North America, Europe and Japan, together with a leveling off of production from capture fisheries, gave rise to high market prices in the 1980s. At the same time, the emergence of new production technologies (e.g. hatchery-reared post larval shrimp (PLS) and improved artificial feeds) allowed for large-scale production (Neiland *et al.*, 2001). However, although the production and financial performance of the shrimp aquaculture industry has been outstanding, its rapid growth has been accompanied by a decrease in shrimp price, due in part to depressed markets and overproduction. As shrimp aquaculture is expected to continue to increase in coming years, shrimp prices are likely to continue to fall as production exceeds demand, thereby challenging the profitability of this industry (Amaya *et al.*, 2007).

Commercial shrimp feed formulations generally include between 25% and 50% fishmeal, which represents the primary and most expensive ingredient (Dersjanti-Li, 2002; Tacon and Barg, 1998; Gonzalez-Rodriguez, 2004). FM and FO have been preferred as the number one protein and lipid source as they are rich in marine oils, essential amino acids, vitamins,
minerals, which all lead to good growth performance in shrimp (Patnaik et al., 2006; Samocha et al., 2004). Fish oils are typically characterized by an array of fatty acids in chain length from $C_{12}$ to $C_{24}$. Fish oils are best known and highly regarded for their high proportions of n-3 LC-PUFA (long chain-polyunsaturated fatty acids). In comparison to oils of terrestrial origin, fish oils contain an abundance of EPA ($20:5n-3$) and DHA ($22:6n-3$). The fatty acid composition of individual fish oils varies considerable, influenced by factors including age, size, species, reproductive status, geographic location and the time of the year when harvested. Fish oils are naturally rich in fat-soluble vitamin A and D. They also contain small concentration of dietary-derived vitamin E, which plays an important role in the prevention of PUFA oxidation (De Silva et al., 2010). Fish meal is a natural source of vitamins such as choline, biotine, B$_{12}$, and includes various trace elements like selenium and iodine. Fish meal of good quality normally contains between 60-72% crude protein by weight with a balanced amount of all essential amino acids (Cho and Kim, 2010). However, limited availability and high demand make FM a costly ingredient (Amaya et al., 2007).

1.2 The use of fish meal and fish oil in aquaculture feeds

By and large, marine FM and FO have been the major feed ingredients used as dietary protein and lipid sources, respectively, in the formulation of commercial aquafeeds. It is estimated that aquaculture feeds currently use about 90% of the global supply of FO and it has been predicted that the demand for FO from the aquaculture industry will imminently outstrip supply (Turchini et al., 2010). In fact, according to Tacon and Metia (2008b) there is a global decrease in reported dietary FM and FO inclusion levels in commercial aquafeeds due to the increasing prices of these commodities since 2000. The cause of the escalating prices are often attributed to several factors, including static global supplies of FM and FO, strong market
demand by the aquaculture and livestock sector in the major importing countries and in particular China (GAIN, 2007). It is evident then, that there is great urgency within the aquafeed industry in finding suitable protein and lipid sources to replace or reduce the use of marine FM and FO, and that economics is one of the principal drivers of using alternative feed ingredients.

Fish meal is a finite global resource and as the human population expands the demand for seafood and overall seafood consumption will increase proportionally. Annual per capita supply from aquaculture has increased from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 6.9 %. From a production of less than 1 million tones per year in the early 1950s, production in 2006 was reported to be 51.7 million tonnes with a value of US$ 78.8 billion, representing an annual growth rate of nearly 7 percent (FAO, 2005). This exceptional growth will require a larger input of feeds, which depend upon higher quantities of various feed ingredients, including protein and lipid sources. Since FM and FO are limited global commodities, the demand for FM and FO in aquafeeds will certainly exceed the global production of these products. This realization has led to significant efforts at research centers throughout the world for the past 15 years or more to develop and test protein and lipid sources to replace FM and FO in the diets of farmed fish (Hardy and Tacon, 2002). These research efforts have focused mainly on the replacement of FM and FO by other protein and lipid sources rather than examining what the animal requirements are and how to thoroughly meet those specific requirements. There is a knowledge gap on the chemical composition and nutritive profiles of some alternative feed ingredients, which will have to be addressed to achieve formulated diets that can be used optimally for farmed fish.

Although FM and FO have been the ingredients for excellence in aquaculture feeds, contamination of farmed fish via that contain FM and FO, which are sometimes derived from the
use of contaminated raw materials, is one of the biggest global concerns for food safety. Some of the contaminants that may be present in FO are the persistent organic pollutants (POP), which consist of a wide range of lipophilic compounds including polychlorinated biphenyls, organochlorine pesticides, polycyclic aromatic hydrocarbons, polybrominated diphenyl ether, and dioxins. Persistent organic pollutants bioaccumulate in the lipid component of fish tissues and are subsequently persevered in extracted FO. Polychlorinated biphenyls represent the highest risk in that they potentially increase the incidence of cancer and affect reproductive viability in humans (De Silva et al., 2010).

Although these drawbacks strongly suggest that it is vital to reduce dependence on FM and FO in aquaculture feeds in order to reduce health risks to humans, Mozaffariam and Rimm (2006) reported that the benefits of fish consumption outweigh the potential risks of the contaminants in fish. However, a key responsibility of an aquaculture feed manufacturer and aquaculture food supplier should always be to ensure food safety and product quality as well as protect the health of consumers.

Fish oils are best known and highly regarded for their abundance of LC-PUFA of the n-3 series-EPA, DPA, and DHA. Fish oils contain high proportions of EPA (20:5n-3) and DHA (22:3n-6). The main species used in the production of FO are the Peruvian anchovy (Engraulis ringens), mackerel (Trachurus/scomber spp.), sand eel (Ammodyte spp.), capeling (Mallotus spp.), menhaden (Brevoorita spp.), herring (Clupa Harrengus) and pollock (Pollachius spp.). These species are known to be pelagic and have a lipid content of 8% or more. Peruvian anchovy has EPA contents that range from 7.6% to 22% and DHA from 9.0 to 12.7%. Capelin oil levels of EPA range from 6.1% to 8.0% and 3.7 to 6.0% for DHA. Herring oil contains levels of EPA and DHA ranging from 3.9 to 15.2% and from 2.0 to 7.8 %, respectively (Turchini et al., 2010).
When fish and shrimp are fed diets containing FO they become a unique rich source of n-3LC-PUFA that are critical components of the human diet. The importance of the fatty acid composition of FO arises from the extensive evidence showing potential health benefits associated with its consumption. Therefore, there is a high demand and increased consumption of FO by humans that could potentially reduce this resource availability for commercial feed production. The consumption of LC omega-3s derived from FO has been shown to help prevent cardiovascular disease, cancer, inflammatory autoimmune diseases, and support brain function and mental health (Ruxton et al., 2007). As for shrimp and other crustacean, it has been shown that lipid content and the associated C18 polyunsaturated fatty acids, linoleic (18:2n-6) and linolenic (18:3n3) as well as n-3 and n-6 HUFA (EPA, DHA, ArA) are required in their feeds.

Another possibly detrimental aspect of continued reliance on FO is the predicated effects of climate change on fisheries, which could affect the availability of this raw material for use as an aquafeed. Of great significance are the predicted changes in ocean circulation patterns that, in turn, will have an influence on the occurrence and frequency of El Nino events. Barret et al. (1995) have documented the devastating effects of El Nino on the Peruvian sardine and anchovy stocks, subsequently impacting global FM and FO supplies and prices. As a potential indication of limited supply, the price of FM roughly tripled from 2002 to 2010, and supply remains limited while the demand for fish feed ingredients is expected to continue to rise (Rust, 2011). In addition, it has been suggested that El Nino events will continue to modify the biomass structure of pelagic resources (Niquen and Bouchon, 2004). In essence, potential climate changes can put key stocks at risk for the production of FM and FO. Needleless to say, the aquaculture sector seems to be compelled to move forward to develop alternatives to FM and FO. If the aquaculture feed industry seeks to replace these commodities with alternatives, pragmatic approaches on the
nutritional benefits of ingredients, including digestibility and palatability, technology, price, market availability and sustainability, will need to be considered.

It has been suggested that one way to reduce shrimp production costs and increase profitability, is to develop plant-protein feeds. In recent years, there had been a global initiative to evaluate a number of different plant alternative sources owing to the great urgency within the aquafeed industry to find sustainable alternative to fish meal and fish oil (Turchini et al., 2010). Nonetheless, the challenge of finding suitable alternative ingredients remains with regards to maximizing sustainability, shrimp performance, and economic benefits.

1.3 Plant protein and oil sources for aquaculture feeds as alternatives

Fish meal and FO derived from wild-harvested whole fish and shellfish including bycatch currently constitute the major aquatic protein and lipid sources available for animal feed (FAO, 2012). However, during 15 years (1994-2009), analysis of data collected by the Food and Agriculture Organization (FAO) of the United Nations has indicated that global FM and FO production from marine capture fisheries has been decreasing at annual average rates of 1.7 and 2.6 %, respectively. It is evident that the increasing demand for these commodities places great pressure on marine ecosystems leading to a depletion of fish stocks. Therefore, there is a drive to adopt more ecologically sound decisions regarding the future of protein and oil sources for aquaculture feeds. It is extremely important to keep a holistic research focus that involves all the components (ingredient digestibility, ingredient palatability, nutrient utilization, final product quality and price) that are necessary to establish the viability of new ingredients.

Hundreds of studies have been conducted to evaluate numerous alternative plant proteins such as soybean meal, corn gluten meal, rice protein, rapeseed protein, and plant oils such as; soybean, rapeseed, linseed, sunflower, peanut, olive and palm oil among others in aquaculture
feeds. For example, Sooking and Davis (2010) found that soy protein concentrate inclusion up to 12% in soybean-based diet can be used in commercial feed formulations for *Litopenaeus vannamei* without causing negative effect on feed conversion ratio (FCR), survival and growth. In another study (Amaya *et al*., 2007), FM replacement using soybean meal at 37% protein level in commercially manufactured feed for the *Litopenaeus vannamei* was performed. These authors demonstrated that FM could be completely replaced using alternative vegetable protein sources without compromising production and economic performance.

Similarly, Lin *et al.* (2012) investigated the replacement of FM with fermented soybean meal in diets for pompano (*Trachinotus ovatus*) at a 46% protein level, and reported that fermented soybean meal did not have a negative effect on fish growth, FCR, and body composition. In another study carried out by Walker *et al.* (2010), partial replacement of FM with soy protein concentrate at a 47% protein level in diets of Atlantic cod was evaluated, with survival and growth not affected.

In addition, the use of soybean as a protein source has been examined for many other commercially important fish species, such as red drum *Sciaenops ocellatus* (McGoogan and Gatlin 1997), Atlantic salmon, *Salmo salar* (Refslie *et al*., 1998), Asian seabass, *Lates calcarifer* (Tantikitti *et al*., 2005), sharpsnout sea bream, *Diplodus puntazzo* (Rondan *et al*., 2004), red sea bream, *Pagrus major* (Biswa *et al*., 2007), and gilthead sea bream, *Sparus aurata* (Venou *et al*., 2006; Bonaldo *et al*., 2008). Compared to other plant proteins, soybean meal is one of the most promising alternatives due to its abundant availability, reasonable price, and high digestibility (Lemos *et al*., 2000). However, in terms of lipid composition, soybean meal contains 90% fewer n-3 fatty acids than FM and contains anti-nutritional factors such as trypsin inhibitors and lectins, which are known to inhibit digestive enzyme activity (Zhou *et al*., 2011).
Another limitation of soybean protein is its amino acid profile, which does not match the nutrient requirements of farmed fish (Wilson, 1989; Floreto et al., 2000); thus limiting its inclusion rate compared to FM (i.e. deficiency in the essential amino acids methionine, lysine and tryptophan), presence of antinutritional factors, and poor palatability (Lim and Dominy, 1990 and Tacon and Akiyama, 1997). Although most of the antinutritional factors do not lead to mortality, they could produce adverse effects and decrease productivity as well as influence the nutritional and organoleptic quality of the final product (Francis et al., 2001). There is a gap in the literature regarding the assessment of organoleptic characteristics of farmed species fed plant-derived ingredients that needs to be assessed.

As for FO replacement, a number of studies have suggested that certain vegetable oils could be considered as good alternative lipid sources in aquaculture feeds. The most widely used vegetable oils for inclusion in animal feeds are soybean, soybean lecithin, corn, cottonseed, and sunflower (Gunstone, 2010). Soybean oil (SBO) and soybean lecithin oil, enriched with linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids, have been used extensively in aquafeeds.

Soybean meal has been used as lipid source for Litopenaeus Vannamei (Hu et al., 2011), common carp (Cyprinus carpio) (Fontagne et al., 2000), Japanese flounder (Paralichthys olivaceus) (Lee et al., 2000), giant fresh water prawn (Macrobrachium rosenbergii) (Labao et al., 1995), Asian seabass (Lates calcarifer) (Catacutan and coloso, 1997), black carp (Mylopharyngodon piceus) (Dai et al., 1988), and red belly tilapia (Tilapia Zillii) (El-sayed and garling, 1998) among others. These studies showed a general acceptance of SBO as a source of dietary lipid for growth. Nonetheless, according to Gunstone (2010), the fatty acid composition of tissues can be altered. This same author has reported that species fed SBO incorporate 16:0, 18:1n-9, and 18:2n-6 into tissue lipids as well as longer chain fatty acids of the same families,
but the n-3 fatty acid concentration in tissues tends to be lower. This in particular brings to light a challenge for the aquaculture industry since aquatic animals are regarded as healthy foods for humans, mostly because of the n-3 fatty acids found in edible portions, but there is an increasing number of farmed species identified with high concentrations of n-6 fatty acids, which are not appropriate from a human nutrition standpoint and promote the pathogenesis of many diseases (Gonzalez-Felix et al., 2010).

Seafood is recommended in human diets to increase the n3/n6 ratio and to promote human health by preventing cardiovascular disease, cancer, inflammatory autoimmune diseases, and supporting brain development and function (Ruxton et al., 2007). For that reason, it is critical to ensure that farmed species retain high levels of n-3 HUFA and high n-3/n6 ratios as found in wild caught species. As diets are continually modified to meet the nutritional requirements of targeted species and least-cost formulations become more common, there is a possibility that diets will provide lower concentrations of n-3 fatty acids (Gunstone, 2010), thereby affecting the final quality (and arguably acceptability) of farmed aquaculture species.

Moreover, the evaluation of sensory characteristics of alternative ingredients for commercial aquaculture species needs further research in order to guarantee final product quality and consumer acceptability. Literature on the impact of FO replacement on sensory quality in farmed fish is rather scarce and mainly related to studies in salmonids. In salmon, replacing FO with SBO had no effect on fillet texture (Rora et al., 2003). In addition, no differences were observed in Atlantic halibut fed either FO or SBO (Haugen et al., 2006), neither in Atlantic cod (Morkore et al., 2007), nor in European bass (Izquierdo et al., 2003).

Flesh pigmentation is an important factor in perception of flesh quality in salmonids (Bell et al., 1998). The flesh levels of lipid-soluble nutrients such as astaxanthin are dependent on the
dietary composition (Lie, 2001), and it has been shown that both total lipid and type of oil can affect carotenoid absorption in Atlantic salmon (Bjerkeng et al., 1999), and color characteristics in raw and smoked Atlantic salmon fillets (Regost et al., 2004). However, the majority of studies show no effects on flesh astaxanthin levels by using up to 100% vegetable oil (Bell et al., 2001; Torstensen et al., 2004), but none of these replaced FO with SBO. As reported by Regust et al. (2004) dietary SBO as a replacement of FO in salmon resulted in reduced flesh astaxanthin and texture (Rora et al., 2003).

Perhaps, special consideration should be directed towards the inclusion of algae products in aquaculture feeds, which are known to have high concentrations of n-3 fatty acids, as well as the development of culture technology with regards to commercial large scale production, in order to assure market availability and sustainability. Alternative ingredients that can provide the n-3 HUFA requirement for human consumption need to be found. Likewise, a comprehensive study of nutrient composition, digestibility, and bioavailability of new ingredients is yet to be conducted.

1.4 Microalgae as an alternative ingredient for aquaculture feeds

Microalgae are valuable in aquaculture and have been used as live feed for larval and juvenile crustaceans and finfish, all bivalve mollusks including oysters, scallops, clams and mussels, and as feed for zooplankton (Ju et al., 2009). Microalgae have received particular interest in the aquaculture sector due to their nutrient profile. Microalgae are rich sources of vitamins, essential amino acids, minerals, essential fatty acids, and carotenoid pigments for aquatic animals (Takeuchi et al., 2002). The chemical composition of different algae sources is now readily available in the literature (Table 1.1; Becker, 2007). Heterotrophically algal groups, such as chrysophytes, cryptophytes and dinoflagellates have been known to produce lipids with
high levels of EPA, DHA and AR (Cohen et al., 1995; Behrens and Kyle 1996). As microalgae biomass is a rich source of nutrients such as n-3 and n-6 fatty acids, (Becker 1994), it has great potential to be an alternative ingredient for sustainable aquaculture feeds.

Micoralgae have diverse uses in aquaculture, their applications are mainly to provide nutrition and to enhance the color of the flesh of salmonids. The most frequently used species are Chlorella, Tetraselmis, Isochrysis, Pavlova, phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema and Thalassiosira (Hemaiswarya et al., 2011). Also, the potential use of DHA-rich oils derived from single-cell microalgae (i.e Schizochytrium spp., Cryptecodinimium cohnii, and Phaeodactylum tricornutum) has been successfully tested on gilthead sea bream (Atalah et al., 2007; Ganaza et al., 2008). Similarly, Schizochytrium spp. oil has been successfully tested on Atlantic salmon (Miller et al., 2007).

Special attention should be paid to understanding the effects of DHA inclusion in the fatty acid composition of aquaculture species. If species fed diets rich in DHA from microalgae allow the retention of this fatty acid in animal’s tissue, microalgae might become one of the most viable alternative ingredients for aquaculture feeds.

Although it is argued that producing algae is expensive, successful commercial utilization of microalgae has been established in the production of nutritional supplements, antioxidants, cosmetics, natural dyes and polyunsaturated fatty acids (PUFA) (Spolaore et al., 2006). To date, several sophisticated technologies are employed worldwide for mass production and processing of microalgae. The annual production of all species is estimated at about 10,000 t/y (Richmond 2004). The blue-green algae Spirulina spp. is used in substantial amounts (over 100 t/y) as a fish and shrimp feed, and even larger markets could be projected as production costs may be reduced in the long term (Hemaiswarya et al., 2011).
Research findings have shown that feeding *Spirulina* spp. results in improvements in animal growth, fertility, aesthetic and final nutritional quality. Intake of *Spirulina* spp. or its extracts intake has also been linked to an improvement in animal health, including accelerated development of the immune system of farmed animals such as chickens, pigs, ruminants, cattle, sheep and rabbits especially during the early stages of their lives (Holman and Malau-Aduli, 2012). Successful inclusion of microalgae in aquaculture feeds has been reported (Zhou *et al.*, 1991; Langdon and Onal 1999). More recently, Palmegiano *et al.* (2005) investigated the use of *Spirulina* spp. as a nutrient source in diets for growing sturgeon (*Acipenser baeri*), and Kiron *et al.* (2012), examined two marine algal products (i.e., *Nanofrustulum* and *Tetraselmis*) for their suitability as FM protein substitutes in feeds of three important aquaculture species (i.e Atlantic salmon, common carp and Pacific white shrimp). Growth performance and feed utilization of these species did not exhibit any differences between those species provided with the algae-based feed and those provided with FM-based feed, indicating that algal meal is an effective replacement of FM.

Diatom (*Thalassiora weissfloggi*) and *Nannochlorpsis* cultures have been added to shrimp feeds, increasing the fatty acid and astaxanthin contents of shrimp tail muscles as well as improving growth rates (Ju *et al.*, 2009). Rincon *et al.* (2012) looked at substitution levels of FM by *Spirulina* maxima in experimental diets for red tilapia fingerlings (*Oreochromis* sp.). They found that FM could be substituted with up to 30% *Spirulina* maxima meal without impacting growth performance in red tilapia fry. These studies provide evidence that microalgae are suitable sources of protein and lipid for some aquaculture species. However, the inclusion of microalgae in feeds should be evaluated at a species level, including a deeper understanding of their digestibility and bio-availability.
Another potential species for aquaculture feeds is *Haematococcus pluvialis*. This alga has frequently been added in diets to test for pigmentation of shrimp, salmon and trout (Chien and Shiau, 2005). Astaxanthin production by this microalga is important to many farmed species since, for example, crustaceans are unable to synthesize carotenoids *de novo*, and astaxanthin or appropriate precursors must be supplied in the diet (Meyers and Latscha, 1997). Dietary supplementation of astaxanthin or its precursors improve the color of penaeids (Liao et al., 1993), especially those intensively cultured for a better market price (Liao and Chien, 1994).

One of the biggest advantages of using microalgae in aquaculture feeds is that in practice, different strategies can be used to improve the PUFA content in microalgae by manipulation of processing conditions such as light intensity, nutrient status and temperature. This in turn allows for the modulation of the lipid composition and consequent optimization of their overall yield productivity (Hemaiswara et al., 2011). The lipid content of microalgae is affected by environmental conditions such as light intensity, nutrient limitation, salinity, temperature, pH and culture age (Solovchenko et al., 2008). Under unfavorable conditions, microalgae growth is arrested; photosynthetic activity decreases, and the excess energy might be stored in form of lipids and carbohydrates (Khozin-Goldber et al., 2011). For example, *Nannochloropsis* cultivated under conditions of nitrogen starvation, is a potent source of saturated and monounsaturated oils (Rodolfi et al., 2009) Apart from this benefit, the high production cost of microalgae remains a constraint. Nonetheless, larger markets could be projected as production costs may be reduced in the long term.

Finally, as suggested by Raja et al. (2004) and Patil et al. (2007), in order to be used in aquaculture, a microalgal strain has to meet various criteria, such as ease of culturing, lack of toxicity, high nutritional value with correct cell size and shape, and a digestible cell wall to make
nutrients available. There is great promise for microalgae to become an alternative ingredient that can greatly contribute to meet the essential nutrient requirements of farmed species and to provide a high quality final product.

This thesis is primarily focused on evaluating the suitability of two types of algae (i.e. *Spirulina* and *Schizochitrium*) as the primary sources of FM and FO replacement in practical diets for Pacific white shrimp *Litopenaus vannamei*, an important and widely produced aquaculture species. The effectiveness of algae meals on the source of dietary protein and fat is normally determined by the estimation of growth rate, feed conversion ratio (FCR) and survival. In addition, the sensory characteristics of shrimp muscle were assessed in order to elucidate if the inclusion of algae meals in shrimp feeds could be commercially viable when considering consumer acceptance.

1.5 Thesis objectives

Given the great need to find suitable alternative ingredients to replace and/or reduce the use of FM and FO in aquaculture feeds, the main objective of this research was to investigate if two species of algae (i.e. *Spirulina* and *Schizochitrium*) could be optimal replacements for FM and FO in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). A further aim was to obtain shrimp of comparable nutritional value to shrimp traditionally fed with FM and FO diets and then to analyze the impact of the inclusion of algae ingredients on the sensory characteristics of shrimp muscle, as one of the most critical factors to consider when evaluating the viability of alternative feeds is that of consumer acceptance of the product.
1.5.1.1 Research questions and hypothesis

1) Is the body chemical composition of shrimp fed algae feeds comparable to shrimp fed FM feeds?

$H_01$: The three essential fatty acids (DHA, EPA and ArA) in the body composition of shrimp will be different between shrimp fed algae and FM feeds.

$H_{A1}$: The three essential fatty acids (DHA, EPA and ArA) in the body composition of shrimp will be the same between shrimp fed algae and FM feeds.

2) Can FM and FO be completely replaced with alternative algae protein and oil sources in shrimp feeds without negatively compromising the production performance of *Litopenaeus vannamei*?

$H_{02}$: Growth parameters (i.e. growth rate, FCR and survival) of *Litopenaeus vannamei* will be different between shrimp fed algae and FM feeds.

$H_{A2}$: Growth parameters (i.e. growth rate, FCR ratio and survival) of *Litopenaeus vannamei* will be the same between shrimp fed algae and FM feeds.

3) Does the inclusion of algae ingredients have an impact on the sensory characteristics of shrimp muscle?

$H_{03}$: The sensory attributes of shrimp muscle will be different between shrimp fed algae and FM feeds.
H_{A3}: The sensory attributes of shrimp muscle will be the same between shrimp fed algae and FM feeds.
2 Algae meals as a substitute for fish meal and fish oil in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*)

2.1 Introduction

In recent years, the increase in cultured production of Pacific white shrimp (*Litopenaeus vannamei*) has led to a number of critical environmental and economic challenges. Consequently, the foremost concerns are with respect to emerging environmental and safety issues associated with the use of potentially contaminated animal by-products in aquaculture feeds and the negative effect of FM production from natural fish stocks (Tacon and Metian, 2008a). Food safety risks in commercial aquafeeds may include salmonellae, mycotoxins, veterinary drug residues, persistent organic pollutants (POP), heavy metals, and excess mineral salts. These contaminants can have a potential effect on the health of cultured species, and may be passed along the food chain via contaminated aquaculture produce to humans (Tacon and Metian, 2008b). Therefore, the use of FM in aquaculture feedshas been criticized due to its negative environmental impact and its limited availability.

Commercial shrimp feeds generally contain between 25% and 50% FM, which accounts for the primary and most costly protein ingredient (Dersjanti-Li, 2002; Tacon and Barg, 1998; Gonzalez-Rodriguez, 2004). These high inclusion rates and associated costs create challenges for the profitability of the shrimp aquaculture industry. Furthermore, the rapid expansion in aquaculture production has led to an increase in demand for marine animal meals and oils which, in turn, has caused the price of fish meal and oil to steadily increase (Pike and Barlow 2003). Collectively this has resulted in an increased need for identification of alternative ingredients to replace marine derived ingredients (Davis and Arnold 2000; Amaya *et al.*, 2007).
In an attempt to address these issues, the aquaculture industry has succeeded in reducing the inclusion of FM and FO in the feeds of farmed aquatic animals (Kiron et al., 2012).

Microalgae have received particular interest, as they are a rich source of vitamins, essential amino acids, minerals, essential fatty acids, and carotenoid pigments for aquatic animals (Takeuchi et al., 2002). Moreover, as microalgae biomass is also a rich source of some essential nutrients such as n-3 and n-6 fatty acids (Becker 1994), it has potential to be an alternative ingredient for sustainable aquaculture feeds. In addition, alternative oil sources for shrimp feed formulations are being pursued given that marine oil sources are limited in supply, and could contain undesirable contaminants (Patnaik et al., 2006). Given that the use of algae as a protein and oil source in aquaculture feeds is a recent concept, the associated research is in its infancy.

Some of the early work was carried out by Zhou et al. (1991), who investigated the application of *Spirulina* mixed feed in the breeding of Bay scallop. These authors demonstrated that *Spirulina* feed proved to promote the normal development of scallop gonads, thereby achieving a higher fecundity and hatchery rate. Palmegiano et al. (2005) investigated the use of *Spirulina* as a nutrient source in diets for growing sturgeon (*Acipenser baeri*), and found that *Spirulina* inclusion improves growth and that an inclusion level of 50% exhibited the greatest growth rate, a more favorable FCR and the highest protein efficiency. More recently, Kiron et al. (2012), examined two marine algal products (i.e. *Nanofructulum* and *Tetraselmis*) for their suitability as FM protein substitutes in feeds for farmed Atlantic salmon, common carp and Pacific white shrimp. Growth performance and feed utilization of all three species were similar between those species provided with the algae-based feed and those provided with fish meal-based feed, indicating that algal meal is an effective replacement of FM. Silva-Neto et al. (2012),
studied the attractiveness of *Spirulina* for *Litopenaeus vannamei* by supplementing experimental diets with *Spirulina* meal and control diets with a FM commercial attractant and reported no differences in growth, but interestingly *Spirulina* meal attractant was preferred more by shrimp than the commercial one.

In another study, Ju *et al.* (2009) evaluated the effects of supplementing two species of marine algae (*Thalassiora weissflogii* and *Nannochloropsis* sp) to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vananamei*). The results indicated that adding algae biomass to the diets improved shrimp growth and survival as well as enhanced pigmentation in shrimp tail muscle. Furthermore, Rincon *et al.* (2012) evaluated three levels of substitution of FM with *Spirulina maxima* meal as a protein source in experimental diets for red tilapia fingerlings (*Oreochromis* sp.) and found that FM can be substituted with up to 30% *Spirulina maxima* meal in diets for red tilapia fry.

Although product costs are high, successful commercial utilization of microalgae has been established in the production of nutritional supplements, antioxidants, cosmetics, natural dyes and PUFA is well established (Spolaore *et al.*, 2006). Worldwide, several sophisticated technologies are employed for mass production and processing of microalgae. The annual production of all species is estimated to be about 10,000t/y (Richmond, 2004). The blue-green algae *Spirulina* used in substantial amounts (over 100 t/y) as a fish and shrimp feed, and even larger markets could be projected as production costs may be reduced in the long term (Hemaiswarya *et al.*, 2011). Research findings have associated *Spirulina* improvements in animal growth, fertility, and nutritional product quality. *Spirulina* intake has also been linked to an improvement in animal health in farmed animals such as chickens, pigs, ruminants, cattle, sheep and rabbits (Holman and Malau-Aduli, 2012). To my knowledge, no study has been
carried out on the use of *Spirulina* and *Schizochytrium* as replacement sources of FM and FO in feeds for *Litopenaeus vannamei*. Therefore, this research was undertaken to investigate the effectiveness of FM and FO substitution using *Spirulina* and *Schizochytrium* as the principal protein and lipid sources in the practical diets of *Litopenaeus vannamei* on growth and production.

2.2 Materials and methods

2.2.1 Source of shrimp and experimental design

Research was conducted at the Colombian Aquaculture Research Center CENIACUA, in Punta Canoa, Bolivar, Colombia. Twenty circular fiberglass tanks composed an outdoor closed system with a volume capacity of 530-L; tanks were filled with green water from a shrimp production pond to replicate pond production conditions. Each tank was fitted with a ring air diffuser connected to a common air supply from two 1-hp regenerative blowers (Sweetwater Aquaculture, Apopka, USA). Specific Pathogen-Resistant (SPR) Pacific white shrimp (mean weight ± SD, 1.31± 0.029) were stocked at a density of 28 shrimp/m². Diets were assigned to the tanks according to a completely randomized design, where each treatment was replicated four times (5 diets × 4 replicates = 20 tanks).

Daily water changes and a cleaning routine (siphoning) were performed. Water quality parameters including pH, salinity, temperature, and dissolved oxygen were monitored twice daily at 0830 and 1600 using a HI 255 Hanna combined meter (Woonsocket, USA), Biomarine ABMTC meter (Hawthorne, USA), and YSI Pro 2030 meter (Yellow springs, USA) respectively. Additionally, ammonium (NH₄⁺) was determined on a weekly basis with a spectrophotometer (Genesys-Thermo Scientific, Massachusetts, USA) using the Indophenol blue method (Tovar and Erazo, 2009).
2.2.2 Feed formulation and management

The main target for the formulation of the experimental diets was the entire replacement of FM, while including algae meal from *Spirulina* and *Schizochytrium* as the main protein and oil sources, respectively. Experimental diets were isolipidic (8%) and designed to contain three protein levels: 1) 37% containing fish meal (FM-37) used as a control, 2) 34% containing *Spirulina* and *Schizochytrium* (ASS-34), 3) 37% containing *Spirulina* and algal oil (AS-37), 4) 48% containing *Spirulina* and *Schizochytrium* (ASS-48). A commercial fish meal feed (Nicovita) was used as control as well (COM-36). The experimental diets were prepared in the nutrition laboratory at the Center for Aquaculture and Environmental Research (CAER), West Vancouver, Canada. Feed was manufactured as follows: dry ingredients were mixed for 15 min in a food mixer (Butcher Hobart, troy, USA), and hot water (60°C) was added and mixed to obtain a consistency appropriate for pelleting. The diet mash was passed through a commercial grinding machine (Butcher Boy, Montebello, USA) with 2.4 mm diameter holes. After pelleting, the feeds were dried targeting moisture content at 10% using an air-drying oven. Finally, feeds were stored in a cold room at 15°C until use.

Initial feed rations were calculated assuming 100% survival, FCR of 1:1.5 and expected growth of 1.5 g/week. Subsequent feed inputs were back calculated, based on an expected weight gain of 1.5 g per week, FCR of 1:1.5 and a mortality rate measured every 2 weeks during a 10-week period. Shrimp growth was monitored on a bi-weekly basis by weighing all the animals and estimating the average weight. Feed was provided twice daily (40% at 0830; 60% at 1530). In addition, four aquariums containing five shrimp each were set up with clear water and fed the experimental diets to confirm the acceptability of feed throughout the study period. At the end of
the growth trial, shrimp production parameters were evaluated by a mean final weight, weekly weight gain, FCR and survival.

2.2.3 Chemical analyses

At the end of the 10-week trial, shrimp were harvested and freeze-dried. Shrimp and feeds were analyzed according to the Association of Official Analytical Chemists (AOAC, 1995). Moisture was determined by drying 2 g samples in an oven (Isotemp-Fisher Scientific, Waltham, USA) at 100°C for 16 h. Ash was obtained by incinerating 0.5 g samples (dried sample) in a muffle furnace (Isotemp-muffle furnace, Fisher Scientific, Waltham, USA) at 600°C for 2 h. Crude protein was determined from total Kjeldahl-N method by means of an automated spectrophotometric flow injection analyzer (FIAstartâ„ў5000, Hillerond, Denmark), using a multiplication factor of 6.25. Gross energy was measured by bomb calorimetry (IKA C 5000 control). Lipids were determined according to the Bligh and Dyer (1959) method. Total carbohydrate content was determined following the phenol sulphuric acid method. Amino acids as well as chemical composition of whole body were analyzed by the laboratory Maxxam Analytics, Burnaby, Canada. Proximate composition of practical diets is shown in Table 2.1.

Fatty acids (FA) were determined based on the one-step method described by Abdulkadir and Tsuchiya (2008). Fatty acid methyl esters (FAME) of diets and shrimp tissue were prepared in two replicates. Butylated hydroxytoluene (BHT) was added to the hexane solvent to prevent lipid oxidation. FAME were quantified using a Varian 3900 Gas Chromatograph (Toronto, Canada) with a Chrompack capillary column (CP-Sil 88 for FAME). Fatty acids were identified by comparison of retention times to those of known standards and they were quantified by comparing the peak area of individual FA in the sample with the peak of the internal standard (Nonadecanoic acid, C19:0).
2.2.4 Sensory evaluation

At harvest, Pacific white shrimp heads were removed and tails were refrigerated (4°C) overnight for sensory analysis the next day. Sensory analysis of shrimp tails was carried out at a commercial shrimp processing plant by a panel of seven expert judges, who conduct shrimp quality analysis on a daily basis. Shrimp tails were cooked for 1.5 min in boiling water, then refrigerated to stop the cooking process before serving (5°C).

Samples from each tank (5 treatments×4 replications) were presented to the judges in a pairwise manner, where each treatment was evaluated with every other treatment repeated four times. There was a total of 10 combinations among the five treatments (FM-37 with AS-37, ASS-48, ASS-34 and COM-38; AS-37 with ASS-48, ASS-34 and COM-38, ASS-48 with ASS-34 and COM-38 and ASS-34 with COM-38).

Shrimp tail samples were served for each treatment on white chinaware plates labeled according to their treatment code (1-5). A sensory ballot was given to each judge consisting of 9-point rating scales, one for each of the desirable (aroma, color, firmness, moistness, fattiness, sweetness) and undesirable (off-odor, fish flavor, earthiness, off-flavor, overall unpleasantness) sensory attributes. Judges evaluated shrimp based on the magnitude of sensory characteristics, relative to the anchor (i.e. reference value) provided (Table 2.8). Immediately following each testing, judges were given water and a piece of cracker to cleanse the palate. Sensory forms were collected at the end of the sensory evaluation.

Descriptive analysis was performed using a collection of six desirable (aroma, color, sweetness, firmness, moisture, fattiness) and five undesirable (off-odor, earthiness, off-flavor and overall unpleasantness) sensory attributes. All quality attributes were evaluated on 9-point structured rating scales, (Botta, 1995), anchored with references to delineate between acceptable
and unacceptable portions of the scale. The desirable attributes: aroma, color, sweetness, firmness, moisture, fattiness, were anchored at 3.0, 3.5, 5.0, 6.0, 6.0 and 4.0, respectively. All the undesirable attributes: off-odor, fish flavor, earthiness, off-flavor and overall pleasantness, were anchored at 1.0, with the exception of fish flavor, which had an anchor at 4.0. These scores were placed as anchors on the linescales, allowing each judge to score the samples relative to previously established reference shrimp values recognized as a point of reference.

### 2.2.5 Statistical analyses

Treatment effects for water quality and production parameters were evaluated using one-way analysis of variance (ANOVA). Significant differences were determined among the treatment means using Tukey test at $\alpha=0.05$. Sensory data were analyzed using 3-way ANOVA, with judge, treatment and replication as main effects and all 2-way interactions (judge $\times$ treatment, judge $\times$ replication, treatment $\times$ replication). These interaction effects were used to evaluate the consistency and reproducibility of the judges, prior to interpretation of the treatment effects (Garcia-Juarez, 2005).

An initial screening of the data revealed that a large portion of the data was missing for one judge; so these data were dropped from further analysis. In addition, preliminary analysis from another judge indicated that their scores were double that of the other judges and therefore these data were also dropped. In the end, data from five judges were analyzed.

Judges evaluated the 11 sensory attributes in the following order: aroma, off-odor, color, firmness, moistness, fattiness, fish flavor, sweetness, earthiness, off-flavor, and overall unpleasantness. For the purpose of interpretation, the attributes were grouped into two categories: desirable (aroma, color, sweetness, firmness, moisture, fattiness) and undesirable (off-odor, fish flavor, earthiness, off-flavor, overall unpleasantness) attributes.
The mean sensory ratings were determined for the desirable and undesirable attributes and compared using the Fischer least-significance-difference (LSD) at α=0.05. Since all the undesirable attributes had an anchor of 1.0, with the exception of fish flavor, sensory scores for fish flavor were re-scaled (i.e. sensory scores were multiplied by a constant) so that the values were equivalent to a scale of 1 through 9.

In addition, the inter-correlation of the sensory attributes was determined by calculating Pearson correlation coefficients (r) from the mean scores. Minitab 16 Version Software (state College PA, USA) was used to carry out the statistical analysis.

2.3 Results and discussion

2.3.1 Water quality

Water quality parameters were maintained at optimum levels for adequate growth and survival of shrimp (Table 2.2). However, ammonium (NH$_4^+$) levels in the tank holding shrimp fed ASS-48 presented higher values than all other groups with an average value of 1.52 mg/L. ASS-48 had the highest protein input (48.34%), which could have contributed to elevated ammonium levels in the water. According to Kureshy and Davis (2002), protein content and availability can affect water quality via shrimp nitrogen excretion. No differences existed among treatments for water quality parameters (P > 0.05).

2.3.2 Growth performance and production parameters

No evidence of disease was observed during the growth trial. Survival was 85-95% and did not differ among treatments (Table 2.3). The high survival rate during the growth trial indicated good health conditions of the shrimp. Based on visual observation of feed consumption using a feeding tray, there were no indicators of feed rejection or reduced palatability. Pacific
white shrimp accepted all of the test diets, demonstrating the palatability of the new ingredients. These observations are consistent with the findings of Ceballos et al. (2005), who reported the high attractiveness of Spirulina for Litopenaus schmitti. Moreover, our results are in line with the findings of Silva-Nieto et al. (2012), who reported that Spirulina meal is an efficient feeding attractant for Litopenaeus vannamei.

Production parameters of Pacific white shrimp are presented in Table 2.3. Shrimp weight increased from 465.7% to 849.9%, relative to initial values. Groups fed FM-37 and COM-36 had higher final weight and weekly weight gain mean values as opposed to mean values for groups fed AS-37, ASS-34, and ASS-48. The average weekly growth rate was 1.15 g for group FM-37, 1.02 g for AS-37, 0.77 g for ASS-48, 0.97 g for ASS-34 and 1.31 g for diet COM-36, indicating that shrimp maintained on the control diets (COM-36) and (FM-37) had the best growth rate. Diet FM-37 contained FM and FO and thus likely were the most digestible resulting in the higher growth performance and nutrient utilization in shrimp. FM is a good source of marine oils that are rich in highly unsaturated fatty acids (HUFA) (Turchini et al., 2010). Lipid content and the associated C18 polyunsaturated fatty acids (PUFA), linoleic (18:2n-6) and linolenic (18:3n-3) as well as n-3 and n-6 HUFA (EPA, DHA, ARA,), are required in shrimp and other crustacean feeds for optimal growth performance (Fennuci et al., 1981; Kanazawa et al., 1997). The minimum requirement of these fatty acids in crustacean feeds is at levels between 5 and 10 g/kg (Akiyama et al., 1992; Gonzales-Felix et al., 2002).

Shrimp fed diets with substitution of FM with algae protein sources exhibited low final mean body weight values, and significant differences in weight among the treatments were observed (Table 2.3). Growth performance of groups fed AS-37, and ASS-34, had the highest mean weight values of the algae based feeds (Figure 2.1). This latter finding could be attributed
to the beneficial properties of DHA from *Schizochytrium* of AS-37 and the combination of *Spirulina* and *Schizochytrium* of ASS-34. The lowest final weight mean value was found in shrimp fed the ASS-48 treatment. It is possible that low performance was affected by a low digestible energy level, which would cause shrimp to utilize protein as a source of energy (Kureshy and Davis, 2002). These latter authors studied the protein requirement for maintenance and maximum weight gain for the Pacific white shrimp by utilizing three practical diets (16%, 32% and 48% dietary protein) and found that the diet containing 48% protein resulted in lower weight gains in juvenile shrimp. Juvenile and sub-adult shrimp exhibited better growth performance when fed the 32% protein diet.

Similarly, Hu *et al.* (2008) investigated the effects of dietary protein to energy ratios on growth rates of juvenile white shrimp and found that protein efficiency decreased with increasing dietary protein from 340 to 420 g/kg, indicating that the surplus protein was degraded rather than used for growth. Usually, low levels of dietary protein are efficiently used for protein synthesis by shrimp. In the present study we speculate that there was an inefficient conversion of protein to growth in the shrimp fed the AM-48 diet due to the continued catabolism of protein in the absence of any protein sparing effect at high protein levels.

The low growth response of shrimp fed ASS-48 may also have been due to reduced digestibility of the diet. Ammonium needs to be maintained at acceptable levels (below 2.3 mg/L (0.09 mg/L for NH₃; Chen *et al.*, 1990) as build-up in shrimp culture systems has been shown to reduce growth performance (Shilo and Rimon 1982). Although this research did not measure NH₃ in the water directly, we could assume that NH₄⁺ concentrations was less than 2.28 mg/L. Our NH₄⁺ concentrations for tanks assigned treatment ASS-48 ranged between 0.0025-4.53 mg/L and had the highest mean value compared to the other treatments (Table 2.2). This may indicate that
there was an excess of nitrogen in the diet, which resulted in high nitrogen excretion as shrimp used excess protein for energy. Research has shown that if protein is in excess relative to energy, excessive dietary protein will be used for energy, resulting in higher Specific Dynamic Action (SDA) and more excreted ammonia nitrogen (Cho et al. (1994); Samantaray and Mohanty (1997), Mathis et al. (2003) and Buffordet al. (2004)). Further research on shrimp diets that contain algae ingredients is needed to investigate nutrient digestibility and establish protein requirements based on alternative ingredients.

Growth performance and feed utilization parameters were affected by dietary treatments (Table 2.3). In the present study FCR ranged between 1.6 and 2.5. Ingested protein was more efficiently utilized for growth by shrimp fed FM-37 or COM-36 (P < 0.05), which had the best FCR (Table 2.3). Conversely, high FCR were observed in treatments fed AS-37, ASS-48 and ASS-34. Groups fed ASS-48 exhibited the highest FCR due most likely to the continued catabolism of protein for energy. Although it is generally accepted that fish and shrimp do not have a specific requirement for dietary carbohydrates, they are widely used in commercial aquaculture feeds. Numerous studies have demonstrated that carbohydrates must be supplied in available form in the diet. The nutritional composition of diet ASS-48 had the lowest carbohydrate levels compared to the rest of the diets (Table 2.1). This low carbohydrate level in diet ASS-48 may have been insufficient to meet the energy requirements of the shrimp. Thus, shrimp possibly converted part of the dietary protein to energy. In turn, this increased protein degradation and increased energy expenditure and ammonium release into the water. Using protein as an energy source is relatively inefficient and reduces the amount of protein available for tissue deposition (Hochachka, 1991).
Overall, the experimental diets met the essential lysine (1.6%) and methionine (0.9%) requirements on an ad libitum basis typical for adequate growth of penaeids (Millamena et al., 1996). Some nutritional requirements of *Litopenaeus vannamei* are known. However, amino acid requirements are more widely established for *Penaeus monodon*.

Millamena et al. (1998) and Xie et al. (2012) claimed that the optimum dietary lysine requirement is 2.0% based on specific growth rates. Fox et al. (1995) reported that the optimal lysine requirement for juvenile *Litopenaeus vannamei* is 1.2-2.3%. Lysine is well known to be an indispensable amino acid for normal growth of shrimp (Cowey and Forster, 1971; Fox et al., 1995; Kanazawa and Teshima, 1981; Richard et al., 2010). Appropriate lysine content could reduce the oxidation of other amino acids by improving the use of other indispensable amino acids (Kerr and Easter, 1995), by promoting a higher growth rate (Xie et al., 2012).

Lysine, arginine and methionine are considered the most limiting indispensable amino acids (IAA) for shrimp in commercial feed formulations (Akiyama et al., 1991). In the present study, lysine levels were below the optimum for the experimental diets AS-37, and ASS-34, which could represent a dietary deficiency that may have led to lower growth and inferior feed efficiency. Methionine and arginine levels were somewhat different from the optimum requirement (Akiyama et al., 1991) (Table 2.4).

Some amino acids (i.e. threonine, arginine, histidine, isoleucine and leucine) were present in diets at concentrations higher than the AA requirements for shrimp proposed by (Millamena et al., 1999) (Table 2.4). Diet ASS-48 exhibited an excess of the optimum requirement of some IAA, which in turn may have adversely affected shrimp growth. Generally, weight gains of shrimp tend to decrease when required amino acid levels are exceeded (Millamena et al., 1999). The reason for the decline in weight gain has not been established, but may be a response to the
shrimp’s intolerance to excess amino acids beyond the optimum dietary level (Mertz 1972). The dietary lipid levels were in the optimum range (5-10%) for adequate growth of white shrimp as reported by Siccardi (2006). The inclusion of DHA in diet AS-37% produced the best growth performance in shrimp out of all the algae based diets, demonstrating the high potential of algal oils as fish oil substitute in aquaculture feeds.

To summarize, shrimp fed the commercial control diet COM-36 had the highest weight gain; whereas, the shrimp fed algae diets had lower weight gains. It is possible that reduced availability of IAA via leaching could have reduced growth in shrimp fed the algae experimental diets. According to Millamena (1996), the feeding behavior of shrimp results in these animals being slow continuous feeders; hence, if feeds are not water stable (i.e. do not retain pellet physical integrity with minimal disintegration and nutrient leaching), crystalline amino acids are quickly leached out and may no longer be present when the feed is consumed. However, we recognize that our laboratory made feeds may not have the same physical properties of commercially extruded manufactured feeds.

2.3.3 Chemical composition of shrimp body

Body chemical composition of shrimp is shown in Table 2.5 and was similar across all treatments. Fatty acid composition of shrimp tails reflected the fatty acid profile of the experimental diets qualitatively (Table 2.6). The addition of Schizochitrium and Spirulina to the experimental diets, in general, did not affect the fatty acid composition of shrimp tails (Table 2.7). Particularly, no differences (P > 0.05) were detected in the content of three essential fatty acids (DHA, EPA and ARA) in the tail of shrimps fed algae based diet, the control diets. Therefore, the HUFA contents did not differ between treatment groups. Total n-3 and n-6 ratios observed in shrimp tissue ranged from 0.61 to 0.72.
Given that fish oil was replaced with alternative oils, it is extremely important to ensure that an animal’s essential fatty acid requirements are met. Our results indicate that the use of *Schizochitrium* and *Spirulina* ingredients contribute to optimal HUFA contents in shrimp tissue, comparable to that of fish oil. Shrimp tails from groups fed algae based feeds exhibited comparable HUFA contents, and high n-3/n-6 ratios in relation to shrimp tails from groups fed fish meal based feeds, which are targeted in seafood production and recommended in the human diet to promote health. Patnaik *et al.* (2006) demonstrated that fish oil can be successfully replaced in diets for *Litopenaeus vannamei* using cells of heterotrophic algae *Schizochytrium* sp. and *Mortierella* sp. obtained from commercial microbial fermentation.

The results of this study reaffirm that *Schizochytrium* is a suitable fish oil substitute (HUFA source) in diets fed to *Litopenaeus vannamei*. However, our results demonstrate that fatty acids cannot be the sole consideration in nutritional studies. In spite of the balanced fatty acid composition of shrimp fed the experimental diets (algae based), the inclusion of algae ingredients did not result in improvements in growth performance. Digestibility studies are needed in order to better understand the metabolic processes that might influence the efficacy of including algae ingredients in shrimp diets. In addition, features of algae feeds, such as water stability, need to be optimized to prevent nutrients from leaching out that would compromise growth performance.

### 2.3.4 Sensory evaluation

The results of sensory evaluation of shrimp tails from *Litopenaeus vannamei* are shown in Table 2.8. The desirable attributes of aroma, color and sweetness differed between treatments (P ≤ 0.05). In contrast, the remaining desirable attributes (firmness, moistness, fattiness) did not exhibit statistical differences (P > 0.05). For the undesirable attributes (off-odor, fish flavor, earthiness, off-flavor and overall unpleasantness) no differences were detected.
Judges were a significant source of variation in all cases (P ≤ 0.05) (Table 2.9). Judge variations are normally attributed to individual taste and scoring differences. Judge-to-judge variation is accepted in sensory evaluation, however, judge consistency as reflected by the interaction (Judge x Sample) is considered more critical (Cliff et al., 1996). In this study, there were differences for the interaction judge x sample for the attributes of aroma and color, suggesting judge inconsistency. It is possible that these terms were not used in the same way by all judges. Nonetheless, F-values were recalculated and new values indicated that in spite of some inconsistency found among the interactions judge x sample for color and aroma, the samples did differ in these two attributes. On the other hand, the interaction judge x replication did not exhibit differences (P > 0.05) which demonstrates reproducibility. Reproducibility indicates how an individual agrees on average with the panel as a whole, showing the homogeneity of the team (Ross, 2001).

Overall the cooked shrimp tail of the groups fed the experimental diets in comparison with the commercial control had a mild aroma (\(\bar{x}=2.65-3.55\)) but no off-odors (\(\bar{x}=1.30-1.60\)). The shrimp muscle was light orange in color (\(\bar{x}=3.00-4.02\)). The texture was of medium firmness (\(\bar{x}=4.65-5.30\)), moistness (\(\bar{x}=5.15-6.05\)), and fattiness (\(\bar{x}=2.95-3.65\)). Flavor wise, shrimp taste had a mild fish flavor (\(\bar{x}=4.50-4.70\)), medium sweetness (\(\bar{x}=3.80-5.15\)). It had almost no earthiness (\(\bar{x}=2.00-2.40\)), was considered to be nearly free of other tastes (\(\bar{x}=1.35-1.95\)), and had low scores for overall unpleasantness (\(\bar{x}=2.95-3.0\)). Desirable attributes including fattiness, firmness and moistness were comparable between shrimp fed the algae diets and the control feeds. These results are somewhat similar to the findings for *Litopenaus vannamei* of Forster et al. (2011), who failed to show differences for these sensory characteristics with dietary inclusion of plant-derived sources (i.e. SBO). In contrast, the attributes aroma, color and
sweetness were different \((P < 0.05)\) between shrimp fed the various diets (Figure 2.2). Shrimp fed FM-37 and COM-36 were considered to be sweeter than AS-34 and ASS-48, which did not differ from each other. In contrast, shrimp fed AS-37 were less sweet than FM-37, but identical in sweetness intensity to the shrimp fed the remaining diets (COM-36, ASS-34, ASS-48). Shrimp fed COM-36 had higher aroma intensity than FM-37 and ASS-34. Also, shrimp fed AS-37 exhibited a stronger aroma than ASS-34. Shrimp fed ASS-48 did not differ from the other diets. Lastly, shrimp fed AS-37, FM-37 and COM-36 did not differ in color. Shrimp fed AS-37 were also similar in color to ASS-34 and ASS-48, which did not differ.

For the undesirable attributes of off-odor, fish flavor, off flavor, earthiness and overall unpleasantness, statistical differences were not identified. However, it is noteworthy that shrimp fed AS-37 exhibited the lowest scores for these attributes, suggesting that judges had a stronger positive sensory experience with this group of shrimp in relation to groups fed the remaining diets (Figure 2.3). The correlation coefficients of sensory attributes are shown in Table 2.10. As expected, firmness and fattiness were highly correlated \((r = 0.92)\). Similarly, perceived fish flavor and overall unpleasantness were correlated \((r = 0.97)\). According to Erickson et al. (2007), shrimp in general are considered to have a mild but distinctive flavor with a texture described as tender and delicate. In this study, mean scores revealed that shrimp were perceived as mild in flavor. Color and sweetness were also correlated \((r = 0.96)\), as well as moistness and earthiness \((r = 0.93)\). The undesirable attributes of fish flavor, off-flavor and overall unpleasantness were negatively correlated with aroma \((r = -0.92, -0.95, -0.97)\), respectively.

Above all, the quality of the shrimp was acceptable based on a reference standard, and dietary inclusion of algae ingredients did not have a negative influence on most sensory characteristics of shrimp muscle. Remarkably, shrimp fed AS-37 were perceived at the lowest
scores for undesirable attributes. For instance diet AS-37 showed greater promise. Our results can provide insight into further designs for adequate algae diets to produce shrimp with more desirable sensory characteristics.

The results of the present study suggest that FM replacement with *Spirulina* and *Schizochytrium* is promising. Although algae diets did not yield improved growth performance in shrimp compared with the reference diet, they could be a key contributor to reduce the use of FM in shrimp feeds. Algae diets showed to be a rich source of HUFA, and shrimp were able retain high levels of HUFA in muscle tissue. Remarkably, diet AS-37 showed great potential as an alternative feed source for *Litopenaeus vannamei*, indicating satisfactory shrimp quality. In the current research, we investigated if Pacific white shrimp can accommodate the algal meals in their feeds. Our findings show that algae meals did not have a negative influence on most sensory attributes, hence when considering consumer acceptance, the inclusion of algae ingredients in shrimp feeds could be commercially viable. Further studies on digestibility of nutrients should be considered when formulating algae meals for their optimization. Another important aspect to consider is the feed processing technique and addition of supplements in order to increase water stability in algae feeds.

### 2.4 Tables

Table 1.1. General composition of different algae (% of dry matter).
<table>
<thead>
<tr>
<th>Alga</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabaena cylindrica</td>
<td>43-56</td>
<td>25-30</td>
<td>4-7</td>
</tr>
<tr>
<td>Aphanizomenon flos-aquae</td>
<td>62</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Chlanydomonas</td>
<td>48</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>rheinhardii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td>57</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>51-58</td>
<td>12-17</td>
<td>14-22</td>
</tr>
<tr>
<td>Dunaliella salina</td>
<td>57</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Euglena gracilis</td>
<td>39-61</td>
<td>14-18</td>
<td>14-20</td>
</tr>
<tr>
<td>Porphyridium</td>
<td>28-39</td>
<td>40-57</td>
<td>9-14</td>
</tr>
<tr>
<td>cruentum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenedesmus obliquus</td>
<td>50-56</td>
<td>10-17</td>
<td>12-14</td>
</tr>
<tr>
<td>Spirogyra sp.</td>
<td>6-20</td>
<td>33-64</td>
<td>11-21</td>
</tr>
<tr>
<td>Arthrosira maxima</td>
<td>60-71</td>
<td>13-16</td>
<td>6-7</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>46-63</td>
<td>8-14</td>
<td>4-9</td>
</tr>
<tr>
<td>Synechococcus sp.</td>
<td>63</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2.1. Nutritional composition of practical diets for *Litopenaeus vannamei*. KJ: Kilojoules/100g. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37
(Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Component</th>
<th>FM-37</th>
<th>AS-37</th>
<th>ASS-48</th>
<th>ASS-34</th>
<th>COM-36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>kj</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>%</td>
<td>37.40</td>
<td>38.30</td>
<td>28.10</td>
<td>42.58</td>
</tr>
<tr>
<td>Protein</td>
<td>%</td>
<td>37.26</td>
<td>37.13</td>
<td>48.34</td>
<td>34.10</td>
</tr>
<tr>
<td>Lipid</td>
<td>%</td>
<td>8.60</td>
<td>8.00</td>
<td>8.40</td>
<td>8.33</td>
</tr>
<tr>
<td>Moisture</td>
<td>%</td>
<td>9.00</td>
<td>10.00</td>
<td>6.30</td>
<td>8.40</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>7.80</td>
<td>6.50</td>
<td>8.80</td>
<td>6.50</td>
</tr>
</tbody>
</table>
Table 2.2. Average of water quality parameters during the 10-week culture period for *Litopenaeus vannamei*. Values are means ± SD. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Value of Water Quality Parameter</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM-37</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>am</td>
<td>28.83 ± 0.76</td>
</tr>
<tr>
<td>pm</td>
<td>30.62 ± 0.99</td>
</tr>
<tr>
<td>Oxygen (mg/L)</td>
<td></td>
</tr>
<tr>
<td>am</td>
<td>5.71 ± 0.62</td>
</tr>
<tr>
<td>pm</td>
<td>5.64 ± 0.62</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>am</td>
<td>8.17 ± 0.19</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>38.6 ± 1.45</td>
</tr>
<tr>
<td>Ammonium (mg/L)</td>
<td>0.63 ± 0.07</td>
</tr>
</tbody>
</table>
Table 2.3. Average production parameters at the end of a 10-week culture period for *Litopenaeus Vannamei* fed practical diets with varying levels of algae meal and fish meal diets\(^1\). Values are means ± SD. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM-37</td>
<td>AS-37</td>
<td>ASS-48</td>
<td>ASS-34</td>
<td>COM-36</td>
<td>P-Value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight (g)</td>
<td>1.72±0.03</td>
<td>1.65±0.07</td>
<td>1.60±0.10</td>
<td>1.6±0.04</td>
<td>1.71±0.09</td>
<td>P&gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight (g)</td>
<td>13.40±0.31(^b)</td>
<td>12.03±0.77(^c)</td>
<td>9.41±0.50(^d)</td>
<td>11.47±0.25(^c)</td>
<td>14.95±0.67(^a)</td>
<td>P≤0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weekly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight gain (g)</td>
<td>1.15±0.03(^b)</td>
<td>1.02±0.08(^c)</td>
<td>0.77±0.05(^d)</td>
<td>0.97±0.0(^c)</td>
<td>1.31±0.07(^a)</td>
<td>P≤0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCR</td>
<td>1.67±0.05(^c)</td>
<td>2.04±0.17(^b)</td>
<td>2.50±0.13(^a)</td>
<td>2.04±0.07(^b)</td>
<td>1.60±0.06(^c)</td>
<td>P≤0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>95</td>
<td>87</td>
<td>85</td>
<td>89</td>
<td>85</td>
<td>P&gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Means within rows that do not share the same letter are significantly different (Tukey, alpha = 0.05). FCR, feed conversion ratio.
Table 2.4. Amino acid composition of practical diets for *Litopenaeus vannamei* (%). FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%).

| Indispensable amino acids | FM-37 | AS-37 | ASS-48 | ASS-34 | Level

| Valine       | 1.33  | 1.71  | 2.19   | 1.56   | -
| Threonine    | 1.23  | 1.61  | 2.26   | 1.46   | 1.40
| Methionine   | 0.68  | 0.62  | 0.95   | 0.58   | 0.90
| Isoleucine   | 1.06  | 1.49  | 1.92   | 1.32   | 1.00
| Leucine      | 2.23  | 2.74  | 3.59   | 2.52   | 1.70
| Phenylalanine| 1.37  | 1.64  | 2.01   | 1.48   | 1.40
| Hsithididine | 1.04  | 0.96  | 1.22   | 0.90   | 0.80
| Lysine       | 1.84  | 1.57  | 2.03   | 1.46   | 2.10
| Arginine     | 1.77  | 2.19  | 2.86   | 2.07   | 1.80
<p>| Tryptophan   | 0.29  | 0.36  | 0.49   | 0.38   | 0.2 |</p>
<table>
<thead>
<tr>
<th>Non-indispensable aminoacids</th>
<th>Diet</th>
<th>Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>1.61</td>
<td>2.24</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.51</td>
<td>2.97</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.41</td>
<td>0.45</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.90</td>
<td>1.75</td>
</tr>
<tr>
<td>Proline</td>
<td>2.36</td>
<td>2.54</td>
</tr>
<tr>
<td>Serine</td>
<td>1.53</td>
<td>2.39</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6.56</td>
<td>7.22</td>
</tr>
</tbody>
</table>

1Amino acid composition of diets were analyzed by a commercial laboratory (Maxxam, Canada)

2Values recommended by Millanema et al. (1999)

- Values not reported
Table 2.5. Whole body chemical composition of *Litopenaeus vannamei* fed various diets\(^1\). KJ: Kilojoules/100g. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Component</th>
<th>Diet</th>
<th>FM-37</th>
<th>AS-37</th>
<th>ASS-48</th>
<th>ASS-34</th>
<th>COM-36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>kj</td>
<td>18.19</td>
<td>15.20</td>
<td>16.76</td>
<td>16.53</td>
<td>17.27</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>%</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.30</td>
</tr>
<tr>
<td>Protein</td>
<td>%</td>
<td>22.21</td>
<td>22.65</td>
<td>22.70</td>
<td>23.25</td>
<td>23.11</td>
</tr>
<tr>
<td>Lipid</td>
<td>%</td>
<td>0.63</td>
<td>0.57</td>
<td>0.39</td>
<td>0.82</td>
<td>0.80</td>
</tr>
<tr>
<td>Moisture</td>
<td>%</td>
<td>75.10</td>
<td>75.00</td>
<td>75.10</td>
<td>74.4</td>
<td>74.0</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>1.90</td>
<td>1.90</td>
<td>2.00</td>
<td>1.90</td>
<td>1.80</td>
</tr>
</tbody>
</table>

\(^1\)Whole body chemical composition of shrimp analyzed by a commercial laboratory (Maxxam, Canada)

\(^2\)Not detected
Table 2.6. Fatty acid composition (% of FAME) of diets<sup>1</sup> FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Selected FA</th>
<th>FM-37</th>
<th>AS-37</th>
<th>ASS-48</th>
<th>ASS-34</th>
<th>COM-36</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>7.60</td>
<td>3.13</td>
<td>6.37</td>
<td>9.51</td>
<td>7.92</td>
</tr>
<tr>
<td>16:0</td>
<td>29.18</td>
<td>40.32</td>
<td>43.82</td>
<td>41.69</td>
<td>30.44</td>
</tr>
<tr>
<td>16:1</td>
<td>6.86</td>
<td>2.93</td>
<td>3.37</td>
<td>1.82</td>
<td>4.63</td>
</tr>
<tr>
<td>18:0</td>
<td>3.50</td>
<td>1.86</td>
<td>1.35</td>
<td>1.39</td>
<td>3.77</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>15.32</td>
<td>9.37</td>
<td>4.53</td>
<td>5.14</td>
<td>11.87</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>8.08</td>
<td>10.32</td>
<td>7.05</td>
<td>7.49</td>
<td>11.12</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>3.87</td>
<td>1.77</td>
<td>0.98</td>
<td>1.50</td>
<td>3.13</td>
</tr>
<tr>
<td>20:3n-3</td>
<td>0.63</td>
<td>-&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.18</td>
<td>0.32</td>
<td>0.44</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.77</td>
<td>-</td>
<td>0.25</td>
<td>0.44</td>
<td>0.10</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.30</td>
<td>0.08</td>
<td>0.15</td>
<td>0.30</td>
<td>0.14</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.12</td>
<td>0.20</td>
<td>0.12</td>
<td>0.23</td>
<td>0.10</td>
</tr>
<tr>
<td>22:0</td>
<td>0.24</td>
<td>0.29</td>
<td>0.23</td>
<td>0.30</td>
<td>0.32</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>4.38</td>
<td>2.62</td>
<td>4.31</td>
<td>8.76</td>
<td>2.86</td>
</tr>
<tr>
<td>24:0</td>
<td>4.64</td>
<td>0.20</td>
<td>0.32</td>
<td>0.61</td>
<td>4.41</td>
</tr>
<tr>
<td>Saturates&lt;sup&gt;4&lt;/sup&gt;</td>
<td>45.15</td>
<td>45.8</td>
<td>52.10</td>
<td>53.51</td>
<td>46.86</td>
</tr>
<tr>
<td>Monounstaturates&lt;sup&gt;4&lt;/sup&gt;</td>
<td>22.18</td>
<td>12.3</td>
<td>7.90</td>
<td>6.96</td>
<td>16.50</td>
</tr>
<tr>
<td>PUFA&lt;sup&gt;5&lt;/sup&gt;</td>
<td>13.34</td>
<td>12.09</td>
<td>8.47</td>
<td>9.75</td>
<td>14.79</td>
</tr>
<tr>
<td>Selected FA</td>
<td>FM-37</td>
<td>AS-37</td>
<td>ASS-48</td>
<td>ASS-34</td>
<td>COM-36</td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>HUFA⁶</td>
<td>4.80</td>
<td>2.90</td>
<td>4.58</td>
<td>9.29</td>
<td>3.10</td>
</tr>
<tr>
<td>Total n-3</td>
<td>9.00</td>
<td>4.59</td>
<td>5.59</td>
<td>10.80</td>
<td>6.53</td>
</tr>
<tr>
<td>Total n-6</td>
<td>9.14</td>
<td>10.40</td>
<td>7.45</td>
<td>8.23</td>
<td>11.36</td>
</tr>
</tbody>
</table>

¹Values represent averages of duplicate samples.

²Not detected.

³Saturates: 14:0, 16:0, 18:0, 22:0, 24:0.

⁴Monosaturates: 16:1, 18:1.

⁵PUFA: 18:2, 18:3, 20:3.


⁸Total n-6: 18:2n-6, 20:3n-6, 20:4n-6
Table 2.7. Fatty acid composition (% FAME) in shrimp muscle tissue. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Selected FA</th>
<th>FM-37</th>
<th>AS-37</th>
<th>ASS-48</th>
<th>ASS-34</th>
<th>COM-36</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>3.16</td>
<td>2.47</td>
<td>2.96</td>
<td>2.09</td>
<td>2.41</td>
</tr>
<tr>
<td>16:0</td>
<td>23.66</td>
<td>26.56</td>
<td>27.08</td>
<td>22.73</td>
<td>24.14</td>
</tr>
<tr>
<td>16:1</td>
<td>1.70</td>
<td>1.01</td>
<td>1.69</td>
<td>1.92</td>
<td>1.51</td>
</tr>
<tr>
<td>18:0</td>
<td>9.69</td>
<td>8.00</td>
<td>8.53</td>
<td>14.05</td>
<td>11.19</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>10.78(^a)</td>
<td>7.32(^{bc})</td>
<td>5.35(^c)</td>
<td>1.89(^d)</td>
<td>9.73(^{ab})</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>14.11(^{ab})</td>
<td>14.57(^{ab})</td>
<td>16.94(^a)</td>
<td>13.2(^{ab})</td>
<td>11.19(^{b})</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>1.27(^a)</td>
<td>0.51(^c)</td>
<td>0.58(^{bc})</td>
<td>0.46(^c)</td>
<td>0.91(^b)</td>
</tr>
<tr>
<td>20:3n-3</td>
<td>2.0(^b)</td>
<td>3.47(^{ab})</td>
<td>4.06(^a)</td>
<td>2.87(^{ab})</td>
<td>1.98(^b)</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.13</td>
<td>0.10</td>
<td>-(^2)</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.14</td>
<td>0.13</td>
<td>0.14</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.11</td>
<td>0.09</td>
<td>0.14</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>22:0</td>
<td>0.53</td>
<td>0.43</td>
<td>0.60</td>
<td>0.77</td>
<td>0.45</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>6.99</td>
<td>4.88</td>
<td>5.70</td>
<td>5.28</td>
<td>5.36</td>
</tr>
<tr>
<td>24:0</td>
<td>8.72(^{ab})</td>
<td>3.83(^c)</td>
<td>4.48(^{bc})</td>
<td>3.61(^c)</td>
<td>9.67(^a)</td>
</tr>
<tr>
<td>Saturates(^3)</td>
<td>45.77</td>
<td>41.29</td>
<td>43.65</td>
<td>43.26</td>
<td>47.85</td>
</tr>
<tr>
<td>Monounstaturates(^4)</td>
<td>12.48(^a)</td>
<td>8.33(^{bc})</td>
<td>7.05(^{cd})</td>
<td>3.81(^d)</td>
<td>11.24(^{ab})</td>
</tr>
<tr>
<td>Selected FA</td>
<td>FM-37</td>
<td>AS-37</td>
<td>ASS-48</td>
<td>ASS-34</td>
<td>COM-36</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>PUFA³</td>
<td>17.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HUFA⁶</td>
<td>7.25</td>
<td>5.25</td>
<td>5.99</td>
<td>5.64</td>
<td>5.59</td>
</tr>
<tr>
<td>Total n-3⁷</td>
<td>10.37</td>
<td>9.11</td>
<td>10.49</td>
<td>8.75</td>
<td>8.34</td>
</tr>
<tr>
<td>Total n-6⁸</td>
<td>14.37</td>
<td>14.8</td>
<td>17.09</td>
<td>13.48</td>
<td>11.44</td>
</tr>
</tbody>
</table>

³⁸See footnotes in Table 6.

¹Values represent averages of duplicate samples. Means within rows that do not share letter are significantly different (Tukey, alpha= 0.05).
Table 2.8. Mean sensory characteristics (n=20; 5 judge ×4 replications) of shrimp at the end of the feeding trial. Values are means ± SE mean. R represents the standard expected value for each attribute. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%).

<table>
<thead>
<tr>
<th>Sensory Attribute</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Numerical anchors on scale), reference anchor (R)</td>
<td>FM-37</td>
</tr>
<tr>
<td>Aroma</td>
<td>2.84 ± 0.23</td>
</tr>
<tr>
<td>(1: mild, 9: strong)</td>
<td>R: 3</td>
</tr>
<tr>
<td>Off-odor</td>
<td>1.250 ± 0.23</td>
</tr>
<tr>
<td>(1: none, 9: strong)</td>
<td>R: 1</td>
</tr>
<tr>
<td>Color</td>
<td>4.05 ± 0.26</td>
</tr>
<tr>
<td>(1: light, 9: dark)</td>
<td>R: 3.5</td>
</tr>
<tr>
<td>Firmness</td>
<td>5.16 ± 0.24</td>
</tr>
<tr>
<td>(1: tender, 9: firm)</td>
<td>R: 6</td>
</tr>
<tr>
<td>Moistness</td>
<td>6.01 ± 0.28</td>
</tr>
<tr>
<td>(1: dry, 9: moist)</td>
<td>R: 6</td>
</tr>
<tr>
<td>Sensory Attribute</td>
<td>Diet</td>
</tr>
<tr>
<td>-------------------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>FM-37</td>
</tr>
<tr>
<td>Fattiness (1: lean, 9: fatty)</td>
<td>R: 4</td>
</tr>
<tr>
<td>Fish Flavor (1: mild, 6: strong)</td>
<td>R: 4</td>
</tr>
<tr>
<td>Sweetness (1: none, 9: strong)</td>
<td>R: 5</td>
</tr>
<tr>
<td>Earthiness (1: none, 9: earthy)</td>
<td>R: 1</td>
</tr>
<tr>
<td>Off-flavor (1: none, 9: strong)</td>
<td>R: 1</td>
</tr>
<tr>
<td>Overall unpleasantness (1: none, 9: strong)</td>
<td>R: 1</td>
</tr>
</tbody>
</table>
Table 2.9. Analysis of variance of sensory characteristics ratings (5 judges): degrees of freedom (df), F-ratios, and error mean squares (MSE). J*S: Judge and sample interaction. J*R: Judge and replicate interaction. S*R: Sample and replicate interaction.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>F-ratios</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Judges</td>
<td>Sample</td>
<td>Replicates</td>
<td>J*S</td>
<td>J*R</td>
<td>S*R</td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Aroma</td>
<td></td>
<td>6.23*</td>
<td>2.94*</td>
<td>0.71</td>
<td>3.59*</td>
<td>1.64</td>
<td>0.82</td>
</tr>
<tr>
<td>Off-odor</td>
<td></td>
<td>8.89*</td>
<td>0.36</td>
<td>0.97</td>
<td>0.43</td>
<td>0.97</td>
<td>1.16</td>
</tr>
<tr>
<td>Color</td>
<td></td>
<td>11.24*</td>
<td>3.59*</td>
<td>1.42</td>
<td>2.35*</td>
<td>1.14</td>
<td>1.07</td>
</tr>
<tr>
<td>Firmness</td>
<td></td>
<td>22.2*</td>
<td>1.20</td>
<td>1.62</td>
<td>1.83</td>
<td>1.16</td>
<td>0.63</td>
</tr>
<tr>
<td>Moistness</td>
<td></td>
<td>6.94*</td>
<td>1.64</td>
<td>0.62</td>
<td>1.08</td>
<td>1.08</td>
<td>1.48</td>
</tr>
<tr>
<td>Fattiness</td>
<td></td>
<td>13.97*</td>
<td>2.44</td>
<td>0.63</td>
<td>1.46</td>
<td>0.36</td>
<td>0.76</td>
</tr>
<tr>
<td>Fish flavor</td>
<td></td>
<td>2.72*</td>
<td>1.08</td>
<td>0.84</td>
<td>2.28</td>
<td>0.91</td>
<td>1.10</td>
</tr>
<tr>
<td>Sweetness</td>
<td></td>
<td>10.17*</td>
<td>5.76*</td>
<td>0.55</td>
<td>1.83</td>
<td>1.32</td>
<td>1.36</td>
</tr>
<tr>
<td>Earthiness</td>
<td></td>
<td>157.52*</td>
<td>0.86</td>
<td>0.05</td>
<td>1.46</td>
<td>0.05</td>
<td>1.17</td>
</tr>
<tr>
<td>Off-flavor</td>
<td></td>
<td>10.64*</td>
<td>1.74</td>
<td>0.43</td>
<td>0.75</td>
<td>0.73</td>
<td>0.62</td>
</tr>
<tr>
<td>Overall unpleasantness</td>
<td>73.1*</td>
<td>0.18</td>
<td>0.38</td>
<td>1.38</td>
<td>0.27</td>
<td>1.29</td>
<td>1.55</td>
</tr>
</tbody>
</table>

| Df | 4  | 4  | 3  | 16 | 12 | 12 | 48 |

* Values within each row that are significantly different at $P \leq 0.05$
Table 2.10. Correlation matrix among the sensory attributes (df=3)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Aroma</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Off-odor</td>
<td>-0.36</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.Color</td>
<td>0.39</td>
<td>-0.93*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.Firmness</td>
<td>-0.43</td>
<td>-0.12</td>
<td>0.08</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.Moistness</td>
<td>-0.88</td>
<td>-0.02</td>
<td>-0.06</td>
<td>0.83</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.Fattiness</td>
<td>-0.43</td>
<td>-0.10</td>
<td>0.21</td>
<td>0.92*</td>
<td>0.79</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.Fish flavor</td>
<td>-0.92*</td>
<td>0.33</td>
<td>-0.23</td>
<td>0.58</td>
<td>0.88*</td>
<td>0.69</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.Sweetness</td>
<td>0.15</td>
<td>-0.85</td>
<td>0.96*</td>
<td>0.15</td>
<td>0.13</td>
<td>0.33</td>
<td>0.02</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.Earthiness</td>
<td>-0.81</td>
<td>-0.04</td>
<td>0.12</td>
<td>0.69</td>
<td>0.93*</td>
<td>0.80</td>
<td>0.93*</td>
<td>0.35</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.Off-flavor</td>
<td>-0.95*</td>
<td>0.17</td>
<td>-0.14</td>
<td>0.34</td>
<td>0.81</td>
<td>0.44</td>
<td>0.91*</td>
<td>0.13</td>
<td>0.88</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>11.Overall unpleasantness</td>
<td>-0.97*</td>
<td>0.39</td>
<td>-0.37</td>
<td>0.59</td>
<td>0.91*</td>
<td>0.61</td>
<td>0.97*</td>
<td>-0.14</td>
<td>0.87</td>
<td>0.91*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Values represent correlation at $p \leq 0.05$
Figure 2.1. Final body weight of Pacific white shrimp fed two control (FM-37; COM-36) and, three experimental (AS-37, ASS-48, ASS-34) diets reared under a green water system. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%). Error bars represent SD.
Figure 2.2. Mean scores for the sensory attributes aroma, color and sweetness. Bars represent aroma, color and sweetness of the various treatments. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: Spirulina at 37%); ASS-48 (Algae based feed, main protein sources: Spirulina and Schizochitrum at 48%); ASS-34 (Algae based feed, main protein sources: Spirulina and Schizochitrum at 34%) and COM -36 (Fish meal based feed, main protein source: fish meal at 36%). Treatments that do not share the same letter are significantly different at $P \leq 0.05$. Error bars represent SE (standard error).
Figure 2.3. Cobweb with descriptive profiles of undesirable attributes for *Litopenaeus vannamei* indicating the results of sensory tests where judges evaluated shrimp that had been reared on experimental and control diets. FM-37 (Fish meal based feed, main protein source: fish meal at 37%); AS-37 (Algae based feed, main protein sources: *Spirulina* at 37%); ASS-48 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 48%); ASS-34 (Algae based feed, main protein sources: *Spirulina* and *Schizochitrium* at 34%) and COM-36 (Fish meal based feed, main protein source: fish meal at 36%). The center of the figure designates low intensity. Intensity of each attribute increases with distance.
3 General discussion, limitations and future directions

3.1 Research contributions

This research undertaken in this dissertation shows that Pacific white shrimp can accommodate algae meals in their diets without compromising survival, fatty acid composition of muscle and final product quality.

My results indicate that the use of *Schizochitrium* and *Spirulina* ingredients contribute to optimal highly unsaturated fatty acid contents in shrimp tissue, key nutrients in human health. Therefore, when considering alternative ingredients for aquaculture feeds, highly unsaturated fatty acid levels in the tissue of farmed species needs to be as good or even better than levels obtained from species that are maintained on a fish oil diet. The results of this study support the findings of Patnaik *et al.* (2006) who reported that fish oil could be successfully replaced in the diet for *Litopenaeus vannamei* using cells of heterotrophic algae *Schizochytrium* sp. This research also adds to the understanding of how to effectively evaluate the effects of feed formulations on the organoleptic properties of shrimp by using a comprehensive statistical approach (3-factor ANOVA). This is a test that would be highly recommended for sensory evaluations of aquaculture products in order to gain insight into consumer acceptability and possible success in the market.

Ultimately, this study provides further information to the body of literature on alternative ingredients for aquaculture feeds. Microalgae has the potential to help reduce and replace the use of fish meal and fish oil in aquaculture feeds.
3.2 Approaches for the evaluation of sensory characteristics in aquaculture products using a three factor Analysis of Variance

This research also adds to the sensory evaluation field of aquaculture products. This study uses a 3-factor ANOVA, which is a more useful approach to interpret panel performance and gain insight into the sensory quality of aquaculture products.

Sensory assessments of aquaculture products have become a key component in studies where new feed formulations are used to evaluate ingredient alternatives that can replace traditional ones. Currently, the aquaculture feed industry is focused on identifying alternative protein and lipid sources (Kiron et al., 2012); key drivers being that sources are cost-effective, eco-friendly and yield excellent growth performance in farmed species. There is also a growing interest to understand the effects of feed formulation on the organoleptic properties of aquaculture products to ensure consumer acceptability and their success in the market.

There is a suite of methods available to describe sensory characteristics in food items (International Organization for Standardization, 2009). Historically this field of science has made use of one-way ANOVA tests to differentiate means for experimental treatments (Garduno-Lugo et al., 2007; Ganesan et al., 2005; Allan and Rowland, 2005; Diler and Gokoglu, 2004). While this approach is typically satisfactory for analytical/compositional variables, it does not provide insight into panel performance.

Another test frequently observed in the literature describing taste test results is the t-student test (Liang et al., 2008; Goulas et al., 2005; Hernandez et al., 2001). When this test is applied to more than two treatments, testing multiple pairs of means inflate the probability of committing a type I error (Whitlock and Schluter, 2009). Thus, in my study use of a 3-way ANOVA approach can be argued to be more applicable for sensory analysis as it allows us
To demonstrate panel performance (panel consistency and reproducibility). To our knowledge this is one of the first studies to show how a 3-way ANOVA can be used to interpret panel performance thus allowing for a better understanding of sensory differences of aquaculture products. As such the work could provide researchers, farm managers and extension agents, the skill to critically evaluate research and make appropriate decisions regarding the sensory quality of new and existing aquaculture products for release into the marketplace.

3.3 Research limitations and future directions

Some of the shortcomings encountered in this study were with regards to feed quality. Generally, the most important properties in feed quality are: feed digestibility, palatability, and water stability. These properties were not measured in this research, and these data could have been useful in explaining performance growth in shrimp. Feed digestibility is a term used to describe only the portion of the feed that is absorbed by the organism (Siccardi, 2006). According to Cordova-Mureta and Garcia-Carreno (2002), feed digestibility is affected by not only the source of the protein, but also the quantity in the diet. Although this property was not measured in this study, one could speculate that the digestibility (i.e. nutrient availability) of the proposed new ingredients, Schizochitrium and Spirulina, could have affected the quality of the feed, thereby influencing shrimp growth performance. Future studies of ingredient digestibility are encouraged as they can provide valuable information to optimize feed formulations and to better understand the future success or failure of incorporating algae ingredients in the practical diets of Pacific white shrimp. Moreover, the optimization of feed formulations can reduce costs and pollutant levels.

Ingredient palatability is defined as acceptable to the taste or sufficiently agreeable in flavor to be eaten (Glencross et al., 2007). While it was difficult to accurately determine the feed
intake rate in this study, owing to the green water system conditions, it is certainly possible that the palatability could have affected feed intake. If non-fish meal ingredients reduce feed intake, it would be of limited use in a feed formulation. Nonetheless, there are strategies to incorporate attractants to avert or resolve palatability issues of feed ingredients using ingredient processing or feeding stimulants (Glencross et al., 2007). Low food attractability is another factor that can lead to decreased feed consumption. Identifying the chemosensory stimuli, which shrimp normally find palatable may assist in improving ingestion of formulated feeds (Holland and Borski, 1993). Finding ways of increasing the rate of feeding in shrimp should also minimize leaching of feed nutrients caused by the animal’s slow feeding behavior (Peñaflorida and Virtanen, 1996). Artificial diets for crustaceans must be chemically attractive to induce their location and feeding, and the addition of small amounts of fish meal chemostimulants might increase ingestion rates and improve growth, survival, and food conversion (Carr, 1998). Future studies that involve testing alternative ingredients should take into account the level of attractability in order to ensure improved feed intake and food conversion.

Pellet water stability is an important quality parameter in the manufacture of aquaculture diets, especially for shrimp, due to their benthic, slow feeding habits, which make sinking water stable feed a necessity for shrimp. High pellet water stability is defined as the retention of pellet physical integrity with minimal disintegration and nutrient leaching while in the water until consumed by the animal. Pellets that break up into small particles and quickly leach nutrients could reduce water quality of the culture environment and lead to poor animal growth, inefficient feed conversion, and low survival (Obaldo et al., 2002). Water stability of experimental diets was not evaluated in this study and is therefore recommended for further investigation.
Lastly, the final quality of feeds depends on variables in the manufacturing process. In this study, the shortcomings of the feed manufacturing (laboratory made feeds) could have been a constraint. Pellet size was bigger than the control COM-36 and not homogeneous. Moreover, based on visual observation, the final product lacked water stability in comparison to the control COM-36. Individually (or collectively) these factors may have influenced the availability of nutrients in feeds via leaching, which could have led to lower growth performance. The pelletization process used in this study represented a challenge in terms of final feed quality. Laboratory made feeds commonly lack the manufacturing and extrusion advantages provided by commercial feeds (Amaya et al., 2007). Extrusion, due to its positive effects on the overall nutritional quality and water stability of replacement feeds (Davis and Arnold, 2000; Hernandez et al., 2004; Samocha et al., 2004), is a more appropriate and sophisticated method that is now widely adopted when producing aquaculture feeds (Carver et al., 1989).

Clearly, an important consideration for future research on alternative ingredients for aquaculture feeds is to give special attention to the engineering involved in feed processing that can lead to selecting the right equipment. This could produce high quality experimental diets, which would be more appropriate to compare to commercial references and therefore accurately evaluate the success of new ingredients such as algae.

3.4 Analysis of thesis work in future research directions

Replacing fish meal and fish oil with algae ingredients in shrimp feeds is promising. This research demonstrates that algae meals can be used in the diets of Pacific white shrimp without affecting their nutritional value. In addition, this study provides insight into evaluating the effect of alternative algae ingredients on the sensory attributes and overall quality of shrimp.

As aquaculture continues to expand, there will be an increasing need to use alternative
and sustainable raw materials in aquaculture feeds. These raw materials will be expected to be competitive in price, abundant in availability and of great nutrition quality, particularly with high n-3 highly unsaturated fatty acid contents. The use of microalgae in aquafeeds may be a suitable alternative since it is rich in n-3 HUFA, yielding high levels of n-3 HUFA in farmed animal’s tissue. This in turn would allow for desirable quality of aquaculture products for human consumption. However, other components of alternative ingredients such as digestibility, palatability, and nutrient utilization need to be further evaluated in order to establish the viability of algae ingredients in aquaculture feeds. Another extremely important aspect to consider in future research is the manufacturing of test diets. This should be given priority as processing techniques play a critical role in the final quality of feeds.

Researches often use laboratory made feeds that may lack the manufacturing and extrusion benefits supplied by commercial feeds (Amaya et al., 2007). Therefore, special attention should be paid to the manufacturing process of experimental feeds in order to obtain high quality feeds and more accurately measure their impact on animal’s growth performance. Defining the focus and objectives of aquaculture research and development is a priority. The focus should be on achieving feed formulations that are cost-effective, eco-friendly and that meet consumer demands.
Bibliography


Diler, I., and Gokoglu, N. 2004. Investigation of the sensory properties of the flesh of rainbow trout (Oncorhynchus mykiss) fed diets with astaxanthin, shrimp waste meal and red pepper meal. European Food Research and Technology 219: 217-222.


Witlock, M.C., and Schluter, D. 2009. The analysis of biological data.Roberts and company
