KRISHNNAPPRIYAA A/P GOPI MASTER OF SCIENCE 2024

PALM KERNEL EXPELLER (PKE) AS AN INERT FEED FOR BRINE SHRIMP *Artemia*: IMPLICATION ON GROWTH, BIOMASS AND MICROBIAL COMPOSITION DURING TANK CULTURE

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Thesis Submitted in Fulfilment of the Requirement for the Master of Science
in the Institute of Climate Adaptation and Marine Biotechnology
Universiti Malaysia Terengganu

2024

DEDICATION

Dedicated this thesis to:

My beloved parents.

Mr Gopi and Madam Saraswathi

I owe you both a big time

Thank you

God bless

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Master of Science

PALM KERNEL EXPELLER (PKE) AS AN INERT FEED FOR BRINE SHRIMP *Artemia*: IMPLICATION ON GROWTH, BIOMASS AND MICROBIAL COMPOSITION DURING TANK CULTURE

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2024

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Artemia is one the most popular live food used in the aquaculture industry. The filter feeding habit of this crustacean species allows them to graze on microalgae, bacteria and detritus as their natural food. In practice, *Artemia* was often raised and cultured with microalgae and various agricultural products. Though microalgae are exceptional food for *Artemia*, their use is costly and laborious. In this study, Palm Kernel Expeller (PKE) a by-product of the oil palm industry has been examined as an alternative feed for *Artemia*. PKE were mixed with water and processed into fine micro-particles through sieving. Feeding with PKE incubated for 1 day at 19cm turbidity contributed to superior *Artemia* growth and biomass. Microscopic observation revealed a full gut, indicating that *Artemia* can ingest PKE and used them as feed. Metagenomics analysis on PKE solution and *Artemia* rearing water revealed the presence of numerous beneficial bacteria such as *Lactobacillus, Bifidobacterium,* and *Candidatus aquiluna* which belong to the Actinobacteriota phylum. The *Artemia* biomass fed with PKE had good nutritional profiles, with 45% protein, 9% lipid, 21% NFE and 23% ash. Supplementing *Artemia* with 3g of yeast with PKE contributed to an even faster growth, reaching 1cm in TL within 9 days and 1kg of biomass upon 14

days of the culture. Overall, this study demonstrated that PKE supplemented with yeast were the best feed combinations for raising brine shrimp *Artemia* during tank culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PALM KERNEL EXPELLER (PKE) SEBAGAI MAKANAN UNTUK *Artemia*: KESAN KEPADA TUMBESARAN, BIOJISIM DAN POPULASI MIKROB SEMASA PENGKULTURAN DALAM TANGKI

KRISHNNAPPRIYAA A/P GOPI

2024

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Artemia ialah salah satu makanan hidup yang paling popular digunakan dalam industri akuakultur. Tabiat penapisan makanan spesies krustasea ini membolehkan mereka memakan mikroalga, bakteria, dan detritus sebagai makanan semula jadi mereka. Dalam amalan, *Artemia* sering dipelihara dan dikultur bersama mikroalga dan pelbagai produk pertanian. Walaupun mikroalga merupakan makanan yang sangat baik untuk *Artemia*, penggunaannya adalah mahal dan memerlukan banyak tenaga kerja. Dalam kajian ini, Palm Kernel Expeller (PKE), iaitu produk sampingan daripada industri kelapa sawit, telah dikaji sebagai makanan alternatif untuk *Artemia.* PKE dicampur dengan air dan diproses menjadi zarah mikro halus melalui penapisan. Pemberian PKE yang diinkubasi selama 1 hari pada kekeruhan 19 cm memberikan pertumbuhan dan biomassa *Artemia* yang unggul. Pemerhatian mikroskopik menunjukkan usus yang penuh, menunjukkan bahawa *Artemia* boleh memakan PKE dan menggunakannya sebagai makanan. Analisis metagenomik pada larutan PKE dan air kultur Artemia mendedahkan kehadiran banyak bakteria bermanfaat seperti *Lactobacillus, Bifidobacterium,* dan *Candidatus aquiluna* yang tergolong dalam filum Actinobacteriota. Biomassa *Artemia* yang diberi makan PKE mempunyai profil

pemakanan yang baik, dengan 45% protein, 9% lipid, 21% NFE, dan 23% abu. Penambahan *Artemia* dengan 3g yis bersama PKE menyumbang kepada pertumbuhan yang lebih cepat, mencapai 1 cm TL dalam 9 hari dan 1 kg biomassa dalam tempoh 14 hari kultur. Secara keseluruhannya, kajian ini menunjukkan bahawa PKE yang ditambah dengan yis adalah gabungan makanan terbaik untuk memelihara udang air masin *Artemia* semasa kultur dalam tangki.

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APPROVALS

I certify that an Examination Committee has met on 13 June 2024 to conduct the final examination of Krishnnappriyaa A/P Gopi, on her Master of Science thesis entitled **"Palm Kernel Expeller (PKE) as an inert feed for Brine Shrimp *Artemia*: Implication on growth, biomass and microbial composition during tank culture."** in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. The members of the Examination Committee are as follows:

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This thesis has been accepted by the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science.

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DECRALATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UMT or other institutions.

____

KRISHNNAPPRIYAA A/P GOPI

Date:

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LIST OF ABBREVIATIONS

PKE	Palm Kernel Expeller
ANOVA	One-way analysis of variance
PRIMER	Plymouth Routines in Multivariate Ecological Research
PCR	Polymerase Chain Reaction
EPA	eicosapentaenoic acid
ARA	arachidonic acid
DHA	docosahexaenoic acid
HUFAs	Highly Unsaturated Fatty Acid
g/L	Gram per litre
mg/kg	Milligram per kilogram
ppt	Part Per Thousand
mm	milimeter
ml	millilitre
µm	micrometer
spp.	species
SPSS	Statistical Package for the Social Sciences
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
°	degree
<	less than
>	more than
%	percentage
±	plus minus

LIST OF FORMULAS

Equation **Page**

1 34

% of dry matters = $\frac{W3-W1}{W2-W1} \times 100$

% of moisture = 100 – % of dry matters

2 34

% of Lipid = $\frac{W3-W1}{W2} \times 100$

3 34

% of Ash = $\frac{W3-W1}{W2-W1} \times 100$

4

% of N in the sample

$$\frac{(T-B) \times N \times 14.007}{W} \times 100$$

% of Protein = % N × F

N = Normality of HCl

F= Protein factor, 6.25

5

% of Fiber =

$$\frac{Wx - (W1 \times C1)}{W2} \times 100$$

Wx = (W3+W4) – W5

C1= $\frac{(W3+W4)-W5}{W1}$

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Artemia, also known as brine shrimp, is the most essential group of live food used in aquaculture (Olesen, 2009). They live in hypersaline waters, coastal lagoons and solar saltworks (Kappas et al., 2004; Persoone and Sorgeloos, 1980). The euryhaline genus of *Artemia*, allows them to have the most effective osmoregulatory mechanism within the animal kingdom (Narciso, 2000; Croghan, 1958). *Artemia* have very effective respiratory pigments which make them tolerate low oxygen levels found in places with high salinities. Great Salt Lake in Utah, contributes almost 90% of the world's commercial harvest of the brine shrimp cysts at their dormant stage. High quality cysts have a good hatching rate, where approximately 200000 to 300000 nauplii are hatched from each gram of the cyst (Treece, 2000). All life stages of *Artemia* namely cysts, nauplii, juveniles, and adults, can be used as feed and they fulfill nutritional requirement of various aquatic species (Sorgeloos, 1980).

Artemia nauplii are small and they are able to survive in euryhaline conditions, making them suitable for many aquaculture species (McConaugha, 1985; Beck and Turingan, 2007). The distinctive quality of *Artemia* is that, they provide an essential food source for early stage of fish, shrimp and other aquatic animals that does not have natural food source, such as during nursery without contaminating the culture water.

Artemia meet the essential nutritional requirements and support their development, reproduction, and enhance the immune systems (Hansen et al., 2021),

On other aspect, adult *Artemia* are favoured for their superior nutritional digestibility, and their contribution to an effective food uptake and earlier satiation of the fish (Dhert et al., 1992). They also have hormonal substances within them that provide additional benefits to increase the fertilization rate and stimulate sexual maturation to various cultured species such as fish and shrimp (Naessens et al., 1997). Several larval stages of the American lobster, *Homarus americanus*, feeds on *Artemia* biomass (Carlberg and Van Olst, 1976). The expanding aquaculture activities offers opportunities for wide use of live, frozen and freeze-dried adult *Artemia* biomass. This leads to a high potential market for *Artemia* biomass in aquaculture (Hoa, 2007; Vinh, et al., 2020).

In 1982, *Artemia franciscana* originated from San Francisco Bay, USA was cultured seasonally in the Mekong Delta of southern Vietnam (Quynh and Nguyen, 1987). The commercial production of *Artemia* cyst in the coastal salt farms in Mekong Delta, has resulted a successful culture providing considerable socio-economic profit (Brands et al., 1995; Anh et al., 1997a). Algae-rich water fertilized from ponds are used as the main food for *Artemia* and inert feeds (rice bran and soybean meal) and animal waste have been used as supplements in their culture. In Bangladesh, *Artemia* have been cultured as human food, contributing high protein sources to local communities which lack proper diets and nutrition (Rahman et al., 2022). *Artemia* are used as one of the main ingredients in food recipes like omelette or to substitute partially in the making of fish, crab, and shrimp cake. The farmers practiced both intensive (stagnant batch culture system) and extensive culture (pond culture) (Islam et al., 2019). Feeds that have been used to feed *Artemia* includes live microalgae *Nanochloropsis* and a wide variety of inert foods including yeast, rice bran, whey, wheat flour, and soybean powder. Cheap food source from the agricultural by-products (rice bran, oil cake, and a small quantity of cod liver oil) have been tested as cost-effective alternatives in the intensive culture of *Artemia* up to adult stage (Sorgeloos et al., 2001).

Because *Artemia* is a nonselective filter feeder, they graze on tiny food particles, which are approximately, 1-50 µm in size**.** *Artemia* can consume a wide range of foods, including bacteria, protozoa, microalgae, and tiny detritus particles (Fernandez, 2001). Microalgae are commonly used as the natural food source for the *Artemia* in large-pond extensive cultures (Zmora et al., 2002). Whereas, by-products from the agriculture and food processing industries such as rice bran, corn bran, soy protein, and whey are used in small-scale intensive cultures (Lavens et al., 1980). Feed like yeast, soybean, rice bran, and corn bran gain their attention in aquaculture because of their small particle size, rich in protein, good water buoyancy and low production cost. According to Coutte et al, 1990, the degree of digestibility and the presence of important nutrients determine the nutritional value of any diet.

1.2 Problem Statement

Artificial or man-made high salinity ecosystems such as seasonal salt ponds or permanent solar salt operations are common culture methods in *Artemia* production, like in Vietnam, the *Artemia* culture depends on the food chains in the pond, which are believed to be a complicated phenomenon. Chicken manure has been used widely in *Artemia* ponds as immediate food and to fertilize the ponds to promote algae bloom. Microalgae are undeniably the best food for *Artemia*, but to produce a unicellular microalgae is costly, labour intensive and unreliable (Figueiredo et al., 2009). It has been crucial to maintain algal bloom and continuously supply microalgae for *Artemia* in an outdoor and indoor culture. Therefore, inert diets such as rice bran, soya bean meal and others, have been utilized in pond culture and tank culture as a supplement food source (Brands et al., 1995; Baert et al., 1997). In pond culture, the ponds are fertilized to stimulate microalgae bloom and microbial compositon as a natural food food for *Artemia*. Providing mix-diet food that consist product like soybean and ground corn produces positive benefits, these diets are costly. An alternative food supply is important to have a sustainable *Artemia* culture. Therefore, it is very important to search different types of food source that could contribute to high production and good quality of *Artemia* biomass. A good, profitable alternative to microalgae in *Artemia* production would be the use of low-cost food source (Anh et al., 2009).

1.3 Significance of Study

In this context, *Artemia* culture is very important to fulfil the increasing demand of brine shrimp in aquaculture industry (Sorgeloos et al., 1998). Increasing demand for *Artemia* has led to overexploitation of its natural sources which resulted in limited availability of cyst and increased the price of this resources. Therefore, *Artemia* culture is crucial to promote a continuous and sustainable supply to market demand (Maldona-Montiel et al., 2003). Besides that, *Artemia* can also be cultured in ponds and hatcheries in coutries like Malaysia, where *Artemia* is not found naturally. The *Artemia* biomass production could economically benefit the aquaculture industry in Malaysia. Utilization of cheap food in *Artemia* culture could significantly reduce the production cost. Agricultureal by-products provide ideal conditions for the growth of suitable microflora, for example, *Pseudomonas* in rice bran, which constitute an important food source in the diet of *Artemia* (Gorospe et al., 1996). The presence of microbes could be an additional food for this continuous filter feeding brine shrimp. Therefore, PKE a cheap agricultural by-product is a potential food that can be used as *Artemia* feed. PKE are easily and widely available in Malaysia which contributes to a continuous availability of its utilization in *Artemia* culture. Although fish meal has been continuously utilizaed as a main food source of dietary protein in commercial aqua feed, PKE consisting plant protein source has captured the attention of farmers in including them partialy or solely in feeding of the aquctic organisms (El-Sayed, 1999).

Objectives

1. To determine the efficacy of PKE as inert feed for *Artemia* and its effect on growth and biomass production.

2. To determine the presence of microbe in PKE and the rearing water of *Artemia* upon incubation.

3. To examine the nutritional values of *Artemia* biomass produced in tank culture systems upon feeding with PKE, PKE supplemented with yeast, and yeast only.

CHAPTER 2

LITERATURE REVIEW

2.1 *Artemia*

2.1.1 Artemia Biology

Artemia is a very unique organism and they possess two reproduction modes, namely ovoviviparous and oviparous. Under favourable conditions, the females release free swimming nauplii and whereas under unfavourable condition, they produce a highly resistant encysted gastrula embryo (Iryani et al., 2020). During the shift of ovoviviparity to oviparity, embryo development stops at gastrula stage. Trehalose is kept as carbohydrate reserve (Clegg, 1962) and each gastrula has its shell coated with a heamatine-like material released by brown shell glands (Dutrieu, 1959; Dutrieu, 1960; De Maeyer-Criel, 1978). These encysted gastrulae, also known as cysts, are then released into the water. The exact mechanisms responsible for the shift from ovoviviparity to oviparity remain unknown but according to Barigozzi, 1939 water salinity was stated as a responsible factor. The link between cysts and haemoglobin synthesis and several environmental factors such as low oxygen levels, high salinities and high population densities was also demonstrated (Dutrieu, 1960; Ballardin and Metalli, 1963) to influences the switch in reproduction mode. Food scarcity, starvation (D’Agostino and Provasoli, 1968) and the presence of iron (Baker, 1966) and chlorophyll (Dutrieu, 1960) in the culture medium induced cysts production in female *Artemia*.

The adult *Artemia* length measures approximately 1cm, with females usually longer than males. For an adult male, the average length is between 8-10mm, whereas for an adult female, is 10-12mm. The body width of both males and females (including legs) is approximately 4mm. Artemia has a segmented body (head, thorax, and abdomen) with broad, leaf-like appendages attached that significantly increases the apparent size of the animal. The head is composed of a single prostomial and five

metameric segments, comprising the median and compound eyes, the labrum, the first and second antennae, the mandibles, the first and second maxillae, and the second maxillae according to order. The thorax has eleven segments, with a pair of swimming legs consisting each segment. The abdomen consists of eight segments. The first abdominal segment, also referred as the genital segments composes the gonopods, which are either the ovisac (female) or paired penes (male). The second abdominal segment are referred as the furca or telson (Criel, 2002). Temperature plays an important role in the development and growth of *Artemia* (Abele, 1982; Hartnoll, 1982; Verhoef et al., 1998; Anger, 2001). The temperature increases the growth rate of *Artemia franciscana*, due to metabolic acceleration, resulting in higher moult frequency (Hochachka and Somero, 1984; Prosser, 1986). Besides temperature, salinity changes accelerate growth (Reeve, 1963; Narciso, 2000) but the the rate brought upon by the effects of temperature is greater (Narciso, 2000).

2.1.2 *Artemia* species

There are seven bisexual species in the genus of *Artemia*, along with large number of parthenogenetic populations (Van Stappen 2002; Eimanifar et al. 2014). *Artemia salina* in Mediterranean basin (Linnaeus, 1758), *Artemia tibetiana* in Qinghai Tibetan Plateau, China (Abatzopoulos et al., 1998), *Artemia sinica* in China and Mongolia (Cai, 1989), and *Artemia urmiana* in Lake Urmia, Iran and Crimean salt lakes, Russia (Gunther, 1899) are the four bisexual *Artemia* species that lives in the Old World. *Artemia franciscana* in North America, Central America and South America (Kellogg, 1906), and *Artemia persimilis* in Argentina and Chile (Piccinelli and Prosdocimi, 1968) are the two natives bisexual *Artemia* species to the New World. *Artemia parthenogenetica* are generally found in many inland salt lakes, lagoons and coastal salterns in Africa, Australia and Eurasia (Van Stappen 2002; Eimanifar et al. 2014).

The brine shrimp *Artemia franciscana* originating from North America's San Francisco Bay and Great Salt Lake, in the year 1950's have been commercially transported worldwide for the purpose of fish farming and aquarium trade. The expansion of aquaculture globally, results in the release of *Artemia franciscana* into

native *Artemia* population. Today, it still exists in several Western Mediterranean places, where it displaces and became dominant over the native populations. However, there is no evidence nor information available on the possible southward displacement of *Artemia persimilis* by the invasive *Artemia franciscana* in Argentina. The population growth and high fecundities of most invasive aquatic species depends on their acceptance to the new environment (Browne, 1980; Browne and Halanych, 1989; Browne and Wanigasekera, 2000; Amat et al., 2005). *Artemia franciscana* can withstand a wide variety of environmental conditions, such as high temperatures, high salinity, low oxygen levels, and ionic changes. Despite the extreme hot weather, *Artemia* have been raised in salt fields in Vinh Chau and Bac-Lieu in the Mekong Delta for over 30 years (Vinh et al., 2020).

2.1.3 DISTRIBUTION OF ARTEMIA

The geographical isolation of *Artemia* populations are reliant upon varying environmental factors, including salinity, temperature, and the ionic composition of the biotope (Bowen et al., 1985, 1988). *Artemia* occurs in hypersaline lakes, salt ponds, man-made saltworks and salt lagoons. They originate from the Great Salt Lake and San Francisco Bay in United States of America, and introduced in countries where they are not found naturally. The high salinity habitat of *Artemia* eliminates food competitors and predators, thus allow them to be cultured in a monoculture conditions (FAO, 1996). Artemia inoculation in saltworks are practiced in countries like Vietnam, Bangladesh, Thailand and others. It is essential to have a continuous availability of this brine shrimps, because if this brine shrimps are no longer available in their natural habitat, they may experience a sharp decline or even worst, go extinct. *Artemia* are incapable of dispersion, therefore migratory birds play a role of vector that carries the *Artemia* cysts in their digestive tracts or attached to their feathers or claws are the main cause of the invasion of these brine shrimps (Lincoln and Peterson, 1979). The distribution of Artemia species in some of the country include: *A. urmiana* (Gunther, 1990) in the Islamic Republic of Iran (Lake Urmia), Crimean salt marshes; *A. sinica* (Cai, 1989) in central and eastern China; *A. tibetiana* (Abatzopoulos et al., 1998) in Lagkor Co Lake, Tibet, China; *A. persimilis* (Piccinelli and Prosdocimi, 1968) in southern South America; *A. franciscana* in North, Central and South America (Verrill,

1869). *A. sorgeloosi* (Asem et al., 2023) in Haiyan Lake, Tibet, China; and *Artemia amati* (Asem et al., 2023) in Kazakhstan.

2.2 CULTURE SYSTEM

A number of culture systems have been introduced for a controlled *Artemia* culture. These culture systems include indoor culture (intensive culture systems, such as semi flow-through systems) and outdoor culture (extensive culture systems, such as high salinity ponds) (Dhert et al., 1992; Landau et el., 1986). *Artemia* is cultured in coastal saltworks in the Philippines, Thailand, Vietnam and others, where there are not found naturally. *Artemia* biomass can be obtained from solar salt ponds and lagoons in numerous tropical and subtropical nations. Both extensive and intensive culture systems have practiced wide range of culture regimes, from batch culture to continuous flow through systems (Brisset et al., 1982). Despite large scale *Artemia* biomass production using salt ponds has expanded successfully in recent years, due to facility-related constrains, hatcheries and laboratories have used indoor tanks or small containers to culture *Artemia* (Van Stappen et al., 2020).

2.2.1 POND CULTURE

Pond production of *Artemia* has been introduced in several tropical nations since the mid-1980s. In the Mekong Delta, Vietnam, pond culture which was integrated in solar salt production ponds has developed into a successful aquaculture activity (Anh et al., 2009; Sui et al., 2013). Vietnam supplies a significant amount of high-quality cysts for domestic use and to some extent, export, to reduce the global reliance on natural resources, they have demonstrated the potential of pond based *Artemia* cyst production during the dry season. Extra source of income from the production of *Artemia* cyst and biomass can be obtained with a proper pond optimization and biological management of saltponds. The pond is prepared with approximately 100 to 150g/L and water depth of 50 to 70 cm. Stocking density of 1 to 10 animals per litre pond water is practiced (Sorgeloos & Kulasekarapandian, 1984). An ovoviviparous mode of reproduction is assured to make sure an increase in population density that is 100 animals or more per litre.

Adult *Artemia* consumes food in the pond not for growth but for reproduction and maintenance of energy. Removing the full-size grown *Artemia* would free the limited space and resources of the ponds for more efficient biomass gain of the nauplii and juvenile stage *Artemia*. Ponds should be fertilized with cheap organic manures such as pig manure and chicken manure to stimulate phytoplankton bloom in the pond before the inoculation of *Artemia*. The yield of *Artemia* biomass can be enhanced by supplementing soy bean and rice bran to the culture ponds. Fertilizers are added when the ponds reach their maximum water depth to ensure phytoplankton bloom. To evaluate food availability in the pond, water turbidity is often measured using a secchi-disc, with ideal levels maintained at approximately 20cm. Green water supplemented with inert feeds are used commonly during *Artemia* pond production. Feeding is applied weekly to biweekly, depending on water turbidity conditions. Limited supply of green water and food supplement results in a significant reduction in the production and quality of *Artemia* biomass.

2.2.2 TANK CULTURE

Commonly, controlled *Artemia* culture are practiced in tanks equipped with a continuous flow-through water system or zero water exchange (batch system). In a flow-through water system, the maximum stocking density of *Artemia* is less than 18 animals per/ml, whereas in a stagnant batch culture system, approximately 20-30 animals per/ml (Dan et al., 2019; Dan, Iwasaki, et al., 2018), this quantity is important to achieve a high survival rate while avoiding the degradation of water quality (Dhont & van Stappen, 2003; Zmora & Shpigel, 2006). In order to produce high yield of *Artemia* biomass, it is worth considering intensive stagnant culture method as it can greatly reduce the constraints of facilities and seawater. In a successful stagnant batch culture system, besides microalgae, inert feed rice bran, soybean meal and yeast are also considered as an ideal food source for *Artemia.* The supplementation of microalgae or other inert feed that can be ingested by *Artemia* nauplii is essential to produce high yield biomass, faster growth and high nutritional value. Culturing *Artemia* in a controlled indoor system has many advantages; (i) season or climate does not affect the culture; (ii) it allows frequent harvesting and reinoculation; and (iii) it provides a much controlled culture over bacteria and outbreak of diseases. A

significant amount of high quality feed is required for the production of *Artemia* biomass under controlled conditions**.** In order to produce *Artemia* in a controlled conditions, the water quality parameters should be kept within an optimum range (salinity between 32-65g/L, oxygen above 2mg/L, temperature between 19-25 °C, and pH between 6.5-8.0).

It was reported that, water quality in the culture tanks were considered optimal as there were no wide variations observed for any of the variables (Dhont, 1996). Lower individual size of *Artemia* were observed in higher salinity. As mentioned before, low temperatures slow down the growth rate of *Artemia* and a temperature beyond 30 °C results in mortality. According to Islam, et al., 2018, salinity is increased with time, from 35 to 65 g/L, in order to control the breakdown of excess feed and bacterial growth. *Artemia* are grown in a favourable environment that provides, constant amount of food and maintained level of dissolved oxyegn. According to the research performed by Zmora & Shpigel (2006), upon feeding mixed diet of diatoms, yast, ciliates and soy protein in a tank culture system with zero exchange of water during the entire cycle results in 50% and 24 % of Artemia survival at 14 and 20 days respectively. The microbial population which develops in the culture tanks are extra food source for *Artemia* (Gorospe and Nakamura, 1996; Milligan et al., 1980).

2.3 Nutritional value of *Artemia*

The newly hatched *Artemia* nauplii and adult *Artemia* (live, frozen and dried forms) contain high amounts of protein, essential amino acids, and fatty acids (Zmora et al., 2002), making them a superior feed for many aquaculture species. Nevertheless, the nutritional value of *Artemia* varies across different regions (Sorgeloos et al., 1998). Because *Artemia* are non-selective filter feeders, they can be used for bioencapsulation to improve its nutritional value. *Artemia* bioencapsulation method have been used to enrich essential nutrients, pigments and therapeutics to the predators (Merchie, 1996). This method has also been identified as an effective way to directly deliver probiotics to the digestive tract of the targeted predators (Gatesoupe, 2002; Suzer et al., 2008). Providing high quality feed to *Artemia* is essential to improve their fatty acid content, this in return would produce high quality *Artemia* (Herawati et al. 2012).

The level of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have important characteristic, are low in *Artemia* (Riberio & Jones, 2008). Therefore *Artemia* require external enrichment or added supplement with high concentration of EPA and DHA. High quality diets that are easily digested and supply the essential nutrients for an optimum survival, growth and immunity are important for a successful larviculture (Giri et al., 2002; Jafari et al., 2011). Fatty acids such as highly unsaturated fatty acid (HUFAs), for example EPA and DHA and arachidonic acid (ARA) must be acquired by the larvae from their diet because they cannot be obtained *de novo* and these nutrients are essential for the larvae growth (Sargent et al., 2002; Berg´e and Barnathan, 2005). According to Léger et al., 1986, the fatty acid composition of *Artemia* are different when they are compared within strains and between batches within the same strain. Generally, locally produced *Artemia* contains 79.91% protein and 8.6% fat, whereas imported *Artemia* contains 70% protein and 6.3% (Herawati, 2014). The difference in the fatty acid composition of the food consumed by the parental population (Vos et al., 1984; Léger et al., 1986; Lavens et al., 1989), the genotype (Navarro and Amat, 1992), or the feed substrate choose for feeding (Ruiz et al., 2007) are responsible for the variations in the fatty acid composition of *Artemia* cysts and nauplii.

Adult *Artemia* biomass from different strains that consume same species of algae has a variation in their fatty acid profile (Gozalbo, 1990). The various components found in the diet have been shown by several researches to justify the fatty acid composition of *Artemia* and other zooplankton (Schauer et al., 1980; Léger et al., 1986; Navarro and Amat 1992; Mura et al., 1997, 1998; Dalsgaard et al., 2003). Therefore, it has been recommended that fatty acids as a helpful trophic indicator that indicate the type of food ingested (Sargent et al., 1988; Napolitano, 1999; Dalsgaard et al., 2003). The highly unsaturated fatty acids, most importantly the n-3 series, has captured the attention in fish larval nutrition studies, as they as essential for larval growth and survival (Watanabe et al., 1983; Lisac et al., 1986; Dendrinos and Thorpe, 1987). Originally, the content of EPA and DHA are low in Artemia, to have an adequate amount of these nutrients they must be enriched with important fatty acid rich lipids to provide the necessary food requirement of fish larvae (Copeman et al., 2002; Hawkyard et al., 2016). The tolerant to stress by the larvae of red seabream

(Pagrus major) (Kanazawa, 1997), red porgy *(Pagrus pagrus)* (Roo et al., 2009), and greater amberjack *(Seriola dumerili)* (Roo et al., 2019) has been correlated to an increase in n-3 HUFA and DHA levels in the nutritional profile of the *Artemia.* According to Lavens and Soegeloos in 1991, Artemia feed with agricultural subproducts contains very small amounts of EPA and DHA in them. However, chemical analysis alone is not sufficient enough to determine the quality of the feed. The growth, feed conversion and survival of the target species should be taken into count to evaluate the real quality of the feed (C.P. Shrimp News, 1995).

2.4 Food for *Artemia*

According to Tunsutapanich (1979), *Artemia* are classified as omnivore. *Artemia* has been fed with variety of food source including microalagae, soy bean meal, rice bran, yeast and others (Sui et al., 2013; Verma, Raghukumar & Naik, 2011). Furthermore, these items can provide natural food for *Artemia* by turning themselves into fertilizer when they decompose. The nonselective feeding behaviour of *Artemia*, enables it to feed on the fertilizer, phytoplankton, detritus, and microorganisms, where all these contributes to its development (Teresita et al., 2003).

The nutritional value of the *Artemia* significantly depends on the culture method and the food source used to feed them. (Gharibi, 2021). Addition of a supplement into the feeding regime is more profitable than using the natural food alone, as it produces twice as much biomass but has the same labour and pond management expenses later on (Anh, 2009). It is known that optimal food size for *Artemia* nauplii, is approximately 16.0µm, however it can vary from 6.8 to 27.5 µm, whereas the adult *Artemia* can consume any particle smaller than 50µm (D'Agostino, 1980; Dobbeleir et al., 1980). The food source used to feed *Artemia* should also have a good buoyancy in water column with minimal solubility, high in nutrition, small in particle size and continuously available throughout the culture with low production cost (Coutteau et al., 1990).

2.4.1 Microalgae

Microalgae is an important live feed in the aquaculture industry and their nutritional value are well established. Their excellent nutritional contents include vitamins, high-quality proteins, pigments, and poly unsaturated fatty acids (PUFAs) (Merchie et al., 1995). Algae generally known to enhance the growth and nutrient utilization of aquatic animals that has been cultured. The supplementation of microalgae into formulated feed, either as a live feed or an additive, improves the stress tolerance, feed utilization, growth-promoting protein acceleration, disease resistance, and carcass quality (Vizcaíno et al., 2014; Rehberg-Haas et al., 2015). Microalgae also promotes pigmentation in cultured species and enhances biological activities. In the past forty years, many algal genera have been studied (Khatoon et al., 2010). A wide range of microalgae has been used as a food source for *Artemia*, that includes dried microalgae such as the genus *Spirulina* and *Scenedesmus* (Person Le Ruyet, 1976) and live microalgae such as genus *Tetraselmis* (Fabregas et al., 1996), *Isochrysis* (Wickins, 1972), and *Dunaliella* (Mason, 1963).

Upon feeding with microalgae *Dunaliella tertiolecta* the length of Artemia reached an average length of approximately 7mm-8mm on the day fourteenth (Agh et al., 2008). Producing a unicellular microalga is laborious and one the main obstacle for culturing aquatic filter feeders. Therefore, microalgae are substitute with other possible feeds that are cheap and not labour intensive. Among the microalgae, not all the unicellular algae can be ingested by *Artemia* (*Chlorella* and *Stichococcus,* as they have thick cell wall that cannot be digested by the brine shrimp. Some dinoflagellates creates toxic compounds, and *Coccochloris* produces gelatinous substances that inhibit the absorption of food. A continues and concentrated algae effluent is required in an intensive *Artemia* culture. When the algae concentration is low, either the *Artemia* density should be reduced (lower production) or high flow rate of algae should be implemented (rise in pumping and heating cost).

2.4.2 Rice bran

Rice bran are known to be a cheap and appropriate food source for intensive *Artemia* culture (Punitha et al., 2007). *Artemia* requires carbohydrates during the first few days of development, therefore the carbohydrates in the rice bran aids the growth of *Artemia* (Johnson, 1980). It is reported that *Artemia* cultivated with rice bran contains low amounts of EPA and DHA (Maldonado et al., 2005). The content of fibre in rice bran is relatively low, and its oil has become an important food for animals and live food. The usage of rice bran as cheap food source for *Artemia* gives a high amount of crude protein and good composition of amino acids in them (Sorgeloos et al., 1980). The protein and ash content of *Artemia* reared upon rice bran were approximately 53.1% and 15.4% respectively (Teresita and Leticia, 2004). During the storing process of the rice bran, the activation of the lipolytic enzyme degrades the quality of the rice bran resulting in loss of crude lipids, this explains the low amount of lipids in the rice bran of 14 -18% (Göhl, 1982). The success of a culture upon feeding with rice bran depends on the dry bathing method of the rice bran. The nutritional composition of the product may degrade following the origin, harvest, processing and there is also a potential of contamination with pesticides which are used during the process of storage. Large ponds in the Philippines utilises rice bran extract as food source for *Artemia* (Brisset et al., 1982). *Artemia* that feeds on rice bran can produce around 41 to 71 eggs per brood pouch (Rehman and Rathinasamy, 1997). Rice bran was used in a batch culture system by Bossuyt and Sorgeloos (1980) to feed the *Artemia* in saturated brine at high densities. Feeding *Artemia* with a mix diet of *Spirulina sp.* and rice bran stimulates oviparity in *Artemia* spp. (Versichele and Sorgeloos, 1980) whereas feeding them with microalgae *Scenedesmus sp*. and *Dunaliella sp* are reported to be less efficient in stimulating oviparity in the *Artemia*. The length of *Artemia* fed with rice bran and algae, *Tetraselmis suecica* was approximately 5.24mm in 15 days of the culture (Terestia and Leticia, 2004).

2.4.3 Soybean meal

Soy bean meal is rich in protein and has a good balance of amino acid composition and crude fiber content that is less than 3%. Nevertheless, it was reported that feeding soybean meal yielded the lowest growth in *Artemia* when compared to rice bran, algae, and wheat bran (Vahdat & Oroujlou, 2021). The raffinose and stachyose content in the soy bean meal resulted in reduced digestion and absorption of nutrients, increased digestive transit rate and bloating. The protein, lipid and ash content of *Artemia urmiana* fed with soybean meal is approximately 48.8%, 16.84% and 18.23% respectively (Ownagh et al. 2015). Whereas according to Vahdat & Oroujlou, (2021), the protein, lipid and ash content of *Artemia franciscana* upon feeding with rice bran was 58.98%, 11.10%, and 15.44% respectively. However, soybeans are not cultivated in tropical countries, thus the availability of soybean meal depends solely on imported resources. The rise in the cost of soyabean meal and unpredictable resourses may affect *Artemia* culture and production.

2.4.4 Bacteria

Bacteria plays a major role as sole food source for herbivorous zooplankton (Rieper, 1978). Bacteria has been known as an essential component of both fresh and marine water environments (Daley & Hobbie, 1975; Ducklow, 1983) and they represent a significant portion of the organic matter in those systems (Wright 1978; Hagstrom et al. 1979; Fuhrman & Azam 1980). According to Intriago and Jones in the year 1993, bacteria have a very small particle size (0.6-3.0 µm) and can be used as an ideal food *Artemia* nauplii that does not have a fully developed filter feeding meachanism (Gorospe et al., 1996). Generally, bacteria are classified as decomposers that are responsible in breaking down and mineralizing organic matter (Ehrlich, 1985), but there is significant evidence that besides playing a major role in regeneration and feed on dissolved nutrients in the water column, the bacteria itself serves as direct food for carnivorous and omnivorous organisms (Rieper, 1978, 1981; Williams, 1981). There have been many reports which highlighted the role of bacteria as feed to organisms belonging to the higher trophic levels, such as zooplanktons (Moriarty, 1986; Leber and Pruder, 1988; Maeda, 1988). D'Agostino, 1980; Napolitano, 1999,

reported bacteria lack PUFAs, which are vital components for aquatic species like *Artemia* (D'Agostino, 1980; Napolitano, 1999). Nevertheless, can be utilized as food for *Artemia* to compensate for an insufficient supply of algae (Toi et al., 2013). According to Intriago and Jones (1993), *Flexibacter sp* was found to be a direct food source for *Artemia*, enhancing growth and survival. Although it has been shown that *Artemia* feeds on bacteria (Intriago and Jones, 1993; Gorospe et al., 1996), it is crucial to differentiate the function of the microbiota in the food and the quality of the food, thus, it is essential to learn their both role in the growth of *Artemia*.

2.4.5 Yeast

It has been more than a century, since researchers began to examine yeast as a food and feed ingredient (Osborne and Mendel, 1919). Yeast has gained the interest in aquaculture as one of the best food for *Artemia* based on few interesting characteristics such as small particle size and good water buoyancy. This allow them to be easily ingested by filter feeding aquatic animals. Since yeast has small particle size, low production cost and high content of protein, they are used as a food (algal substitute) for many filter feeding organisms such as rotifers (Hirayama and Watanabe 1973; Fukusho 1980; Lemilinaire 1984), bivalve molluscs (Epifanio 1979; Urban and Langdon 1984) and Artemia (Shimaya et al. 1967; Talloen 1978; Johnson 1980; Nimmannit and Assawamunkong 1985). The rigid cell wall of the yeast holds them in a place and prevents them from leaching into the culture medium which could deteriorate the water quality. Yeast having a high protein content can be easily produced based on various raw material, regardless of the climate and at comparatively low production costs (Kihlberg, 1972).

Several products of yeast e.g. baker's yeast (Talloen, 1978; James & Makkeya, 1981), brewer's yeast and methanol yeasts (Robin et al., 1987) and *Kluyveromyces* (Lavens et al., 1987; Lavens & Sorgeloos, 1991), has been added as a protein source in mixed diets for the production of Artemia biomass. The use of marine yeast results in a poor culture (Johnson, 1980; Nimmanit & Assawamunkong, 1985) but not when the culture is run-in small-scale systems with low animal densities (Shimaya et al., 1967; Kawano et al., 1976; Johnson, 1980). However, James et al., 1987 reported

feeding Artemia with *Candida* yeast in a batch culture system of $10m^3$ without water renewal results in high yield of the animal. The culture conditions then, were favourable for the development of microbial blooms, which has the potential to serve as a food supplement for Artemia and in a way fill in the dietary gap. When Artemia were fed with yeast, their fecundity measured in terms of either the number of broods per animal or the number of offspring per animal was higher than that of Artemia fed with rice bran (Brintha, 2016). In contrary, Coutteau et al., 1990 reported, feeding these brine shrimps with baker's yeast (*Saccharomyces cerevisiae*) only results in a poor culture. Several researches have reported, that supplementing yeast with *Spirulina* (James & Makkeya, 1981; Robin et al., 1981) and agricultural waste (Talloen, 1978; Lavens et al., 1987) has improved the growth and survival of the brine shrimp.

2.5 PALM KERNEL EXTRUDER (PKE)

The palm tree, *Elaeis guineensis*, Jacq originates from West Africa. They are popularly being cultivated today in many countries around the world (Khatun et al., 2017). Palm kernel expeller (PKE) by-product of palm oil, has a promising dietary fibre resource of 23g/100g (Akinyeye, Adeyeye, Fasakin and Agboola, 2011) and produced approximately 40 million tons per annum globally (Pereira, Souza, Jr., and Rosa de Freitas, 2020). The fruits basically grow in bunches and have soft outer skin. When they become ripe, they turn reddish. The structure of the fruit compromise of few parts known as, mesocarp, endocarp and the kernel. Two types of oils can be produced from the fruit. The first oil known as crude palm oil (CPO) extracted from the mesocrap of the fruit. CPO is commonly used as cooking oil. A hard nut (endocarp) also known as the shell that encloses the kernel inside the mesocarp. This endocarp itself contains the second oil type named palm kernel oil (PKO). The fatty acid composition of the PKO is mainly saturated fatty acid, which is 16% of myristic acid (C14:0), 48% of lauric acid (C12:0), and 15% of oleic acid (C18:0). They are commonly used as one of the ingredients to produce margarine, confectionery, animal food and cosmetics (soaps and creams). In order to produce PKO, breaking the endocarp (the hard shell) and separating them from the kernel would be the first step (Akubuo and Eje, 2002). The solid part from palm kernel that remains after the

extraction of PKO by means of mechanical screw pressing is the Palm Kernel Meal (PKM). Generally, this palm kernel meal is also referred as Palm Kernel Cake (PKC) or Palm kernel Expeller (PKE). They have a nutritional composition of crude lipid (12-20%) and crude protein (18-20%). The nutritional values differs according to the extraction process (mechanical or addition of solvent in the extraction process) (Chin, 2002). The nutritional level is reported to be high (18% of proteins and 20% of lipids), if only extraction is made with mechanical screw pressing.

According to Perez, (1997) the nutritional values of the PKE can be enhanced by fermentation (Iluyemi et al., 2006) or supplementation with any possible lysine-rich sources (molasses or vegetable oil). Several studies were done, to include PKE as a feed ingredient in the diets of *Oreochromis niloticus* (Omoregie and Ogbemudia, 1993), *Labeo senegalensis* (Omoregie, 2001), Red hybrid tilapia (Ng et al., 2002; Ng and Chong, 2002), hybrid *C. macrocephalus* × *C. gariepinus* (Ng and Chen, 2002), Red tilapia (Iluyemi et al., 2010), Nile tilapia juveniles (Carvalho et al., 2012), *Clarius gariepinus* (Udo et al., 2012), sex reversed Nile tilapia (Thongprajukaew et al., 2015) and *O. niloticus* (Adjanke et al., 2016). Ng and Chen, 2002 has concluded PKE as a partial replacement for soybean meal in the diets of hybrid Asian-African catfish. The amount of PKE added in the fish diet varies among the species yet poor digestibility of PKE were reported (Omoregie, 2001). These can be explained by the presence of high concentration of non-starch polysaccharides (NSP) in the cell wall materials (Dusterhoft, 1993). The NSP is referred as an anti-nutritional factor that inhibit digestion of nutrients in PKE (Choct, 2001). PKE is recognised as a best feed for ruminats as it contains valuable nutritional composition of protein, amino acids, NFE and fiber. Besides ruminant, they are also a valuable feed ingredient in poultry and swine diets (Ng, 2003). Only the ruminants stands a high chance to utilize the nutrient of PKE with the aid of microorganisms (bacteria and fungus) and their enzymes that plays a role in breaking down the cellulose walls of the cells (Lim et al., 2001).

Compared to the other agricultural by-products, taking into count the percentage of proteins in PKE and price of PKE, the cost effectiveness of proteins are considered cheapest in PKE (Shayne, 2000). PKE is easily and widely available throughout Malaysia. The continuous availability and low cost of this by product in

many tropical nation where aquaculture is practiced have developed the interest in its use as a potential feed for aquatic organisms. PKE which is also an energy-feed, is known to have similar composition as rice bran, copra meal, and corn gluten feed. PKE being rich in fibre, has a high potential in being commonly used as feed ingredient in ruminants diet (Wan Zahari et al. 2012). The amount of protein is sufficient for most ruminants but slightly higher for non-ruminants (Ismail et al., 2019). The fast growing oil palm industry in 2017 itself produced about 25.64 million tonnes of oil palm products, such as oil palm fronds (OPF), palm kernel cake (PKC) and palm oil mill effluent (POME). Astoundingly, 2.2 million tonnes from this production in 2017, are palm kernel cake (PKC) (Kushairi et al., 2018). The agro-industry has growing interest in aquaculture due to the beneficial nutritional value, cheap price, and readily usable after reprocess.

Table 2.1: Feed compatibility to be used as food for *Artemia*

Component	Microalgae	Yeast	Soy bean meal	Rice Bran	Bacteria	Palm Kernel Expeller
Particle Size	~1-50 µm	~500 µm-2mm	~500 µm-2mm	~500 µm-2mm	~0.3-60 µm	~50-500 µm
Buoyancy	Buoyant	Slightly Buoyant	Sinks	Sinks	Varies	Sinks
Price in Malaysia	Moderate to high	Moderate	Moderate	Low to moderate	Price varies	Low to moderate
Preparation time	Long (requires cultivation)	Short (ready to use)	Short (Processed meal)	Short	Varies (depends on cultivation)	Short (processed expeller)
Availability	Low	High	High	High	Varies	High
Suitability for *Artemia*	Excellent (e.g., Spirulina, Chlorella)	Good (e.g., Saccharomyces cerevisiae)	Fair (not ideal, can be used sparingly)	Fair (not ideal, can be used sparingly)	Good (e.g., Lactobacillus spp., Bacillus spp.)	Have not been tested as food for *Artemia*

2.6 Metagenomics

The development in metagenomics has gained their attention and expectations worldwide, in recent times. The introduction to metagenomics and their progress over the decade has become of the most noticeable evolution in the study of microbial ecology. Metagenomics is known as the straight forward genetic analysis of genomes or genetic information within an environmental sample. Cloning of the environmental DNA was the first initiated in metagenomics, subsequently by functional expression screening (Handelsman et al., 1998) and was then enhanced by direct random shotgun sequencing of environmental DNA (Venter et al, 2004, Tyson et al., 2004). In past studies, they do not only illustrate the concept of the metagenomics methods, but also revealed the functional gene diversity in the microbial world around us (Simon and Daniel, 2007). The approach of phylogenetic research in metagenomics is relatively constrained as they focus solely on the diversity of one gene (eg. 16S rRNA gene), which allows access to the functional gene composition of microbial population. Metagenomics is an essential pathway for producing novel hypotheses of microbial functions, extraordinary findings of proteorhodopsin-based photoheterotrophy or ammonia-oxidizing Archaea agrees to this truth (Beja et al., 2008).

The swift and significant drop in expenses associated with next-generation sequencing has dramatically raised the development of sequence-based metagenomics. Resulting a sudden increase in metagenome shotgun sequence datasets in recent years. Looking ahead, metagenomics composed 16S rRNA gene fingerprinting methods in characterizing microbial community profiles. Consequently, it is expected to evolve into a principle application for many laboratories and scientists working in microbial ecology studies. Prior to microbial population, metagenomics includes a few essential processes and methods which includes the extraction of DNA of the desired sample, followed by sequencing utilizing modern technologies. In binning and annotation, to construct genomes and determine the community members, the sequencing data is assembled and analysed. Besides statistical analysis that aids in interpretations of the data, experimental design itself is crucial to have a reproducable results.

The reproducibility of metagenomics studies depends on the efficiency of one to share and produce reliable results. A metagenomc analysis on PKE reveals that's it comprises a huge population of *Lactobacilli that* are well-known for their probiotic effects (Navidshad et al., 2016). They are beneficial bacteria that play a crucial role in maintaining a healthy balance in the gut microbiota of humans and animals alike. Lactic acid, produced as a result of their fermentation process, creates an acidic environment that can inhibit the growth of harmful pathogens. This mechanism not only helps in maintaining gut health but also contributes to overall immune function and digestion. Consequently, *Lactobacilli* are commonly used in probiotic supplements, fermented foods, and in some cases, even in agricultural and aquacultural applications to promote health and prevent disease.

CHAPTER 3

METHODOLOGY

3.1 Culture system

The same procedure and condition were applied for *Artemia* hatching and *Artemia* rearing from nauplii to adult stage in 14 days of culture.

3.1.1 *Artemia* hatching

The experiments were carried out at the *Artemia* hatchery of University Malaysia Terengganu, in Kuala Nerus, Malaysia. Cysts from Great Salt Lake, Utah USA, *Artemia franciscana* were purchased and hatched in 28g/l seawater with continuous aeration, pH of 8 and temperature of 28°C (Sorgeloos et al., 2001).

1.3.2 *Artemia* culture

Upon 20h of incubation, freshly hatched nauplii with a final density of approximately 1 gram were transferred to 1 tonne rearing tanks with an initial water volume of 400L. The rearing tanks were filled with 400L of seawater and four air stones attached at the tank for a continuous aeration under stagnant batch culture conditions. *Artemia* were grown to 14 days to adult stage at 28°C to 30°C.

3.2 Preparation of Palm Kernel Expeller as Artemia inert feed

PKE were purchased from Nutrivet Sdn. Bhd. Negeri Sembilan. The PKE were added to seawater PKE at the ratio of 4L of seawater into 1kg of PKE. The PKE solution were then sieved through a 50µm plankton net. The preparation of PKE were same for all the experiment.

Objective 1

3.3 Experiment 1

3.3.1 Optimization of PKE

In order to identify the ideal turbidity level of PKE for *Artemia* feeding, an experiment involving varying turbidity levels of 13cm, 15cm, 17cm, 19cm and 21cm were conducted in triplicates. The turbidity levels were measured using a ruler, functioning as a turbidity stick (Van Stappen, 1996). A disc of white and black colour was attached to the bottom of the ruler (eg. Secchi-disk). The ruler was first submerged, then the feed was added in the culture tanks. The turbidity of the culture water was adjusted and maintained for 14 days. The growth and yield of *Artemia* biomass fed with different turbidity of PKE were determined.

3.3.2 Length of *Artemia* and Yield of Biomass

The length of the *Artemia* were measured daily for 14 days (10 replicates per tank), from the anterior margin of the head to the base of caudal furca using a profile projector (Nikon V-12B) (Abreu-Grobois, 1991; Coutteau et al.,1992; Lavens et al., 1987; Naegel, 1999). The yield of the biomass was weighed using an electronic precision balance (A&D, GX-10001A, Built-In calibration weight, max-10.2kg).

1.4 Experiment 2

1.4.1 Incubation of PKE

The PKE were prepared as mentioned in method 3.2 and were incubated for 0 day (no incubation), 1 day (one day incubation), 2 days (two day incubation) and 3 days (three day incubation). Biological catalyst were not added during the incubation period and throughout the experiment. Optimized amount of PKE from method 3.3.1, was applied in this experiment. A turbidity of 19cm were maintained throughout the culture for 14 days. The daily growth and weight of the biomass at end of the 14th day of culture was measured as mentioned in method 3.3.2.

Objective 2

3.5 Metagenomic Analysis

The microbial composition was analysed in both, PKE over various incubation periods and in the rearing water of *Artemia* (30g/L) before and after adding 1-day incubated PKE on days 1, 7, and 14 of the culture. *Artemia* was fed with PKE incubated for 1 day with a turbidity of 19cm everyday (14 days).

3.5.1 Water sample collection and DNA extraction preparation

The water samples were collected in an autoclaved 1L borosilicate, blue cap bottle (BOMEX). Water samples was collected from PKE incubated over different time period (an additional sample PKE incubated for 14 days, mentioned N days were included) and from the rearing water of *Artemia* before (control) and after the addition of PKE (incubated overnight), all in separate autoclaved bottles. The samples were collected in triplicates. The water samples were filtered using membrane filter sized 0.22 µM (DURAPORE). The membrane filters were then kept in -20°C prior to DNA extraction.

3.5.2 DNA extraction and detection using gel electrophoresis

The DNA extraction was done for each of the samples using ZymoBIOMICS DNA Miniprep kit according to manufacturer's instructions (Zymo Research, US). To validate the size and fragmentation level of the extracted DNA, a fosmid DNA from meridian bioscience, USA was used with a concentration of 100ng/L and sized 40Kb. An electrophoresis run was done on the the fosmid DNA and extracted DNA in a 1% TBE buffered agarose gel prior to 90Volt, for 60 minutes. The TBE buffer were stained with SYBR^{TM} Safe DNA Gel Stain (Invitrogen, USA). The gel was then, observed under Gel Imaging System Model: Omega (Gel Company, USA). The purity of the extracted DNA was then evaluated by calculating using an absorbance ratio at 260/280nm using Spectrophotometer (Eppendorf AG, Germany).

3.5.3 PCR

The 16S metagenomics sequencing was done referring to the 16S metagenomics sequencing library provided by Illumina, USA. The 16S V3-V4 rRNA region was amplified using forward and reverse primers containing Illumina overhang adapters (Forward Primer = 5' TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG and 16S Amplicon PCR Reverse Primer = 5' GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C). The PCR was performed in a thermal cycler utilizing the program of 95°C for 3 minutes, 25 cycles (95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds), 72°C for 30 seconds and 72°C for 5 minutes and hold at 4°C. The amplified product was then purified using AMPure XP beads. The library was prepared by attaching illumine indices (Illumina, CA, USA).

3.5.4 DNA sequencing

The samples were sequenced with approximately 100,000 reads, 250 base pairs in length. The sequence reads were then delivered into CLC genomic workbench

pipeline (Qiagen, Germany) for bioinformatics analysis. In the metagenomics workflow, the organisms were classified from V3 and V4 amplicon utilizing the database of 16S rRNA data. They were classified based on the Greengenes database. The output of the workflow was a classification of reads at several taxonomic levels: kingdom, phylum, class, order, family, genus, and species. The analysis output included (i) Cluster graph: A number of raw clusters, clusters passing filter, clusters that did not align, clusters not associated with an index, and dupicates were shown; (ii) Sample table: The sequencing results for each sample were summarized and (iii) Cluster pie chart: The break down of each sample was classified in a graphical representation.

3.5.5 Downstream data analysis

A total of 42 paired-end raw reads from 21 libraries (**Supplementary table S1**) were imported into QIIME 2 (https://github.com/qiime2/qiime2) for downstream data processing. Raw reads were trimmed by q2-cutadapt plugin (https://github.com/qiime2/q2-cutadapt) and denoised by DADA2 plugin (https://github.com/qiime2/q2-dada2) to generate Amplicon Sequence Variant (ASV) table and representative sequences. Taxonomic classification of the representative sequences was conducted by using q2-feature-classifier plugin (https://github.com/qiime2/q2-feature-classifier) with pre-trained Silva 138 (16S/18S rRNA) references (https://docs.qiime2.org/).

The ASV quantification and taxonomic classification tables were imported into Phyloseq (https://joey711.github.io/phyloseq/) for alpha (Observed, Chao1, and Shannon indices) and beta diversity (Bray-Curtis dissimilarity) analysis. Microbial composition was normalized and analysed at phylum and genus levels. Multivariate Association with Linear Models algorithm (MaAsLin2) (https://huttenhower.sph.harvard.edu/maaslin/) was used to identify differential microbial genera between experimental groups. Microbial functional community analysis was predicted by PICRUSt2 and microbial functional community quantification was imported into STAMP for pairwise comparisons (https://kiwi.cs.dal.ca/Software/STAMP).

Objective 3

3.6 Experiment 1

3.6.1 Optimization of Baker's yeast as feed for *Artemia*

Baker's Yeast (Brand: Mauripan) purchased from a local market were used in this experiment. PKE were maintained at a turbidity of 19cm with Baker's yeast cells as supplement. Four different amount of yeast powder 1g, 2g, 3g, and 4g were used in this study. Yeast powder was mixed with 1L of seawater for cell activation prior to be applied into the culture tank. Baker's yeast was supplemented to *Artemia* once per day for 14 days. The growth and yield of *Artemia* biomass were recorded as mentioned in method 3.3.2.

3.7 Experiment 2

3.7.1 *Artemia* feed preparation and feeding regime

In this study, different types of feed were used to test the growth of *Artemia*, yield of biomass, proximate composition and fatty acid profile of the *Artemia* biomass. The treatments were Baker's Yeast, PKE and PKE supplemented with yeast.

Each treatment was carried out in triplicates. The *Artemia* were fed daily for 14 days. Turbidity of the culture water were used to determine the amount of feed given to the *Artemia*. Turbidity of the culture water were maintained at the level of 19cm. Optimized baker's yeast of 3g from method 3.6 was used to supplement the *Artemia*. Baker's yeast was prepared by mixing the yeast with 1L of sea water, before adding them into the culture tank. The growth and yield of *Artemia* biomass was recorded as mentioned in method 3.3.2.

3.8 The gut content of *Artemia*

The gut content of *Artemia* was observed under a dissecting microscope at the magnification of 100x.

3.9 Nutritional Analysis

3.9.1 Sample preparation

Artemia biomass fed with Baker's Yeast, Palm kernel expeller (PKE), and Palm kernel Expeller supplemented with yeast was further analysed for the content of moisture, ash, lipid, protein, fibre and fatty acid profile. The *Artemia* biomass was freeze-dried in a freeze dryer at -34 to -45°C for 48 hours. The freeze-dried samples were then ground into a fine powder prior to use. Each analysis was done in triplicates (AOAC, 1995).

3.9.2 Moisture

The crucible was dried for 30 minutes at 100°C in the oven. After being dried and cooled in a desiccator, the crucible was weighed and its weight was recorded as W1. Approximately, 2 grams of freeze dried *Artemia* sample was weighed in the crucible and its weight was recorded as W2. The samples are dried in an oven for 6 hours at 100°C. The crucible are then cooled in a desiccator, the crucible was then weighed and its weight is recorded as W3 (AOAC, 1995). The moisture was calculated using the formula:

$$\text{\% of dry matters} = \frac{W3 - W1}{W2 - W1} \times 100$$

% of moisture = 100 – % of dry matters

3.9.3 Lipid

Aluminium extraction cup was dried in an oven for an hour at 100°C. It was then cooled in a desiccator and its weight was then recorded as W1. Approximately 2 grams of freeze dried *Artemia* sample was weighed (recorded as W2) and placed in extraction thimble. The surface of the thimble is covered with a thin layer of cotton wool and fixed into the Soxtec machine (Soxtec^{TM}2043, FOSS). 50ml of hexane were added into the extraction cup and placed on the heating mantle at the Soxtec machine. The extraction of the lipid were done 15 minutes in "Boiling" position and 30 minutes in "Rinsing" position, The Crude lipid collected in the extraction cup was then dried in an oven for 2 hours at 100°C (AOAC, 1995). The extraction cup was then allowed to cool in a desiccator and weighed (recorded as W3). The lipid content was calculated using the formula:

$$\% \text{ of Lipid} = \frac{W3 - W1}{W2} \times 100$$

3.9.4 Ash (Marsham, Scott & Tobin, 2007)

The crucible was dried in an oven set to 100°C for 30 minutes. The crucible was weighed after being cooled in a desiccator and its weight was noted as W1. 2 grams of freeze dried Artemia sample was weighed in the crucible and its weight was recorded as W2. The crucible was then placed in a muffle furnace (Carbolit/ELF11), for 3 hours at 600°C. At this temperature, only the minerals were left behind as all organic matter will be burnt. The crucible was then cooled in a desiccator, before weighing them (AOAC, 1995). Its weight then was recorded as W3. The Ash content was then calculated as below:

$$\% \text{ of Ash} = \frac{W3 - W1}{W2 - W1} \times 100$$

3.9.5 Protein

Approximately 0.2g of freeze dried *Artemia* biomass is weighed (recorded as W) and placed into digestion tubes.

3.9.5.1 Digestion

Kjeldahl tablets is added into all the digestion tubes (including blank), followed by 5ml of sulphuric acid added into the same tubes. The scrubber unit were turned on and the air flow regulating valve were fully opened. The exhaust manifold were fit on the tubes in the rack. The rack is then placed in the digestion unit. Digestion takes place for 40 minutes. Once the digestion was complete, the hot rack and manifold were carefully removed from the digestion unit and were placed on a hot resistant surface. The tubes were them allowed to cool. Once the tubes were cooled, 40 ml of distilled water were added to each tube.

3.9.5.2 Distillation

30 ml of Boric acid (4%) were prepared and placed in a 250 ml conical flask. 8 drops of indicator were added to the boric acid. 30 ml of NaOH were prepared and added into the digestion tubes. Distillation was done in Kjeldahl machine (KT 200 Kjeltec) unit for 5 minutes.

3.9.5.3 Titration

The distilled product were then titrated with 0.1N HCl until there is a change of colour from green to pink. The volume of HCl used were recorded as T. The same titration procedure was done for the distilled product from blank, the volume of HCl used were recorded as B.

The content of protein was calculated using the formula (AOAC, 1995):

% of N in the sample

$$\frac{(T - B) \times N \times 14.007}{W} \times 100$$

% of Protein = % N × F

N = Normality of HCl

F = Protein factor, 6.25

3.9.6 Nitrogen-free extract (NFE)

The amount of nitrogen-free extract was estimated by subtracting the percentage of the total content of protein, lipid, fat, fibre, and ash from 100%

3.9.7 Fibre

The filter bags are weighed and its weight is recorded as W1. Approximately 1g of freeze dried *Artemia* sample were weighed and recorded as W2 (one filter bag is left empty without sample and noted as blank). The weighed samples were placed into the filter bags and sealed within 0.5cm from the open edge using a heat sealer. The fat is extracted from the sample by soaking all the filter bags including blank in petroleum ether for 10 minutes. The filter bags were then removed from petroleum ether and allowed to air dry for approximately 5 minutes. The samples in the bag are spread evenly by slightly flicking the bag to avoid clumping of the sample. The fibre bags were placed in the bag suspender trays (ANKOM Technology). Three bags per tray were placed (9 trays, 24 bags total). The bag suspender weight was placed on top of

the 9th tray to keep the bag suspender stay submerged. 1900-2000ml of sulphuric acid were added (1500ml for samples less than 20 bags, 100ml/bag must be added for samples more than 20 bags) to ANKOM fiber analyser vessel. Timer for 45 minutes was set, once the Agitation and Heat were turned on. Once it was confirmed that the bag suspender is agitating, the lid was tightly sealed. The Heat and Agitation were turned off, after 45 minutes. The exhaust valve was opened and the hot solution were released before opening the lid. Once the solution has been exhausted, the exhaust valve was closed and the lid was opened. 1900ml of hot water (90-100ºC) were added and agitated for 5 minutes (rinsing with hot water were repeated 2 times). The steps bolded were repeated using Sodium hydroxide (NaOH). The filter bags were removed from the suspender and the excess water were gently pressed out. The bags were placed in a 250ml beaker, followed by the addition of acetone until all bags are covered. The bags were allowed to be soaked for 3 to 5 minutes. The bags were then removed and the excess acetone were lightly pressed out. The bags were then dried in oven at 105 ºC. Bags were cooled in desiccator and weighed (recorded as W3). The bag was placed in a crucible that has been dried and weighed (W4). It was then placed in muffle furnace for 2 hours at 600 ºC. The ash sample were then cooled in a desiccator and weighed (W5). The content of fibre was calculated using the formula:

% of Fiber =

$$\frac{Wx - (W1 \times C1)}{W2} \times 100$$

$Wx = (W3+W4) – W5$

$C1= \frac{(W3+W4)-W5}{W1}$

3.9.8 Fatty acid analysis

Fatty acid analysis was performed using Fatty Acids Methyl Ester (FAME) analysis, utilizing an optimized procedure (Lepage & Roy, 1984). This method suggested a direct transesterification, where hexane was utilized for FAME extraction, subsequently by solvent drying. The resulting FAME compounds of 1.5ml were injected into the (Gas chromatography) GC machine. Nitrogen gas was used as the carrier gas at a pressure of 100 kPa, and recognition was performed using a Flame Ionization Detector (FID). The temperature was programmed to an initial temperature that was set at 85°C, increased to 150°C (30°C/min), then to 152°C (0.1°C/min), further increased to 172°C (0.6/min) and last at 187°C (25°C). The analyses were done in triplicates.

4.0 Statistical analysis

All the data were analysed with ANOVA (Analysis of Variance) via a computer software SPSS (Statistical Package of Social Science, version 20). Significant difference in the average mean values were compared with Turkey's multiple range test. A significance level of 95% ($p<0.05$) was considered as significantly different.

CHAPTER 4

RESULTS

Objective 1 (Experimet 1)

4.1 Growth and biomass production of *Artemia* upon feeding with different turbidities of PKE

The initial growth of the *Artemia* (day 1) in all treatments were approximately 0.5mm. Upon feeding *Artemia* with PKE at different turbidities, the *Artemia* growth in all treatments showed an increasing trend in a range of 0.5mm to 1.5mm from day 1 to day 14 of the culture ($p>0.05$). An average growth of 9mm to 10mm was obtained in all the treatments at day 14 of the culture ($p>0.05$).

Feeding *Artemia* with PKE at turbidity 19cm yielded 693g of adult biomass upon 14 days of culture from an initial incubation of 1g nauplii. This condition was best across all the feeding regimes examined in this study. Feeding PKE at turbidity 13cm produced the lowest yield, with total harvested adult biomass of 289g ($p<0.05$), a weight three times lesser than the 19cm turbidity.

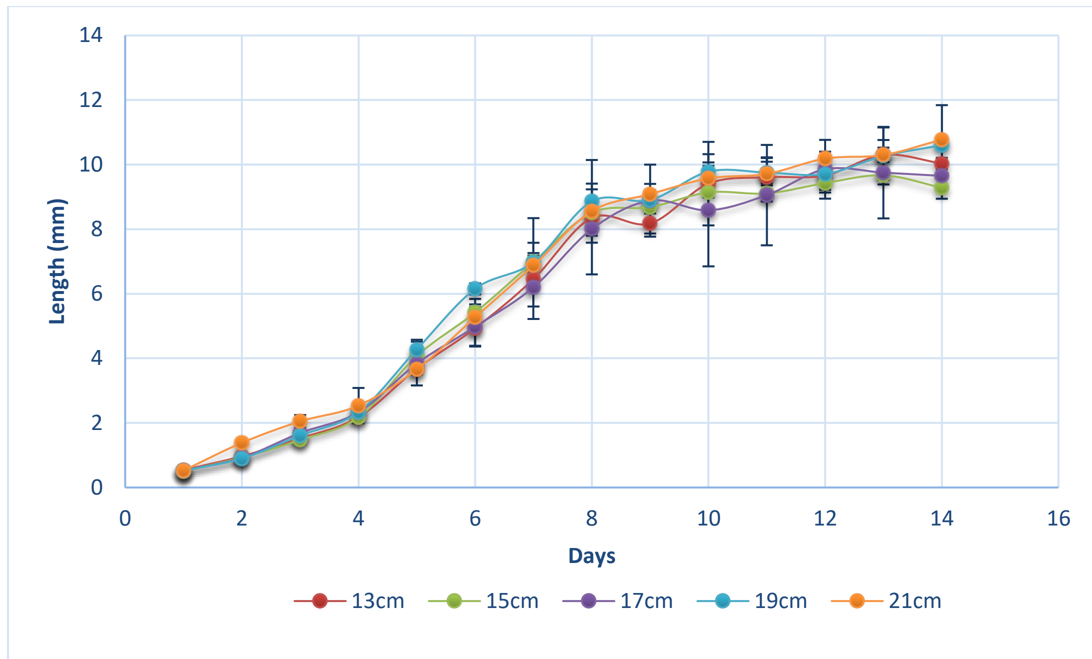

Figure 4.1a: Growth of *Artemia* upon feeding with different turbidities of PKE. The error bars stands for standard deviation.

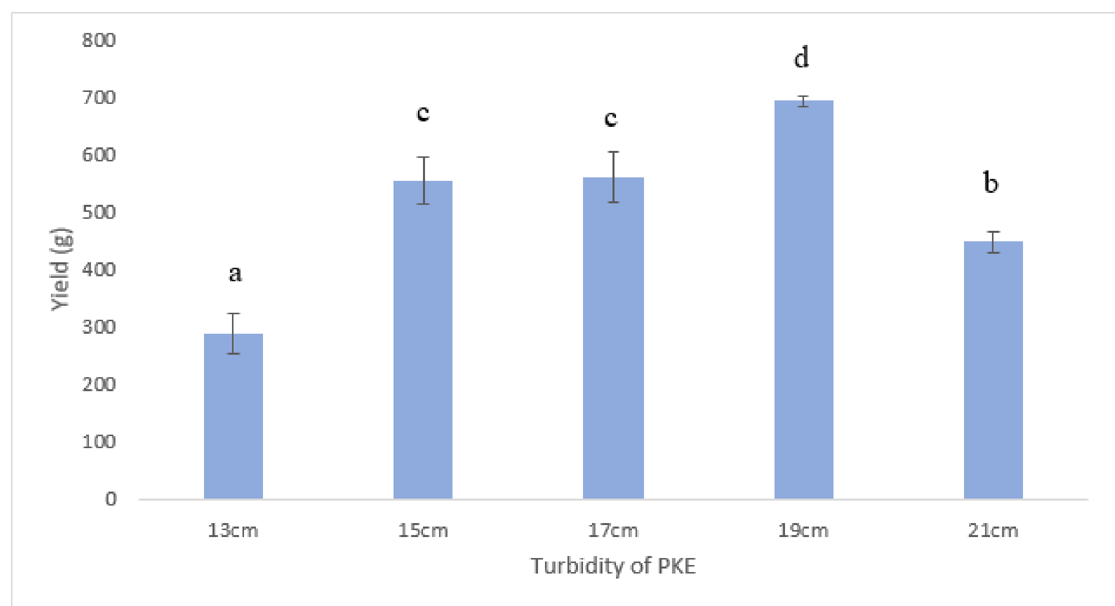

Figure 4.1b: The average yield of *Artemia* biomass (g) reared upon different turbidities of PKE (13cm, 15cm, 17cm, 19cm, 21cm) in the end of 14 days of culture. The error bars stands for standard deviation. Different letters indicates significant differences ($P<0.05$) among the treatments.

Objective 1 (Experiment 2)

4.2 The growth and *Artemia* biomass production upon feeding with PKE incubated at different duration.

The length of *Artemia* fed with PKE without incubation measured approximately 4.2mm and 9.1mm at day 7 and 14, respectively, whereas those fed with PKE incubated 1 day reached approximately 6.9mm and 10.8mm. *Artemia* fed with PKE incubated for 2 days grew to 3.9mm and 8.6mm at similar time points while those fed with PKE incubated for 3 days reached 5.3mm and 8.9mm, respectively. At day 12, upon feeding *Artemia* with PKE incubated 1 day the length of *Artemia* reached an optimum growth of 10.2mm, whereas those fed with PKE without incubation, 2 days incubation and 3 days incubation measured approximately 8.1mm, 8.2mm and 7.9mm respectively.

Feeding PKE without incubation to *Artemia* yielded approximately 197g of biomass upon 14 days of culture (Figure 4.2b) whereas application of PKE incubated 1 day, 2 days and 3 days yielded 635, 577 and 479g, respectively. Overall, the use of PKE incubated 1 day at 19cm turbidity was the best condition to boost *Artemia* biomass production when compared to other treatments examined in this study during culture ($P<0.05$). This condition was applied for use in subsequent experiments.

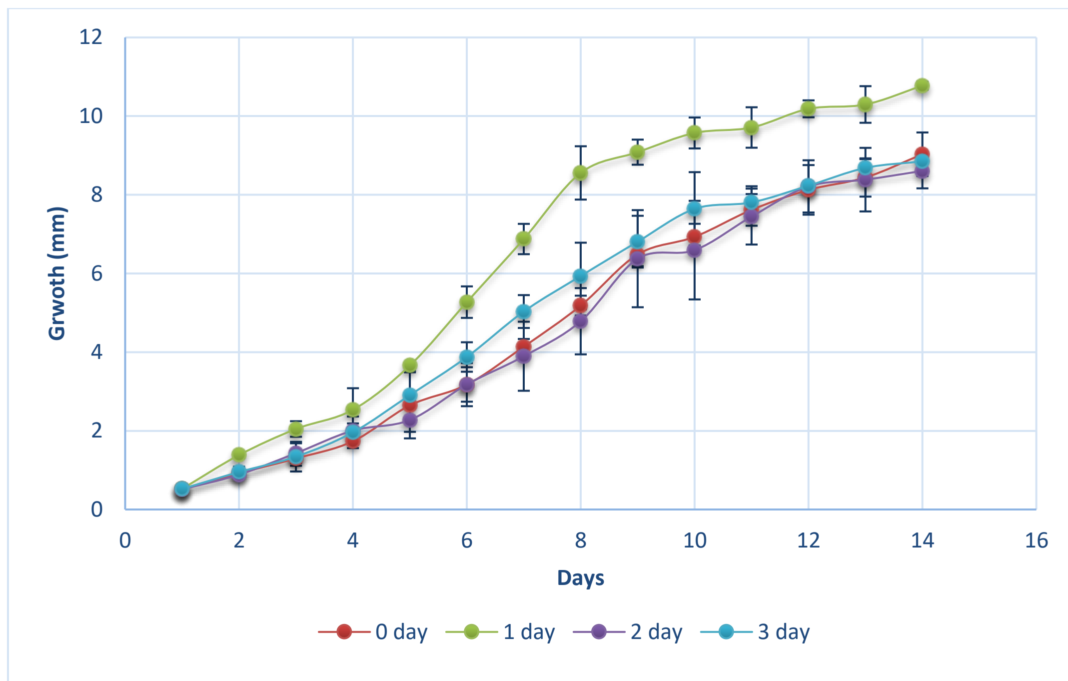

Figure 4.2a: The growth of *Artemia* upon feeding with PKE incubated at different duration: 0 day (no incubation), 1 day incubation, 2 days incubation, and 3 days incubation for 14 days. The error bars stand for standard deviation.

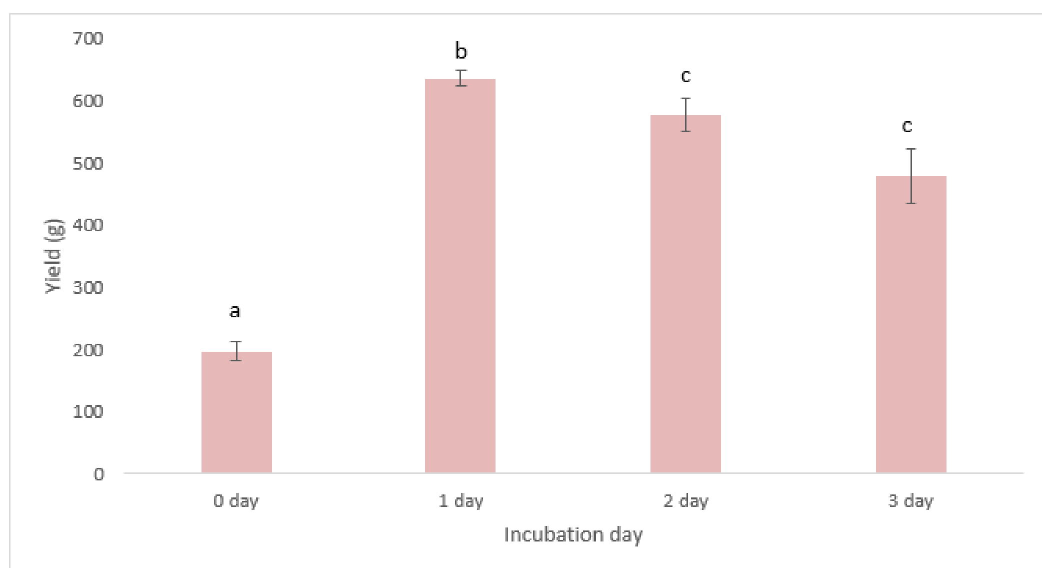

Figure 4.2b: The yield of *Artemia* biomass upon feeding with PKE incubated at different duration: 0 day (no incubation), 1 day incubation, 2 days incubation, and 3 days incubation in the end of 14 days culture. The error bars stand for standard

deviation. Different letters indicate significant differences (P<0.05) among the treatments.

Objective 2

4.3 The microbiome analysis of PKE solutions and *Artemia* rearing water.

4.3.1 Alpha and beta diversity indices

The 16S amplicon sequencing of all 21 libraries had generated an average of 16475 features per sample and the subsequent clustering produced a total of 1527 ASVs (**Supplementary table S1**). The number of unique ASVs detected in PKE solutions were in order of PKE3D>PKE1D>PKED>PKE2D. Both alpha diversity indices, Chao1 and Shannon, (**Fig. 4.3.1a**) showed that PKE3D has the highest species richness and diversity. PKED and PKE2D were with higher species diversity, but lower species richness than PKE1D (**Fig. 4.3.1a**). Meanwhile, the number of unique ASVs in PKE1D-enriched *Artemia* rearing water (RD1, RD7, and RD14) was ranged from 113 to 280 (**Supplementary table S1**), where species richness and diversity were declined from the first day (RD1) to seventh day (RD7), and plateaued until the end of trial (**Fig. 4.3.1a**).

Bray-Curtis dissimilarity analysis had separated all experimental samples into three clusters (**Fig. 4.3.1b**). The microbiome profile of PKE3D samples was distant from other two clusters, whereas PKED, PKE1D and PKE2D were clustered together, implied that microbiome profile changed the most in PKE3D. All PKE1D-enriched *Artemia* rearing water samples (RD1, RD7, and RD14) formed a cluster, indicate that the microbiome community in PKE1D-added rearing water did not change much from Day 1 to Day 14 of *Artemia* culture. All biological replicates were clustered together, indicating that intragroup samples were closely related and the data is reliable to proceed for downstream analysis.

4.3.2 Microbiota community composition changes

The changes in microbial composition were analyzed at phylum and genus levels (**Fig. 4.3.2**). Overall, the top five abundant phyla in all PKE solutions were Proteobacteria, Campilobacterota, Firmicutes, Actinobacteriota, and Patescibacteria. Proteobacteria was the most abundant phylum in PKED, but its dominancy in terms of relative abundance were reduced from 59% to 8% 3-days after incubation (PKE3D) (**Fig. 4.3.2a**). The relative abundance of Campilobacterota increased from 34% to 53% 1-day after incubation, then started to decrease to 43% and 3% respectively after 2 (PKE2D) and 3 days (PKE3D) (**Fig. 4.3.2a**). Conversely, the proportion of Firmicutes increased starting from PKE2D, where its relative abundance raised from 0.7% in PKE1D to 77% in PKE3D (**Fig. 4.3.2a**).

The top five dominant phyla in PKE1D-added *Artemia* rearing water were Proteobacteria, Actinobacteriota, Campilobacterota, Patescibacteria and Bdellovibrionota. The relative abundance of Proteobacteria in PKE1D-added *Artemia* rearing water demonstrated a decreasing trend, where the composition went from 93% in RD1 to 76% in RD7, and then to 50% in RD14. Meanwhile, Actinobacteriota was growing continuously from 3% in RD1 to 41% in RD14 (**Fig. 4.3.2a**). The abundance of Campilobacterota rose from RD1 to RD14, where its composition was elevated from 0.03% to 7% (**Fig. 4.3.2a**).

The top 20 genera were accounted for approximately 88% of the total community (**Fig. 4.3.2b**). *Halarcobacter* was the most abundant genus in all PKE solutions, except PKE3D which *Anaeroplasma* occupied approximately 68%. Whereas, the relative abundance of *Thioclava* declined from 47% in PKED to 0% in PKE3D. On the other hand, consistent application of PKE1D induced massive changes in microbial genera composition of *Artemia* rearing water (**Fig. 4.3.2b**). The top 3 dominant genera in RD1 (*Yangia*, *Pseudooceanicola*, *Candidatus_Actinomarina*) were shifted to *Marivivens*, *Candidatus_Aquiluna,* and *Kordiimonasi* in RD7, as well as *Candidatus_Aquiluna, Kordiimonasi,* and *Thalassospira* in RD14.

4.3.3 Differential abundance of microbial genera in response to PKE

Differential abundance of 9 and 1 genera were detected in PKE1D and PKE2D, while no differential microbial genera was significant (FDR<0.05) in PKE3D, when compared to PKED (FDR<0.05) (**Fig. 4.3.3**). The abundance of 6 genera, including beneficial *Bifidobacterium* and *Lactobacillus*, were significantly (FDR>0.05) higher in PKE1D, whereas 2 genera (*Clade_1a*, and *Thioclava*) were reduced (FDR<0.05) in PKE1D (**Fig. 4.3.3a**). Only *Lactobacillus* grew more dominantly in PKE2D (**Fig. 4.3.3a**).

A total of 7 and 4 microbial genera abundance were altered exclusively (FDR<0.05) in RD7 and RD14 (**Fig. 4.3.3b**). The abundance of *Candidatus Aquiluna* showed an increasing trend, conversely *AB1*, *Pseudooceannicola*, and *Yangia* demonstrated a significant (FDR<0.05) decreasing trend, from RD1, to RD7 and RD14 (**Fig. 4.3.3b**).

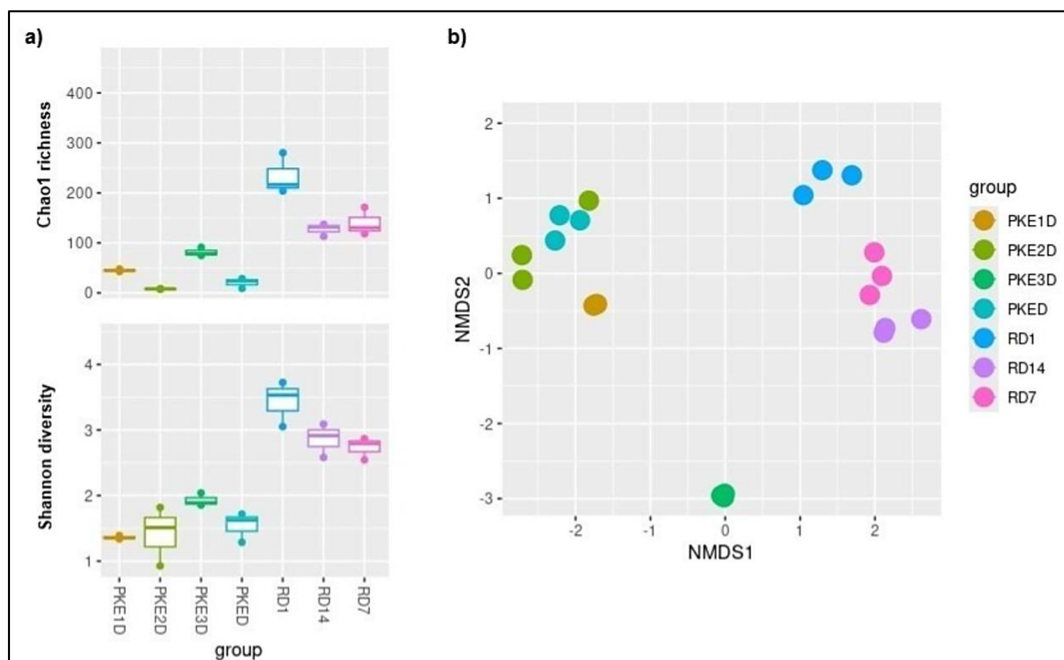

Figure 4.3.1 (a) Alpha (Chao1 and Shannon), and (b) beta (Bray-Curties disimilarity) diversity indices of the experimental samples in 16S metagenomic analysis. PKED, non-incubated PKE solution; PKE1D, 1 day-incubated PKE solution; PKE2D, 2 days-

incubated PKE solution; PKE3D, 3 days-incubated PKE solution; RD1, RD 7, and RD14 refer to Day 1, 7, and 14 of *Artemia* rearing water containing PKE1D.

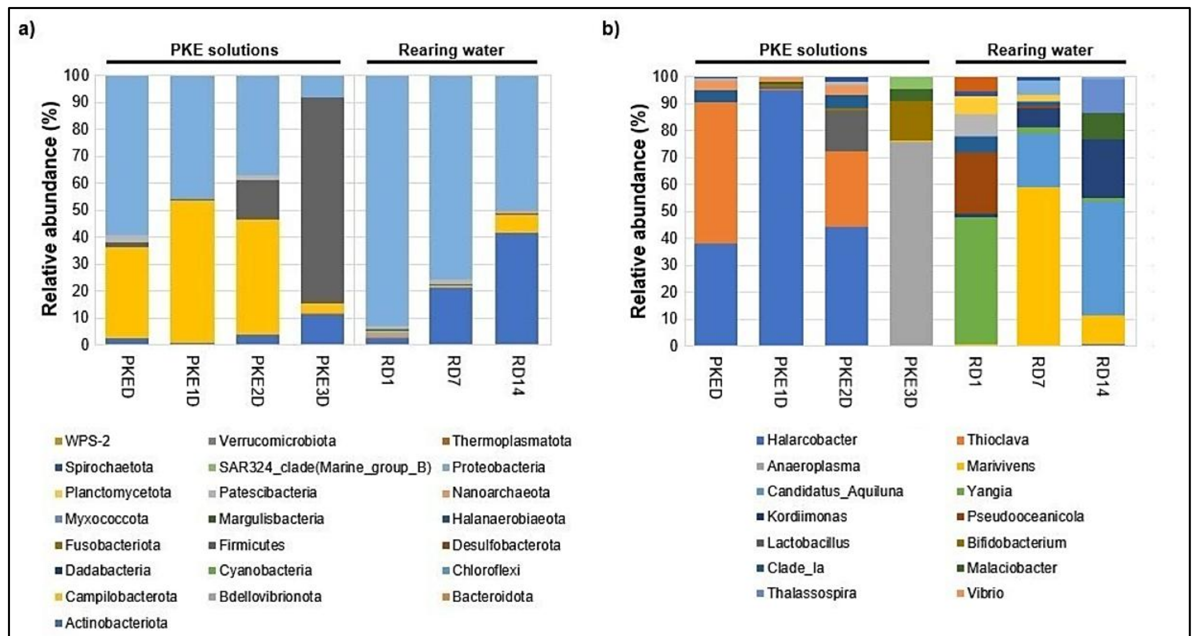

Figure 4.3.2 The microbial composition analysed at (a) phylum, and (b) genus levels. PKED, non-incubated PKE solution; PKE1D, 1 day-incubated PKE solution; PKE2D, 2 days-incubated PKE solution; PKE3D, 3 days-incubated PKE solution; RD1, RD 7, and RD14 refer to Day 1, 7, and 14 of *Artemia* rearing water containing PKE1D.

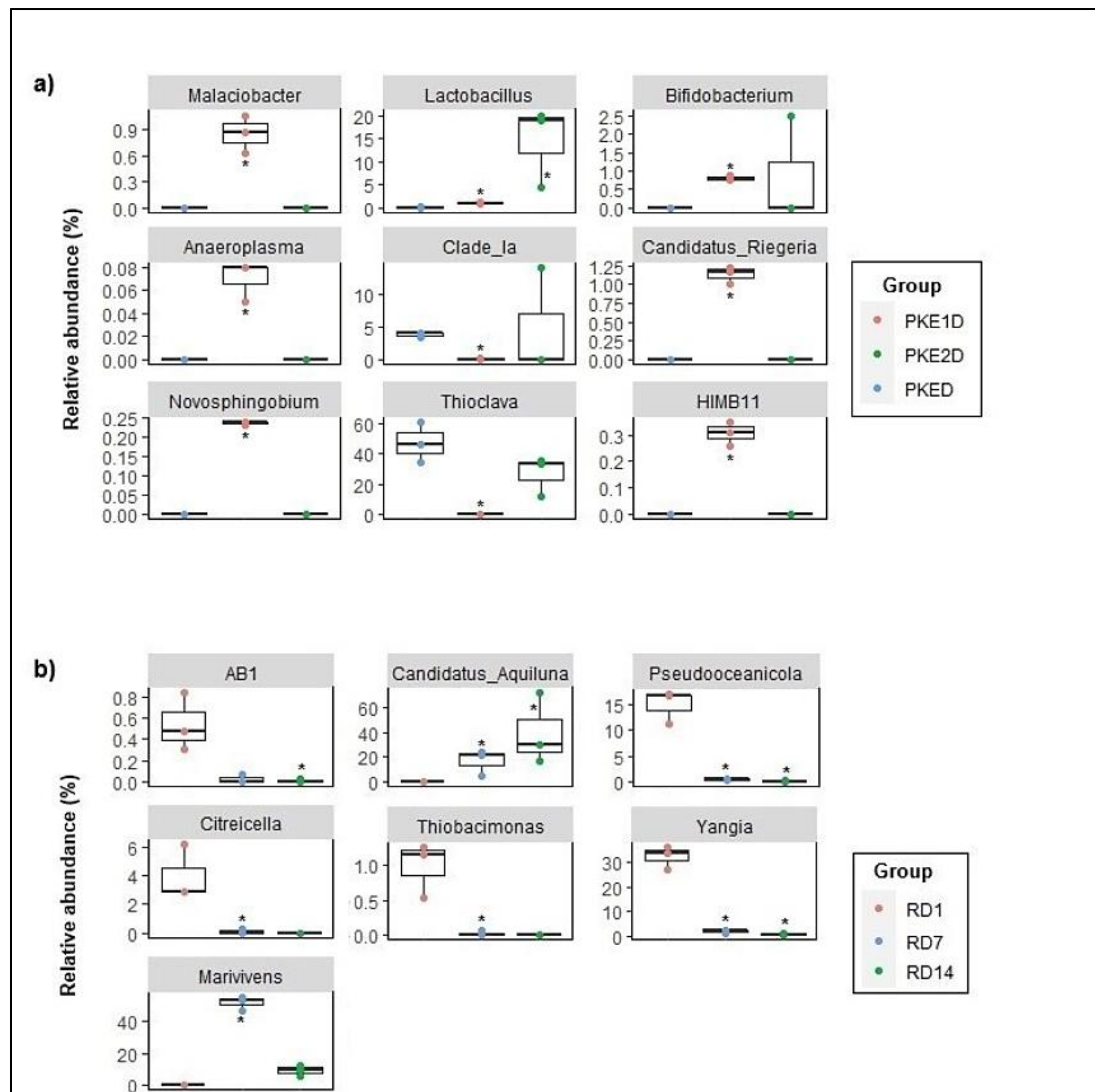

Figure 4.3.3 Differential genera flagged in (a) PKE solutions, and (b) *Artemia* rearing water, by MAasLin2, when compared to the control groups (PKED and RD1). PKED, non-incubated PKE solution; PKE1D, 1 day-incubated PKE solution; PKE2D, 2 days-incubated PKE solution; PKE3D, 3 days-incubated PKE solution; RD1, RD 7, and RD14 refer to Day 1, 7, and 14 of *Artemia* rearing water containing PKE1D.

Objective 3 (Experiment 1)

4.4 The length and biomass of *Artemia* upon supplementing with yeast.

The initial growth of the *Artemia* in all treatments were approximately 0.5mm. Upon supplementing *Artemia* with yeast at different concentrations, the length of *Artemia* increased in a range of 0.5mm to 2.5mm from day 1 to day 14 of the culture ($p>0.05$). At day 9, upon supplementing *Artemia* with 3g yeast, the length of *Artemia* reached an optimum growth of 10.2mm, whereas those supplemented with 1g, 2g, and 3g yeast measured 8.6mm, 9.3mm and 9.2mm respectively. *Artemia* supplemented with 3g yeast measured 11.2mm at day 14 of the culture ($p<0.05$), whereas those supplemented 1g, 2g and 4g yeast measured 9.5mm, 10.7mm and 10.6mm respectively.

Artemia in all the treatments yielded approximately 900g to 1.2kg biomass ($p>0.05$) upon 14 days of culture (Figure 4.4b). Overall, *Artemia* supplemented with 3g yeast was the best condition to boost *Artemia* length to 10.2mm in 9 days and 11.2mm in 14 days when compared to other treatments examined in this study. This condition was applied for the use in subsequent experiments.

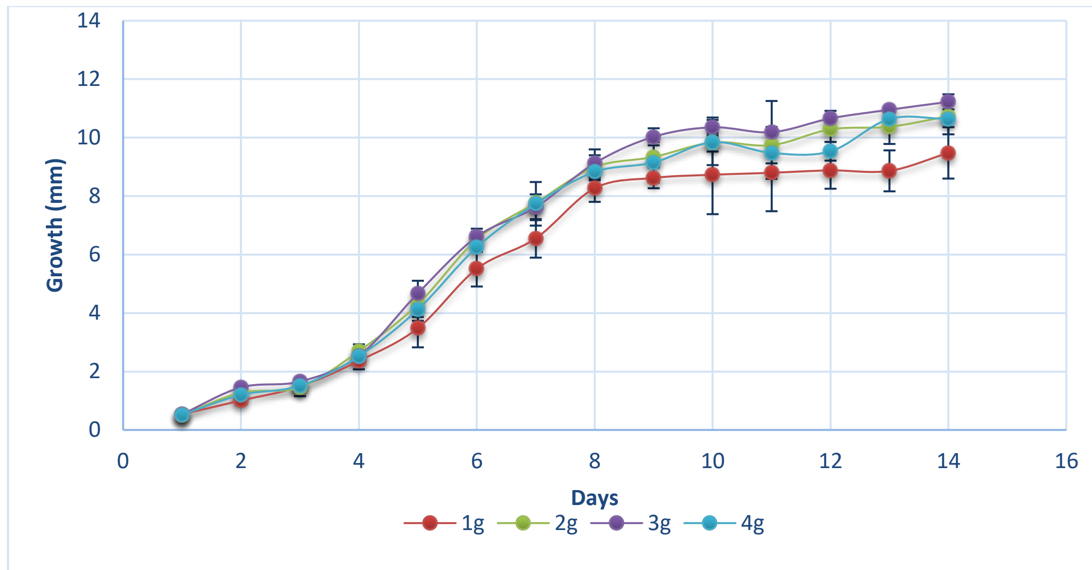

Figure 4.4a: The total average growth of *Artemia* upon supplementation of yeast with PKE at a concentration of 1g, 2g, 3g and 4g. The standard error stands for Standard deviation.

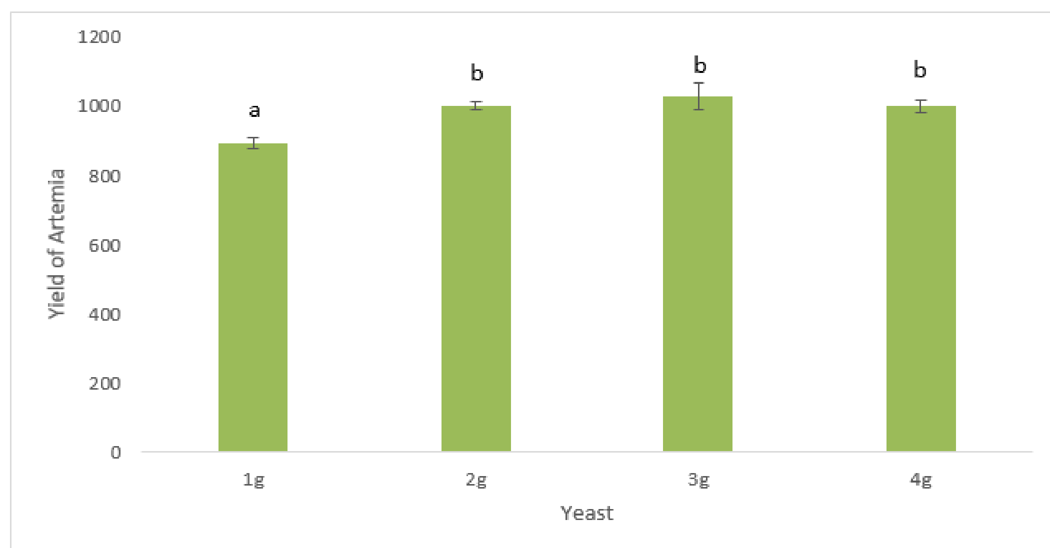

Figure 4.4b: The yield of *Artemia* upon supplementation of Yeast with PKE at a concentration of 1g, 2g, 3g and 4g of yeast. The standard error stands for Standard deviation. Different letters indicates significant differences (P<0.05) among the treatments.

Objective 3 (Experiment 2)

4.5 The length and biomass production of *Artemia* upon feeding with Yeast, PKE supplemented with yeast and PKE

The initial length of *Artemia* in all the treatments were approximately 0.5mm. *Artemia* fed with PKE supplemented with yeast reached an optimum length of 10mm in 9 days of culture ($p<0.05$), whereas *Artemia* fed with PKE and yeast measured 7.7mm and 7.1mm respectively. At day 14, the *Artemia* fed with PKE supplemented with yeast measured 11mm ($p<0.05$), whereas those fed with PKE and yeast measured 10.2mm and 9.9mm respectively. *Artemia* fed with PKE supplemented with yeast showed the fastest growth in a range 0.8mm – 1.8 mm in 14 days culture, whereas *Artemia* fed with PKE and yeast grew in a range of 0.5mm - 1.0mm in 14 days culture.

Feeding *Artemia* with PKE supplemented with yeast yielded approximately 1kg of biomass upon 14 days of culture (Figure 4.5b), whereas feeding them with PKE and yeast alone yielded 674g and 394g respectively. Therefore, supplementing yeast with PKE to *Artemia* contributed a faster growth and boost *Artemia* biomass production compared to other treatements examined in this study ($p<0.05$).

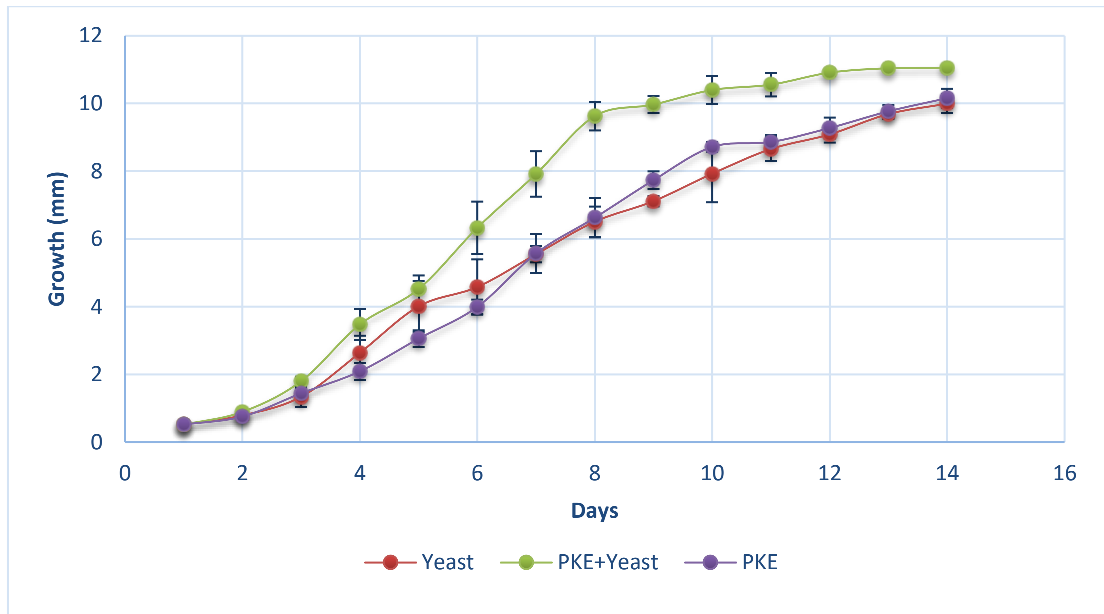

Figure 4.5a: The total average growth of *Artemia* upon feeding with Yeast, PKE supplemented with Yeast and PKE in 14 days of culture. The standard error stands for standard deviation.

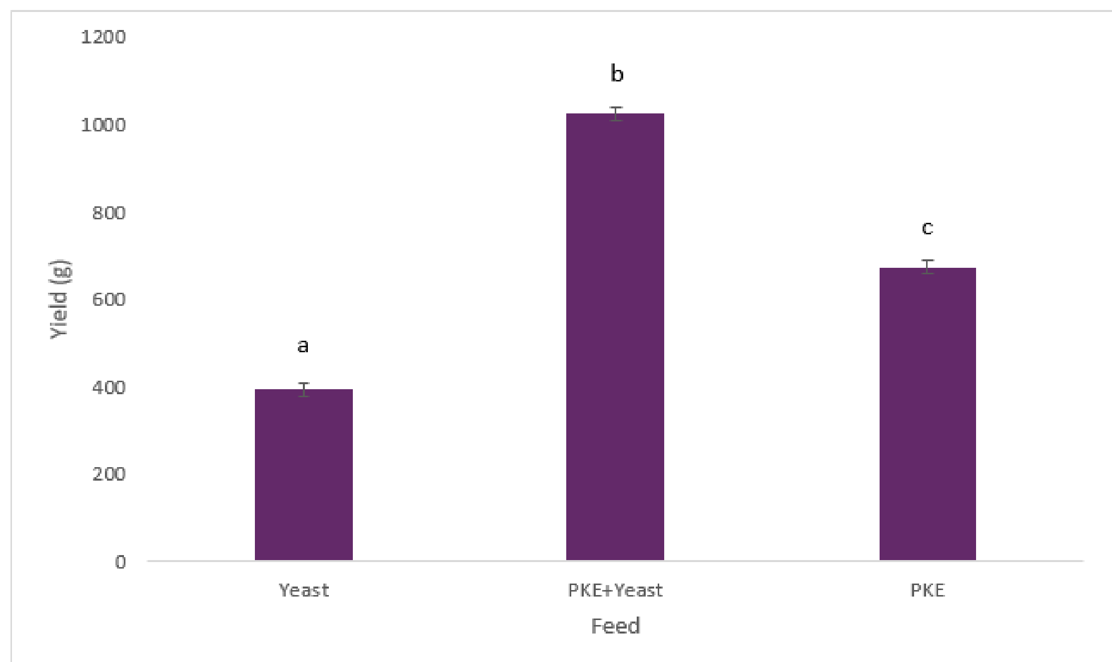

Figure 4.5b: The yield of *Artemia* biomass upon feeding with Yeast, PKE supplemented with Yeast and PKE in 14 days of culture. The standard error stands for standard deviation. Different letters indicates significant differences ($P<0.05$) among the treatments.

4.6 The nutritional composition of *Artemia* upon feeding with yeast, PKE supplemented with yeast and PKE solely.

The highest crude lipid content in *Artemia* was 11% upon feeding with PKE supplemented with yeast, whereas those fed with PKE and yeast was 9% and 7% respectively ($p<0.05$). The crude protein content in *Artemia* fed with yeast and PKE supplemented with yeast were approximately 52% ($p>0.05$) in each treatment, with the content 7% lower in *Artemia* fed with PKE solely. The fibre content was approximately 1% for all the treatments ($p>0.05$). On the other hand, the ash content in the *Artemia* fed with PKE was 23%, with the content 3% higher than those fed solely with Yeast and PKE supplemented with yeast ($P<0.05$).

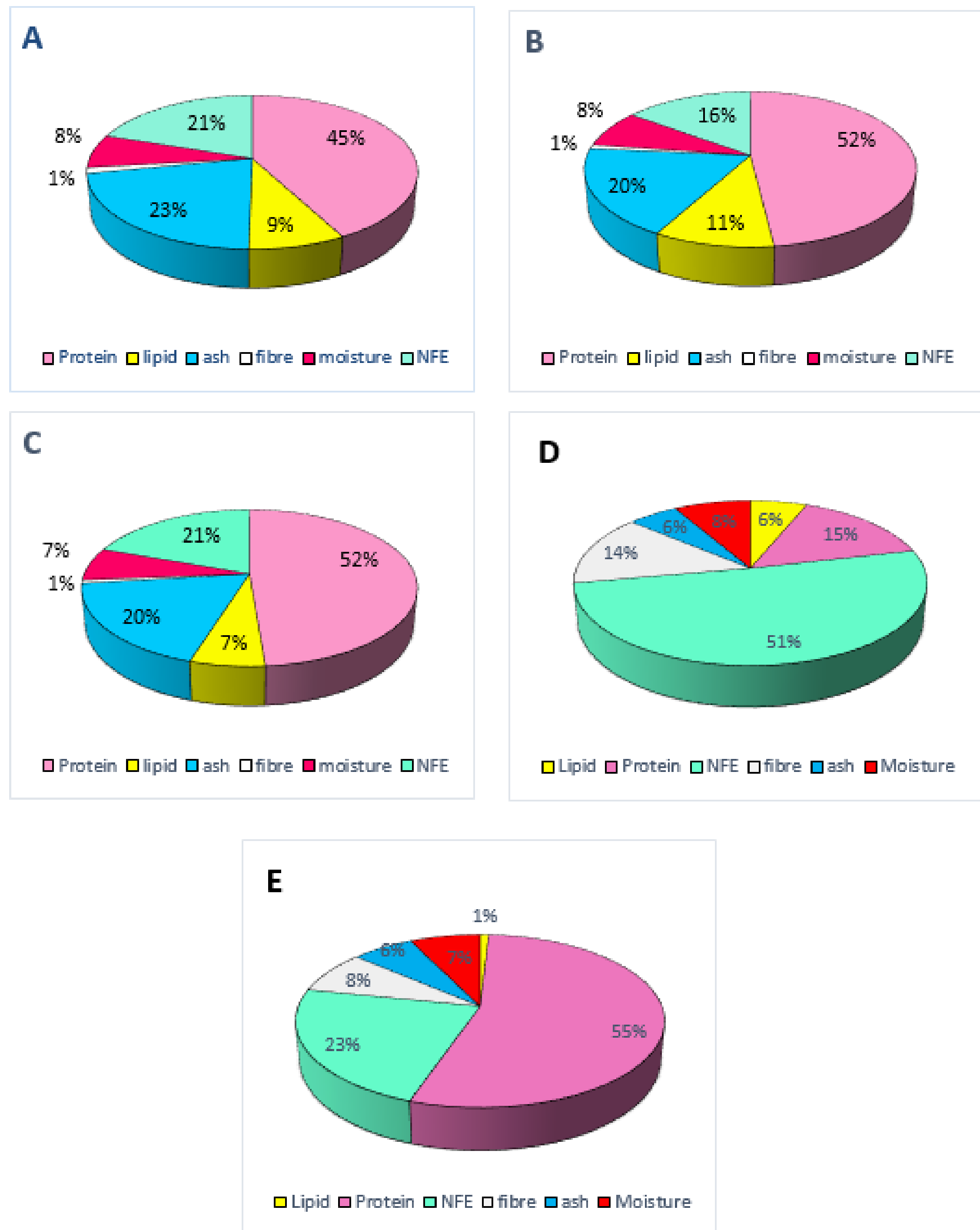

Figure 4.6: The pie chart A, B, C, D and E shows the nutritional composition of the *Artemia* upon feeding with PKE, PKE supplemented with yeast, Yeast in 14 days of culture and the feed PKE and yeast.

4.7 The gut content of *Artemia*.

The gut of *Artemia* was dark and full upon feeding with PKE supplemented with yeast, whereas those fed with PKE and yeast solely displayed a partial filled gut. Empty gut was seen in unfed *Artemia* (Figure 4.7).

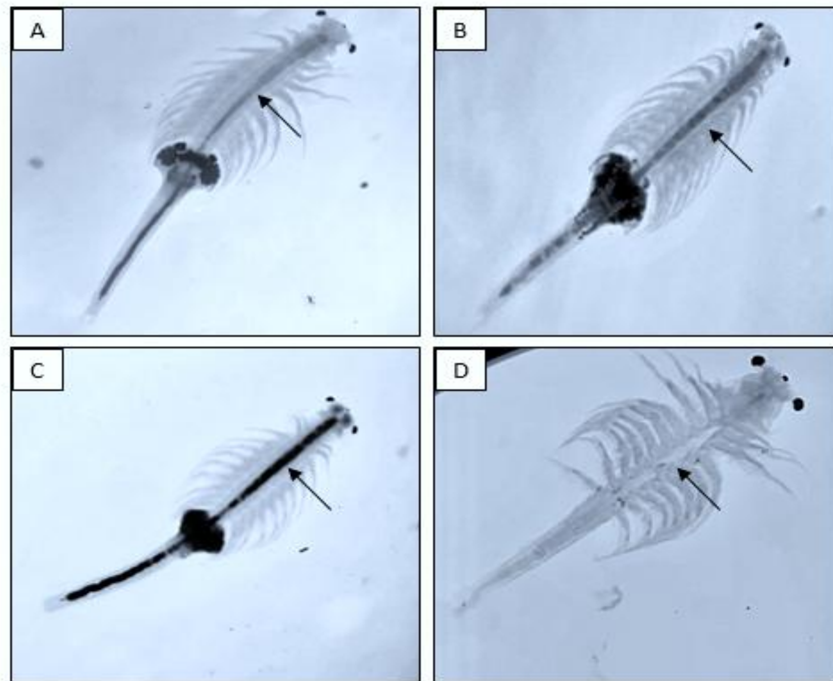

Figure 4.7: Arrow shows the gut of *Artemia* fed with Yeast, PKE, and PKE supplemented with yeast on day 14 of the culture. A: Gut of *Artemia* fed with yeast, B: Gut of *Artemia* fed with PKE, C: Gut of *Artemia* fed with PKE supplemented with yeast and D: Gut of unfed Artemia.

4.8 The fatty acid profiles of *Artemia*

Table 4.1: The fatty acid profiles of *Artemia* fed solely with yeast, PKE supplemented with yeast and PKE.

Fatty Acid (%)	Yeast	PKE supplemented with yeast	PKE
C14:0	0.65±0.06a	1.37±0.07b	4.80±0.09c
C16:0	4.44±0.38a	9.54±0.31b	11.60±0.17c
C18:0	3.39±0.22a	5.83±0.54b	5.73±0.11b
C20:0	0.06±0.05a	0.19±0.32a	0.19±0.19a
C22:0	0.00±0.00a	0.88±0.76a	0.30±0.02a
SFA	8.53±0.45a	17.80±1.19b	22.63±0.29c
C16:1n-7	14.63±0.27a	13.31±0.47a	13.27±0.18b
C18:1n-9	23.61±0.63c	21.76±1.06b	10.67±0.15a
C18:1n-7	1.61±0.15a	17.49±1.00b	19.26±0.32c
C20:1n-9	0.00±0.00a	0.12±0.01b	0.36±0.01c
MUFA	39.85±0.36a	52.69±2.52b	43.56±0.37a
C18:2n-6	4.24±0.08b	0.83±0.56a	1.53±0.08a
C18:3n-3	0.78±0.05b	0.74±0.08b	0.25±0.05a
C20:2n-6	0.02±0.02a	0.05±0.02a	0.02±0.03a
C20:4n-6	0.97±0.15a	1.54±0.12b	1.15±0.03a
C20:3n-3	0.00±0.00a	0.02±0.03a	0.00±0.00a
C20:5n-3	0.30±0.05a	1.65±0.26b	0.32±0.02a
C22:6n-3	0.25±0.30a	0.34±0.07a	0.35±0.11a
PUFA	6.55±0.51c	5.16±0.71b	3.61±0.17a

The highest EPA content in *Artemia* upon feeding them with PKE supplemented with yeast was 1.65% ($p<0.05$) whereas those fed with PKE and yeast solely was 0.32% and 0.30%, respectively. The DHA content in *Artemia* was relatively low in all treatements, with 0.35%, 0.34% and 0.25% in *Artemia* fed with PKE, PKE supplementd with yeast and yeast. The ARA content in *Artemia* upon feeding with PKE supplemented with yeast was 1.54%, whereas in *Artemia* fed with PKE and yeast was 1.15% and 0.97% respectively. The LA content in *Artemia* fed with yeast was 4.24% significantly higher than those fed with PKE with 1.53% and yeast 0.83%. The ALA content in *Artemia* upon feeding with yeast showed 0.78%, whereas those fed with PKE supplemented with yeast and PKE showed 0.74% and 0.25%.

CHAPTER 5

DISCUSSION

Microalgae is undoubtly one of the best foods for *Artemia*. The use of microalgae as feed for raising *Artemia* from nauplii to adult stage has been well-documented. Several potential microalgae species can be used for mass culture and this includes *Nanochloropsis, Tetraselmis, Dunaleilla,* and *Spirulina*. Microalgae cells are high in protein content and they boost *Artemia* nauplii to adult stage in 15 days. Though the use of microalgae is superior, the culture and upscaling process is costly and labour intensive (Sivaji, 2016; Vartak and Joshi, 2002). A substantial amount of funding must be invested prior to starting a commercial production system for *Artemia*. This facility includes a laboratory to maintain microalgae isolates and culture stocks, and large area for tank/pond set up for upscale. Uncontrolled upscaling conditions may result in contamination, causing microalgae culture to crash. In addition, contaminated microalgae such as those with fungus may bring about fungal diseases, causing mortalities to *Artemia* during culture. The use of dried microalgae on the other hand, is not economical because they are very costly and they have been shown to contaminate the rearing water if a protein skimmer is not installed during *Artemia* culture. It is therefore crucial to find a more sustainable alternatives to culture *Artemia,* particularly in tank/indoor production.

Several types of agricultural by-product such as oatmeal, rice bran, corn bran, wheat bran and soymeal have been shown to be useful to raise *Artemia* for biomass production. They are easily available and cost effective than microalgae. If these by-products are fed in sufficient amount they can be used to replace 90-100 percent of live algal (Vahdat and Oroujlou, 2021). Nevertheless, prior to feeding these feeds to *Artemia*, certain factors must be taken into count such as the size of the food particle, solubility in water, feed availability and cost, buyoncy in water colum, impact on water quality, nutritional values and digestibility. Since *Artemia* feed on paricles <50µm, these feed must be homogenized and passed through a 50µm filter before it is offered to *Artemia*.

In this study, PKE was examined as a potential feed for *Artemia.* This agricultural by-product from the oil palm industry (Ng and Chong, 2002) have been commonly used and incorporated into ruminant diet for cow and cattle farming (Chia et al., 2009; McGrath et al., 2018). The cheap production cost and continuous availability of PKE have gained the interest as a potential alternative in fish diets in the last decade (Ng and Chen, 2002). The application of PKE as an *Artemia* feed requires several processing steps. This includes 1) mixing the appropriate amount of PKE in water, 2) incubating the mixture and 3) sieving the particles through plankton nets with mesh less than 50 microns prior to use for feeding *Artemia* nauplii. Feeding *Artemia* with PKE resulted in an average growth of 9mm to 10mm upon 14 days of culture, comparable to the use of microalgae. The use of *Tetraselmis sp.* promoted *Artemia* growth from nauplii to adult stage within 15 days of culture, resulting in a total average length of 9.5mm (Vartak and Joshi, 2002). These findings indicated that PKE is as good as microalgae as a feed for *Artemia*.

It has been shown that to boost growth, the appropriate amount of feed and nutrition must be provided to *Artemia* (D'Agostino, 1980). In the trials where optimization of the amount of PKE was performed, feeding *Artemia* with 13cm turbidity resulted in poor yield, perhaps due to over-feeding. Over feeding could result in high mortality due to water deterioration. The unconsumed food by *Artemia* degrades in the rearing water, thus increases the ammonia and nitrite level that might be harmful to *Artemia.* In a study perfomed by Nimura (1980), overfeeding of Artemia results in uneven growth rate, short gut retention time and insufficient nutrition assimilation. The growth of the surviving animals was slightly higher because there are more space and feed in the culture tanks (Figure 4.1a). Of all, feeding *Artemia* with 19cm PKE turbidity contributed to highest biomass production. This feed concentration was used as a reference for stagnant culture system in tanks without water replacement. It should be noted that feed turbidity may vary according to the culture systems used to culture *Artemia* biomass. The gut of *Artemia* (Figure 4.7) showed that PKE was consumed by them, this also indicates that PKE particle size of ≤50μm upon being filtered in a 50μm filter can be ingested by *Artemia*. According to Lavens and Sorgeloos (1996), feeding *Artemia* with sufficient amount of feed, ensures a fully filled gut and tends to release compact faecal pellets, whereas in cases of

insufficient feed, they have an empty or incomplete gut that releases loose faecal pellets. PKE is also known to be high digestible by *Artemia* as there was no any thread like faecal observed in *Artemia* upon feeding them with PKE.

A metagenomics analysis conducted on PKE solutions after varying incubation durations revealed the presence of potential supportive microbial community that is induced in PKE after incubation. It is worth noting that, apart from the PKE particles, the abundant Campilobacterota in PKE1D may have maintained the dominance of this phylum during the 14-days *Artemia* rearing and transition into adult (Figure 4.3.2). Precedent study showed that Campilobacterota is uniquely resided in the gut of adult shrimp *Rimicaris kairei*, implying its crucial roles in fulfilling adult shrimp physiological demands (Qi et al., 2022). Besides that, increment of beneficial Actinobacteriota population, especially *Lactobacillus* and *Bifidobacterium*, was most significant in 1 and 2-days-incubated PKE solutions (Figure 4.3.3). The elevated abundance of gut Actinobacteriota often an indicator for health improvement in shrimp model (Zhou et al., 2023; Huang et al., 2022), which supported our findings, where the feeding of PKE containing higher proportion of Actinobacteriota led to greater *Artemia* growth and biomass productivity (Figure. 4.1).

PKE1D outperformed PKE2D and PKE3D in *Artemia* rearing trial, probably owing to higher abundance of microbiota with bioremediation capabilities in the feed, such as *Novosphingobium* and *Candidatus riegeria* (Figure 4.3.3). Past studies had reported that *Novosphingobium* and *Candidatus riegeria* are nitrogen-removal and sulfur-oxidizing symbionts (Jäckle et al., 2019; Xiao et al. 2024) and they may contribute to improving the quality of the rearing water. This explained why water change was not required during the entire rearing process, with minimal water quality deterioration. PKE1D inclusion in the *Artemia* rearing water had also consistently enriched the growth of Actinobacteria *Candidatus Aquiluna* (Figure 4.3.3b), a vital genus in maintaining healthy bacterioplankton community in shrimp culture (Yang et al. 2018). In addition, PKE1D ought to stabilize the microbiota functions, where no significant change of microbial functional profile was taking place in *Artemia* rearing water from Day 1 to end of the trial. Unlike in *Tetraselmis*-fed Artemia rearing water, where *Vibrio* sp. count remained predominant (Turcihan et al., 2021).

Apart from the particles, beneficial bacteria which are present in PKE may served as food for *Artemia* (Intriago and Jones, 1993). Bacteria cells are nutritious because they have a good nutritional composition of 63% protein and 11% carbohydrates (Young and Scrimshaw, 1975). Although they have low lipid content of 2.5-9.0% and lack in PUFA, they are still widely utilized as food in aquaculture (Brown et al., 1996). However, certain bacteria species (mostly halophilic bacteria) are still able to produce EPA (20:5n-3), DHA (22:6n-3), and ARA (20:4n-6) by their own (Watanabe et al. 1997). It has been demonstrated several times on the efficiency of *Artemia* to survive on bacteria alone (Yasuda & Taga 1980; Intriago & Jones 1993; Gorospe & Nakamura 1996; Verschuere et al. 2000). In addition, bacteria aids in the digestive performance of *Artemia* by supplying enzymes that breaks down large food particles and makes them easier to be absorbed by *Artemia* (Toi et al., 2013; Moriarty, 1998). For example, it has been demonstrated that *Artemia* uses bacterial enzymes for the digestion of algae cells (Intriago and Jones, 1993). Somehow, PKE incubation beyond one day was not recommended for the application in *Artemia* production, as the findings showed that the amount of beneficial bacteria were reduced (Figure 4.1). Moreover, the increase of firmicutes population in PKE3D might lead to off-balance Proteobacteria to Firmicutes ratio and impacted the health of *Artemia*, as reported in other shrimp species such as *Penaeus vannamei* when infected by white faeces disease (*Kurniawinata* et al. 2022).

In this study, to aid the efficiency of PKE in the growth and production of *Artemia* biomass, yeast is supplemented with PKE to *Artemia*. Upon supplementing *Artemia* with yeast, the length of *Artemia* reached approximately 10mm on the eighth day of the culture, this indicates better feed intake and availability in the culture, whereas in a controlled *Artemia* culture, it was reported that the length of *Artemia* reared with different food sources like rice bran and soybean reached up to 9.50mm to 8.20mm on the fifteenth day of the culture (Vartak et al., 2002). Similar results were obtained by Mo (2020), where the animals fed with yeast supplemented diets revealed better growth rate and biomass to those without yeast. The nutritional composition of the *Artemia* differs in every life stage (Manassah, 2012). Supplementation of yeast to *Artemia* gives a better yield of lipid and protein.

Supplemental feeds provide extra food such as highly nutritious mixed algae and microbes that aids in faster growth and maturation rate (Wurtsbaugh and Gliwicz, 2001). This illustrates the faster growth and high production of *Artemia* biomass in this study upon increasing food availability in the tanks where yeast is supplemented. It was suggested by Brintha, 2016 that yeast is one of the best feeds that contributes to a faster growth, high survival and reproduction of the aquatic animals. The determination of the best food to be used in a culture includes buoyancy of the feed, size, palatability, odour, continuous availability and cost of the feed (Olguin, 2005). Yeast stimulates the development of other possible food source like bacteria and algae in the culture medium (Coutteau et al., 1992). According to Coutteau et al., 1990 feeding *Artemia* with baker's yeast alone results in poor culture (growth and yield of *Artemia*). The researcher also reported, the addition of yeast as a supplement results in a bigger size of *Artemia* (7.10mm-7.90mm) after 14 days. The growth rate, yield of biomass and maturation rate of *Artemia* is highly dependent on the quantity and quality of the food combined with the temperature (Anh et al., 2009).

In this study, protein has the major portion of 45-52% in the nutritional composition of *Artemia* upon feeding with yeast, PKE and PKE supplemented with yeast. Similar protein content was obtained, upon feeding *Artemia* with rice bran produces 52.25% (Agh and Hosseini Ghatre, 2002), upon feeding with *Chaetoceros*, Nestum, and enrichment Nestum produces 56.4%, 42.87% and 41.16% respectively (Naegel, 1999). The lipid content in *Artemia* upon feeding with PKE supplementing with yeast gives the best results of 11%. Similar results were obtained in a study performed by Leger et al (1986), 10.6% of lipid were obtained upon feeding *Artemia* with a mix diet of *Tetraselmis sp.* and rice bran. The lipid and protein are required for the development of the sexual organs in *Artemia*, but these nutrients are not essential for *Artemia* that are not ready for gametogenesis. The lipid composition in the diet of *Artemia* is essential for a continued fertility of the animal (Johnson, 1980). The addition of yeast as a supplement for *Artemia* initiates the development of microbes which plays an important role in the nutritional properties of the food for *Artemia*. This condition could be a result of enhanced digestibility characteristic or nutritional supplementation (Douillet, 1987).

The lipid and fatty acid profile of *Artemia* are largely dependent on factors such as nutritional composition of the feed, type of strain, and environmental factors (Léger et al., 1986). *Artemia* generally lack in essential polyunsaturated fatty acids (PUFAs): EPA, DHA and ARA. In this study, supplementing yeast to *Artemia* gave a better EPA content of 1.65% compared to feeding with PKE and yeast with 0.32% and 0.30%. However, the DHA level in *Artemia* remains low across all diets. It should be noted that fish and shrimp require 3.2% EPA and 0.9% DHA for optimal growth (Brown, 2002). The content of EPA and DHA in *Artemia* obtained by feeding PKE are still below the recommended levels, so enrichment is required to increase their fatty acid content.

CHAPTER 6

CONCLUSIONS

The results of this study revealed that PKE is a good food for *Artemia*. The particle size of PKE that is <50µm can be consumed by *Artemia*, this was confirmed by observing the gut of *Artemia* upon feeding them with PKE. Feeding *Artemia* with 19cm PKE turbidity upon 1-day incubation contributed to *Artemia* measuring 1cm in length within 14 days of culture and highest biomass production. PKE incubated 1 day contributed to composition of beneficial microbes like *Lactoplantibacillius* that dominated 42% of the total microbial population, and also helped to inhibit pathogens in the culture. Supplementing 3g of yeast with PKE to *Artemia* enhanced the growth to 1cm in 9 days of the culture and produced the highest biomass of 1kg in 14 days culture. The utilization of yeast in *Artemia* culture is very convenient as the preparation is very easy, cheap, it has good buoyancy in the water column and small particle size that is less than 50 µm. Feeding PKE to *Artemia* produced a good nutritional composition of 45% protein, 21% NFE, 9% lipid, 23% ash, 8% moisture and 1% fiber. In addition, supplementation of yeast enhanced the nutritional composition to 52% protein, 16% NFE, 11% lipid, 20% ash, 8% moisture and 1% fibre. The EPA value of *Artemia* was enhanced from 0.32% upon feeding with PKE to 1.65% upon supplementing with yeast. This study contributes to the development of commercial products such as live *Artemia* biomass, frozen *Artemia* and PKE as a commercial food that can be used to raise *Artemia* from nauplii to adult stage in 14 days culture.

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APPENDICES

Appendix 1

1. Statistical analysis of the growth of Artemia upon feeding with different turbidity of PKE.

Day	13cm	15cm	17cm	19cm	21cm
1	0.5390±0.01[a]	0.5093±0.01 [a]	0.5253±0.01[a]	0.5236±0.02[a]	0.5238±0.01[a]
2	0.9662±0.07 [a]	0.9293±0.06 [a]	0.9189±0.09 [a]	0.8952±0.04[a]	1.3838±0.08[b]
3	1.5197±0.17[a]	1.4736±0.08[a]	1.6886±0.25[ab]	1.6100±0.07 [ab]	2.0468±0.20 [b]
4	2.1799±0.07 [a]	2.1737±0.08 [a]	2.3302±0.11 [a]	2.3370±0.15 [a]	2.5353±0.55 [a]
5	3.6778±0.17 [a]	4.0728±0.50 [a]	3.8376±0.68 [a]	4.2743±0.10 [a]	3.6617±0.06 [a]
6	4.9326±0.54 [a]	5.4202±0.42 [a]	4.9781±0.61 [a]	6.1516±0.17 [a]	5.2713±0.40 [a]
7	6.4532±0.33 [a]	6.9854±0.59 [a]	6.2014±0.98 [a]	6.9735±1.37 [a]	6.8758±0.39 [a]
8	8.3489±0.55 [a]	8.5086±0.42 [a]	8.0046±1.41 [a]	8.8619±1.28 [a]	8.5563±0.68 [a]
9	8.1762±0.31 [a]	8.6813±0.46 [a]	8.8838±1.12 [a]	8.9023±0.30 [a]	9.0836±0.32 [a]
10	9.4097±1.29 [a]	9.1394±0.16 [a]	8.5853±1.74 [a]	9.7807±0.29 [a]	9.5718±0.39 [a]
11	9.6079±0.59 [a]	9.1067±0.25 [a]	9.0548±1.55 [a]	9.7541±0.33 [a]	9.7105±0.51 [a]
12	9.6626±0.53 [a]	9.4268±0.20 [a]	9.8550±0.91 [a]	9.7041±0.49 [a]	10.1852±0.22 [a]
13	10.3013±0.22 [a]	9.6679±0.15 [a]	9.7511±1.42 [a]	10.2670±0.88 [a]	10.2960±0.47 [a]
14	10.0349±0.17 [a]	9.2836±.34 [a]	9.6508±0.40 [a]	10.5984±1.24 [a]	10.7737±0.10 [a]

Appendix 2

2. Statistical analysis of the average yield of Artemia biomass reared upon different turbidity of PKE 14 days of culture.

Turbidity	13cm	15cm	17cm	19cm	21cm
Yield	288.8±34.23[a]	555.8±40.23[c]	562.2±44.09[c]	693.3±8.26[d]	448.9±18.35[b]

Appendix 3

3. Statistical analysis of the growth of Artemia upon feeding with PKE that has been incubated on different period of time.

Day	0 day	1 day	2 day	3 day
1	0.5227±0.02a	0.5238±0.01a	0.5126±0.01a	0.5219±0.01a
2	0.9221±0.07a	1.3838±0.08b	0.8860±0.04a	0.9530±0.14a
3	1.2988±0.19a	2.0468±0.20b	1.4320±0.25ab	1.3436±0.38a
4	1.7344±0.18a	2.5353±0.55a	2.0068±0.36a	1.9617±0.23a
5	2.6474±0.84a	3.6617±0.06a	2.2756±0.30a	2.9080±0.61a
6	3.1737±0.55a	5.2713±0.40b	3.1787±0.44a	3.8766±0.37a
7	4.1383±0.20a	6.8758±0.39b	3.8958±0.88a	5.0323±0.42a
8	5.1834±0.25a	8.5563±0.68b	4.7854±0.84a	5.9355±0.84a
9	6.4836±0.30a	9.0836±0.32b	6.3758±1.23a	6.8084±0.66a
10	6.9316±0.33a	9.5718±0.39b	6.5932±1.25a	7.6421±0.93ab
11	7.6162±0.40a	9.7106±0.51b	7.4460±0.71a	7.8066±0.41a
12	8.1248±0.63a	10.1852±0.22b	8.2139±0.66a	8.2315±0.03a
13	8.4401±0.49a	10.2960±0.47b	8.3847±0.81a	8.6833±0.22a
14	9.0295±0.55a	10.7737±0.10b	8.6064 ±0.44a	8.8506±0.03a

Appendix 4

4. Statistical analysis of the yield of Artemia biomass upon feeding with PKE that has been incubated on different time period.

Incubation Day	0 day	1 day	2 day	3 day
Yield (g)	197.3±15.0a	635.2±12.5b	577.0±25.4c	479.1±43.6c

Appendix 5

5. Statistical analysis of the the microbial composition in PKE incubated on different time period.

Bacteria	0 day	1 day	2 day	3 day	N day
Vibrio tritonius	32±14.84a	20±2.0a	38±17.0a	13±0.6a	35±1.0a
Lactiplantibacillius	2±2.08a	42±3.6b	23±21.1a	1±0a	6±0.6a
Vibrip sp	41±20.74b	2±0.6a	23±11.0b	0±0a	0±0a
Mangrovibacter	4±5.20a	28±1.5b	6±9.8a	0±0a	0±0a
Ligilactobacillius	0±0a	4±2.6b	1±1.2ab	20±1.0c	17±0.6c
Pseudodessulfovibrio	0±0a	0±0a	0±0a	32±4.0c	6±0.6b

Appendix 6

6. Statistical analysis of the microbial composition in the rearing water of Artemia (30g/l) on day 1, 2 and 7 upon feeding with PKE (incubated overnight).

Bacteria	Control	Day 1	Day 7	Day 14
Pseudoalteromonas	0±0.0a	9±11.9ab	33±11.5bc	48±13.6c
Muricauda	1±1.5a	7±4.6ab	1±0a	19±11.1b
Pelagibacter	30±7.8b	3±2.5a	1±0a	0±0a
Phaeocystidibacter	4±1.7a	3±1.5a	12±10.4a	6±7.2a
Alteromonas	3±4.4a	4±2.5a	14±11.4a	1±0.6a

Appendix 7

7. Statistical analysis of the microbial composition in the rearing water of Artemia (150g/L) on day 1, 2 and 7 upon feeding with PKE (incubated overnight).

Bacteria	Control	Day 1	Day 7	Day 14
Leeuwenhoekiella	6±7.0a	25±18.5a	19±24.8a	6±5.5a
Alteromonas	18±8.0a	8±6.1a	11±7.9a	4±1.2a
Idiomarina	1±0a	21±5.5b	1±0.6a	1±0.6a
Actibacterium	3±2.3ab	11±4.2b	2±1.2a	3±2.9a
Thalassospira indica	0±0a	0±0a	0±0.0a	17±20.2a
Thalassospira povalilytica	0±0a	0±0a	0±0a	15±26.6a

Appendix 8

8. Statistical analysis of the growth of Artemia upon supplementation of Yeast with PKE at a concentration of 1g, 2g, 3g and 4g of yeast.

Day	1g	2g	3g	4g
1	0.5394±0.02a	0.5169±0.02a	0.5371±0.01a	0.5222±0.01a
2	1.0063±0.14a	1.2742±0.27ab	1.4560±0.08b	1.1979±0.04ab
3	1.4877±0.04	1.4611±0.31a	1.6554±0.02a	1.5107±0.02a
4	2.3640±0.29a	2.6960±0.23a	2.5320±0.07a	2.5226±0.15a
5	3.4818±0.66a	4.2983±0.43a	4.6747±0.43a	4.1199±0.39a
6	5.5147±0.61a	6.5168±0.37b	6.6118±0.17b	6.2503±0.16ab
7	6.5543±0.66a	7.7693±0.11a	7.6199±0.44a	7.7368±0.75a
8	8.2698±0.47a	8.9907±0.41a	9.1250±0.47a	8.8299±0.29a
9	8.6238±0.36a	9.3392±0.62ab	10.0288±0.29b	9.1534±0.69ab
10	8.7301±1.35a	9.8342±0.77a	10.3526±0.34a	9.8412±0.32a
11	8.7980±1.32a	9.7411±0.07a	10.1870±1.07a	9.4773±0.89a
12	8.8793±0.63a	10.2864±0.43b	10.6582±0.26b	9.5330±0.32ab
13	08.8640±0.70a	10.3720±0.59b	10.9547±0.02b	10.6394±0.25b
14	9.4797±0.88a	10.7337±0.62ab	11.2247±0.26b	10.6349±0.02ab

Appendix 9

9. Statistical analysis of the yield of Artemia upon supplementation of Yeast with PKE at a concentration of 1g, 2g, 3g and 4g of yeast.

Yeast	1g	2g	3g	4g
mean	892.9±16.17a	1000.7±10.27b	1028.3±36.49b	999.9±17.12b

Appendix 10

10. Statistical analysis of the growth of Artemia upon feeding with Yeast, PKE supplemented with Yeast and PKE in 14 days of culture.

Day	Yeast	PKE+Yeast	PKE
1	$0.5278±0.03^{a}$	$0.5194±0.02^{a}$	$0.5222±0.02^{a}$
2	$0.7916±0.06^{a}$	$0.8964±0.05^{a}$	$0.7590±0.11^{a}$
3	$1.3419±0.29^{a}$	$1.8027±0.13^{a}$	$1.4458±0.15^{a}$
4	$2.6382±0.51^{ab}$	$3.4762±0.45^{b}$	$2.0923±0.25^{a}$
5	$4.0112±0.75^{ab}$	$4.5211±0.40^{b}$	$3.0579±0.24^{a}$
6	$4.5820±0.82^{a}$	$6.3299±0.77^{b}$	$3.9950±0.22^{a}$
7	$5.5491±0.24^{a}$	$7.9184±0.67^{b}$	$5.5740±0.58^{a}$
8	$6.5102±0.45^{a}$	$9.6262±0.42^{b}$	$6.6258±0.58^{a}$
9	$7.1153±0.16^{a}$	$9.9666±0.25^{c}$	$7.7351±0.26^{b}$
10	$7.9260±0.84^{a}$	$10.3977±0.41^{b}$	$8.7169±0.14^{a}$
11	$8.6554±0.36^{a}$	$10.5549±0.35^{b}$	$8.8613±0.21^{a}$
12	$9.0859±0.24^{a}$	$10.9094±0.10^{b}$	$9.2751±0.31^{a}$
13	$9.6874±0.14^{a}$	$11.0373±0.05^{b}$	$9.7673±0.19^{a}$
14	$9.9936±0.28^{a}$	$11.0479±0.07^{b}$	$10.1552±0.28^{a}$

Appendix 11

11. Statistical analysis of the yield of Artemia biomass upon feeding with Yeast, PKE supplemented with Yeast and PKE in 14 days of culture.

Treatment	Yeast	PKE+Yeast	PKE
Yield	394.2±15.23[a]	1024.5±14.69[b]	673.7±15.54[c]

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