CHAPTER-V
DISCUSSION

EXPERIMENT-1:
EVALUATION OF THE NUTRITION QUALITY OF SEAWEED
Ulva reticulata AND Sargassum cinctum AS DIETARY INGREDIENT

5.1 NUTRITIONAL COMPOSITION

Seaweeds were reported to contain significant quantities of proteins, lipids, carbohydrates, minerals, amino acids and vitamins (Kumar and Kaladharan 2007; Manivannan et al., 2008; Thinakaran and Sivakumar, 2012).

5.1.1 Proximate composition

Burtin (2003) reported that the protein content of green and brown seaweed are generally ranging 10 to 30% and 5 to 15% respectively. The protein content of U. reticulata (12.21%) is similar to those obtained in Ulva spp. by Manivannan et al. (2009) but higher than those obtained by Manivannan et al. (2008) whereas in S. cinctum (10.29%) is similar to those obtained in Sargassum spp. by Rohani-Ghadikolaei et al. (2012) and Manivannan et al. (2008). Seaweeds are relatively low in lipid (1–5% of dry weight) (Burtin, 2003; Polat and Ozogul, 2008). In the present study crude lipid content of U. reticulata (3.1%) and S. cinctum (2.84%) is similar to those reported by Rohani-Ghadikolaei et al. (2012) but higher than those estimated by Manivannan et al. (2008) for same genus of both seaweeds. In U. reticulata the ash content (26.38%) and moisture content (6.53%) are comparatively higher and similar to those obtained in Ulva spp. by Rohani-Ghadikolaei et al. (2012), whereas in Sargassum cinctum ash (21.86 %) and moisture (6.59 %) contents are comparatively lower than Sargassum spp. those obtained by Rohani-Ghadikolaei et al. (2012). Carbohydrates was the major component in the proximate composition of U. reticulata (51.78%) comparatively lower to those obtained by Ortiz et al. (2006) and Rohani-Ghadikolaei et al. (2012) for Ulva spp., whereas in S. cinctum (58.42%) it is similar to Sargassum spp. obtained by Kumar et al. (2017). These results are
comparatively higher than those reported for same genus by Chakraborty and Santra (2008) and Manivannan et al. (2008).

5.1.2 Amino Acid Profiling

Tabarsa et al., (2012) reported pronounced differences between amino acid profiles of various seaweeds. Chakraborty and Bhattacharrya (2012) reported amino acid composition dependent upon the physico chemical condition of the growing environment. In the present study, the amount of total amino acid was 10.30 and 9.79 mg/100 mg (DW) in U. reticulata and S. cinctum respectively. Both the seaweeds U. reticulata and S. cinctum contained relatively higher percentage of glutamic acids (11.53, 11.02 %) and aspartic acid (10.45, 10.16 %) respectively which are responsible for the special flavour and taste of seaweeds. Similar results were obtained in previous studies also (Wong and Cheung, 2000; Kumar and Kaladharan, 2007; Gressler et al., 2010).

The quantity of essential amino acid to total amino acids of U. reticulata and S. cinctum were accounted 5.22 and 5.39 mg/100 mg (DW) reflecting the distribution of more than 50 % essential amino acids among both the seaweed species whereas in fishmeal and soybean meal which is 35% and 28.75% respectively. Presence of 45-49% of essential amino acid in K. alvarezii and H. musciformis and more than 40% in C. lentillifera and U. reticulata (Ratana-arporn and Chirapart, 2006; Kumar and Kaladhara, 2007) occurred in various regions of the sea, corroborate with the present study.

The essential amino acids such as valine, isoleucine, phenylalanine, histidine, methionine, arginine, threonine, lysine, leucine and tryptophan are not synthesized in fish and shrimps which are the absolute dietary requirements (Cowey and Forster, 1971; Nose, 1974). All these amino acids except threonine and tryptophan (not tested) are present in both seaweeds making it a good feed supplement. The results of the study suggested that these species could be used as alternative nutrient sources of protein and amino acids for human and animal consumption.

5.1.3 Fatty Acid Profiling

Seaweeds contain many essential fatty acids, which may add to their efficacy as a dietary supplement or as part of a balanced diet. Fatty acid compositions of algal
lipids vary widely with species, habitat, light, salinity, pollution and environmental conditions (Kim et al., 1996; Ratana-arpon and Chirapart, 2006). In the present study quantity of unsaturated fatty acid in seaweeds U. reticulata (70.34%) and S. cinctum (68.74%) was higher than saturated fatty acids is in agreement with previous reports (Khotimchenko and Svetashev, 1987; Khotimchenko and Kulikova, 2000), whereas in fishmeal and soybean meal is 50.91% and 43.57% respectively.

Among the SFAs the amount to TFAs of Palmitic acid was the most abundant in U. reticulata (24.67%) and S. cinctum (26.47%) as well as fishmeal (39.50) and soybean (47.65 %) than other SFAs to TFAs. Dominance of palmitic acid of both seaweeds is in agreement, but Heptadecenoic acid (4.53%) in U. reticulata is relatively higher compared to previous reports for same genus spp. (Rohani-Ghadikolaei et al., 2007; Debarma et al., 2016). Debarma et al. (2016) reported that Oleic acid was the most dominant monounsaturated fatty acid (MUFA) in the seaweeds whereas in present study Eladic Acid (2.34 and 4.66 %) dominated as MUFAs.

Although macro algae were reported to have low lipid content their polyunsaturated fatty acid composition is superior to those of terrestrial vegetables in regard to human diet (Goecke et al., 2010; Kumari et al., 2010). In present investigation contribution of PUFA to total amino acid was relatively higher in both seaweeds, U. reticulata (67.04%), S. cinctum (60.78%) than fish meal (43.30%) and soybean meal (20.79%) which is in agreement with previous report that marine algae are rich in polyunsaturated fatty acids (PUFAs) (Wood, 1988; Kayama et al., 1989). In present investigation the health-beneficial PUFA eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) of both seaweed are relatively higher; AA is higher in U. reticulata and lower in S. cinctum and the lower concentration of essential PUFAs Linolenic acid in both seaweeds and Linoleic acid in U. reticulata whereas the higher concentration of Linoleic acid in S. cinctum are obtained than those reported by Matanjun et al. (2009), Chakraborty and Santra (2008), Rohani-Ghadikolaei et al. (2007); Debarma et al. (2016), Ortiz et al. (2006) for the species of same genus.

Consumption of long-chain omega-3 PUFA are known to be beneficial to human health (Ruxton et al., 2005) and together with a low dietary n-6/n 3 ratio, are fundamental for a diet with cardio-protective benefits. In the present study both
seaweeds contained the highest total n-3 PUFA with less than 1 n-6/n-3 ratio is considered as good source of fatty acid for animal and human nutrition.

5.2 **In-vitro ANTI-BACTERIAL ACTIVITY OF SEAWEEDS**

Seaweed has been proven to be a potential source of antibacterial compounds towards both Gram-negative and Gram-positive pathogenic bacteria (Kolajinathan et al., 2009). In the present study, an experiment was conducted to determine the antibacterial activity of Methanol extracts of *U. reticulata* and *S. cinctum* against *Vibrio parahaemolyticus* which is known as Vibriosis causing bacteria in shrimp culture. Methanol extracts of seaweeds were used in the study to assess the antibacterial activity as it has been reported that methanol is the most suitable solvent for preparation of seaweed to study the inhibitory effects against bacterial pathogens (Rajasekar et al., 2012).

The result indicated that the zone of inhibition also correspondingly increased with increased seaweeds concentration with highest 15.0 ± 0.35 mm in *U. reticulata* and 16.37 ± 0.12 in *S. cinctum* which is relatively higher than which was obtained by Ravikumar et al. (2016) of *n*-butanol extract of *U. reticulata* from Kanyakumari coast and Manivannan et al. (2011) of methanol extract of *S. tenerrimum* from Vedalai coast respectively. These results are in agreement with Kandhasamy and Arunachalam (2008) that variation in antibacterial activity is probably due to the method of extraction and location from where samples were collected. Antibacterial activity of *S. cinctum* is probably higher than *U. reticulata* which in agreement with Natrah et al. (2015) who noted that the brown seaweed was found to be more active than the red and green seaweeds.

Many workers have revealed that crude extracts of Indian seaweeds are active against pathogenic bacteria (Hellio et al., 2000 and Lima-Filho et al., 2002). In present investigation antibacterial activity of both seaweeds could be source of antibacterial compound with potential use in aquaculture in order to control *V. parahaemolyticus* infection.
EXPERIMENT-2:
EFFECT OF *Ulva reticulata* AS DIETARY INGREDIENT ON GROWTH PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

AND

EXPERIMENT-3:
EFFECT OF *Sargassum cinctum* AS DIETARY INGREDIENTS ON GROWTH PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

5.3 GROWTH PARAMETER

5.3.1 Wet Weight

In Experiment-2, final average wet weight was higher in UT3 (73.20±1.14 g) and UT2 (72.60±4.32) fed 6% and 3% level of *U. reticulata* respectively than UT1-control (68.80±2.72), UT4 (65.57±3.11) and UT5 (61.42±5.92) involving 0%, 9% and 12% level of *U. reticulata* respectively. Whereas, final mean weight gain ranged between 3.439±0.275 to 4.005±0.203 g with highest in UT2 (3%), UT3 (6%) and lowest in UT4 (9%) and UT5 (12%) than UT1 (0%). In Experiment-3, final average wet weight was higher in ST2 (72.44±3.90 g) fed 3% level of *S. cinctum* than ST1-control (69.59±4.02) followed by ST3 (64.40±2.63), ST5 (55.68±3.46) and ST4 involving 0%, 6%, 12% and 9%, level of *S. cinctum* respectively. The final mean weight gain ranged between 2.946±0.089 to 4.019±0.348 g with highest in ST2 (3%) ST1 (0%) followed by ST3 (6%), ST5 (12%) and ST4 (9%). Results of both the experiments are similar to Rodríguez-González et al. (2014) who reported final average mean weight and wet weight gain of *L. vannamei* higher with 5% level of *U. lactuca* meal than control and higher levels (10% and 15%). Briggs and Funge-Smith (1996) also found that using diets with a higher content of *Gracilaria spp.* resulted in a significant reduction in growth of *P. monodon.*

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5.3.2 Specific Growth Rate (SGR)

The specific growth rate of Experiment-2 ranged between 3.821±0.30 to 4.450±0.226 % with highest in UT2 (3%) and UT3 (6%) followed by UT1 (0%), UT4 (9%) and UT5 (12%). In Experiment-3, the specific growth rate ranged between 3.273±0.099 to 4.466±0.38 % with highest in ST2 (3%) and lowest in ST4 (9%) as compared to other diets. The specific growth rate of all the treatment diets of Experiment-2 and Experiment-3 was slightly higher than those obtained by Rodríguez-González et al. (2014) which was range between 3.11±0.18 to 3.23±0.12 % for *L. vannamei* fed with 5, 10, 15% levels of *U. lactuca* meal and *G. parvispora* meal. The specific growth rate of control diet in both experiments was almost similar to those obtained by Hafezieh et al. (2014) for *L. vannamei*.

5.3.3 Feed Conversion Ratio (FCR)

In Experiment-2, Feed conversion ratio (FCR) was better with lowest (1.668±0.170) in UT2 (3%); highest (2.101±0.086) in UT5 (12%) and 2.063±0.082 in UT4 (9%) respectively. It was significantly different (P<0.05) from UT1 (0%) treatment diet, whereas in UT3 (6%) 1.916±0.115 it was slightly higher than UT1 (0%). In Experiment-3, feed conversion ratio (FCR) was recorded lowest (1.693±0.121) in ST2 (3%) and highest (2.199±0.056) in ST5 (12%) followed by ST4 (9%) (1.953±0.060) it significantly differed (P<0.05) from ST1 (0%) (1.756±0.03), whereas in ST3 (6%) (1.866±0.023) it was slightly higher than ST1 (0%) fed treatment diet. Feed conversion ratio (FCR) of present investigation in control diet of both the experiments was little higher to those obtained by Hafezieh et al. (2014) for *L. vannamei* fed with soy flour and fish meal as major diet ingredients whereas lower than those obtained by Sudaryono et al.(2015) for feeding commercial diet. Hafezieh et al. (2014) recorded that FCR of 5% level of *S. illicifolium* was slightly higher than control which is in agreement with present study FCR of 6% level of both seaweeds which is slightly higher compared to control diet.

5.3.4 Protein Efficiency Ratio (PER)

In Experiment-2, protein efficiency ratio (PER) of all the treatments ranged between 1.406±0.016 to 1.596±0.086 with highest in UT2 (3%) followed by UT1 (0%), UT3 (6%), UT4 (9%) and UT5 (12%). In Experiment-3, protein efficiency ratio (PER) of all the treatments did not differ significantly. It ranged between 1.456±0.046
to 1.568±0.052 with highest ST2 (3%) followed by ST4 (9%) and ST3 (6%), whereas lowest in ST5 (12%). Almost similar range of protein efficiency ratio between (1.13±0.05 to 1.56±0.22) treatment diet was also observed by Sudaryono et al. (2015) using different levels of *S. cristaefolium* diets.

### 5.4 SURVIVAL

In Experiment-2, average survival percent recorded did not differ significantly (P<0.05) at ranged between 83.33±1.66 to 88.33±1.66 % with highest in UT5 (12%) followed by UT3 (6%) and UT4 (9%), whereas lowest in UT2 (3%). In Experiment-3 also average survival percent recorded was not different significantly (P<0.05) ranging between 86.67±1.66 to 91.67±1.66 % with highest ST3 (6%) as well as ST4 (9%) followed by ST2 (3%) and ST5 (12%) and ST1 (0%). Almost similar range of survival was obtained in *L. vannamei* fed with different concentrations of *Sargassum cristaefolium* fed diets than those fed control diet by Sudaryono et al. (2015). Hafezieh et al. (2014) obtained high survival rate of *L. vannamei* in *Sargassum illicifolium* included treatment diet than control diet but it was little higher than result obtained in this study.

### 5.5 PHYSICO-CHEMICAL WATER PARAMETERS

*L. vannamei* is one of the hardiest shrimp being cultured; it can withstand water quality conditions and physical handling that would create serious challenges for other species. Excess feed, fecal matter and metabolites will exert tremendous influence on the water quality of shrimp farm (Soundarapandian and Gunalan, 2008). The physico-chemical water parameters analyzed during this study were found within the permissible range.

#### 5.5.1 Temperature

Temperature can effect shrimp growth directly controlled by food consumption and nutrients availability in the food. The cultured shrimp grow best in a temperature range from 24-32°C (Fast and Lannan, 1992). Temperature has pervasive controlling effect on growth (Wootton, 1996; Das and Saksena, 2001). In the present study, the temperature remained within range 27.71°C to 28.33°C and 27.92°C to 29.01°C in Experiment-2 and Experiment-3 respectively which were ideal range for the shrimp growth.
5.5.2 pH

Wang et al. (2004) reported favorable pH range of 7.6-8.6 for L. vannamei. In the present study the pH was found to be in the range of 7.96 - 8.45 and 7.84 - 8.36 in Experiment-2 and Experiment-3 respectively which was suitable for rearing of shrimp.

5.5.3 Dissolved Oxygen (DO)

Chen (1985) reported that less than 3.7 ppm of DO concentrations seems to be critical point in shrimp culture system. However, in the present study, the dissolved oxygen values were found within the optimum range for the entire period ranging between 6.17 to 6.35 ppm and 6.23 to 6.41 ppm in Experiment-2 and Experiment-3 respectively.

5.5.4 Salinity

Several works have reported good growth and survival of L.vannamei in brackish water range of 10-35ppt which is considered ideal for shrimp culture (Balakrishnan et al., 2011). In the present study the rearing water salinity was maintained 30 ppt for both Experiment-2 and Experiment-3.

5.5.5 Nitrate (NO₃⁻)

In the present study, it ranged from 0.14 to 0.18 mg/L for Experiment-2 and Experiment-3. The Nitrate content was found to be within the optimum range during all the sampling days. Gupta et al. (2004) suggested that optimum nitrate level of less than 0.2 to 0.5 mg/L which does not affect the growth of the culture shrimp.

5.5.6 Nitrite (NO₂⁻)

Gupta et al. (2004) reported that high concentrations of ammonia and nitrite reduce shrimp growth and in extreme cases cause mortality. The desired level is less than 0.20 mg/l for maximum production in brackish water culture system. During the entire rearing period in the present study, the nitrite was found to be within the range from 0.042 to 0.051 mg/L and 0.038 to 0.046 mg/L for Experiment-2 and Experiment-3 respectively. Thus, the nitrite content remained within the acceptable range.
5.5.7 Alkalinity

Gupta et al. (2004) suggested that suitable alkalinity value in the culture system is less than 200 to 250mg/L. The alkalinity recorded in the present study was in the range of 127.27 to 134.7 mg/L and 128.70 to 135.1 mg/L, which was found suitable for rearing.

5.5.8 Hardness

Gupta et al. (2004) suggested that in brackishwater, alkalinity and hardness are usually high, so these variables are seldom important in management of shrimp farms. In the present study the hardness of water ranged from 163.2 to 177.5 mg/L and 166.18 to 175.5 mg/L for Experiment-2 and Experiment-3 respectively.

5.6 NUTRITIONAL COMPOSITION OF L. vannamei FLESH

5.6.1 Proximate composition

It has been reported that protein content of shrimp ranged between 17 and 21% depending on shrimp species (Yanar and Celik, 2006; Sriket et al., 2007). In present investigation similar range of protein content was recorded in Experiment-2 (17.54 to 19.19%) with highest in UT2 (3%) and lowest in UT5 (12%) U. reticulata fed diet as well as Experiment-3 (16.96 to 18.79 %) with highest in ST2 (3%) and lowest in ST4 (9%) S. cinctum fed diets compared to control fed diet.

In general, lipid act as major food reserves along with protein and subjected to periodic fluctuations influenced by environmental variables like temperature (Pillay and Nair, 1973). But this does not affect the lipid composition of muscle tissue to any great extent. In present investigation range of crude lipid content observed in Experiment-2 was 1.66 to 2.19 % with highest in UT3 (6%) and lowest in UT4 (9%) U. reticulata fed diet as well as in Experiment-3, 1.49 to 2.77 % with highest in ST5 (12%) and lowest in ST4 (9%) S. cinctum fed diets compare to control fed diet. Similar range of crude lipid content of L. vannamei flesh was also observed by Ch et al. (2015). In present investigation range of carbohydrate content observed in Experiment-2 was 3.61 to 4.21% which was similar to those obtained by Ch et al. (2015) whereas in Experiment-3 it was 3.73 to 4.27 % which exhibited almost an inverse relationship with protein content. Similar findings were recorded by Silva and Chamul (2000). In Experiment-2 and Experiment-3 ash content ranged between 1.19
to 1.86% and 1.06 to 1.74% respectively whereas moisture content between 73.83 to 75.10% and 73.92 to 76.07% respectively which were close to the finding by Yanar and Celik (2006) and Gokoglu et al. (2008).

5.6.2 Amino Acid Profiling

The amino acid profiles detected amino acids are the building blocks of protein. Amino acids play an important role in human nutrition and health promotion. The amount of amino acid content varies by intrinsic (species, size, and sexual maturity) and extrinsic factors (food resources, fishing season, water salinity, and temperature) (Akiyama et al., 1997; Limin et al., 2006). In present investigation in both experiment-2 and experiment-3 the valine, lysine, leucine, arginine dominated as essential amino acid whereas glycine, proline, glutamine and alanine as non-essential amino acids which is close to the finding of Cobb et al. (1975) and Gunalan et al. (2013). Valine is involved in many metabolic pathways, protein synthesis and optimal growth (Wilson, 2002). In present investigation the quantity of Valine in Experiment-2 was highest in UT3 (6%) whereas Experiment-3 in ST2 (3%) than control diet. Histidine aids osmoregulation and metabolic pathways during certain emergencies/harsh conditions (Abe and Ohmama, 1987) which is highest in all the treatments of both seaweed included diets compared to control diet. Lysine is very important during fish spoilage which was highest in ST2, ST3 and ST4 compared to control diet. According to Erkan et al. (2010) serine is an important amino acid necessary for fatty acid metabolism and the immune system which was highest in UT3 in Experiment-2 and ST3, ST4 and ST5 in Experiment-3.

5.6.3 Fatty Acid Profiling

In the present study, among saturated fatty acids palmitic acid was dominant in all the treatments of both experiments which were close to findings for L. vannamei (Gunalan et al., 2013) and for P. monodon (Akintola et al., 2013). Eladic acid dominated as MUFA in all the treatments of both experiments which differs from the previous report by Gunalan et al. (2013) who found Oleic acid was dominated in flesh of L. vannamei. Gunalan et al. (2013) reported that concentration of polyunsaturated fatty acids was highest in flesh of L. vannamei followed by saturated fatty acids and mono unsaturated fatty acids. Similar result was observed in all the treatments of both experiments in present study. In the present study, the presence of n-3 PUFA,
particularly linoleic, EPA and DHA indicate better growth and survival of *L. vannamei* in the culture pond. The higher levels of EPA and DHA would increase stress tolerance and membrane permeability (Watanabe, 1993; Lin *et al.*, 2003). In present investigation EPA was highest in UT4 and ST2, ST5 in Experiment 2 and Experiment 3 respectively whereas DHA was higher in UT4 of Experiment-2 and almost all the treatments except ST4 of Experiment 3 compared to control diet. In Experiment-2 the concentration of EPA was lower than DHA in all the treatment except UT4 similar to those obtained by Gunalan *et al.* (2013) whereas, in Experiment-3 EPA was higher than DHA in ST2, ST3 and ST5 which is in agreement with Ackman (1989) who reported that the shellfish tend to have EPA greater than DHA.

### 5.7 Vibrio spp. CHALLENGE TEST

In present study, the *Vibrio spp.* bacterial load in control treatment rearing water and hepatopancreas tissue samples was $168.5 \pm 1.5 \times 10^1$ and $33.80 \pm 0.80$ respectively in Experiment-2, whereas $163.50 \pm 1.50 \times 10^1$ and $27.86 \pm 2.14 \times 10^1$ CFU/g respectively in Experiment-3. *Vibrio spp.* bacterial load of seaweed treated rearing water and hepatopancreas tissue samples of Experiment-2 was highest ($141 \pm 1.0 \times 10^1$, $28.67 \pm 0.29 \times 10^1$ CFU/g respectively) in UT2 and lowest ($54 \pm 1.0 \times 10^1$, $3.61 \pm 0.28 \times 10^1$ CFU/g respectively) in UT5 whereas Experiment-3 it was highest in ST2 ($137.50 \pm 1.50 \times 10^1$, $23.70 \pm 1.52 \times 10^1$ CFU/g respectively) and lowest ($47.00 \pm 1.0 \times 10^1$, $1.67 \pm 1.11 \times 10^1$ CFU/g respectively) in ST5. These decreased gradually from 3% to 12% inclusion of *U. reticulata* (Experiment-2) and *S. cinctum* (Experiment-3). These finding is in according to Immanuel *et al.* (2004) who reported the *Vibrio* load in hepatopancreas and muscle tissues of *P. indicus* fed with seaweed extracts from *U. lactuca* and *S. wightii* when challenged with *V. parahaemolyticus*. 