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Comparison of proximate composition level in *Litopenaeus vannamei* cultured in various Stocking density during summer crop in province of Gujarat states in India

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Abstract

Culture shrimp sample of *Litopenaeus vannamei* were collected from commercial pond at Datardi village, Rajula (Gujarat). All pond harvested shrimp were collected in concern chill dead tank, from this, shrimp sample were randomly selected for biochemical composition. The tests were implemented three times. The present study was conducted to evaluate the effect of stocking density on *L. vannamei* with the emphasis on proximate, nutritional, amino acids and fatty acid composition. The proximate composition of *L. vannamei* was higher protein (%) in ST1 (30 nos/m²) treatment followed by crude fat (%), carbohydrate (%) and Ash (%) in ST1 (30 pc/m²). Comparing all treatment major elements like calcium content was higher in ST5 (70 pc/m²) with 24.52±2.12 than potassium (K) in ST6 (80 pc/m²), sodium (Na) in ST6 (80 pc/m²) and magnesium (Mg) in ST1 (30 pc/m²) whereas minor elements predicted were Copper (Cu) followed by iron (Fe), Zinc (Zn), Manganese (Mn) and Chromium (Cr). Totally 19 amino acids were detected, among these, arginine, histidine, isoleucine, threonine, leucine, methionine, phenylalanine, tryptophan, lysine and valine are essential amino acids and alanine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine are non-essential amino acids. In total essential amino acids recorded in ST4 (60 pc/m²) followed by ST1 (30 pc/m²), ST3 (50 pc/m²), ST6 (80 pc/m²), ST2 (40 pc/m²) and ST5 (70 pc/m²) whereas the total non essential amino acid (NEAAs) in treatment ST5 (70 pc/m²) followed by ST2 (40 pc/m²), ST6 (80 pc/m²), ST3 (50 pc/m²), ST1 (30 pc/m²) and low in ST4 (60 pc/m²). Total 18 fatty acid were detected in all treatment. The results indicated that *L. vannamei* shrimp has higher volume of SFA, PUFA, MUFA. The total unsaturated fatty acids (USFAs) was higher in treatment ST2 with (66.15 µg/g of FAME) followed by ST3 (66.00µg/g of FAME), ST6 (65.58 µg/g of FAME), ST4 (63.04 µg/g of FAME), ST1 (62.75 µg/g of FAME) and ST5 (62.08 µg/g of FAME). Comparing all the treatment there is clear evident that stocking density affect the proximate, nutritive, amino acid and fatty acid level in *L. vannamei* cultured shrimp.

Keywords: *Litopenaeus vannamei*, fatty acid profile, amino acid profile, shrimp, nutritional value, summer crop

Introduction

Shrimp is one of the world's widely preferred seafood because of its most popular flavor and taste. Among Seafood products shellfishes have attracted huge crowd as important sources of nutrients in the human diet. Apart from their delicacy, the Decapoda order of crustacean group like prawns, lobsters, and crabs, and has received special demand because it is a food rich in lipids, proteins, and major and minor minerals and vitamins^[1, 2].

Fish is accepted universally as a rich source of nutrition for millions. It is considered as a remedy to set nutritional deficiencies and body's nutritional balance. Fish consumption is therefore increasing rapidly in many countries, but the importance of fish in daily diet is not yet fully realized since, it is still a supplemental diet to a large section of Indian population. Therefore, the annual per capita fish consumption in India is very low i.e., 8kg against the world average of 10kg. At the same time, according to the WHO standards a person needs 11kg of fish to fulfill the minimum nutritional requirement^[3]. In India, millions of people are suffering from malnutrition. Protein deficiency may be minimized for some extent by making available cheaper fish items like shrimps and crabs, which are rich in protein, minerals and vitamins A and D. It also serves as a good source for iodine, phosphorus, magnesium, iron, copper, sulphur and calcium which are essential to keep up the health and stamina^[4].

Nutritive values directly reflect the biochemical compositions of any edible organisms, there are five basic nutrients such as protein, lipids, ash and carbohydrates. For any living organisms, proteins are vital nutrients for the structural and functional. The entire living animal on earth, proteins are regularly used for growth and repair of tissues, a continuous supply of proteins or its basic component amino acids is required. Amino acids are the micro molecules and building blocks of proteins. Protein and lipids, also contain several dietary major and minor minerals such as calcium, iron etc., which plays and significant roles for wear and tear of physiological and biochemical activities in human. *Penaeid* shrimp may not have a definite lipid requirement but for commercial shrimp farming, feeds range from 6% to 7.5% and a maximum level of 10% [5]. Minerals are necessary for physiological and biochemical process for human body, acquires assimilates as utilized food to maintain health and activity. Below normal level of mineral causes some health problem. The facts on the carbohydrate nutrition of crustaceans is meagre with weight gain and specific growth rate were lowest and the feed conversion ratio was poorest in shrimp fed having 35% of this carbohydrate source. So studies on minerals in chief source like shrimps are essential. Therefore, the present study was under taken with following objectives comparison of carcass and proximate composition of cultured *Litopenaeus vannamei* (Boone, 1931) with different stocking density during summer crop.

Materials and Methods

The experiment was conducted in commercial shrimp pond at Kavaya Aqua Farm, Datardi village, Taluka: Rajula, Dist: Amreli, Gujarat, India. (Latitude 20° 57'35.38" N and 71° 32'35.60" E and Longitude 20° 57'35.93" N and 71° 32'32.03" E) (Plate 1). Total 22 numbers of ponds in the farm out of this 18 were culture ponds and 2 were sedimentation ponds and 2 were reservoirs. The size of the culture pond was 0.5 ha and depth was 1.8 m. The experiment was conducted in as completely randomized design (CRD) with 6 treatments with 3 replications. Summer crop treatment was represented as ST1 with stocking density of 30 nos/m² was denoted as ST1 30 nos/m² as on for 2nd treatment ST2 with 40 nos/m², treatment ST3 50 nos/m², treatment ST4 60 nos/m², treatment ST5 70 nos/m², and in treatment ST6 80 nos/m² was maintained. The experiment was carried out for total 120 DOC (days of culture). The culture ponds were prepared as per standard procedures [6].

The shrimp, *Litopenaeus vannamei* post larvae (PL 09) were procured from commercial shrimp hatchery Grobest Hatchery Pvt. Ltd. Chennai. Post larvae were packed in oxygenated polythene bags and brought to commercial pond at Kavaya Aqua Farm at Datardi. *L. vannamei* seeds were PCR tested. The PL were acclimatized in the farm with adequate aeration. The initial average weight of post larvae was 0.06±0.01. The experimental pond management was carried out by following steps given below during both the season. Drying, tilting of dry pond, lime application, pond water filling with filter bags, disinfection, blooming, stocking post larvae as per design, check tray observation, water quality management, paddle wheel aerators, sampling, sludge removal, harvesting and ice killing. *L.vannamei* shrimp sample were randomly collected from the final harvested from pond through sluice gate. Shrimps were collected from each treatment pond and accordingly packed in marked polythene bags, placed in

thermocouple box with crushed ice. Samples were brought to the Fisheries Research and Training Centre, J.A.U., Mahuva. The samples were washed with deionized water to remove any adhering contamination, drained under folds of filter paper. The fresh shrimp samples (Plate 2) collected were segregated individually as per polythene bags, placed in petri dish. Samples were placed at 45°C in oven drying for two to three days (Plate 3). Dried sample were and crushed and powdered (Plate 4). The dried powdered shrimp samples were packed and sent to Food Technology Laboratory, J.A.U., Junagadh for amino acid and fatty acid profiling and proximate composition analysis.

Proximate and Mineral Composition Analysis

The crude protein, crude lipid and carbohydrate were estimated as per standard methods A.O.A.C. (1990). 1 g of powdered shrimp tissue was taken in a porcelain crucible was kept in a muffle furnace at 60°C for 4 h. The residue ash content was weighed and the percentage was calculated. Moisture content was estimated by hot air oven method and minerals were analyzed by following the method of A.O.A.C. (1990). Quintuplicate sample reading were taken and the formula are as mentioned below.

Crude protein (CP)

Percentage of protein was calculated by multiplying the percent of nitrogen found with a factor of 6.25.

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Crude lipid

$$\text{Crude lipid (\%)} = \frac{\text{Weight of the ether extract}}{\text{Weight of sample}} \times 100$$

Moisture

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample} - \text{Dried weight of sample}}{\text{Wet weight of sample}} \times 100$$

Ash

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Total Carbohydrate

Total carbohydrate was calculated by difference method given by the formula:

$$\text{Total carbohydrate} = 100 - (\text{CP} + \text{CL} + \text{Moisture} + \text{Ash})$$

Mineral

L. vannamei shrimp mineral composition was determined by using MPAES 4200 (Microwave Plasma Atomic Emission Spectrophotometer). Dry shrimp powder sample (1.0 g) was weighed into a precision digital balance, 30 mL of Di acid (HNO₃: HClO₄), predigest for 1 hr and further digested by heating the sample in a hot plate up to it remain just 4-5 mL. Cool and makeup 100 mL by Di Water by several wash to flask. The mineral composition was determined in triplicate using a spectrophotometer.

Table 1: The method of Element and Wavelength

Sr. no	Element	Wavelength (nm)	Method
1	Calcium	445.478	MP AES
2	Magnesium	383.829	MP AES
3	Copper	324.754	MP AES
4	Iron	259.940	MP AES
5	Manganese	257.610	MP AES
6	Zinc	213.857	MP AES
7	Cobalt	340.512	MP AES
8	Sodium	-	Flame photometric method
9	Potassium	-	Flame photometric method

Amino acid profiling

- a. **Hydrolysis:** Sample biomass (3-10 mg dry weight and 10-20 mg wet weight) was taken in a heat stable test tube; added 100 μ l of 0.1N HCL, 800 μ l of 6N HCL, 100 μ l of Nor-Leucine st (1000 ppm) and 10 μ l of phenol and sealed the tube. The hydrolysis was carried out at 110 °C for 16 hrs with dry bath. After hydrolysis, the contents were transferred to 10 ml standard flask. Added 0.5 ml of 50% NaOH in standard flask and made volume up to 10 ml with diluents (0.1N HCL).
- b. **Derivatization:** The 100 μ l of hydrolysates were taken into 10 ml falcon tube; added 900 μ l of borate buffer, 1ml of FMOC and mixed thoroughly; added 4ml of n-Hexane and vortexed for 45 second. Two layers were formed, the upper layer was discarded and the lower layer was collected into the UHPLC injection tube or vial and seal.
- c. **UHPLC Analysis:** UHPLC vial with collected sample was loaded into the tray of auto sampler. Then 25 μ l of sample was injected to an amino acid analyzer equipped with column (C 18⁺ 4.6 X 25 mm, 5 μ m packing) and diode array detector (265nm Wavelength). The Column was run with mobile phase A and B at flow rate of 1.5 ml/min. The column gradient was maintained as 10 - 50% B for 45 min., 50% B for 5 min., 90% B for 10 min., 100% B for 2 min., 100% B for 5 min., 10 % B for 2 min., 10% B for 6 min. Standard amino acid mixture (25 μ l) was also run separately, and then the chromatograms of standard and sample were compared and quantified.

Fatty acid profiling

Fatty acids of samples were identified and quantified as methyl esters using GC-MS (Gas Chromatography Mass Spectrophotometer) unit.

1. Lipid extraction

- 1 Gram of each sample powder was extracted with chloroform-methanol mixture (2:1 v/v)
- The homogenate were filtrated to recover the liquid phase
- The filtrate were washed with 0.2 volumes (4 ml for 20 ml) of 0.85% NaCl solution.
- The mixture was allowed to decant.
- The lower chloroform phase containing lipids were collected and evaporated under vacuum in a rotary evaporator to bring down to a concentration of 2-3 ml.
- Additional evaporation of chloroform was carried out under oven and residue was weighed to quantify the amount of lipid extracted.
- The lipid residue was re-dissolved in chloroform/methanol (2:1, v/v) and then stored in a 25

mL conical flask with glass stopper at -20°C until needed.

2. Preparation of Fatty Acid Methyl Esters (FAME)

- The isolated lipids obtained by heating at 45°C with adding 2ml of 0.5N Methanolic NaOH for 5 min.
- 2 ml of BF₃ was added for esterification for 2 min
- 5ml of n-Heptan to recover the methyl esters in organic phase for 8 min using Heating Mental.
- Mixture was washed with saturated NaCl solution
- Two phases was formed.
- The upper layer, an organic phase containing the fatty acid methyl ester (FAMES) was collected out and stored in 10 ml all glass vials for further analysis.

3. GC-MS Analysis

- TRACE 1310 GC fitted with TSQ 8000 EVO MS system and Triplus auto analyzer (Thermo, US).
- The system parameter for analysis were, the oven temperature 1 min initial hold at temp. 50°C, raised to 50-150°C @ 20°C min⁻¹ and hold of 15 min. at 150°C, again raised from 150-240°C @ 20°C min⁻¹ and a final hold of 2 min at 240°C.
- Helium was used as a carrier gas with column flow rate 1ml.
- The MS conditions were ionization voltage 70 ev, MS transfer line temperature 250°C, ion source temperature 230°C, foreline pressure 70 m torr, mass range of 40-500 and the scan time equal to GC run time.

Results and Discussion

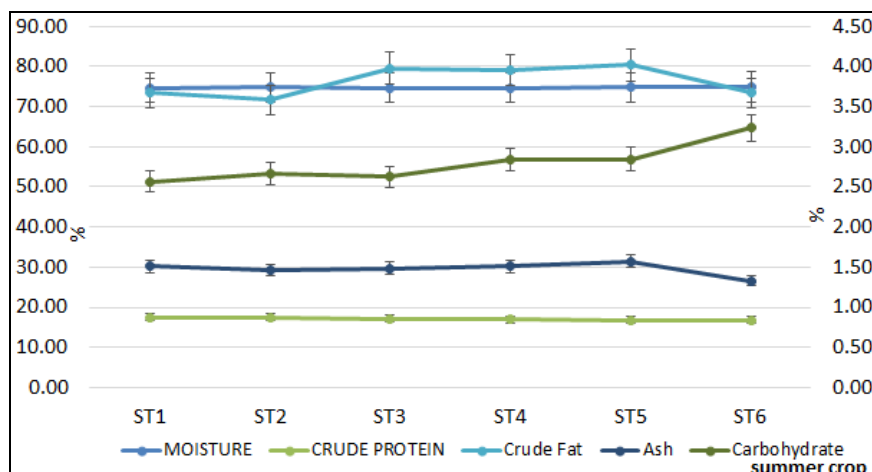
Biochemical composition is the key to measure and assess the nutritional quality of food sources. Any body composition of aquaculture animals has been found to correlate with dietary nutrients. The proximate body composition including moisture, fat, protein and ash are good indicators of physiological condition of organism. The most preferred and excellent source of nutritive food and flavor for human being are shrimp, lobsters and crabs.

1. Proximate composition of *L. vannamei* shrimp

The proximate composition detected from the flesh of the *L. vannamei* stocked with different stocking density during summer crop is shown in (Table 2. Fig. 1). The level of crude protein content within the cultured shrimp during summer crop varied from 16.81% to 17.58%.The highest concentration of protein was recorded in ST1 (17.58±0.49) followed by ST2 (17.49±0.30), ST3 (17.23±0.14), ST4 (16.96±0.33), ST6 (16.86±0.28) and ST5 (16.81±0.06). Crude fat highest concentration in ST5 (4.02 ± 0.06%) followed by ST3 (3.98 ± 0.04), ST4 (3.95 ± 0.06%), ST6 (3.68 ± 0.07%), ST1 (3.67 ± 0.04%) and ST2 (3.59 ± 0.11%). Highest carbohydrate in treatment ST6 (3.24 ± 0.50) followed by ST5 (2.85 ± 0.34), ST4 (2.83 ± 0.36), ST2 (2.67 ± 0.39), ST3 (2.63 ± 0.19) and ST6 (2.56 ± 0.62), highest ash content in treatment ST5 (1.57 ± 0.18) followed by ST4 (1.53 ± 0.06), ST1 (1.52 ± 0.04), ST4 (1.53 ± 0.06), ST2 (1.46 ± 0.02) and ST6 (1.33 ± 0.08) whereas highest moisture% ST6 (74.90±0.23) followed by ST2 (74.79±0.10), ST4 (74.73±0.12), ST5 (74.75±0.15), ST3 (74.68±0.10) and low in ST1 (74.67±0.19).

Table 2: Proximate composition (%) in the flesh of *L. vannamei* shrimp during summer crop

SD (nos/m ²)	Proximate composition (%) (n= 5)				
	Moisture	Crude protein	Crude Fat	Carbohydrate	Ash
ST1	74.67±0.19	17.58±0.49	3.67±0.04	2.56±0.62	1.52±0.04
ST2	74.79±0.10	17.49±0.30	3.59±0.11	2.67±0.39	1.46±0.02
ST3	74.68±0.10	17.23±0.14	3.98±0.04	2.63±0.19	1.48±0.05
ST4	74.73±0.12	16.96±0.33	3.95±0.06	2.83±0.36	1.53±0.06
ST5	74.75±0.15	16.81±0.06	4.02±0.06	2.85±0.34	1.57±0.18
ST6	74.90±0.23	16.86±0.28	3.68±0.07	3.24±0.50	1.33±0.08

**Fig 1:** Proximate composition (%) in the flesh of *L. vannamei* shrimp during summer crop.

Mohanta *et al.* [7] stated that the protein level of aquaculture animals initially increased then decreased with increasing dietary protein levels. [8] Sriraman and Reddy stated that biochemical composition of any living organisms varies with season, size of animal, stages of maturity and availability of food, zonation and temperature, etc. [9] Shaikmahmud and Magar observed that an increase in age and size presumed to increase in protein content, resulted of accumulation and storage. Shrimp is considered as a high-range protein covering nutrient like fish, which contain 8-20% protein. [8] Sriraman and Reddy reported in shrimp muscle, protein is the dominant biochemical constituent. In the present investigation, *L. vannamei* shrimp protein ranged between 16.81 to 17.58% (in all treatment). This statement is in agreement with [10, 11] Yanar and Celik; Sriket *et al.* stating that shrimp protein content ranged between 17-21% depending on shrimp species. As per [12] Sambhu and Jayaprakas report, the protein level in *P. indicus* was varied from 44.62 to 80.87%. The high protein content in the lowest size groups may be attributed to increased protein synthesis during the nursery growth phase as it has been observed elsewhere in shrimps and mantis shrimps [13, 14, 15]. [13] Achuthankutty and Parulekar stated that higher protein concentration recorded in juveniles than in adults, but shrimps collected from wild.

The proteins are essential for normal function, growth, and maintenance of body tissue. This indicates that the high protein content confirms a comparatively low heat of nutrient metabolism, also called specific dynamic action (SDA) and the limited amounts of inexpensive carbohydrate as source of energy. Carbohydrates serve as precursors for the synthesis of dispensable amino acids and certain nutrients, which in free and bound state along with proteins as protein-bound sugars and glycogen. Carbohydrates are considered to be the first substances to be utilized for the synthesis of energy required for metabolic and physiological activities. Carbohydrate content showed an inverse relationship with protein content.

Similar findings were recorded by [12, 16, 17, 18, 19] Silva and Chamul (2000); Sriraman (1978); Nair and Prabhu (1990); Reddy and Shanbhogue (1994); Ravichandran (2000). Lipid act as chief source of food storage along with protein and subjected to periodic fluctuations influenced by environmental variables like temperature [20]. [21] Pillay and Nair recorded an inverse relationship between lipids and moisture content, but this does not affect the lipid composition of muscle tissue in a body. Lipid content of *L. vannamei* quantitatively a good source of human diet, so in the present study the amount of lipid level provide enough fat for human diet.

[22] Gopakumar reported that in some shrimp species, moisture varied 79.3-83.6% which correlates the results from the present study of 74.67 to 74.90%. Moisture of fresh shrimp is generally reported as 75-80% [11, 23]. In present study 74.67 to 74.90% moisture was recorded. Ash is one of the least studied biochemical constituents of crustaceans in general and *L. vannamei* in particular. Ash content of shrimp is generally 1-1.5%. The ash content of the *L. vannamei* was calculated as 1.33 to 1.52% in the present study. High amount of ash indicates the richness of the food in terms of element composition. [11] Sriket *et al.* calculated the amount of ash in black tiger and white shrimps were 0.95 and 1.47%, respectively. Similar stated by [24] Nargis these values were very close to the present study findings. [25] Suneetha *et al.* stated that different reproductive stages, there is change in the proximate chemical composition, fresh mass, water content, ash content, organic constituents, lipid and protein contents and energy levels of penaeid prawn, *Penaeus monodon*. Changes in chemical composition lead to differences in the nutritional value, sensory attributes, and shelf life of shrimps [11, 26].

The decrease in water content resulted in the relative increase in protein, fat and ash content that is highly correlating the present study.

2. Minerals composition of *L. vannamei* shrimp during summer crop

Mineral composition of shrimp is important for nutritional point for selecting the best product for health. [27] Agusa *et al.* reported that minerals are excellent for growth and nourishment of human body and prevents several nutritional deficiency diseases. Minerals also constitute important mechanisms of enzymes, hormones and enzyme activators [28].

2.1 Major elements of *L. vannamei* shrimp during summer crop

The minerals of the *L. vannamei* flesh are shown in (Table 3, Fig. 2 and 3). Comparing major elements (g kg^{-1}) availability in all treatment, Calcium (Ca) content was maximum in ST5 ($24.52 \pm 2.12\text{g}$) followed ST3 ($22.57 \pm 5.5\text{g}$) than all other treatment. Potassium (K) maximum concentration of elements was recorded in ST6 ($9.10 \pm 0.35\text{g}$) followed by ST2 ($8.94 \pm 0.35\text{g}$). Sodium (Na) recorded in ST6 ($9.08 \pm 0.92\text{g}$) followed by ST2 ($8.84 \pm 0.53\text{g}$). Magnesium (Mg) in ST1 ($0.98 \pm 0.06\text{g}$) followed by ST2 ($0.97 \pm 0.06\text{g}$).

Minerals such as calcium, magnesium, potassium and sodium increased gradually with increase of salinity in experimental waters. The ratio of Mg: Ca and Na: K was directly proportional to the culture medium and ionic changes on growth rate and body compositions. Fish and shellfish contain significant amounts of minerals such as, calcium, magnesium, phosphorus, potassium and sodium [29, 30]. In the present study during investigation the mineral like Ca, Mg, K, P, Mn, Na, Zn, Cu, Fe and Cr were detected in the edible part of *L. vannamei*. Calcium levels varies from 19.35 to 24.52 gKg^{-1} , which is higher (59.5 mg) than green tiger shrimp [10], sea bass (63.6 mg) and sea bream (19.2 mg) [31]. [32] Lovell stated that calcium is crucial elements for hard tissue structure, blood clotting, muscle contraction, nerve transmission, osmoregulation and as a cofactor for enzymatic procession. Additionally, several Ca supplements contain lead, which impairs health in numerous ways. [33] Whithney stated that Ca interferes with the absorption and action of lead in the body system. The shrimp waste is well-known in high calcium contain [34].

Sodium is the principal cation of the extra cellular fluid and regulator of its volume. [33] Whithney stated that sodium helps to maintain acid-base balance and is essential for nerve system, additionally he noted that level of Na in flesh of *P. longirostris* and *P. martia* was found as 876 and 574 mg g^{-1} respectively, whereas [35] Gunalan *et al.* stated that 67.7 mg g^{-1} in *L. vannamei* shrimp. In present investigation, Na in flesh of *L. vannamei* was noted between 7.01 to 9.08 g kg^{-1} , which is higher.

Potassium plays a major role in maintaining fluid and electrolyte balance and cell integrity. During the nerve transmission and muscle contraction, potassium and calcium briefly exchange places across the cell membrane. Potassium requirement for human is about 2gday^{-1} . [30] Abdullah *et al.* reported that deep seawater rose shrimp and golden shrimp 996 and 644 $\text{mg}/100\text{g}$ respectively, [35] Gunalan *et al.* stated in farm shrimp *L. vannamei* 56.7 mg/g . In present investigation, K contents of *L. vannamei* were found in between 7.69 to 9.10 g kg^{-1} , which is higher than but less than that reported by [10] Yanar and Celik for green tiger shrimp and [31] Erkan and Ozden stated for sea bass and sea bream.

Magnesium content of *L. vannamei* was about 8.7 to 9.8 mg g^{-1} . Magnesium is key for human nutrition and it is required for body's enzyme system and maintain bone health, whereas [33] reported that magnesium acts in all cells of soft tissues, where it forms part of the protein-making machinery and necessary for energy metabolism.

2.2 Minor elements of *L. vannamei* shrimp during summer crop

Comparing minor elements (mg g^{-1}) availability in all treatment (Table 3, Fig 3), Copper (Cu) concentration was maximum in ST6 (87.79 ± 1.22) followed by ST5 (85.38 ± 3.08) than all other treatment. Iron (Fe) high concentration recorded in treatment ST6 (91.71 ± 1.07) followed by ST5 (86.71 ± 0.59). Zinc (Zn) higher conc. was in treatment ST3 (78.35 ± 1.59) followed by ST6 (77.18 ± 1.42). Manganese (Mn) in treatment ST5 (9.92 ± 0.29) followed by ST2 (9.75 ± 0.48). Chromium (Cr) high in ST5 (8.40 ± 0.74) followed by ST6 (8.28 ± 1.23).

Vital and most essential trace elements is iron (Fe) in human system. It serves as a carrier of oxygen to tissues from the lungs by red blood cell. Adequate Fe in diet is very important for avoiding some major health problems [28, 36]. Iron content of shrimps used for this study was 4.54 mg . However, the value stated for *L. vannamei* was higher than those stated by [37]. Whereas it was less in green tiger shrimp (1.48 mg), speckled shrimp (1.55 mg) [10] Sriket *et al.* and *Aris teusantennatus* (0.9 mg) [38]. In the present investigation, Fe range between 78.6 to 91.71 mg g^{-1} .

Other essential elements such as copper, zinc and manganese are valuable for all living animals because they play important roles in many physiological functions. [39] White and Rainbow reported that high amounts of copper are present in crustaceans, decapods that use copper to carry oxygen to their tissues. Copper has been known one of the major catalysts for oxidation [40]. [41] Oehlschlager stated that despite the high copper concentrations in aquatic food present no problem for human health. [37] Gokoglu *et al.* reported levels of Cu and Zn for *P. longirostris* as 1.33 and 14, 57 mg g^{-1} , in the present study, the range of Cu, Zn in *L. vannamei* shrimp was (80.30 to 87.79 mg g^{-1}) and Zn (63.41 to 78.35 mg g^{-1}), comparing these results Zn level of this study value was higher than all. According to Agency for Toxic Substances and Disease Registry (Anon, 2004) a very high intake can cause some health problems, such as liver and kidney damage. Cu, Cd and Zn were found as 2.2, 0.7 and 6.1 mg g^{-1} for *P. longirostris* respectively. In this present study, copper was found in range between 80.30 to 87.79 mg g^{-1} , which is higher than reported earlier. The level of Cd, Zn, Cu are higher in this area may be due to ship breaking yard and Pipava port.

Concentrations values of chloride, sulfate, calcium, magnesium, potassium, and sodium were lower than values recorded in deep seawater [43, 44]. In the present investigation the results were similar.

Shellfish are usually higher in minerals Ca, Mg, K, Na, Zn, Cu, Fe, Mn and Cr than fish. Heavy metals, like Zn, Cu and Cr may be present in seafood depending on how they feed and where they live.

The mineral composition in *L. vannamei* shrimp flesh cultured during summer crop was in the following order: $\text{Ca} > \text{K} > \text{Na} > \text{Mg} > \text{Cu} > \text{Fe} > \text{Zn} > \text{Mn} > \text{Cr}$.

Table 3: Mineral composition in the flesh of *L. vannamei* shrimp harvest during summer crop

n=5	Stocking Density Nos/m ²					
	ST1	ST2	ST3	ST4	ST5	ST6
Major Elements (g kg⁻¹)						
Ca	20.14±2.7	19.97±1.9	22.57±5.5	21.37±3.8	24.52±2.12	19.35±1.73
Mg	0.98±0.06	0.97 ± 0.06	0.92± 0.06	0.97±0.06	0.93±0.08	0.87±1.02
K	7.69±0.95	8.94±0.35	8.05±0.8	7.82±0.7	7.91±0.60	9.10±0.35
Na	7.01±0.95	8.84±0.53	7.57±0.8	8.31±0.9	7.41±0.81	9.08±0.92
Minor Elements (mg g⁻¹)						
Zn	63.41±0.84	66.05±0.96	78.35±1.59	69.77±0.5	70.54±0.74	77.18±1.42
Cu	80.30±2.16	80.71±1.71	85.17±3.97	84.38±5.6	85.38±3.08	87.79±1.22
Fe	78.63±0.70	82.71±0.27	82.68±0.72	78.78±0.76	86.71±0.59	91.71±1.07
Mn	8.91±0.64	9.75±0.48	9.35±0.25	9.01±0.15	9.92±0.29	9.18±0.07
Cr	6.64±0.76	6.66±1.35	7.28±0.80	7.22±1.73	8.40±0.74	8.28±1.23

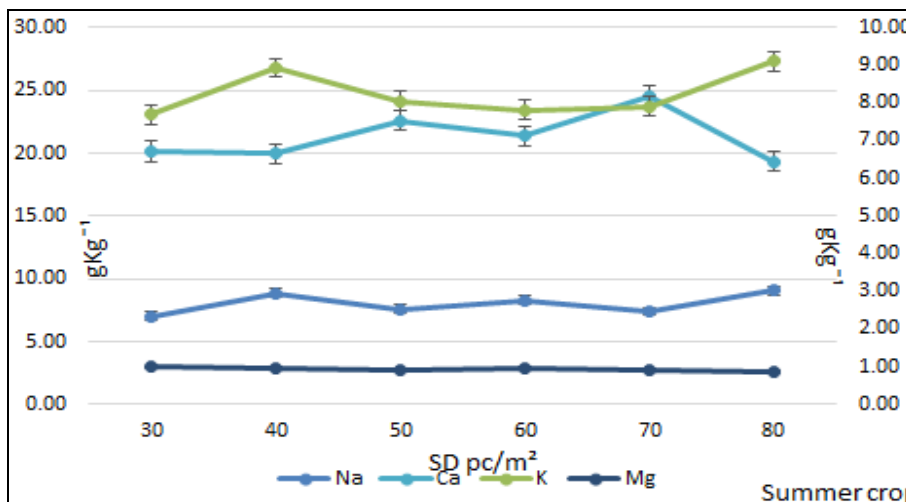


Fig 2: Major elements composition in the flesh of *L. vannamei* shrimp cultured during summer crop.

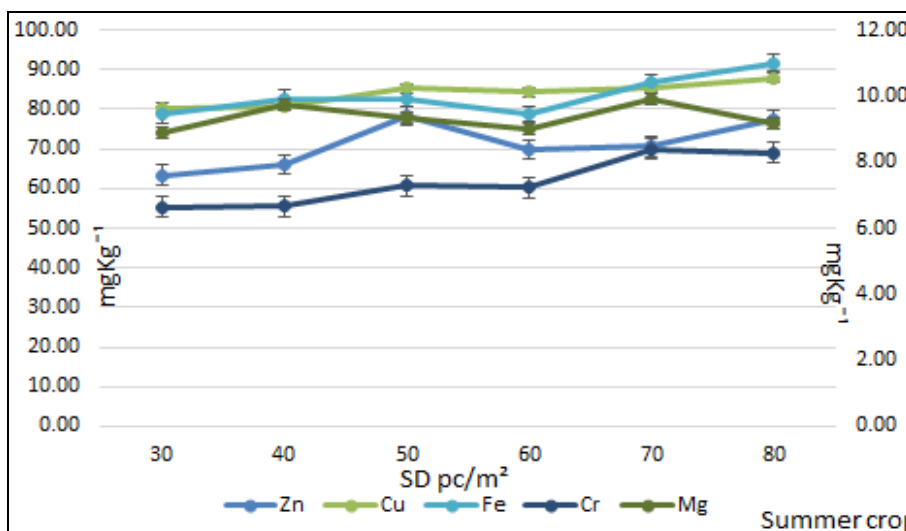


Fig 3: Minor elements composition in the flesh of *L. vannamei* shrimp cultured during summer crop.

3. Fatty acid profile

Fatty acid profiling of experimental shrimp at harvested were detected using Fatty Acid Methyl Ester by Gas Chromatography (GC-MS). Fatty acid composition and chromatogram of fatty acid profile are shown in (Table 4. and Fig 4 to 9). The total saturated fatty acid (SFAs) composition of *L. vannamei* shrimp ranged within treatment is 33.85 to 37.92 µg/g of FAME, total Monounsaturated fatty acid (MUFAs) within treatment was 23.23 to 30.34 µg/g of FAME, total Polyunsaturated fatty acid (PUFAs) ranges between 32.41 to 40.62 µg/g of FAME.

In all treatment, the palmitic acid (C16:0) was dominated than other SFAs in all, with range between 20.16 to 25.78 µg/g of FAME, the highest quantity of palmitic acid (C16:0) was in ST1 treatment 25.78 µg/g of FAME followed by ST5 (22.64), ST3 (20.99), ST4 (20.95), ST2 (20.53) and low in ST6 20.16 µg/g of FAME. Other SFAs have been detected namely lauric acid, myristic acid, pentadecyclic acid, margaric acid, Stearic acid, archidic acid and behenic acid, which totally ranged between 0.07 to 12.33ug/g of FAME.

The quantity of Un-Saturated Fatty Acid (USFAs) ranged between 62.08 to 66.15 µg/g of FAME. Monounsaturated

fatty acid ranged between 23.23 to 30.34 $\mu\text{g/g}$ of FAME. Oleic acid was present relatively higher among MUFAs with range between 18.10 to 28.78 $\mu\text{g/g}$ of FAME. The quantity of polyunsaturated fatty acid (PUFAs) range between 32.41 to 40.62 $\mu\text{g/g}$ of FAME with highest in ST6 and lowest in ST1 treatment. Gamma- linolenic acid (GLA) was highest in ST3

(20.96 $\mu\text{g/g}$ of FAME) and lowest in ST6 (16.88 $\mu\text{g/g}$ of FAME). Other USFAs have been detected namely palmitoleic acid, oleic acid, elaidic acid, eicosenoic acid, gamma linolenic acid, eicosadienoic acid, arachidonic acid, alpha linolenic acid, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA).

Table 4: Fatty Acids in the flesh of *L. vannamei* shrimp cultured

Fatty acid ($\mu\text{g/g}$ FAME)	ST1	ST2	ST3	ST4	ST5	ST6
Lauric acid C12:0	0.12	0.18	0.16	0.08	0.07	0.06
Myristic acid C14:0	0.35	0.56	0.57	0.45	0.38	0.49
Pentadecylicacid C15:0	0.20	0.37	0.33	0.35	0.31	0.00
Palmitic acid C16:0	25.78	20.53	20.99	20.95	22.64	20.16
Margaric acid C17:0	0.72	1.03	0.95	1.30	1.12	0.00
Stearic acid C18:0	9.45	10.64	9.71	12.33	11.91	11.26
Arachidic acid C20:0	0.63	0.54	0.57	0.59	0.63	2.45
Behenic acid C22:0	0.00	0.00	0.72	0.91	0.86	0.00
Σ Saturated FAs	37.25	33.85	34.00	36.96	37.92	34.42
Palmitoleic acid C16:1 (n-7)	0.93	1.16	1.25	1.12	0.98	0.00
Oleic acid C18:1 (n-9 cis)	28.78	25.84	23.87	18.10	19.17	19.65
Elaidic acid C18:1 (n-9tran)	0.00	0.00	2.23	2.85	2.40	2.86
Eicosenoic acid	0.63	0.90	0.98	1.16	0.90	2.45
Σ MUFAs	30.34	27.90	28.33	23.23	23.45	24.96
Omega-6 fatty acids						
Gamma-linolenic acid (GLA) 18:3(n-6)	19.64	20.96	21.71	17.29	17.56	16.88
Eicosadienoic acid 20:2 (n-6)	1.04	1.61	1.74	2.12	1.56	1.84
Arachidonic acid (AA) 20:4 (n-6)	1.34	2.08	1.74	2.98	2.8	2.38
Omega-3 fatty acids						
Alpha-linolenic acid (ALA) 18:3(n-3)	2.21	1.11	1.14	0.96	1.37	1.30
Eicosapentaenoic acid (EPA)20:5 (n-3)	3.86	5.79	5.17	7.77	7.44	7.68
Docosahexaenoic acid (DHA)22:6(n-3)	4.32	6.70	6.17	8.69	7.90	10.54
Σ PUFAs	32.41	38.25	37.67	39.81	38.63	40.62
Σ Unsaturated fatty acid	62.75	66.15	66.00	63.04	62.08	65.58

Many authors reported that the superior nutritional value of marine oils of sardine, pollack, short-necked clam and cod liver oils over plant oils or animal fats for *Penaeus japonicus*, *Penaeus monodon* and *Penaeus vannamei* [45, 46, 47, 48]. In the present study, shrimp sample among SFAs group palmitic acid was dominating followed by stearic and margaric acids. The concentration of palmitic acid in *L. vannamei* was 20.16 to 25.78%. This statement is in agreement by [49] Bragagnolo and Rodriguez-Amaya, *M. carcinus* [50] Simon *et al.*, *F. schimitti* [51] Moura *et al.* stated that the C16:0 has pinpointed as the key fatty acid (FA) in white shrimp, similar to that reported for other crustaceans species such as *Penaeus brasiliensis*, *Penaeus schimitti* and *Xiphopenaeus kroyeri* and six shrimp species marketed in China [52] Li *et al.* additionally, [11] Sriket *et al.* reported 22.2% in black tiger shrimp and 21.8% in white tiger shrimp respectively. [53] Rosa and Nunes reported 17.3% in golden shrimp, 18.0% in pink shrimp and 17.6% in Norway shrimp. It has been stated that combination of essential fatty acids in the diet reflect with better growth rate and survival in aquaculture [54, 55, 56]. [57] Osborn and Akoh reported in their report that n-9 fatty acids, found as oleic acids (C18:1 n-9) plays a moderate role in the body. Moreover, n-6 fatty acids cannot be synthesized by humans and are therefore considered as essential fatty acids.

In the present study, the presence of n-3 PUFA particularly, linoleic, EPA and DHA indicates better growth and survival of *L. vannamei* in the culture pond. The concentration of EPA and DHA was in the range of 3.86 to 7.77 and 4.32 to 10.54 $\mu\text{g/g}$ of FAME respectively, this statement is supported by [58, 59] that higher levels of EPA and DHA would increase stress tolerance and membrane permeability in shrimp. The

foremost Fatty acid found in all the treatment were palmitic acid (C16: 0), docosahexaenoic acid (DHA, C22: 6n-3), eicosapentaenoic acid (EPA, C20: 5n-3), and stearic acid (C18:0) and oleic (C18: 1n -9cis), similar to that reported by [53] Rosa and Nunes, who also found these predominant FA in *A. antennatus* and *P. longirostris* marine species.

In the present study among the monounsaturated fatty acids (MUFA), the oleic acid C18: 1n-9cis was in the majority ranging between (18.10 to 28.78 $\mu\text{g/g}$), as observed in other shrimp species including *P. brasiliensis* and *X. kroyeri* [49], *P. monodon* and *P. vannamei* [11], and *X. kroyeri* [60]. Findings of C18:1 n-9 cis of *L. vannamei* species in this study were similar to previously reported findings of white and black tiger shrimps [11, 61]. In addition, DHA and EPA, belonging to n-3 fatty acids family, are considered as essential [62]. The proportion of omega 6 to omega 3 should be as 1- 2 [63]. EPA and DHA are two of the major PUFA, higher% of PUFA was found ST6 7.6% & 10.54% followed by ST4 (7.77 & 8.69. In the present study, the value of EPA was lower than that of DHA in *L. vannamei* although [64] Ackman reported that, the shellfish tend to have EPA greater than DHA. Furthermore, n-3 fatty acids are essential in growth and development throughout the human life cycle and should be included in the diet. [65] Gonçalves reported the rate of fatty acid in *P.vannamei* shrimp at 9.7%.Linolenic acid (C18:3 W3) and Docosahexaenoic (DHA – C22: 6W3) are the most important and significant unsaturated fatty acid with double band which human body is unable to synthesize them. In the present study polyunsaturated fatty acids (PUFA) were predominant (32.41 to 40.62 $\mu\text{g/g}$ of FAME) in the samples of the present study, and C22: 6n-3, DHA was observed at higher concentrations

(10.54 to 4.32 µg/g of FAME), followed by C20: 5n-3, EPA (7.68 to 3.86 µg/g of FAME). There is no consensus on the predominant FA in shrimps. Although SFA appear in higher concentrations in some species, there may be differences within the same species. [51] Found higher SFA levels in *L. vannamei*. Environmental changes and diet altogether are the factors that most influence the FA composition in crustacean muscles [52].

4. Amino acid profiling of *L. vannamei* shrimp

The total quantities of amino acids concentration ranges from 0.35mg to 11.82mg amino acids/100g (DW). The total essential amino acids (EAA) concentration was highest in treatment ST4 (66.08%) followed by ST1 (64.54%), ST3 (63.95%), ST6 (63.57%), ST2 (62.49%) and low in ST5 (61.08%) whereas highest total NEAA concentration recorded was in treatment ST5 (38.92%) followed by ST2 (37.51%), ST6 (36.43%), ST3 (36.05%), ST5 (35.46%) and lowest concentration in ST1 (35.46%) amino acid/100g(DW) (Table 5. and Fig. 10 - 16).

Highest concentration of individual EAA amino acid was from treatment ST1 Lysine (11.82%) followed by ST2 Isoleucine (10.76%), ST4 Isoleucine (10.5%) remaining all amino acid of this group range between 10.36 to 1.73%/100g (DW).

Highest total concentration of individual NEAA was from ST5 Glycine (10.66%) followed by ST6 Glycine (8.88%) other all remaining amino acid of this group range between 8.86 to 0.35%/100g (DW).

Amino acids are the building blocks of proteins and serve as protein builders. They are utilized to develop various cell structures and function, they are key components and serve as source of energy [66]. Amino acids are basic need and plays important role in human nutrition and health promotion. The level of amino acid content varies by intrinsic (species, size, and sexual maturity) and extrinsic factors (food resources, fishing season, water salinity, and temperature) [67, 68]. Crustacean muscles comprise high concentration of free amino acids, such as arginine, glycine, proline, glutamine and alanine [69]. Isoleucine, Histidine, Methionine, Valine, Glycine, Proline, Cysteine, Tyrosine etc. [69, 70, 71, 72]. The free amino acids have been shown to function in osmoregulation [73]. Neurotransmitter [74] Mullen and Martin stated metabolic pathways of growth including protein synthesis [70, 72, 75] Padma; Bhavani; Wilson stated allergic and inflammatory reactions. In the present investigation total 19 amino acids were categories in Essential Amino Acid (EAAs), Non-essential Amino Acid (NEAAs), both essential-amino acids and non-essential amino acids in the muscle tissue were quantified (Table 5). In summer crop, under Essential-Amino Acids (EAAs) category 10 amino acids in which the mean concentration of Isoleucine was dominating followed by methionine in all treatment whereas 09 amino acid under Non-Essential Amino Acid (NEAAs) in which mean concentration of glycine was higher followed by glutamic acid in all treatment. Isoleucine and methionine are active amino acid during summer season and hence predicted in maximum (mg/100g (DW)).

Table 5: Essential amino acid (EAA) and Non-Essential Amino Acids (NEAA) composition (mg/100g (DW) recorded from *L.vannamei* shrimp flesh during summer crop in different treatments

List of Amino Acid (%)	Abbreviation	Stocking density (Nos/m ²)					
		ST1	ST2	ST3	ST4	ST5	ST6
Alanine	Ala	2.12	3.50	2.58	1.73	2.92	1.97
Asparagine	Asn	2.37	2.45	1.77	1.09	2.44	1.97
Aspartic Acid	Asp	3.67	2.39	2.81	2.94	2.51	3.31
Cysteine	Cys	6.08	7.02	7.16	6.83	6.39	7.10
Glutamic Acid	Glu	7.77	6.63	8.86	7.54	7.62	7.26
Glycine	Gly	8.37	8.29	5.69	7.52	10.66	8.88
Proline	Pro	1.81	2.55	2.34	2.50	3.55	2.19
Serine	Ser	1.43	2.22	2.26	1.45	0.35	1.38
Tyrosine	Tyr	1.83	2.45	2.58	2.32	2.49	2.38
Total NEAA (%) in sample		35.46	37.51	36.05	33.92	38.92	36.43
Threonine	Thr	4.28	4.96	6.01	6.39	6.98	6.95
Arginine	Arg	4.94	4.82	5.60	4.41	4.95	4.12
Histidine	His	5.84	5.82	9.59	5.56	3.53	10.36
Valine	Val	6.02	5.80	6.50	6.87	6.08	5.28
Methionine	Met	6.98	6.07	7.26	6.19	6.65	6.07
Iso leucine	Ile	10.30	10.76	10.35	10.50	9.78	9.33
Phenylalanine	Phe	6.06	5.76	6.75	6.57	6.96	6.17
Leucine	Leu	2.31	2.37	1.73	8.51	3.70	8.33
Lysine	Lys	11.82	8.31	2.07	4.62	2.88	1.79
Tryptophan	Trp	5.98	7.82	8.10	6.45	9.57	5.17
Total EAA (%) in sample		64.54	62.49	63.95	66.08	61.08	63.57

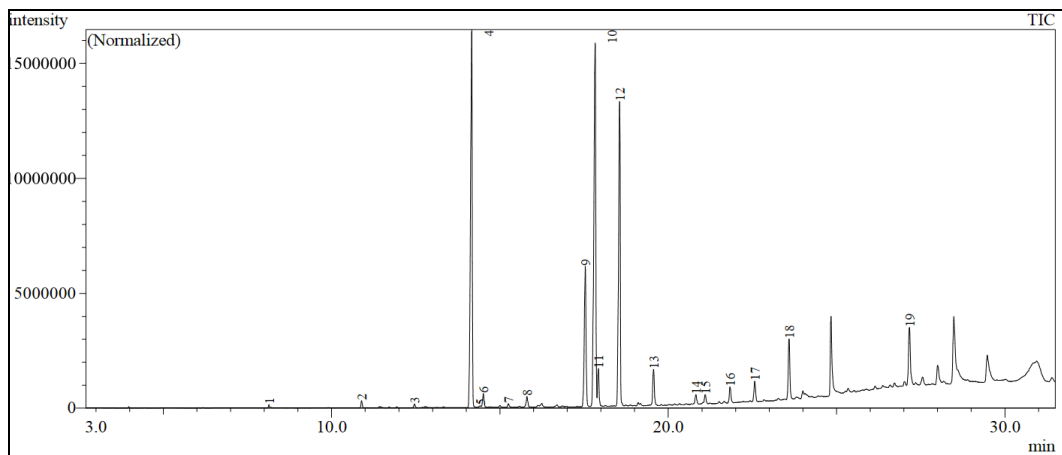


Fig 4: Chromatogram of fatty acid profiling of *L. vannaemii* during summer crop at the end of 120 DOC of ST1

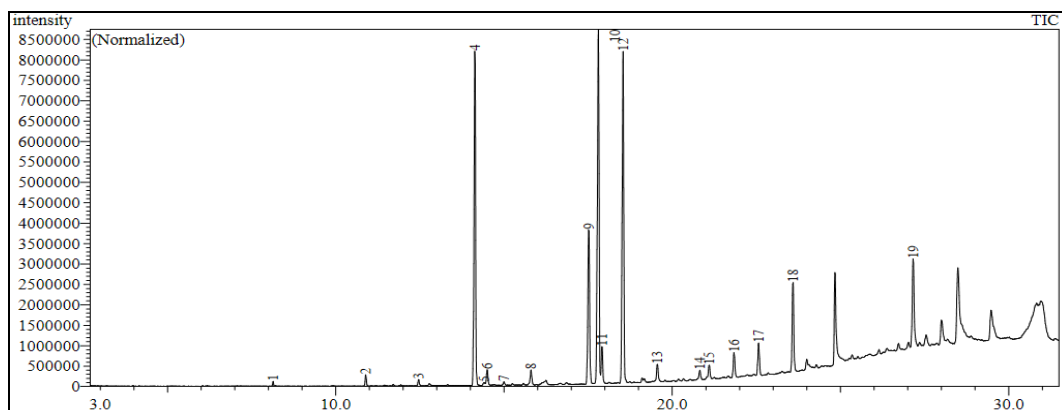


Fig 5: Chromatogram of fatty acid profiling of *L. vannaemii* during summer crop at the end of 120 DOC of ST2

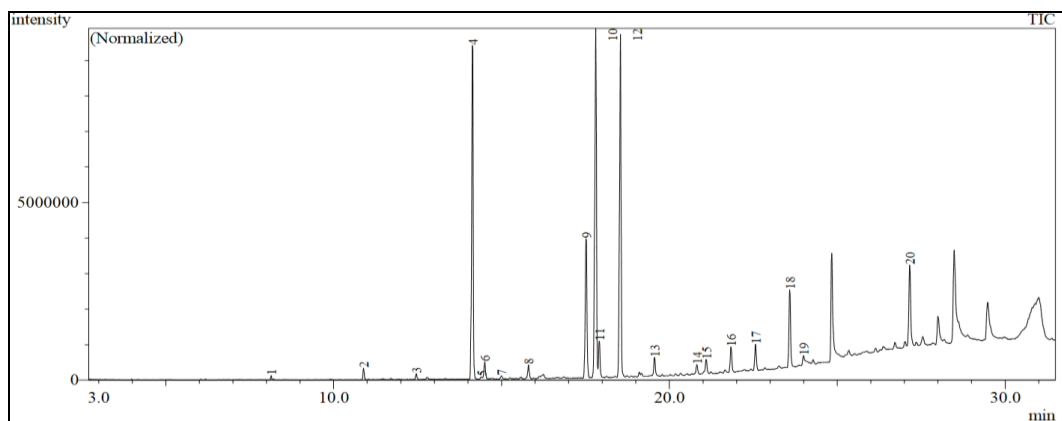


Fig 6: Chromatogram of fatty acid profiling of *L. vannaemii* during summer crop at the end of 120 DOC of ST3

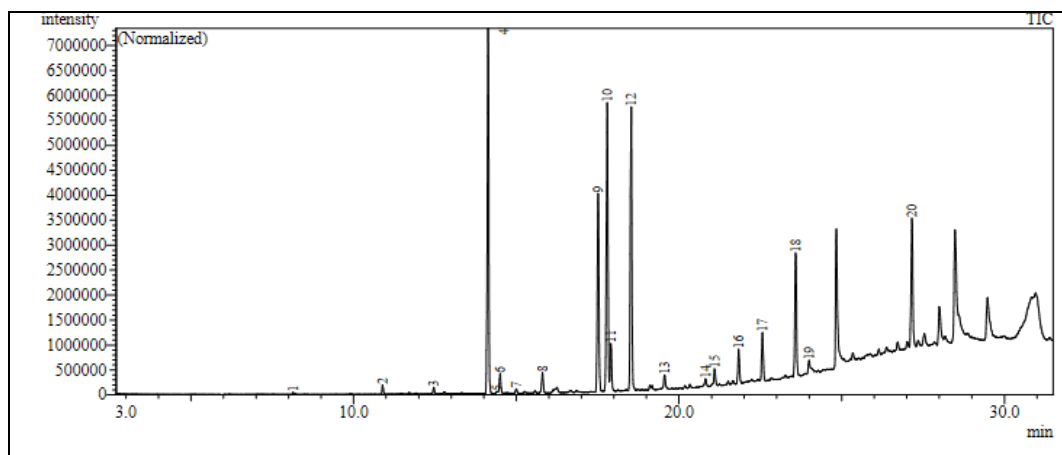


Fig 7: Chromatogram of fatty acid profiling of *L. vannaemii* during summer crop at the end of 120 DOC of ST4

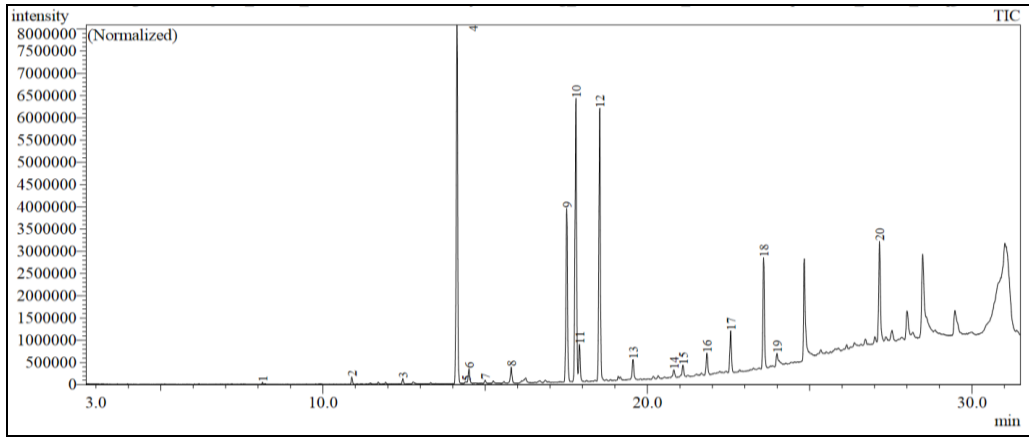


Fig 8: Chromatogram of fatty acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST5

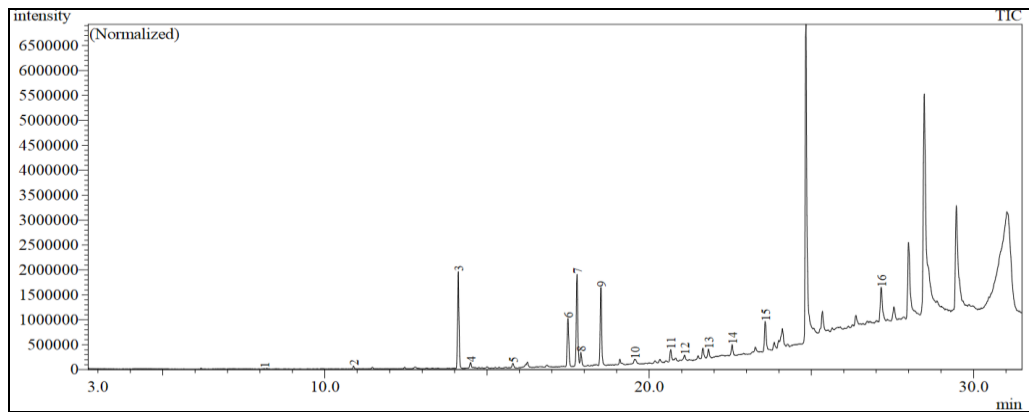


Fig 9: Chromatogram of fatty acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST6

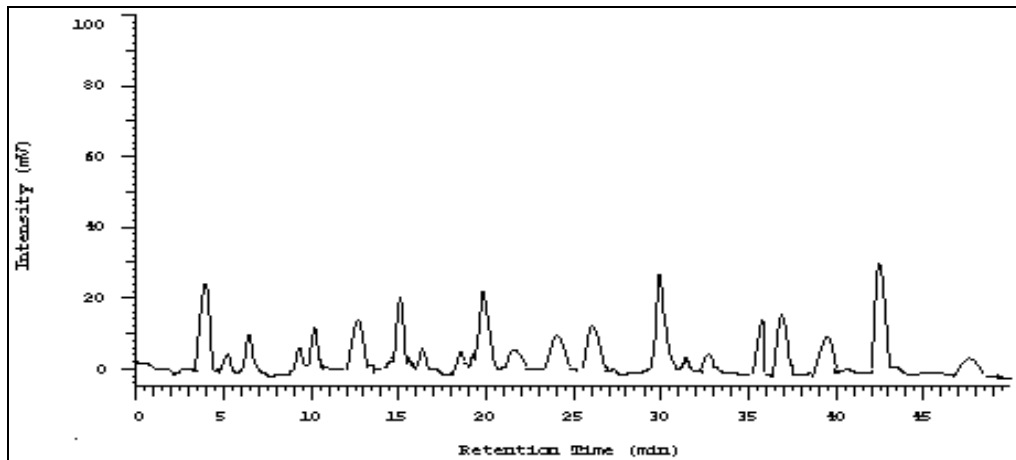


Fig 10: Chromatogram of amino acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST1

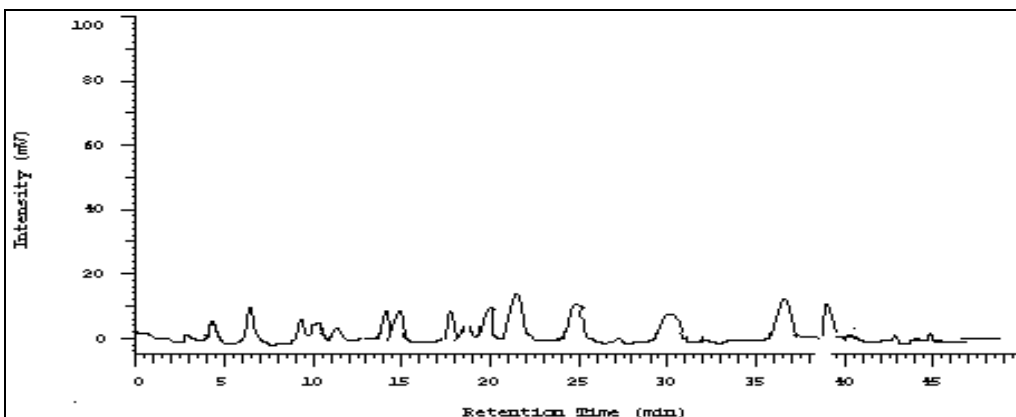


Fig 11: Chromatogram of amino acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST2

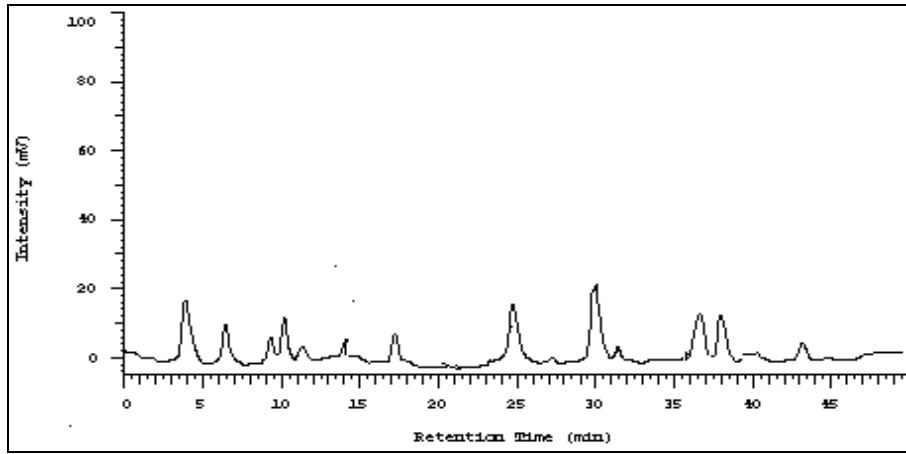


Fig 12: Chromatogram of amino acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST3

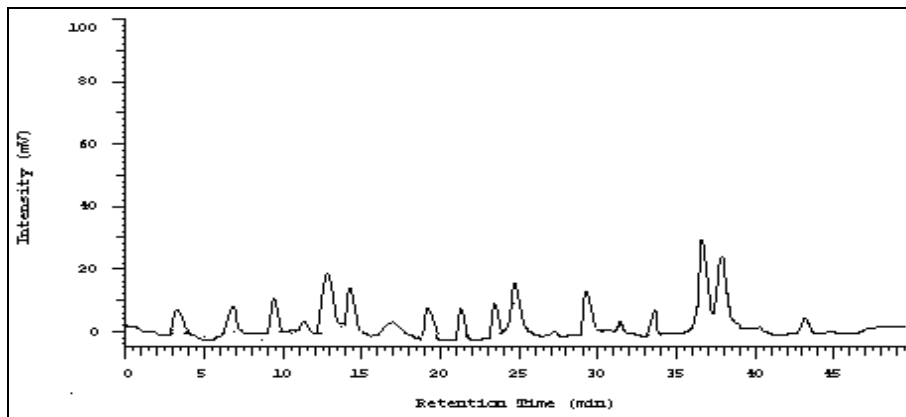


Fig 13: Chromatogram of amino acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST4

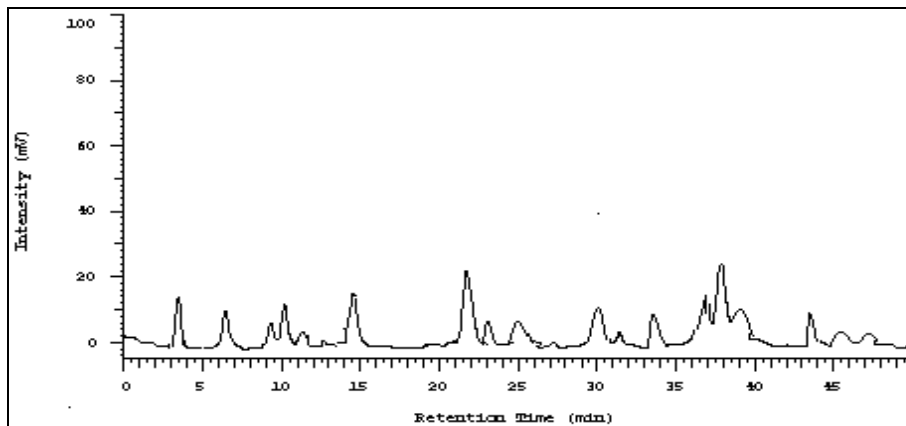


Fig 14: Chromatogram of amino acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST5

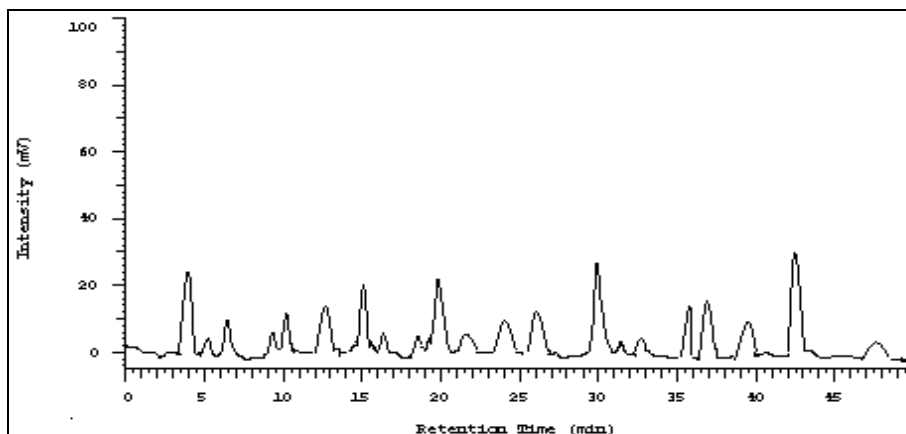


Fig 15: Chromatogram of amino acid profiling of *L. vannamei* during summer crop at the end of 120 DOC of ST6

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