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A Study on the Fatty Acid Enrichment of *Artemia franciscana* for the Healthy Rearing of *Penaeus vannamei* Post-larvae

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ABSTRACT

Background: The study aimed to investigate the growth performance of *Penaeus vannamei* PL through the supplementation of fatty acid enriched *Artemia franciscana.*

Methods: The animals were stocked at 3000PL/m^3 in triplicates. The experimental diet (100nauplii/PL/day) fed initially for 7days include *Artemia franciscana* (unenriched) as control and Fatty acid (0.5%, 1%, 2% and 3%) enriched *nauplii* as treatments (T1, T2, T3 and T4) and later switched to commercial diet for 18 days.

Result: No significant difference in water quality parameters between the experimental groups was observed. Highest weight gain percentage and survival was found in T3. Fatty acid profile of *Artemia franciscana* and shrimp at day 7 and 25 were analysed. Higher W-6 fatty acid accumulation was found in shrimp on day 7. Histological analysis of muscles, hepatopancreas and intestine showed different fatty acids levels reflected structural changes in epithelial cells. The current study revealed that *Artemia franciscana* enriched with 2% fatty acid emulsion can be used as an optimum level to improve the zootechnical performance of *P. Vannamei.*

Key words: *Artemia franciscana,* Epithelial cells, Fatty acids, Growth, *Penaeus vannamei.*

INTRODUCTION

The increased shrimp farming posed the demand for the production of post-larvae from hatcheries. The major challenge in shrimp larval rearing is the rearing of early larval stages as it will be highly depending on the live foods due to the incomplete development of digestive systems (Agh and Sorgeloos, 2005). These larvae have the tendency to prey upon live food organisms and encounter problems in accepting artificial diets (Kolkovski, 2001; Sargent *et al*. 1995). The use of artificial larval diet leads to the reduced growth rate, the lack of stability of culture water which leads to leaching, poor water quality and bacterial growth (Liao *et al*. 1988; Jones *et al*. 1989). Hence the supplementations of live feed like *Artemia* and Rotifer (*Brachionus plicatilis*) is important in shrimp culture. Typically, marine shrimp and fish larvae are fed with live feeds such as algae, zooplankton, Rotifers and *Artemia*. Due to various influencing factors, the fatty acid profile of rotifers and *Artemia* remains inadequate (Léger *et al*. 1987), particularly to the HUFA profile. Thus the practice of enrichment has been developed as a means of overcoming this nutritional deficiency. Also, artificial feed contains more than 90% of dry matter whereas *Artemia* nauplii have only about 10%, which helps in easy digestion. In practice, *Artemia* have been used as a live food during the mysis and post-larval development stages for more than 30 years due to its year-round availability (Leger *et al*. 1987). Nutritional compounds like essential amino acids (EAA) and unsaturated fattyacids play a major role in improving the growth and survival of crustaceans and larval finfish. EPA plays a major role in increasing the survival rate by maintaining the flexibility of biological membrane while DHA is essential for enhancing the growth performance and larval quality and helps in the development of tissues in the nervous system (Dhont and Sorgeloos, 2002; Sorgeloos *et al*. 2001). DHA/ EPA ratio greater than two is essential to promote growth performance, pigmentation and resistance to stress. A diet containing high levels of HUFA has improved the survival rate of post-larvae which were prone to salinity stress test (Kontara *et al*. 1997). Since, the concentration of DHA and EPA in *Artemia nauplii* is low (Suprayudi *et al*. 2004) (containing 0.33% EPA, while DHA was at an undetectable level) it can only be achieved through enrichment with a mixture of fatty acids like oleic acid, linoleic acid and linolenic acid and vitamin C. *Penaeus* spp. and *Macrobrachium rosenbergii* (Bengtson *et al*. 1991), *Farfantepenaeus paulensis*, (Martin *et al*. 2006) and *Penaeus monodon* (Immanuel *et al*. 2011) showed increased growth and survival on the supplementation of enriched *Artemia*. The course of *Artemia* feeding at different life stages

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of *P.vannamei* revealed the fact of supplementing enriched *Artemia* at the Post-larval stages, however the difference in nutrient absorption and retention by the shrimp larvae have not been studied much. The use of different enrichment products in the form of microparticulate products, self-emulsifying concentrates and in various emulsified preparations are widely used by the hatchery operators. However, the information on the supplementation of emulsified concentrates of enriched *Artemia* before and after the supplementation of non-enriched *Artemia* is scanty. Therefore, the ultimate aim of the study was to investigate the effect of growth performance on *Penaeus vannamei* through dietary supplementation of *Artemia nauplii* enriched with different concentration of fatty acids. The histological changes of shrimp juvenile tissues on the intake of enriched *Artemia sp.,* was also not reported much which could actually help in the understanding of the nutrient absorption and accumulation by the shrimp tissues.

MATERIALS AND METHODS

Site and experimental system

The study was performed in Advanced Research Farm Facility at Madhavaram, TNJFU, Chennai. *Penaeus vannamei* (PL_5) were purchased from the Aqua Nova Hatcheries. After five days of acclimatisation, the animals were stocked in aquarium tanks of 70 litres capacity in triplicates (3000PL/m^3). The experimental diet includes unenriched *Artemia franciscana* (Leach) *nauplii* as control (C), *Artemia franciscana* nauplii enriched with different concentrations of 0.5%, 1%, 2% and 3% fatty acid as treatments (T1, T2, T3 and T4).

Experimental diet

The experimental animals were supplemented four times with 100 *nauplii* / PL/ day for seven days. After seven days, the culture animals were fed with commercial diet for 18 days at 15% of total biomass. The proximate composition of the pelleted feed used in the experimental trial was mentioned in the Table 1.

Hatching and Enrichment of *Artemia franciscana* (Leach) *nauplii*

Artemia nauplii were enriched with fatty acids mixture (Oleic acid, linoleic acid and linolenic acid) and vitamin C (10%) as per the protocol of Akbary *et al*. (2011) with minor modification.

Newly hatched *Artemia* nauplii (200,000 nauplii/L) were divided in batches in 5 liter plexiglass tanks. 30g of Gelatin was dissolved in 800 ml of boiled deionized water as enrichment suspension. After cooling, 160 ml of fatty acid mixed with ascorbic acid (Vit-C) (16g) was added to this enrichment suspension. From this, 0.5 ml of this mixture was added for one liter of water at the onset of the enrichment period in *Artemia* culture. Another 0.5 ml/l of the enrichment diet was added 12 hours before harvesting and *Artemia franciscana* were harvested after 24 hours.

Table 1: Proximate composition of the commercial diets.

Nutrient	Composition (%)
Protein	39.1
Lipids	12.7
Ash	8.3
Fiber	1

Water quality parameters

Temperature and pH were determined on daily basis. According to APHA (2008) Alkalinity, Hardness, Salinity and dissolved oxygen were measured weekly. Nitrite-nitrogen and Ammonia-nitrogen were analysed using Resorcinol and Phenol hypochlorite method.

Growth performance

The growth performances for all the animals in the experimental groups were calculated based on the following equations:

Specific growth rate (SGR, % day^{-1}) =

$$[\ln W_t - \ln W_0] / t \times 100$$

FCR =

Feed offered (dried weight)/weight gain (wet weight)

$$\text{Survival rate (\%)} = (Nt \times 100) / N_0$$

$$\text{Weight gain (\%)} = (W_t - W_0 / W_0) \times 100$$

Where

W_0 and W_t are the initial weight (g) and final weight (g) of the experimental animals, N_0 and N_t are initial and final number of shrimp and t represents culture time in days.

Fatty acid profile

Lipid extraction from the experimental animals was done using Folch method (Folch *et al*.1957) with slight modifications. The extracted lipid was subjected for FAME preparation (AOAC, 1995) and analysed in GC-MS for fatty acid profile.

Histological study

In order to study the histological changes occurred, the shrimp post-larvae were collected from all the experimental tanks on 7^{th} and 25^{th} day. Samples of muscle, hepatopancreas and intestine were fixed in Davidsons' fixative (95% ethanol, acetic acid, formalin, de-ionized water) and passed through the process of dehydration, block making, sectioning, staining and microscopic observations. Slides were examined under a Phase contrast microscope (Model: Zeiss, Scope A1, Germany).

Statistical analysis

SGR, FCR, survival, weight gain percentage and water quality parameters were subjected to independent T test using SPSS (version 20.0) at 5% confidence level.

RESULTS AND DISCUSSION

The various physio chemical parameters analysed during the culture trial was shown in Table 2 along with the statistical

Table 2: Results of the physico chemical parameters of water in the culture tanks.

Parameters	Control	Treatment 1 (0.5%)	Treatment 2 (1%)	Treatment 3 (2%)	Treatment 4 (3%)
Temperature (°)	27±0.2[a]	27±0.3[a]	27±0.2[a]	27±0.4[a]	27±0.3[a]
	(26-29)	(26-29)	(26-29)	(26-29)	(26-29)
Salinity (ppt)	7±0.2[a]	7±0.1[a]	7±0.2[a]	7±0.1[a]	7±0.2[a]
pH	7.8±0.01[a]	7.8±0.04[a]	7.8±0.05[a]	7.8±0.02[a]	7.9±0.03[a]
	(7.6-7.9)	(7.6-7.9)	(7.6-7.9)	(7.6-7.9)	(7.6-7.9)
Alkalinity (ppm)	124±2.2[a]	127±5.1[a]	125±3.5[a]	129±4.4[a]	138±2.0[a]
	(80-140)	(112-149)	(112-149)	(112-149)	(112-149)
Dissolved Oxygen (ppm)	5.8±0.02[a]	6.2±0.04[a]	6.3±0.06[a]	6.1±0.02[a]	6.2±0.03[a]
	(5.2-6.3)	(5.2-6.3)	(5.2-6.3)	(5.2-6.3)	(5.2-6.3)
Ammonia (ppm)	0.24±0.01[a]	0.1±0.02[a]	0.1±0.04[a]	0.1±0.03[a]	0.28±0.02[a]
	(0.01-0.24)	(0.01-0.28)	(0.01-0.28)	(0.01-0.28)	(0.01-0.28)
Nitrite (ppm)	0.01±0.04[a]	0.01±0.02[a]	0.02±0.03[a]	0.01±0.02[a]	0.01±0.04[a]
	(0.01-0.2)	(0.01-0.4)	(0.01-0.4)	(0.01-0.4)	(0.01-0.4)
Hardness (ppm)	822±35[a]	874±28[a]	886±21[a]	851±24[a]	869±21[a]
	(650-820)	(723-890)	(723-990)	(723-890)	(723-899)

Table 3: Zoo-technical performance of the shrimp post larvae reared for 25 days in the experimental trial.

Parameters	Control	Treatment-1 (0.5%)	Treatment-2 (1%)	Treatment-3 (2%)	Treatment-4 (3%)
Initial weight (mg/individual)	8±2.2	8±2.3	8±2.1	8±2.3	8±2.2
Final weight (mg/individual)	98±16[c]	102±28[c]	104±25[b]	133±26[a]	112±19[b]
Survival	86.3±1.4[c]	90.6±1.9[b]	92.6±2.2[b]	96±1.5[a]	94.9±1.8[a]
Weight gain (%)	746.21±241.25[bc]	754±511.47[c]	759±233.21[c]	799.97±549.25[a]	772±431.25[b]
FCR	0.81±0.02[c]	0.75±0.02[b]	0.77±0.03[b]	0.66±0.02[a]	0.74±0.01[b]
SGR (%/day)	9.58±0.12[bc]	9.71±0.17[b]	9.76±0.21[b]	9.96±0.19[a]	9.81±0.08[b]

analysis. No significant difference was observed between the control and treatments for all the water quality parameters and were maintained at an optimal range throughout the experimental trial.

Zoo- technical performance of the shrimp post-larvae

No significant difference was observed in SGR for control, T1, T2 and T3 whereas control and T2 were found to be significantly different. Significant difference (P>0.05) in feed conversion ratio and survival rate were found between the experimental groups. Highest weight gain percentage was observed in T3. The growth performance of shrimp post-larvae was shown in Table 3. Shrimp post-larvae fed with enriched *Artemia* exhibited enhanced growth and survival than control. Similar reports were observed by Zelaya *et al*. (2007) who reported an improved growth performance in the diets of *L. vannamei* supplemented with enriched fatty acids along with the commercial feed. This may be due to the increased fatty acid metabolism of the shrimp post-larvae leading to the faster growth rate (Hurtado *et al*. 2007; Martins *et al*. 2006; Palacios *et al*. 2004b). The dietary requirements of essential fatty acids differ between penaeid species. The requirement of n-3 PUFA in *L. vannamei, P. Monodon* and post-larval penaeid were found to be 0.5%, 0.5-1% and 1% (Gonzalez-Felix *et al*. 2002; Chen and Tsai, 1986; Kanazawa *et al*. 1979a). The lower growth rate in T4 compared to T3 may be due to the higher levels of dietary n-3 HUFA as reported in the studies of Rees *et al*. (1994). The fattyacid supplementation >2% in the diet can lead to the declined growth performance and survival in *L. vannamei* (Glencross and Smith, 2001). The increase in body weight and survival in T3 showed the better result in terms of feed conversion ratio and weight gain.

Fatty acid composition of *Artemia franciscana*

The fattyacid profile of the experimental diet reveals that higher levels of EPA (4.7%) were found in T4 than T3 (4.3%). The linoleic (18:2n-6) and linolenic acid (18:3n-3) levels in treatments were higher when compared to control. The fatty acid enriched with *Artemia* nauplii affects the ∑n-3 HUFA levels ranging from 26.78% to 31.86% in treatments whereas in the control it was around <19.11% (Table 4). The total ether extract content of the experimental group ranges from 6.86% to 9.94%. The EPA/DHA ratio ranged from 12 to 28.66 in treatment groups. The recommended levels of EPA, DHA and other essential fatty acids were recorded in T1 and T2 as reported by Merican and Shim, 1997 in *L. vannamei* and Kanazawa *et al*. 1979b; Xu *et al*. 1993 in *Marsupenaeus japonicas* and *Fraxinus chinensis.* In the current study, the accumulation of saturated fatty acids was almost similar

between the treatments. The fattyacid profile of the enriched and unenriched *Artemia* nauplii revealed the absorption of fatty acid emulsions at different concentrations and absorption rate of nutrients were found in the inclined trend for the increased concentrations.

Fattyacid composition of shrimp post-larvae on 7th and 25th day sampling

On 7th day, animals fed with enriched diet showed higher w-6 fatty acid accumulation in tissues and ∑n-3 HUFA ranged from 21.3 % to 25.07% whereas EPA/DHA ratios ranged from 1.297% to 2.612% (Table 5). On 25th day, w-6 fattyacid accumulation in tissues were same in T2 and T3 with ∑n-3 HUFA ranging from 14.38 % to 20.54% and EPA /DHA ratios ranging from 1.297% to 2.612% (Table 6). However due to the inherit capability of *Artemia sp.,* in DHA catabolism, EPA/DHA concentration was declining in the increased fatty acid concentration in the post-larvae on 7thday. The fatty acid composition of the shrimp larvae on 7th day was found higher at the increased concentrations which indicate the characteristic absorption of post-larvae. After a course of 7 days feeding of enriched *Artemia*, the shrimp post-larvae were fed with commercial diet for 18 days. Interestingly, the fatty acid composition of the juveniles was higher up to 2% and absorption capacity of the shrimp juveniles were found lesser at 3% enrichment at the end of the trial. The retarding growth of the juveniles was found in T1 and T2 which might be due to the lack of adequate essential nutrients. The decline in growth rate of T4 compared to T3 might be due to the deposition of excessive fatty acid supplement leading to inhibition effect on the mRNA of fatty acid synthase (FAS) (Chen *et al*. 2015). The findings of the present study revealed that *P. vannamei* when fed with an excess HUFA do not enhance the growth of the animals and the utilization of short chain fatty acids were pronounced in all treatments in case of their deficiency, the animal prefer to use long chain fatty acids. Similar results were also found in the studies of

Table 4: Fatty acid composition of enriched and unenriched *Artemia franciscana* used in the culture trial.

Fatty acid composition (%)	C	T1 (0.5%)	T2 (1%)	T3 (2%)	T4 (3%)
Ether extract	6.86	9.22	9.41	9.63	9.94
Myristic acid (C14:0)	2.94	1.08	1.13	1.25	1.19
Palmitic acid (C16:0)	22.15	18.64	20.96	17.99	21.58
Stearic acid (C18:0)	8.7	7.82	7.75	6.97	6.99
Oleic acid (C18:1(n-9))	25.48	30.62	29.14	32.04	30.09
Linoleic acid (C18:2(n-6))	5.76	5.99	6.72	7.87	6.94
Linolenic acid (C18:3(n-3))	18.81	25.48	26.91	26.13	26.95
Arachidic acid (C20:0)	0.5	1.7	1.7	1.8	1.9
Behenic acid (C22:0)	0.3	0.4	0.5	0.6	0.4
Eicosapentaenoic acid (20:5n-3)	0.2	1.2	2.3	4.3	4.7
Docosahexaenoic acid (22:6n-3)	<0.1	0.1	0.1	0.15	0.2
Palmitoleic acid (C22:6n-3)	5.2	3.07	3.45	4.68	4.02
Others	4.35	5.06	4.85	4.97	4.56
∑n-3 HUFA	<19.11	26.78	29.31	30.93	31.86
EPA/DHA	<2	12	23	28.66	23.5

Table 5: 7th day sampling of shrimp post-larvae.

Fatty acids composition (%)	C	T1 (0.5%)	T2 (1%)	T3 (2%)	T4 (3%)
Ether extract	7.56	6.37	7.49	5.27	6.87
Myristic acid (C14:0)	2.83	1.83	2.16	1.23	2.06
Palmitic acid (C16:0)	23.12	23.37	21.98	19.65	23.45
Stearic acid (C18:0)	8.91	10.76	9.94	13.17	10.39
Oleic acid (C18:1(n-9))	19.15	18.43	19.32	18.03	19.56
Linoleic acid (C18:2(n-6))	7.6	7.53	7.43	11.17	8.58
Linolenic acid (C18:3(n-3))	8.7	6.93	8.6	2.76	6.62
Arachidic acid (C20:0)	0.65	0.65	0.69	0.94	0.68
Behenic acid (C22:0)	6.91	7.1	7.42	6.47	6.17
Eicosapentaenoic acid (20:5n-3)	12.6	10.16	10.54	9.38	10.18
Docosahexaenoic acid (22:6n-3)	9.71	4.67	4.71	3.59	4.5
Palmitoleic acid (C22:6n-3)	7.39	6.41	6.47	2.14	5.48
Others	1.69	2.09	0.69	2.06	2.27
∑n-3 HUFA	21.67	21.76	23.85	25.07	21.3
EPA/DHA	1.29	2.17	2.23	2.61	2.26

Table 6: 25th day sampling of shrimp post-larvae.

Fatty acids composition (%)	C	T1 (0.5%)	T2 (1%)	T3 (2%)	T4 (3%)
Ether extract	5.54	5.13	5.33	4.82	4.48
Myristic acid (C14:0)	1.51	1.81	2.39	1.13	1.35
Palmitic acid (C16:0)	24.57	24.29	34.55	22.95	23.05
Stearic acid (C18:0)	10.8	11.42	10.65	11.46	11.73
Oleic acid (C18:1(n-9))	20.21	18.65	19.09	21.19	19.2
Linoleic acid (C18:2(n-6))	12.06	12.39	12.53	12.53	12.22
Linolenic acid (C18:3(n-3))	1.63	1.01	1.45	2.72	1.76
Arachidic acid (C20:0)	0.93	0.86	0.7	0.87	0.82
Behenic acid (C22:0)	5.12	5.41	5.49	4.75	5.83
Eicosapentaenoic acid (20:5n-3)	6.84	9.82	9.95	11.47	11.54
Docosahexaenoic acid (22:6n-3)	5.91	5.7	5.54	6.35	6.68
Palmitoleic acid (C22:6n-3)	4.92	4.64	5.12	3.68	3.97
Others	2.01	2.27	2.49	2.16	2.57
∑n-3 HUFA	14.38	16.53	16.94	20.54	19.98
EPA/DHA	1.15	1.72	1.79	1.80	1.72

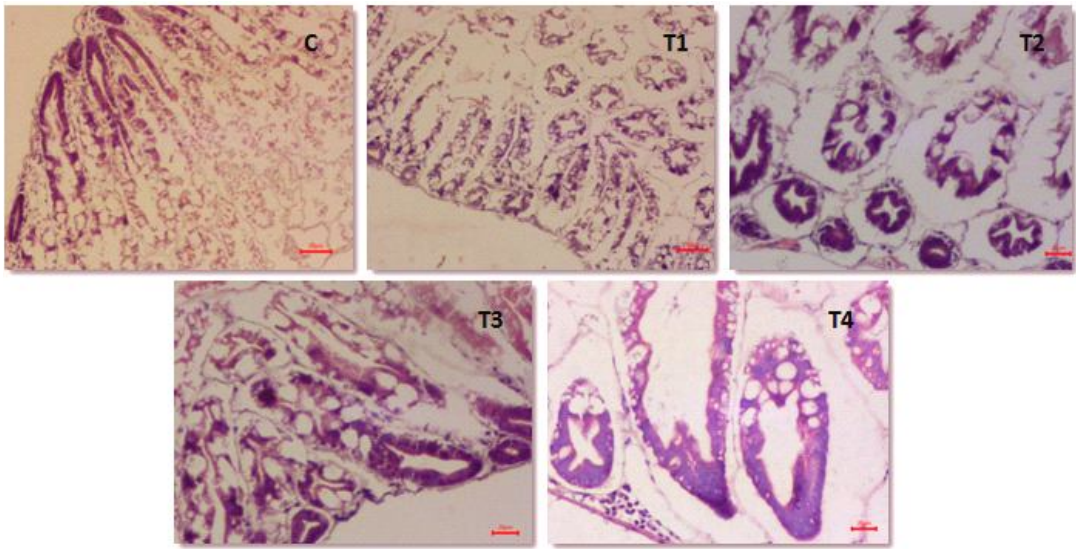

Fig 1: Histological changes of hepatopancreas of *P. vannamei.*

Deering *et al*. 1997; Palacios *et al*. 2004 who reported *P.monodon* used shorter-chain fatty acids for their metabolism and were able to retain long-chain unsaturated fatty acids.

Histoarchitecture of the Hepatopancreas of *P. vannamei*

The numbers of hepatopancreas Basal cells (B cells) were higher in T4 than T3 but the size of the cells was normal. The normal architecture was observed with differential cells at each concentration. The interstitial sinuses between tubules were normal and in T4 well-organized glandular tubular structure were observed (Fig 1).

Histoarchitecture of the Intestine of *P. vannamei*

In the control group the epithelial cells and cellular structure of villi are columnar in shape, showing normal intestinal crypts, villi and laminar propria. The mean height of epithelial cells in abdominal segment was higher in T1, T3 and T4 than control. The shrimp mid-gut showed mild degeneration of intestinal epithelial cells (Fig 2).

Histoarchitecture of muscle

In T4, the muscle fibres displayed a variety of morphological changes of progressive myofibrillar degeneration and necroitic myopathy, due to deposition of heavy fats on the tissue (Fig 3).

No attempts were made till now to describe the histological changes of different tissues such as hepatopancreas, muscle and intestine of *P. vannamei* reared in fatty acid enriched *artemia* diet. The structure of hepatopancreas was normal in control however the number of B cells and R cells were significantly less in control. T1, T2, T3 and T4 groups tend to show the improved cellular architecture compared to control as the number of reserve cells (R- cells) was much higher. Similarly, in the present study it was found that different concentrations of fatty acids,

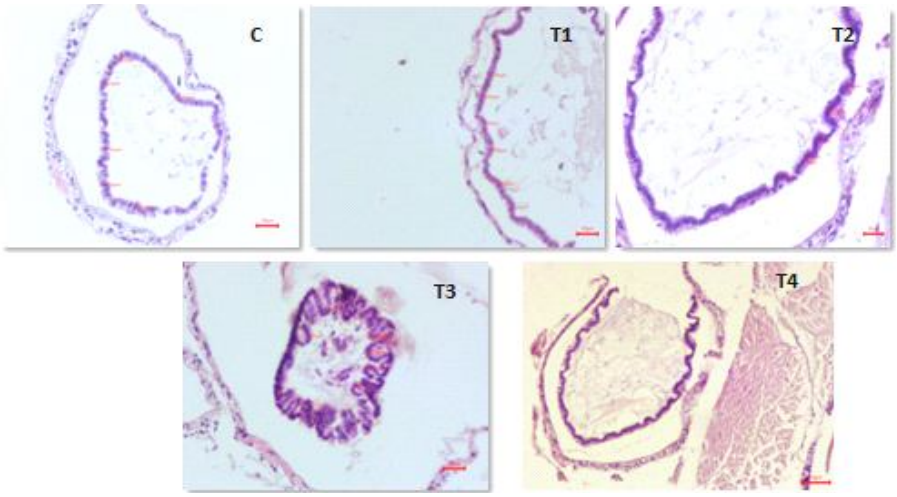

Fig 2: Histological changes of intestine of *P. vannamei.*

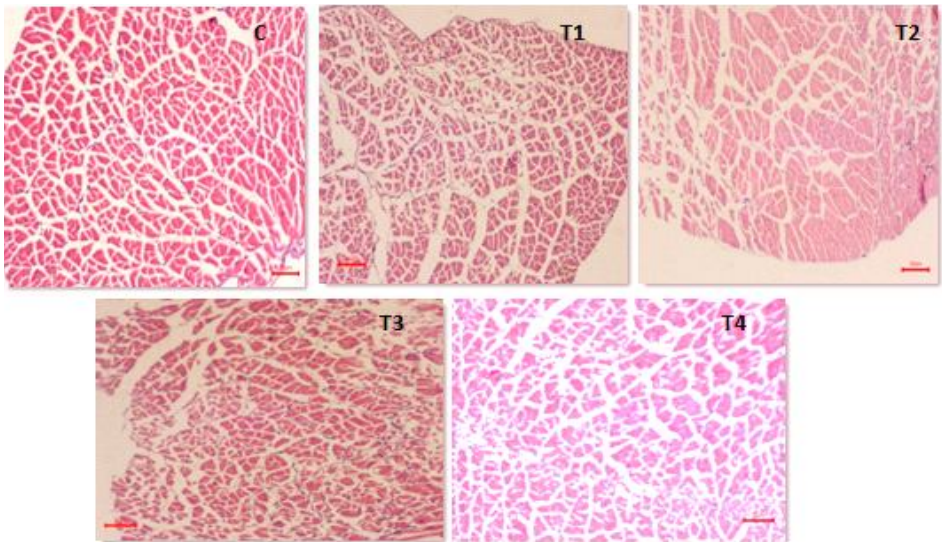

Fig 3: Histological changes of muscle of *P. vannamei.*

reflected alterations in structure of epithelial cells and tubules of hepatopancreas. The number of R-cells was found in increased numbers in treatments than control which might be due to the high energy demand for osmoregulation which utilizes reserved nutrients from R-cells (Li *et al*. 2008). This suggested that 3% fatty acid enriched diet has impact on the physiology in turn on the histological alterations in shrimp. The number of hepatopancreatic B-cells significantly increased in T2 and T3 compared to T4 with respect to the increased levels of EPA and DHA. Histological study of intestine is an important factor to be analysed as different morphological structure can be correlated with physiological functions such as digestion, assimilation and adaptation to varying salinity (Yamamoto and Hiraro, 1978; Cataldi *et al*. 1998). The digestive tract of crustacean arises from an ectodermal stomodeum, an endodermal mesenteron and an ectodermal proctodeum (Vonk, 1960; Shiino, 1968; Johnson, 1980). Similarly, in the current experiment, alterations were observed in the height of epithelial cells. Those shrimps supplemented with fatty acid enriched diet showed best recovery in the structure of intestinal epithelial cells especially in T3 group which might be due to the persistence of higher levels of essential HUFA and EPA/ DHA. Prominent changes in the muscle tissues underlying the exoskeleton were not seen between treatment and control fishes.

Therefore, from the histological findings it is suggested that the aquatic environment with 1%, 2% and 3% fatty acid enriched *Artemia* feeding make tremendous changes in the organizational structure of hepatopancreas and intestine. Though the number of reserve cells and basal cells are high in 3% fatty acid (T4) enriched hepatopancreas tissue, the occurrence of myofibrillar degeneration in the muscles of the animals suggests the restriction of percentage enrichment up to 2%. Histologically, it is proved to enrich the *Artemia franciscana* up to 2% of fatty acid for rearing the healthy shrimp post-larvae. Further, the study indicated that the enrichment of 2% fatty acid to *Artemia* is

highly beneficial to maintain the regular structure of hepatopancreas, muscle and intestine of shrimp post-larvae, which were also concurrent with the declined growth rate in the T4 restricting the growth performance.

CONCLUSION

P. vannamei post-larvae fed with optimum levels of PUFAs achieved enhanced growth performance. Better growth performance was obtained in the shrimp post-larvae fed with 2% fatty acid emulsion and found to have a beneficial effect in the growth and survival. The study also recommends the fatty acid enriched diet for the healthy rearing of post-larvae in the commercial hatcheries to improve the growth and survival. As this study was carried out in 7ppt salinity, this paves the way to apply the research findings of this study in low saline shrimp post- larvae hatchery production.

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