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ROLE OF HUFA ENRICHED *ARTEMIA* ON MOULTING FREQUENCY AND HUFA ENRICHMENT *MACROBRACHIUM ROSENBERGII* (DE MAN)

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ABSTRACT
A 30 day experiment was conducted to investigate the effects of enrichment on fatty acid composition of adult *Artemia* through n-3 HUFA emulsions containing EPA and DHA and to study the impact of n-3 HUFA enriched Adult *Artemia* on fatty acid composition of giant freshwater prawn *Macrobrachium rosenbergii*. The prepared emulsions were found efficient in enriched adult *Artemia* by increasing to level of EPA and DHA up to a maximum of 5.69 and 3.78% respectively in 2h enrichment. The fatty acids profiles of enriched adult *Artemia* fed *M. rosenbergii* sub adults (T2) have an enhanced total value of EPA and DHA 3.98 and 3.24% respectively. *M. rosenbergii* sub adults fed with enriched adult *Artemia* showed higher moulting cycle (15.17 ± 1.5) and Moulting rate/ animal/quarter (6 ± 0.2). The present study indicates that, the nutritional quality of *Artemia* in relation to Fatty acids can also be increased by enrichment which can influence to moulting cycle and fatty acid profile of freshwater prawn.

INTRODUCTION

India is blessed with 5.4 million ha of freshwater resources including reservoirs, tanks and ponds and 1,71,334 km of rivers and canals, suitable for giant freshwater prawn culture (Bojan and Viswakumar, 2003). The prawns of the genus *Macrobrachium* are considered to be in an active process of intrusion into freshwater from their marine origin (Jalihal *et al*., 1993). Therefore, like any marine decopods, *Macrobrachium spp* may also show an inclination towards n-3 HUFA for better performance and production. Direct feeding of n-3 HUFA might not be feasible for *M. Rosenbergii* as the prawn being an omnivore, preferring solid feeds due to morphological features of its mouth parts and feeding behaviour. *Artemia* spp are a live-food and considered to be a preferred feed for *Macrobrachium spp* at all stages. Adult *Artemia* can also be enriched in the same way as nauplii, but due to higher filteration efficiency (Leger *et al*., 1986 and Dhont *et al*., 1993). The use of adult *Artemia* as feed for the sub-adult giant freshwater prawn is also possible due to bigger size and palability. This fact could vouch for the use of adult *Artemia* for enrichment (Dhont and Lavens, 1996). Crustaceans, the rate of growth is a function of both the frequency with which they moult and the size increment per moult (Wickins, 1982 and Kibria, 1985). Moulting cycle of ecdysis the prawn is inactive and does not feeds. It therefore has to utilize the reserve, particularly the lipids stored during the prior inter-molt stage. The lipid content will increase at mid pre-molt. As there is a scarcity of information in the literature regarding these important biological aspects in the largest Giant fresh water prawn (*M. rosenbergii*), the present study was undertaken with the objectives of investigating the moulting frequency and fatty acids of sub-adults *M. rosenbergii* with emphasis on the influences of diets.

MATERIALS AND METHODS

Culture and enrichment of adult *Artemia*

Four round FRP tanks (10 l capacity) were arranged for culture of *Artemia* with the stocking density of 5000 nauplii/l (Dhont *et al*., 1993). Requirement of *Artemia* was calculated as per the wet weight of *Artemia*. Adult *Artemia* was kept in 15 l capacity FRP cones containing 3 l of water and enriched emulsion was added (Table. 1) at the rate of 0.6 g/l of water prescribed by(Dhont *et al*., 1993 and Leger *et al*., 1986 . The enrichment was conducted in emulsions containing n-3 HUFA for a time period of 2 h with aeration. After enrichment the adult *Artemia* kept under refrigeration condition.

Experimental animals

M. rosenbergii sub-adults with mean body weight of 5.0783 $\pm$ 0.74 g were collected and sorted out into 3 sub-groups under the experimental code C, T_1 and T_2 for maintaining triplicates, each with 8 uniform sized animals.

The feeding was done for 30 days under confined condition with n-3 HUFA enriched *Artemia*, non-enriched *Artemia* and a broodstock maturation diet. At the end of 30 days one animal from each group was taken randomly and sacrificed for gonad observation as well as lipid extraction for fatty acid analysis. Sub-adults were fed adult *Artemia* at the rate of 8% per kg body weight.

Analysis of performance

Fatty acid analysis

Total lipids were extracted from *Artemia* as well as from the muscle of *M.rosenbergii* sub-adults following the standard methods (Floch *et al*., 1957). The AOAC (1995) was followed to esterify the lipid extract. Fatty acids analysis was done in Gas chromatography.

Handling of experimental data

The following formulae (modified after Kibria,1985) were applied to analyze data relevant to moulting, moulting frequency and growth rate.

Moulting rate/ animal / quarter: $M_r = M/N \times 3$

where M = Number of molts and N = number of living prawns.

Moult /animal/ quarter M_q = M/q,

where q = Quarter (90days)

Moult cycle: $M_c = N/M_d$,

where N = average number of living prawns and M_d = moult per day.

Statistical analysis

The mean and standard error were estimated for all the parameters in each enrichment and treatment group. The results were subjected to one- way analysis of variance (ANOVA).

RESULTS

Enrichment of *Artemia*

Table 1:

Sl. No.	Fatty acids	Source oil (Mega-3 oil)
1	Butyric(C4:0)	0.00
2	Hexanoic(C6:0)	0.2
3	Tridecanoic(C13:0)	0.09
4	Myristic(C14:0)	0.00
5	Myristdeic(C14:1)	0.1
6	Pentadeconic(C15:0)	0.08
7	Palmitic(C16:0)	1.26
8	Palmitdeic(C16:1)	0.05
9	Heptadecanoic(C17:0)	0.35
10	Oleic (C18:0)	11.6
11	Linoleic(C18:2)	2.22
12	Linolenic(C18:3)	8.56
13	Arachidic(C20:0)	2.80
14	Arachidonic(C20:4)	2.20
15	EPA(C20:5)	38.00
16	Behenic(C22:0)	0.56
17	Decosadienic(C22:2)	2.32
18	DHA(C22:6)	26.00
19	Lignoceric(C24:0)	0.34
20	Nervonic(C24:1)	0.06

The fatty acids profile (% of different fatty acids) of source (Mega-3) of n-3 HUFA used for enrichment process is presented in Table.1.

The EPA (20:5n-3) content in the cultured adult *Artemia* was 0.64% and DHA content in the cultured adult *Artemia* was zero before the commencement of enrichment process. The EPA and DHA content after 1, 2, 3 and 4h of enrichment in the adult *Artemia* was observed. The observed values of EPA and DHA were further processed and presented in Fig.1.

Fatty acids profiles of *M. rosenbergii*sub-adults after fed with 3 different diets.

The EPA and DHA contents in is *M.rosenbergii* sub-adults were zero at the start of the experiment and the level rose to 2.00, 2.56 and 3.98%, respectively for C, T1 and T2 groups for EPA and 0.18, 1.74 and 3.24%, respectively for C, T1 and T2 groups for DHA as presented in Table.2.

Influence of enriched and unenriched adult *Artemia*on the moulting cycle of *M. rosenbergii* sub-adults

M.rosenbergii sub-adults when fed with three different diets under C, T1 and T2 groups, showed remarkable differences in their moulting cycle as presented in Table. 2.

DISCUSSION

Enrichment of *Artemia*

Production of *Artemia* is much simpler than culturing other organisms and therefore, it is preferred over other live food organisms (Leger and Sorgeloos, 1992). Sargent *et al*. (1999) reported that crustaceans cannot synthesise n-3 HUFA by their own and therefore the fatty acids need to be supplemented. Nevertheless, their accumulation and retention in *Artemia* are found to vary based on the duration of enrichment.

The higher level of EPA and DHA contents (5.69 and 3.78%) were found to be reached in 2h enriched adult *Artemia*. However, DHA content was decreased from 3.58 to 1.53% when the enrichment period was increased to 3h. Evjemo *et al*. (1997) observed that *Artemia* rapidly metabolized DHA for energy production and therefore their level could be reduced during enrichment. This was further certified by loss of DHA in *Artemia* during enrichment period due to metabolic retroconversion. This could be the reason for the decrease in the n-3 HUFA content in *Artemia* beyond 2h of enrichment in this study. This is in accordance with Naessens *et al*. (1997) who had stated that enrichment of adult *Artemia* was generally carried out for 2h. But incompatible to that of Legar *et al*. (1986) and Dhont *et al*. (1993). Miguel *et al*., 2015 observed that no significance difference between initial hour to 6 hours enrichment of AMP Na/Ca as a vitamin source.

Fatty acid profile and moulting studies of *M. rosenbergii* fed with enriched adult *Artemia*

There is an established relationship between the moulting cycle and the nutrition. However, only scanty reports are available onthe influence of n-3 HUFA on the moulting cycles and its rhythum. In the present study, the fatty acids profile of *M. rosenbergii* fed with n-3 HUFA enriched *Artemia* exhibited the presence of higher content of n-3 HUFA (Table.2) and there was clear difference in the moulting cycle, moulting rate and moult per quarter of the *M. rosenbergii* fed with different

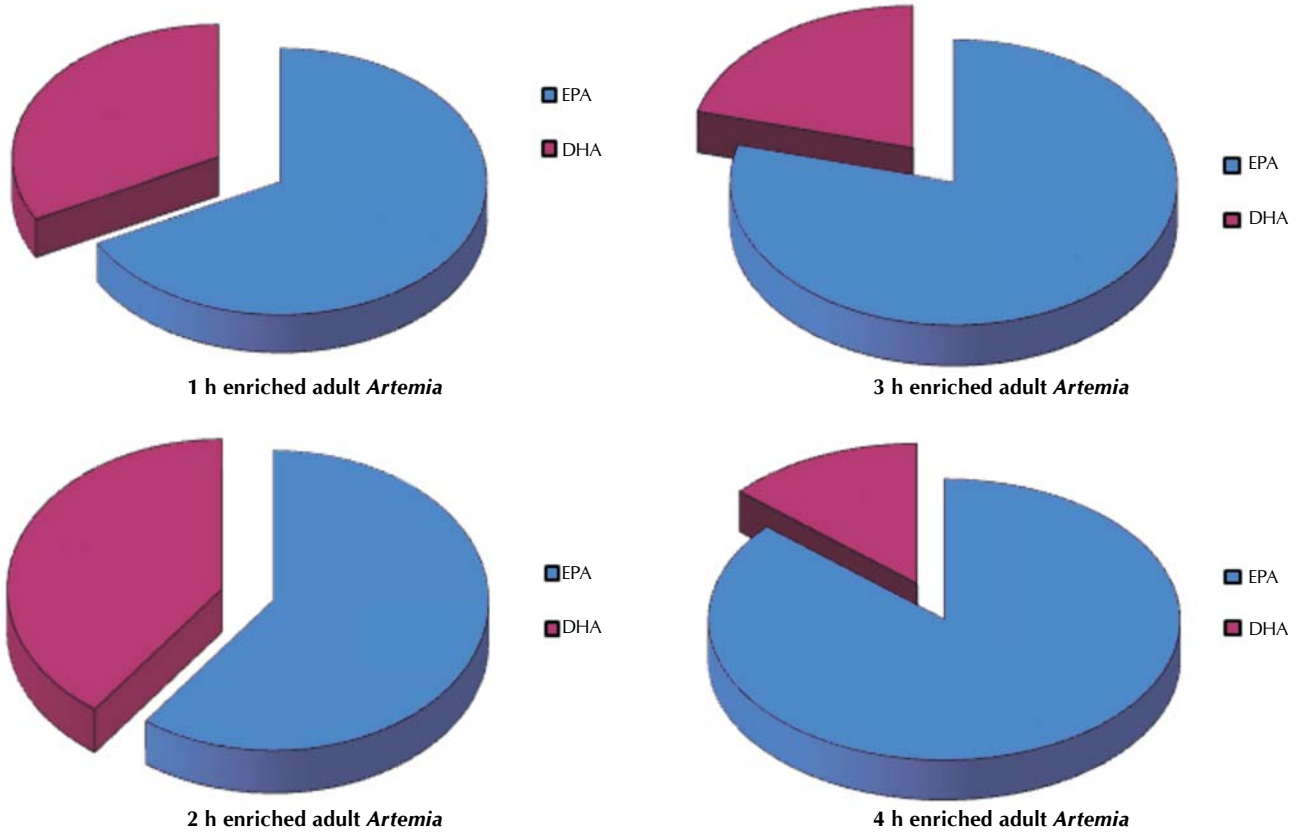

Figure 1: Percentage of EPA and DHA(in EPA + DHA) in enriched adult *Artemia* after different hours of enrichment

Table 2: Fatty acids profiles of *M.rosenbergii* sub-adults after fed with 3 different feeds

Sl. No.	Fatty acids	Initial (%)	Control(%)	Treatment1(%)	Treatment2(%)
1	Butyric(C4:0)	0.09	0.45	0.38	0.58
2	Hexanoic(C6:0)	1.57	0.37	0.38	0.71
3	Tridecanoic(C13:0)	0.19	0.33	2.56	2.63
4	Myristic(C14:0)	0.31	8.35	0.83	8.55
5	Myristdeic(C14:1)	1.98	2.95	2.74	2.44
6	Pentadeconic(C15:0)	0.48	0.47	0.39	1.33
7	Palmitic(C16:0)	21.41	47.02	41.38	39.45
8	Palmitdeic(C16:1)	6.75	7.02	0.01	0.00
9	Heptadecanoic(C17:0)	0.24	0.00	0.00	1.48
10	Oleic (C18:0)	0.14	0.34	0.58	0.86
11	Linoleic(C18:2)	0.45	0.86	14.39	18.69
12	Linolenic(C18:3)	0.18	1.34	1.77	1.36
13	Arachidic(C20:0)	0.41	0.00	0.00	0.45
14	Arachidonic(C20:4)	0.21	0.05	0.00	0.04
15	EPA(C20:5)	0.00	2.00	2.56	3.98
16	Behenic(C22:0)	0.28	0.06	0.00	0.07
17	Decosadienic(C22:2)	2.79	3.71	1.11	0.05
18	DHA(C22:6)	0.00	0.18	1.74	3.24
19	Lignoceric(C24:0)	0.28	0.04	0.58	0.64
20	Nervonic(C24:1)	0.42	0.12	0.08	0.12

diets with varied level of n-3 HUFA content. In the present study, compared to initial fatty acid contents of *M. rosenbergii* sub-adults, the status of n-3 HUFA at the end of the moulting experiment in enrichment in enriched *Artemia* fed group (T2) was higher (Table.3), which confirmed that the fatty acids were transferred from enriched *Artemia* To the targeted *M. rosenbergii* sub-adults**.** Similar method was observed by Anantharaj (2007) for *M. rosenbergii* post larvae. According to Xu *et al.*,(1993), penaeus chinensis when fed with the diet containing 1% 22:6 n-3 exhibited better survival rate, moulting frequency and weight gain among the groups fed the other diets. Manosathiyadevan and Selvisabhanayakam, (2011),

Table 3: Moulting cycle of *Macrobrachium rosenbergii* after fed with 3 different feeds

Treatment	Moulting rate(Mr)	Moulting per day (Md)	Moulting cycle (Mc)
C	1.25 ± 0.2	0.166 ± 0.02	24.72 ± 4.1
T_1	1.33 ± 0.3	0.177 ± 0.04	23.77 ± 5.2
T_2	2 ± 0.2	0.266 ± 0.02	15.17 ± 1.5

stated that pollution can alter the glycogen content in body tissue of *M.rosenbergii*. A marked variation in the moulting cycle of the fresh water prawn, *Palaemon paucidens* was reported by Teshima and Kanazawa, (1983), When the concentration and fatty acids composition of some lipid classes were altered in the diet. The total of EPA and DHA contents of *M. rosenbergii* sub-adult in C,T1 and T2 groups were 2.18, 4.3 and 7.22% respectively. Lena mahalingam *et al*., 2009 was reported that the total lipid content was found to be more in the hepatopancreas when compared to muscles and gills in both juveniles and three groups of sub adults. Fatty acid composition of the animal body tissue, mainly n-3 HUFA'P, is correlated with their susceptibility to various diseases i.e. immunity, and ability to tolerate the unfavorable environmental factors. If their ability to synthesize those fatty acids is lacking and/or very poor, providing those fatty acids exogenously (Watanabe *et al*. 1974; Pillai *et al*. 2003) will minimize the problem. The higher moulting rate (2 ± 0.2) of *M. rosenbergii* fed with T2 diet indicated that the n-3 HUFA content in their food influenced the moulting, which is also statistically significant ($P > 0.01$). the lesser intermoult period (15.17 ± 1.5 days) in t2 could substantiate the frequency of moulting when enriched *Artemia* was given as feed. From the above study, it can be concluded that more research is needed or moulting, in particular with sub-adult *M.rosenbergii* in order to obtain higher growth and to lower the mortality rate that is associate with moulting. The consum er is also at the receiving end of the benefits that come along with n-3 HUFA accumulation in the body tissue of *M.rosenbergii*.

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