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Study on the Effects of Fertilization on the Fatty Acid Profile of *Artemia* Cysts

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Abstract: A study was undertaken to find out the effect of fertlization on the fatty acid profile of *Artemia* cysts (GSL strain, Utah, USA origin) produced from the modified traditional solar salt works of Bangladesh during winter months (January-March) through different fertilization treatments (T_1,T_2,T_3). Application of fertilizer for T_1 was 50 kg urea + 20 kg TSP/ha, T_2 was 500 kg dried and powdered chicken manure/ha and T_3 was 1000 kg dried and powdered chicken manure/ha with dress up weekly/bi-weekly fertilization in all the cases. Palmitic, Linolenic, Eicosapantaenonic and Docohexaenoic acids (mg/g.DW) were found highest for the cysts in T_1 (16.0±1.36%), T_2 (14.7±0.47%), T_2 (4.7±0.40%) and T_2 (0.7±0.06%) treatments, respectively. High amount of 18:3(n-3) acids in the cysts of all sources proves to be freshwater type of the cysts. The presence of marine type essential fatty acids in the cysts of all sources were found low for 20:5n-3 (3.7-4.7%) and very low for 22:6n-3 (0.09-0.7%). No significant variation was observed for 16:0 acids within the treatments, but for 18:3(n-3) acid, the variation was found highly significant (P= 0.0052) between T_2 and T_3. For 20:5(n-3), only variation between T_2 and T_3 was found insignificant (P=0.1161), but between other treatments, significant variation was observed between T_2 and T_3 (P=0.0241), T_2 and T_3 (P=0.0022) and T_1 and T_3 (P=0.0161).

Key words: Fertilization, unsaturated, fatty acid, *Artemia*, cysts

Introduction

A detailed biochemical and commercial analysis of *Artemia* from the different sources (olney *et al.*, 1980; Schauer *et al.*, 1980; Seidal *et al.*, 1980, 1982; Soejima *et al.*, 1980; Leger *et al.*, 1985) revealed only one factor, namely the presence of highly unsaturated fatty acids (HUFA) in *Artemia* that unambiguously related to the feed intake of the animals during their grow up (Leger *et al.*, 1985, 1986a,b). Watanabe *et al.* (1978a, 1980) and Leger *et al.* (1986a) found that the content of 20:5w3 in *Artemia* appears to very not only from strain to strain but also within same strain and its presence is considered as the principal factor for the food value of *Artemia* (Watanabe *et al.* 1978b) as low levels of the HUFA 20:5w3 in *Artemia* results in low survival and poor growth in all marine fish and crustacean larvae. It has been shown that food condition greatly determines the fatty acid profile of *Artemia* offspring. Though HUFA levels of *Artemia* cysts produced in lakes and large ponds (i.e. all commercial operations) are completely determined by nature, but manipulation of food conditions aiming to increase HUFA levels in *Artemia* off springs are however, possible for small system, e.g. intensive pond and tank cultures (Vos *et al.*, 1984; Lavens *et al.*, 1986). As there is no natural source of *Artemia* in Bangladesh, so solar salt works in the winter months can be used as a potential source for integrated production of salt and *Artemia* (biomass and cysts) by using exotic mother strains, where they can be manipulated for increasing the food value also. As the culture environment and feed ingested by the parental population has great influence on cyst and biomass quality, so the production of *Artemia* and verification for their qualitative difference due to difference in fertilization treatment is also important to understand the effect of local culture environment on the cyst quality from the cysts of origin.

Materials and Methods

Cysts source: Cysts were produced from the traditional solar salt works of Bangladesh in the winter months (January-March) through integrated culture with salt by using Great Salt Lake (GSL, Utah, USA) origin mother cysts through various fertilization treatments as follows:

T_1 = Initial application: 50 kg urea +20 kg TSP/ha.
Dress up: 25 kg urea+20 kg TSP/ha/every 7 days.

T_2 = Initial application: 500 kg dried and powdered chicken manure/ha
Dress up: 250 kg dried chicken manure/ha/3-4 days interval.

T_3 = Initial application:1000 kg dried and powdered chicken manure/ha
Dress up: 500 kg dried chicken manure/ha/3-4 days interval.

Fatty acid profile analysis: Fatty acid profile analysis of the cysts were done on decapsulation basis followed the ICES standard methodology.

Total lipid extraction: Total lipid was extracted according to the procedure of Ways and Hanahan (1964) with some modification. Extraction was performed using a solvent mix made of 2 parts of $CHCL_3$ (chloroform) and 1 part CH_3OH (methanol). CH_2CL_2 was replaced from the original procedure by $CHCL_3$ because lipid may contaminated by water after extraction when CH_2CL_2 used. Finally the amount of fatty acid on dry weight basis was calculated and the lipid after extraction (1 ml of solvent mix consisting of 3 parts of CH_3OH and 2 parts $C_6H_6CH_3$) flushed with nitrogen and closed well and stored in freezer.

Exterification: Exterification was done according to the modification procedure of Lepage and Roy (1984), where total lipid was esterified directly. (Therefore, the saponification step is superflous). The purpose of this step is to modify the fatty acids into their methyl esters (FAME= Fatty Acid Methyl Ester). Finally FAMEs were dissolved with 1 ml iso-octane and transfer into a 2 ml vial with screw cap and teflon-faced silicon septaliner and finally flushed with nitrogen and stored in a freezer at -30°C.

Gas-Chromatography analysis: Gaschromatograph was done in Carlo Erba HRGS 5160 mega series apparatus (carrier gas hydrogen at a pressure of 50 KPa) with Chrompack WCOT fused silica cappilary column (stationary) phase- CP-Sil-88. The standby temperature at 105°C on-column injector was used with a Carlo Erba OC on-column control unit.

Data expression: The data for each FAME was expressed as percentage of total FAMEs (relative values) and as mg per g dry weight of tissues (absolute values).

Results

Fatty acid analysis (area% and mg/g DW) data of the cysts of all the sources are presented in Table 1. 16.0 acid for treatments T_1, T_2 and T_3 was found as 16.0, 16.0 and 15 mg/g (DW) respectively. Analysis of variance for palmitic acid, i.e. 16:0 showed F ratio and propability within T_1 and T_2, T_2 and T_3, T_1 and T_3 as 1.0000, 0.3739; 3.3601, 0.1407; 1.5610, 0.2796 respectively.

For linolenic acid, i.e. 18:3 (n-3) treatmentwise content was 14, 15 and 13 mg/g (DW) for T_1, T_2 and T_3 respectively and F ratio and probability within T_1 and T_2 was found 2.0773 and 0.2230, within T_2 and T_3, 30.5636, and 0.0052 (highly significant), and withinT_1 and T_3, 1.9620 and 0.2339 respectively.

In case of eicosapentaenoic acid, i. e. 20:5 (n-3), the content was 4, 5 and 5 mg/g (DW) for T_1, T_2 and T_3 respectively and analysis of variance between the treatments showed significant variation between T_1 and T_2 ($F = 75.7033$ and $P = 0.0010$), between T_2 and T_3 ($F = 41.1835$ and $P = 0.0030$), between T_1 and T_3 ($F = 8.0000$ and $P = 0.04747$).

22:6(n-3) content for T_1, T_2 and T_3 was found 0.15, 0.7 and o.! mg/g (DW) respectively. Significant variation for docosahexaenoic acid, i. e. 22:6 (n-3) was observed between T_2 and T_3 ($F = 49.0000$ and $P = 0.0022$) and between T_1 and T_3 ($F = 16.0000$ and $P = 0.0161$). F-ratio and probability for other treatments was found between T_1 and T_2 as 4.5000 and 0.1012.

Significant variation in HUFA content between T_1 and T_2 ($F = 63.8790$ and $P = 0.0013$) and between T_2 and T_3 ($F = 53.1250$ and $P = 0.0019$) was observed. But the variation was not significant between T_1 and T_3, where F ratio and probability was found as 0.4211 and 0.5518, respectively.

Table 1: Fatty acid content (area % and mg/g of DW) of the cysts produced through various fertilization treatments.

Code	Peak	Treatments					
		T_1		T_2		T_3	
		Area%	mg/g DW	Area%	mg/g DW	Area%	mg/g DW
*1 R	14:0	1.6±0.30	1.6±0.46	1.7±0.17	1.7±0.10	1.4±0.10	1.2±0.06
*2	14:1(n-5)	1.3±0.00	1.2±0.10	1.2±0.10	1.2±0.12	1.4±0.00	1.1±0.06
*3	15:0	0.3±0.06	0.3±0.06	0.4±0.05	0.4±0.00	0.2±0.00	0.2±0.00
*4	15:1(n-5)	0.8±0.06	0.7±0.06	0.7±0.00	0.7±0.06	0.9±0.00	0.7±0.06
*5	14:2	0.3±0.07	0.2±0.00	0.3±0.00	0.2±0.00	0.3±0.00	0.2±0.00
*6 R	16:0	17.7±0.49	16.0±1.36	17.2±0.80	15.6±3.54	18.2±0.59	14.8±0.98
*7	16:1(n-7)	6.0±0.92	5.5±1.37	6.6±0.78	6.9±0.62	5.0±0.10	4.1±0.09
*8	17:0	1.1±0.05	1.5±0.17	1.0±0.05	1.1±0.00	1.1±0.06	0.9±0.10
*9	17:1(n-7)	1.6±0.10	1.5±0.17	1.6±0.10	1.6±0.06	1.7±0.06	1.4±0.10
*10	18:0	4.9±0.16	4.5±0.35	4.6±0.50	4.8±0.44	5.7±0.59	4.4±0.23
*11 R	18:1(n-9)	21.5±1.00	19.9±1.07	23.3±5.10	24.1±5.02	26.5±4.39	21.8±2.57
*12	18:1(n-7)	7.4 ±0.20	7.0±0.36	7.4±0.52	7.5±0.65	7.5±0.36	6.6±0.52
*13	18:2(n-6)-t	0.3 ±0.00	0.3±0.00	0.3±0.00	0.3±0.00	0.4±0.00	0.3±0.06
*14 R	18:2(n-6)-c	5.5 ±0.14	5.1±0.40	5.4±0.30	5.6±0.23	5.9±0.15	4.8±0.42
*15	19:0					0.1±0.00	0.1±0.00
*16	18:3(n-6)	0.5 ±0.12	0.5±0.17	0.5±0.06	0.6±0.05	0.4±0.12	0.4±0.06
*17	19:1(n-9)			0.09±0.00	0.1±0.00		
*18	18:3(n-3)	14.1±0.78	13.8±0.62	14.2±0.40	14.7±0.47	15.6±0.10	12.9±0.91
*19	18:4+19:2	2.7±0.10	2.5±0.15	2.8±0.15	2.5±0.25	2.8±0.15	2.2±0.15
*20	20:0						
*21	20:1(n-9)	0.4±0.06	0.4±0.05	0.4±0.10	0.4±0.10	0.5±0.12	0.4±0.12
*22 R	Internal Standard						
*23	21:0						
*24	20:3(n-6)	0.08±0.01	0.08±0.00	0.09±0.01	0.08±0.00	0.10±0.00	0.10±0.00
*25	20:4(n-6)	0.50±0.15	0.50±0.20	0.70±0.06	0.70±0.00	0.10±0.01	0.10±0.00
*26	20:3(n-3)	0.40±0.06	0.30±0.06	0.30±0.06	0.40±0.06	0.40±0.00	0.3±0.06
*27	21:5	0.40±0.00	0.40±0.07	0.40±0.00	0.40±0.06	0.40±0.00	0.3±0.00
*28	22:0	0.30±0.07	0.20±0.00	0.40±0.07	0.30±0.07	0.30±0.00	0.2±0.00
*29 R	20:5(n-3)	2.60±0.06	3.70±0.38	3.50±0.02	4.70±0.40	2.80±0.12	3.8±0.21
*30	22:1(n-9)	0.13±0.06	0.10±0.06	0.10±0.06	0.10±0.07	0.10±0.00	0.1±0.00
*31	23:0						
*32	23:1(n-9)						
*33	22:4(n-6)						
*34	22:3(n-3)						
*35	24:0						
*36	22:5(n-3)	0.30±0.06	0.40±0.10	0.40±0.06	0.60±0.00	0.10±0.00	0.1±0.00
*37	24:1(n-9)						
*38 R	22:6(n-3)	0.20±0.05	0.30±0.15	0.30±0.06	0.70±0.06	0.10±0.00	0.1±0.00
∑HUFA w3>20:3(n-3)		3.00±0.12	4.50±0.36	4.30±0.31	6.10±0.57	3.10±0.29	4.1±0.2

Highest values for areas (%) and mg/g (DW) of palmitic, linolenic, eicosapentaenoic, docosahexaenoic and HUFA of the cysts of treatments T_1,T_2 and T_3 was found as follows: 18.2±0.59 (T_3 cysts), 16.0±1.36 (T_1 cysts), 15.6±0.10 (T_3 cysts), 14.7±0.47 (T_2 cysts), 3.5±0.02 (T_2 cysts), 4.7±0.40 (T_2 cysts), 0.3±0.006 (T_2 cysts), 0.7±0.06 (T_2 cysts) and 4.0±0.31 (T_2 cysts), 6.0±0.57 (T_2 cysts).

Discussion

Except the Essential Fatty Acids (EFA), most of other acids were found comparatively higher in the cysts produced through T_2 and few acids were found higher in the cysts of T_3. Generally the food value of *Artemia* produced after inoculation in another biotope is not necessarily the same as the food value of the inoculation materials (Leger,1989), because the main factor influencing the nutritional value of *Artemia* is the content of essential fatty acid 20:5n-3 and the level of contamination by chlorinated hydrocarbons. The most important fatty acids found in cysts, nauplii and adults of *Artemia* are 16:0 (palmitic), 16:1n-3 (palmitoleic), 18:1n-9 (oleic), 18:2n-6 (linoleic) and 20:5n-3 (eicosapentaenoic). The fatty acid profile in *Artemia* offspring in mainly determined by the composition of the diet ingested by the parental population, which explains that the food present in the various natural biotopes of *Artemia* is not identical and that the composition of food in one biotope may change in function of time (Leger, 1989). So the present finding is in full agreement with the above findings except the variation observed in case of 16:0 fatty acid content of the cysts (variation were found insignificant). This might because of the common environmental condition of the production ponds (Lavens and Sorgeloos, 1984). Categorisation of *Artemia* cysts on the basis of fatty acid contents (Watanabe *et al.*, 1978a; 1978b; 1980) led to place all the present produced cysts into freshwater type as they have high content of 18:3n-3 (Watanabe *et al.*, 1978a). However as the minimum content of 20:5n-3 acid in cyst for marine larvae has been determined and recommended as 4 per cent by Navarro *et al.* (1988), so the lower level of requirement for marine predators (for early feeding of fish and shrimp larvae) can also be fulfilled by the present produced cysts, as the content of 20:5n-3 in the present produced cysts is ranging between 3.7-4.7 per cent and they are also capable to modify the fatty acid in their diet to produce unsaturated fatty acid which they require. Similar observation was reported by Kayama *et al.* (1963), where authors observed conversion of linolenic acid 18:3 (9,12,18) into EFA like eicosapentaenoic and docosahexaenoic acid. Findings like, presence of eicosapentaenoic acid and presence of docosahexaenoic acid in very small amounts in the cysts is
in full agreement with Schauer *et al.* (1980); Leger *et al.* (1986a); Cowgill *et al.* (1987); Navarro (1990). And generally, the presence of higher amount of 20:5n-3 in *Artemia* is associated with a lack of 18:3n-3 (Benijts *et al.*
1975; Claus *et al.*, 1977; 1979; Schauer *et al.*, 1980) and was confirmed by the present finding as the cysts of all sources contained higher amount of 18:3n-3 with lower amount of 20:5n-3 acids. Like present finding, rich content of 18:3n-3 and low content of 20:5n-3 in GSL mother strain cysts were also reported by Leger *et al.* (1986a) and placed the cyst under freshwater type. The proportion of 18:2n-6 was found relatively higher in all sources of cysts (agreed with Watanabe ;1978a with lower content of 20:5n-3 acid (3.7 - 4.7%). Study reveals that the general HUFA content characteristics, i.e. freshwater type of the cysts is remain unaffected due to environmental and fertilization difference during culture and production of cysts, though a significant variation in the fatty acid content between the treatments were observed in several cases.

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