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Enrichment of *Artemia* with free methionine

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Abstract

Different enrichment procedures of the free amino acid (FAA) methionine were tested for *Artemia* nauplii. A direct enrichment protocol (methionine dissolved in the culture water) was compared with liposome enrichment protocols that varied in their membrane composition. During 16 h of direct enrichment in 5.3 mM methionine, the nauplii increased their content of free methionine between 20 and 30 times compared to the unenriched control (43.1 ± 1.2 and 68.4 ± 3.8 pmol · nauplius^{-1} in two separate experiments vs. 2.4 ± 1.0 pmol · nauplius^{-1} in control). However, by encapsulating the identical amount of methionine into liposomes made from pure egg yolk phosphatidylcholine (PC) (> 99% PC) and cholesterol, the nauplii content of free methionine reached 148.8 ± 27.6 pmol · nauplius^{-1}, which is approximately 60 times more than in the unenriched control. Another liposome composition tested, made from crude egg yolk PC (> 60% PC) and cholesterol, resulted in 90.5 ± 4.1 pmol · nauplius^{-1}. The enriched nauplii still retained 80% of the free methionine after 8 h of incubation at conditions simulating feeding for Atlantic halibut larvae (13°C, 33.5 g · l^{-1}).

In conclusion: (1) *Artemia* nauplii can successfully be enriched with free methionine, (2) the high retention of free methionine in the *Artemia* nauplii following transfer to fish tanks shows that it is possible to offer fish larvae a feed with a high level of FAA, based on enrichment of *Artemia* nauplii.

Keywords: *Artemia*; Enrichment; Free amino acids; Liposomes

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1. Introduction

Artemia nauplii and rotifers are commonly used as the start feed for fish larvae worldwide. Although feeding *Artemia* provides reasonable growth during the initial feeding stages in marine fish species in culture, diets based solely on *Artemia* throughout the entire live food period have resulted in a high degree of abnormalities such as malpigmentation and incomplete eye migration in cultured species of flatfish (Seikai, 1985; Næss et al., 1995; Næss and Lie, 1998). On the other hand, these abnormalities are absent or less frequent when different species and developmental stages of copepods harvested from the sea or cultivated in ponds are used (Seikai, 1985; Næss et al., 1995; Næss and Lie, 1998).

Stoichiometric studies have shown that amino acids are the major substrates of aerobic metabolism during the development of embryo and yolk sack larvae of marine species which have pelagic eggs (Fyhn, 1989; Rønnestad et al., 1992, 1994, 1999; Finn et al., 1995; Seoka et al., 1997; Sivaloganathan et al., 1998). In Atlantic halibut amino acids contribute to as much as 60% of the energy substrate at the time around start feeding regardless if the larvae are in the endogenous or the exogenous feeding mode (Rønnestad and Fyhn, 1993; Rønnestad and Naas, 1993). Due to the rapid larval growth and development, there is a large amino acid requirement both to maintain the appropriate concentration in the tissues necessary to obtain an optimal growth rate and amino acid utilization (Tacon and Cowey, 1985), and to supply the fuel for energy metabolism. Protein synthesis itself is energetically expensive, with a theoretical minimum of 50 mmol ATP per g protein required (Waterlow et al., 1978). However, the real costs may be several times higher (Stouthamer, 1979; Houlihan et al., 1995). In addition, the provision of these amino acids may be problematic as studies have suggested that the fish larval gut has limited ability to digest proteins (Rust, 1995; Rønnestad et al., 1999). However, dietary free amino acids (FAA) can be readily absorbed and utilized for protein synthesis (Rust, 1995) suggesting that marine fish larvae should be offered a diet with a high and balanced content of FAA (Fyhn, 1989; Rønnestad et al., 1999).

The need for dietary FAA of marine fish larvae is also suggested by the large pool of FAA found in marine invertebrates such as copepods, which are the natural food for the larvae in the sea (Yancey et al., 1982; Fyhn et al., 1993). In contrast, *Artemia* nauplii, and especially nauplii of *Artemia fransiscana*, which is the common strain used in aquaculture, contain markedly lower levels of FAA compared to wild copepods (Fyhn et al., 1993; Helland, 1995). This is especially true for the essential amino acid methionine, which in *Artemia* nauplii exists at trace or low levels only (Fyhn et al., 1993; Helland, 1995). This suggests that methionine is a limiting amino acid for fish larvae when fed *Artemia* nauplii (Conceição et al., 1997). Based on these findings, it would be pertinent to study the possibility of enriching *Artemia* with FAA in order to improve their nutritional value for marine fish larvae.

One approach to enrich *Artemia* with water-soluble substances, such as FAA, is to use liposomes (Hontoria et al., 1994; Ozkizilcik and Chu, 1994; Touraki et al., 1995; McEvoy et al., 1996). Liposomes are spherical lipid vesicles (10 nm to 20 μm) containing an aqueous volume enclosed by a phospholipid membrane. These vesicles are potent delivery vectors for hydrophilic (dissolved in the aqueous volume) as well as

hydrophobic (embedded in the lipid membrane) nutrients and are potential carriers for FAA. By encapsulating water-soluble substances into suitably sized liposomes, these vesicles may be readily ingested by *Artemia* nauplii similarly to the non-selective filter feeding of micelles in lipid emulsion enrichment.

The aim of this study was to compare different enrichment protocols for their efficacy to enrich *Artemia* with FAA. Direct enrichment (FAA directly dissolved in the culture water) was compared with liposome enrichment protocols (FAA encapsulated in liposomes before added to the culture water) that varied in their membrane compositions. To reduce the complexity of the experiments only methionine was used since this essential amino acid only exists at trace or low levels in nauplii of *Artemia*.

2. Materials and methods

Enrichment of the nauplii of *Artemia* with free methionine was studied in two series of experiments. In both series, the content of free methionine in the nauplii was studied during a 16-h enrichment period with a 5.3-mM free methionine solution of culture water. The retention of free methionine following enrichment was also monitored. In the first series, two experiments investigating the direct approach for the uptake of methionine by the nauplii were carried out. In the first experiment of series I, nauplii were enriched with methionine directly dissolved in the culture water and the nauplii were analyzed for FAA. However, this experiment did not take into consideration the further metabolism of methionine into other metabolites. Consequently, in the second experiment of series I, nauplii were enriched with an identical concentration of free methionine, but which contained a fraction of ^{35}S-methionine to determine the amount of methionine absorbed from the culture medium.

In series II, a comparison was made between direct enrichment and two different liposome enrichment protocols that differed in their liposome membrane composition.

2.1. Series I: the nauplii absorption of methionine compared with the accumulation of free methionine

Four grams of *A. fransiscana* cysts (E.G. grade, INVE, Belgium) were incubated in 1 ℓ filtered (0.2 μm) seawater (about 33.5 $g \cdot l^{-1}$) at 29°C under continuous aeration and illumination. After 24 h, the nauplii were collected and washed with tap water. The non-radioactive enrichment was performed in a 5-l round bottom beaker containing 3000 ml of filtered seawater while the radioactive enrichment was performed in a 8-ml plastic tube filled with 4 ml of filtered seawater. In both experiments, *Artemia* nauplii were added to give a density of 600 nauplii $\cdot ml^{-1}$, and a gentle aeration ensured a homogeneous distribution of the nauplii during the enrichment. L-Methionine (> 98%, Sigma, USA) dissolved in distilled water (300 mM) was added in both experiments to achieve a methionine concentration of 5.3 mM in the culture water. In the radioactive

experiment a small amount of L-^{35}S-methionine (in 0.1% 2-mercaptoethanol, Amersham, UK)(< 1% of the total methionine) was added, which gave a final specific radioactivity of methionine equal to 0.34 Bq · pmol^{-1}. The nauplii from both experiments were kept at 29 ± 1°C during the 16-h enrichment.

In the non-radioactive experiment, samples of nauplii in cultured water (40 ml) were collected for FAA analyses in three parallels prior to the addition of methionine, immediately following the methionine addition and then every fourth hour during the enrichment period. The samples were filtered and washed with tap water on a plankton net (50 μm) and the nauplii loaded in pre-weighed cryo-tubes. The tubes were immediately frozen on dry ice before stored at −20°C until analyses. At every sampling point, five 1.0-ml samples were also collected and fixative added to preserve the nauplii for later counting of the nauplii density of the culture.

In the radioactive experiment, samples of nauplii in cultured water (20 μl) were collected for detection of radioactivity, in triplicates, at every fourth hour during the enrichment. The samples were filtered (50 μm) and washed with tap water and the exact number of individuals counted before the samples were transferred into 20-ml liquid scintillation counting (LSC)-vials. The samples were then solubilized in 1.5 ml of Soluene 350 (Packard, USA) for one h at 60°C, before added 10 ml of Ultima Gold (Packard) scintillation cocktail.

In order to determine the methionine retention in the nauplii following the 16-h enrichment period, the nauplii from both experiments were washed in tap water and then transferred to tanks containing filtered seawater (33.5 g · l^{-1}) at 13 ± 1°C. These conditions are similar to those in tanks of Atlantic halibut larvae during first feeding. The nauplii from the non-radioactive experiment were placed in a beaker filled with 10 l of seawater (70 nauplii · ml^{-1}), while the nauplii from the radioactive experiment were placed in a beaker filled with 1.0 l seawater (2.3 nauplii · ml^{-1}). In both experiments, the nauplii samples were collected and stored by the same procedure as during enrichment. Samples were taken immediately after transfer and then at increasing intervals during the next 16 h. Water samples were also collected in three parallels of 3.0 ml to calculate the release of free methionine and ^{35}S from the nauplii during the period after enrichment.

2.2. Series II: comparison of direct enrichment and liposome enrichment

2.2.1. Preparation of liposomes

Crude egg yolk phosphatidylcholine (PC) (> 60% PC), purified egg yolk PC (> 99% PC) and cholesterol (> 99%) were obtained from Sigma. Two kinds of liposome membrane compositions were tested; 1) crude egg yolk PC: cholesterol (10:4 w/w) and 2) purified egg yolk PC and cholesterol (10:4 w/w). To a 250-ml round bottom flask was added 100 mg of egg yolk PC and 40 mg of cholesterol in a small volume of chloroform: methanol (2:1 v/v). The solvents were evaporated in a Rotavapor R110 (Büchi, Switzerland) at 35°C. The resulting lipid film was further dried for 30 min in a vacuum desiccator, before adding 5 ml of 300 mM methionine in 530 mM NaCl (isosmotic to diluted seawater at 25 g · l^{-1}) and rotated slowly for 30 min at 35°C. The

resulting liposome suspension was extruded through a polycarbonate filter (0.6 μm) using a LiposoFast liposome extruder (Avesdin, Canada).

2.2.2. Enrichment protocols

A. fransiscana cysts (Neptun Industries, Salt Lake City) were hydrated, decapsulated and incubated at 29°C under continuous aeration and illumination. After 24 h the nauplii were collected, washed and stored at 20°C for 5 h before transferred to 50-ml plastic centrifuge tubes. Each tube contained 35 ml of seawater (25 $g \cdot l^{-1}$) and ca. 300 nauplii ml^{-1}. The tubes were gently aerated through syringe needles and placed in a water bath to maintain a temperature at 29 ± 1°C during the experiment.

Three enrichment protocols were tested and each treatment contained 12 tubes, which allowed four samples of three parallels each. The direct enrichment protocol was carried out by enriching the *Artemia* nauplii in the tubes with 0.625 ml of 300 mM methionine. In contrast, the liposome enrichment protocols were carried out by enriching the tubes with 0.625 ml of their respective liposome suspensions (300 mM methionine) thereby ensuring that all the enriched groups received the identical amount of 1.9 mmol of methionine in each tube (5.3 mM). As a control, one group of tubes was not enriched.

After 16 h of enrichment, the nauplii were washed with tap water and transferred to 1-l beakers supplied with seawater at 25 $g \cdot l^{-1}$ and 22 ± 1°C, with an exchange rate of approximately 0.25 $l \cdot h^{-1}$, to simulate larval feeding conditions for gilthead seabream.

Samples for FAA analyses were collected by emptying the content of each tube/beaker through a filter (50 μm) which were then washed well with distilled water. The nauplii were loaded into pre-weighed cryo-tubes and immediately stored at −30°C until lyophilization and analyses. To calculate individual dry weight, between 50 and 200 nauplii were loaded onto pre-weighed glass-fiber filters. The nauplii were dried over night at 70°C and weighed before the exact number of individuals was counted.

2.3. Analyses and calculations

Nauplii samples for FAA analyses were lyophilized before extracted in 6% tricloro acetic acid (TCA) for 24 h at 4°C. The FAA were separated by cation-exchange chromatography (automatic high pressure sample loading), detected by post column derivation with orthophthaldialdehyde and quantified with a fluorimeter, using a Chromaspec J 180 (Rank Hilger Analytical, England) amino acid analyzer. External standards (20 amino acids) of 0.5 nmol per amino acid were ran for every eighth sample. Water samples for FAA analyses were applied directly on the amino acid analyzer without any extraction in TCA.

Nauplii samples for ^{35}S detection were counted twice (5 min) in a Packard Tri-Carb 300 within 48 h from mixing. The level of absorbed methionine was calculated as:

$$\text{Absorbed methionine (pmol nauplii}^{-1}\text{)} = \frac{\text{radioactivity of nauplii individuals (Bq)}}{\text{specific radioactivity of methionine (Bq pmol}^{-1}\text{)}}.$$

The level of sulfur released to the water in form of sulfur-containing compounds derived from the previous absorbed free methionine was calculated as:

$$\text{Released sulfur (pmol nauplii}^{-1}) = \frac{\text{radioactivity of water sample (Bq)}}{\text{number of nauplii in the beaker} \times \text{specific radioactivity of methionine (Bq pmol}^{-1})}.$$

2.4. Statistics

Differences between treatments and the homogeneity of the variance were tested by Tukey's HSD test (one-way ANOVA) and Levene's test, respectively, performed by Stat soft Statistica version 5.0. Calculations of standard deviation were performed by Microsoft Excel version 5.0. All data are presented as mean ± SD, $n = 3$.

3. Results

In the directly enriched *Artemia* nauplii of series I, the content of free methionine reached 43.1 ± 1.2 pmol nauplius^{-1} at the end of the 16-h enrichment period (Fig. 1),

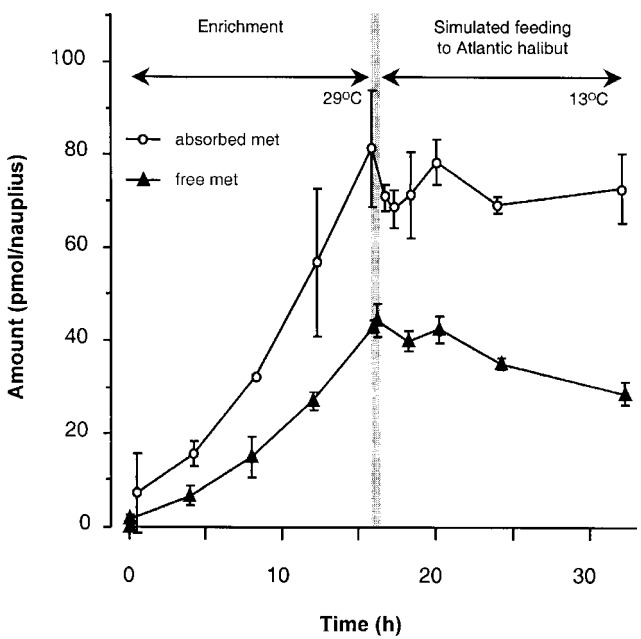

Fig. 1. The content of free methionine and the amount of absorbed methionine in *A. fransiscana* nauplii during 16 h. Values are presented as mean ± SD, $n = 3$ of direct free methionine enrichment (5.3 mM) followed by simulated feeding to fish tanks. Values are presented as mean ± SD, $n = 3$.

which was nearly 20 times higher than in the unenriched control of series II (2.4 ± 1.0 pmol nauplius^{-1}). The level of methionine absorbed from the culture water was at every sampling during the enrichment period approximately twice as high as the nauplii content of free methionine (Fig. 1), and at the end of the 16-h enrichment, the ^{35}S-activity in the nauplii corresponded to an absorption of 81.3 ± 12.5 pmol of methionine nauplius^{-1}. In total, the absorbed methionine represents approximately 1% of the methionine added to the enrichment culture.

During the 16 h of incubation in clean seawater at conditions which simulate feeding to Atlantic halibut larvae (13°C), the content of free methionine was reduced by one-third to 28.8 ± 2.3 pmol · nauplius^{-1} giving a retention of 67%. However, no free methionine and only a small fraction of ^{35}S, corresponding to 5 pmol · nauplius^{-1}, was found in the water at the end of the incubation period (Fig. 2).

In the series II experiments, all the enrichment protocols increased the content of free methionine in the nauplii (Fig. 3), while the content of most other FAA showed only smaller changes (Fig. 4). The nauplii enriched by the direct enrichment protocol contained 68.4 ± 3.8 pmol · nauplius^{-1} of free methionine after 16-h enrichment, which was nearly 30 times greater than levels found in the unenriched *Artemia* (2.4 ± 1.0 pmol · nauplius^{-1}). On a dry weight basis, this represents a relative content of free methionine equal to 42.5 pmol · μg^{-1}. However, at the identical enrichment time, levels of approximately 60 and 40 times the control level were found in the nauplii enriched by the purified PC liposome enrichment protocol (148.8 ± 28 pmol · nauplius^{-1}) and the crude PC liposome enrichment protocol (90.5 ± 4.1 pmol · nauplius^{-1}), respectively. At all sampling times, the pure PC liposome enriched *Artemia* contained a significant ($P < 0.05$) higher level of free methionine compared to the other treatments. Likewise, the unenriched *Artemia* contained a significant ($P < 0.05$) lower level of free methionine compared to the other treatments. However, no significant ($P < 0.05$) different content of free methionine was found between the crude PC liposome enriched *Artemia* and the direct enriched *Artemia*, at any sampling time, even though there is a clear

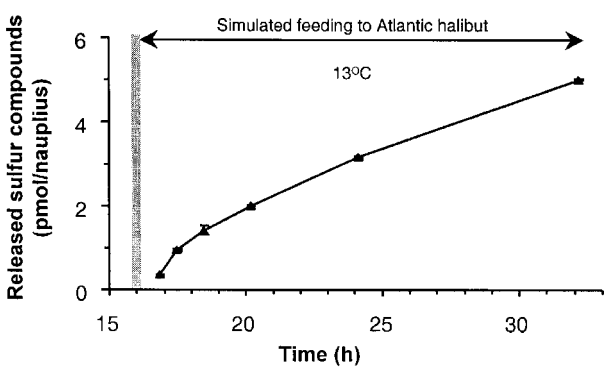

Fig. 2. The amount of sulfur containing metabolites derived from the previous absorbed ^{35}S-methionine (0.34 Bq pmol^{-1}) that were released from the *A. fransiscana* nauplii during 16 h. Values are presented as mean ± SD, $n = 3$ incubation in clean seawater (simulated feeding to fish tanks). Values are in pmol nauplii^{-1} of sulfur and are presented as mean ± SD, $n = 3$.

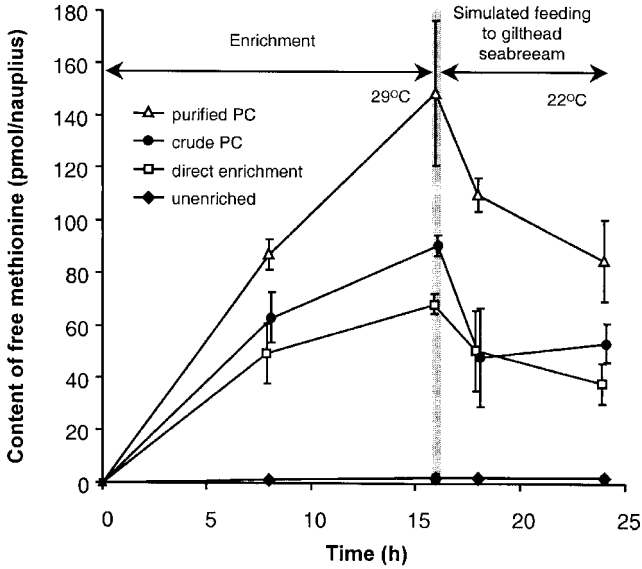

Fig. 3. The content of free methionine in *A. fransiscana* nauplii during 16 h of enrichment with 5.3 mM of free methionine using three different enrichment protocols, followed by simulated feeding to fish tanks. The protocols were: (1) purified PC: liposome enrichment protocol using liposomes made from purified egg yolk PC (> 99% PC) and cholesterol; (2) crude PC: liposome enrichment protocol using liposomes made from crude egg yolk PC (> 60% PC) and cholesterol; (3) direct enrichment: enrichment by dissolving the methionine directly into the culture water. Data are slightly adjusted laterally to separate concurring points. Values are presented as mean ± SD, $n = 3$.

tendency of a higher level of free methionine in the crude PC liposome enriched *Artemia*. Homogeniety of variance ($P > 0.05$) was found at all sampling times.

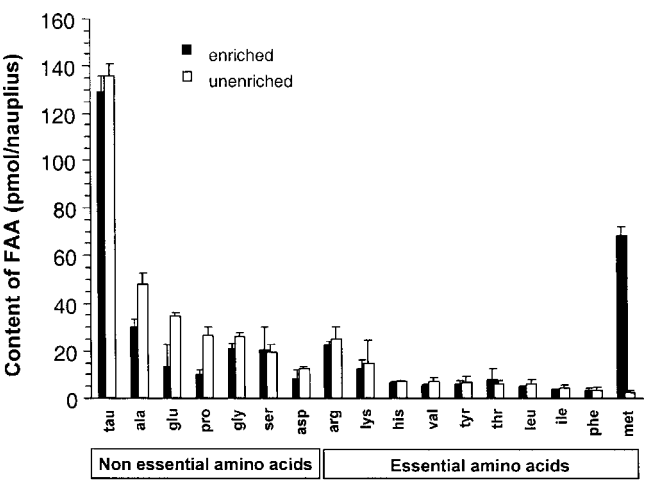

Fig. 4. Content of FAA in *A. fransiscana* nauplii after 16 h of direct enrichment in 5.3 mM of free methionine compared with the unenriched nauplii (control).

During the simulated feeding, all enriched groups reduced their content of methionine, but the methionine values were still 10 fold higher than the unenriched group after 8 h. The directly enriched group and the groups enriched with purified PC liposome and crude PC liposome showed methionine retention of 56%, 57% and 59%, respectively, suggesting a similar retention efficiency of free methionine between the direct enrichment and liposome enrichment protocols.

4. Discussion

The results demonstrate that the free methionine content in *Artemia* can be significantly increased by enrichment. However, in spite of the rapid increasing content of free methionine in the nauplii during the non-radioactive enrichment, the labeled methionine was absorbed by the nauplii approximately twice as fast as the accumulation of free methionine, suggesting that approximately 50% of the absorbed methionine was converted to other sulfur containing compounds.

The drinking rate of the *Artemia* nauplii in seawater (34 $g \cdot l^{-1}$) at 30°C has been reported to be 10.8% of body weight $\cdot h^{-1}$ (Navarro et al., 1993) which during the 16-h enrichment represents approximately 25 nl of culture water and 130 pmol of methionine. This approximates the measured uptake of labeled methionine, suggesting that the accumulation of methionine in the nauplii in the direct enrichment protocol was achieved by drinking. A nauplii gut full of the culture medium (5.3 mM free methionine) would represent less than 5.5 pmol of methionine based on approximate calculations of the nauplii gut volume. Thus, to reach a level of 43 pmol of free methionine in the nauplii, free methionine must have been concentrated in the gut, or more likely, absorbed from the gut and entered the tissues and body fluids where it was partly maintained in its free form.

The metabolites derived from ingested free methionine would consist of other sulfur containing compounds such as protein bound methionine, specific amino acids (cysteine and taurine), sulfate or other compounds from methionine–cysteine degradation. Of these compounds only the free and the protein bound methionine may contribute to the methionine requirement of the predating fish larvae.

Compared to the direct enrichment protocol, up to twice the levels of free methionine in the nauplii were achieved when the *Artemia* nauplii were enriched with free methionine encapsulated in liposomes. This suggests that liposomes were actively filtered from the water and ingested by the nauplii, thus increasing the rate of ingestion of free methionine compared to the direct enriched nauplii. The results also showed that the two different liposome treatments resulted in significantly ($P < 0.05$) different nauplii content of free methionine. The highest content of free methionine was demonstrated in *Artemia* enriched with liposomes made from purified egg yolk PC. Unlike the purified PC, the crude egg yolk PC contained a mix of ca. 68% PC, ca. 19% phosphatidylethanolamine (PE), and other phospholipids. The different phospholipid groups in the crude egg yolk lecithin have different head groups (polar regions) that vary in size and charge distribution. For instance choline, the head group of PC, is significantly larger than ethanolamine, the head group of PE (New, 1990). Conse-

quently, the non-uniform distribution of head groups in the crude egg yolk PC may have resulted in phospholipid packing abnormalities leading to the leakage of entrapped substances (Hauser et al., 1981; New, 1990).

The enrichment by liposomes of purified egg yolk PC and cholesterol gave 149 pmol · nauplius^{-1} of free methionine. This represent an enrichment efficiency (total content of free methionine in *Artemia* · total methionine added to culture^{-1} · 100) of about 0.9%, while the enrichment by liposomes comprised of crude egg lecithin and cholesterol, which gave 91 pmol · nauplius^{-1}, showed an enrichment efficiency of approximately 0.5%. These enrichment efficiencies are difficult to compare with other liposome enrichment studies (Hontoria et al., 1994; Ozkizilcik and Chu, 1994; Touraki et al., 1995; McEvoy et al., 1996) due to differences in the enrichment substance and experimental design. Ozkizilcik and Chu (1994) reported that 16% of the glycine added to the enrichment culture was taken up by the nauplii during 24 h of enrichment. However, in their experiment, only glycine entrapped by liposomes was added to the enrichment culture (20% of the glycine was entrapped), while the enrichment efficiency of the present experiment considered both the entrapped and the non-entrapped methionine added. In addition, these authors included glycine assimilated into the nauplii protein and glycine transformed to other substances while the present study considered only the content of free methionine in the nauplii. But more important, Ozkizilcik and Chu (1994) used significantly lower levels of glycine (8.5×10^{-5} mM) compared to the concentration of methionine in the present study (5.3 mM), which may have resulted in more efficient absorption and assimilation of the FAA.

The liposome suspensions from both treatments were exposed to seawater and observed in high concentrations on glass slides by a phase contrast microscope. An immediate change from small (< 1 μm) free moving particles to large continuous aggregations was seen, a behavior attributed to interactions between the polar head groups of PC in the liposome membranes and Ca^{2+} in the seawater (Matsumura et al., 1995). However, during observation, smaller fragments (with a typical diameter of 10 to 50 μm) were loosening from these large continuous aggregates and were floating away before re-adhering to another large aggregate. This indicates a reversible liposome aggregation and suggests that the large aggregations may have formed aggregates of a more moderate size in the culture water compared to the conditions during the observations. This can be envisaged due to the reduced liposome density and the turbulent culture water. Particles filtered from the water by the *Artemia* nauplii ranges in size from 1 to 50 μm (Lavens and Sorgeloos, 1996), thus it is unclear whether the aggregating behavior of the liposomes was an advantage or a disadvantage as liposomes less than 1 μm may have formed aggregates within the filtered range, as well as the largest aggregates formed may have been out of the range for an optimal filtration and ingestion by the nauplii.

The high temperature, alkaline pH and high level of metal ions (Na^+, Ca^{2+}, Mg^{2+}) in the culture water of *Artemia* enrichment cultures, together with the constant illumination and high concentration of O_2, represents a strongly oxidative environment (Hunt and Tsang, 1981). Thus, oxidation of unsaturated fatty acids in the liposome membranes ($\sim$ 45% of total fatty acids in egg yolk is unsaturated; New, 1990) may have resulted in breakage and subsequent release of the entrapped content (Hunt and Tsang,

1981). More effective enrichment protocols might have been achieved by inclusion of antioxidants into the liposome membranes, or by using fully saturated membrane lipids (Hernández-Casseles et al., 1990).

No significant difference in retention of free methionine was observed between the direct enrichment and the liposome enrichment protocols. This agreed with Hontoria et al. (1994) who concluded that the liposomes were spontaneously degraded in the nauplii digestive tract with a subsequent release of the liposome contents. This would resemble the ingestion of free methionine in the digestive tract of nauplii in the direct enrichment protocol.

An effective retention of free methionine in *Artemia* during the simulated feeding to fish tanks was found in both series of experiments. However, a higher retention of free methionine was found in the nauplii at incubation at 13°C (series I, approximately 80% after 8 h), compared to the nauplii incubated at 22°C (series II, 56–59% after 8 h), suggesting a temperature dependent turnover of methionine. Although the liposome enriched treatment gave the highest methionine levels, the least effective direct enrichment protocol (series I) alone might have ensured from 43 to 35 pmol of free methionine from each nauplii if ingested by fish larvae during the first 8 h after enrichment. This is about 20 times higher than unenriched *Artemia* or *Artemia* enriched on commercial preparations, and also represents a higher relative content of free methionine than what is found in the commonly used copepod *Temora longicornis* (calculated from Fyhn et al., 1993; Helland et al., 2000). And since the liposome enrichments did not improve the cost efficiency of the enrichment, due to the high expense of the liposome lipid itself, the liposome technique does not seem to be recommendable in enrichment of *Artemia* with free methionine for a nutritional purpose. However, liposome technique may be useful in enrichment of *Artemia* with other water-soluble compounds, in cases where the enrichment efficiency is of high importance due to high expense or restrictions of the component, or if an adequate enrichment cannot be achieved by direct enrichment.

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