E-ISSN 2149-0236

Journal of Aquaculture Engineering and Fisheries Research

FULL PAPER TAM MAKALE

THE CHANGES IN THE MOLECULAR WEIGHT PROFILES AND BIOCHEMICAL COMPOSITIONS OF POTENTIAL FEED INGREDIENTS FOR SUSTAINABLE AQUACULTURE

Mevlüt Aktaş, Munevver Ayçe Genç, Ahmet Bozkurt, Ercüment Genç, Mehmet Naz

Cite this article as:
Aktaş M, Genç MA, Bozkurt A, Genç E, Naz M (2019) The Changes in the Molecular Weight Profiles and Biochemical Compositions of Potential Feed Ingredients for Sustainable Aquaculture. Journal of Aquaculture Engineering and Fisheries Research 5(1), 1-11

[1]Department of Aquaculture, Marine Science and Technology Faculty, Iskenderun Technical University, Hatay, Turkey
[2]Department of Aquaculture Fisheries and Aquaculture Engineering, Faculty of Agriculture, Ankara University, Ankara, Turkey

Submitted: 22.02.2019

Accepted: 11.03.2019

Published online: 10.04.2019

Correspondence:

Mehmet NAZ

E-mail:
mehmet.naz@iste.edu.tr

Journal abbreviation:

J Aquacult Eng Fish Res

ABSTRACT

The objective of study was to evaluate the differences in the molecular weight profiles and the biochemical compositions of potential feed ingredients such as fish meal (FM), Artemia nauplii (ArtN), Artemia metanauplii (ArtMn), copepod meal (CopM), dry Daphnia sp. (dryD), Daphnia magna (freshD) and Tubifex. The lowest and highest ash (ArtN 5.84±0.05%, 45.18±1.17% dryD) lipid (dryD 4.71±0.06%, ArtN 30.19±0.65%) and protein contents (45.45±3.90% freshD, 66.74±0.2% CopM) were detected ($p<0.05$). The highest palmitic acid and oleic acid levels were found in dryD and ArtMn, respectively. Except for freshD, the linoleic acid levels were relatively low. Linolenic acid level was just found Tubifex and dryD. The highest docosahexaenoic acid and eicosapentaenoic acid levels were observed in fish meal and copepod meal, respectively. The highest and lowest levels of feed ingredients used were determined in 2532>= Da and 2532-13000 Da, respectively. The highest molecular weight profile belongs to 67000<=Da was observed in Tubifex.

In conclusion, accorded to their nutritional levels of tested ingredients can make important contributions to microdiet formulations. Considering this molecular weight profiles data results cautioned that the use of all feed ingredients except for Tubifex in microdiet formulations may cause the high leaching ratios containing 2532>= Da molecular weight.

Keywords: Feed ingredients, Live foods, Proximate compositions, Fatty acids, Molecular weight profiles

Introduction

Related to the increase observed in global aquaculture production, the demand for aqua-feeds is growing. Aquaculture sector is highly dependent to fish meal and fish oil supply from wild fisheries. Actually, fish meal is regarded as the best dietary protein source due to the excellent balance of essential amino acids and essential fatty acids (Tacon 1993). However, fish meal is expensive due to high demand and limited global supply. The sustainability of aquaculture sector may be threatened by its present over-dependence on fish meal and fish oil (FAO 2002). Therefore, for sustainable aquaculture as the mentioned by Higgs et al. (1995), replacing fishmeal with more sustainable ingredients of either animal or vegetable sources is a necessary. According to Lovell (1998), feed ingredients containing 20% or more crude protein are considered as protein sources.

The feeding procedure of cultured marine fish larvae has a critical importance. The larval stages of marine fish larvae require live food organisms to meet optimal requirements. The dependence to live feed during early feeding stages of fish larvae is considered to be inevitable for most fish species. In this context, live feeds such as Artemia and rotifers have been used widely in the culture of fish larvae. Difficulties in feeding rotifers have been reported because of their small size, nutritional variability, and the susceptibility of rotifer culture to crashing (Kovalenko et al. 2002). The biggest disadvantages of Artemia are marked variation in cost, physical properties, and nutritional quality among different sources.

Copepods are important crustaceans studied because of their key role in aquaculture. In marine hatchery, copepods can complement the nutrient supply from rotifers and *Artemia nauplii* as they contain higher levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and other polyunsaturated fatty acids (PUFA) that are essential for the growth and health of marine fish larvae (Støttrup 2000; McKinnon et al. 2003). Therefore, there is an increasing interest on the use of copepods to improve larval fish nutrition (Evjemo et al. 2003). In addition, the growth stages of calanoid copepods from first nauplius to mature size provide a broad spectrum of prey sizes (80 to N900 μm in length and 3-5 μg in dry weight) (Schipp et al. 1999).

On the other hand, hatchery for freshwater fish (for human consumption or ornamental purposes) are still heavily dependent on the natural food for early development, such as Daphnia sp. and tubicid worms (*Tubifex* sp.), etc. *Daphnia magna* is one of the most important live feed organisms for feeding of freshwater fish larvae (Macedo and Pinto-Coelho 2001).

Aquatic oligochaetes worms play an important role in commercial aquaculture as a live food source. Because, they are rich in protein and contain polyunsaturated fatty acid (PUFA) which is essential for aquaculture (Hilton 1983; Graney et al. 1986; Vineetha 2001; Das et al. 2012). Most widely used aquatic oligochaetes for aquaculture feed are contributed by freshwater oligochaetes, *Tubifex* sp. (Lietz 1987; Mollah et al. 2012), Branchiura sowerbyi and Enchytraeus sp. (Watanabe and Kiron 1994; Memis et al. 2009; Hossain et al. 2012). This oligochaetes worm, *Tubifex* sp. is one of the best and cheapest live feed for fish, prawns and frogs (Marian and Pandian 1984).) It has been reported to be an important live feed in larval rearing of hatchery-produced catfishes, ornamental fishes, prawns, etc. (Hossain et al. 2011).

It has been determined that the forms of proteins are presented in artificial diets has an important role on the development of the digestive system of larvae (Cahu and Zambonino Infante 1995; Zambonino Infante et al. 1997). Therefore, the identification of the forms of proteins in alternative feed ingredients may contribute towards the formulation of more appropriate artificial diets for critical larval stages of fish (Ronnestad et al. 1999; Holt 2000). The biochemical characterization and functional properties of fish meal, *Artemia nauplii*, *Artemia metanauplii*, copepod meal, fresh *Daphnia magna* and *Tubifex* sp. have been described by some researchers (Colombo-Hixon et al. 2013; Cauchie et al. 1999; Herewati et al. 2016; Naz 2008; Diken, 2017). However, a combined analysis of the biochemical compositions and molecular weight profiles of feed ingredients such as fish meal, *Artemia nauplii*,

Artemia metanauplii, copepod meal, dry *Daphnia sp*., fresh *Daphnia magna* and *Tubifex* is not available.

The study aimed to reveal the molecular weight profile of fish meal (FM), *Artemia nauplii* (ArtN), *Artemia metanauplii* (ArtMn), copepod meal (CopM), dry *Daphnia* sp. (dryD), *Daphnia magna* (freshD) and *Tubifex*. In addition, biochemical compositions of the above mentioned feed ingredients were determined.

Materials and Methods

Fish meal (FM), copepod meal (CopM), dry *Daphnia* sp. (dryD) and *Tubifex* were purchased from commercial companies. Fresh *Daphnia magna* (freshD), *Artemia nauplii* (ArtN) and *Artemia metanauplii* (ArtMn) were obtained from Aquaculture Research Unit, Faculty of Marine Science and Technology, Iskenderun Technical University, Iskenderun, Hatay, Turkey.

Daphnia magna culture

Fresh *Daphnia magna* (freshD) was obtained from the pond culture unit. The study was carried out in two concrete ponds measuring 6×2×1.5 m. To encourage phytoplankton growth such as *Chlorella* spp., *Scenedesmus* sp., *Nannochloropsis* sp., *Chaetoceros* sp., *Tetraselmis* sp. and *Dunaliella* sp., 2 kg of farm manure-fertilizer (sheep and chicken) and 0.5 kg of inorganic fertilizer ($(NH_4)_2SO_4$) were added into the pounds. Approximately, after one week, water colour turned green and then *Daphnia magna* culture was added. During production period, water temperature ranged from 20°C to 28°C. In addition, yeast was given as nutritional supplement to *Daphnia magna* culture environment. One month later, approximately 5 kg of freshD was harvested, washed with tap water and preserved at -80°C up to the biochemical and molecular weight analyses.

Hatching of *Artemia cysts* and incubation procedures

Artemia cysts (1 g L^{-1}) (E. G. *Artemia* System SA, INVE, Ghent, Belgium) were incubated in a 150 L tank included sterilized seawater (35-36 ppt, 30°C) under continuous aeration and illumination. After 24 h, the nauplii were collected and washed with tap water. Enrichment was performed in a 150 L tank. *Artemia nauplii* were added to give a density of 400 nauplii mL^{-1} and gentle aeration ensured a homogenous distribution of the nauplii during the enrichment (S. Presso, INVE, Ghent, Belgium). The composition of the commercial enrichment emulsion is shown in Table 1. The enrichment diet was added at the start of incubation time (time (0) and again 12 h later (both times 0.5 g L^{-1}).

Analytical Methods

Proximate compositions of feed ingredients

Proximate compositions such as ash and protein values of samples taken from potential feed ingredients such as FM, ArtN, ArtMn, CopM, dryD, freshD and *Tubifex* were tested according to the AOAC (2000) procedures and also, lipid analyses were performed according to the chloroform-methanol extraction method described by Bligh and Dyer (1959).

Fatty acid compositions of feed ingredients

Fatty acid contents of tested potential feed ingredients were performed Garces and Mancha (1993) method. Analysis of the fatty acid methyl esters were carried out on a gas chromatography mass spectrometer (GC-MS) equipped with an HP-INNOWAX capillary column (HP 19091N-136 Model-0.25 mm × 60 m × 0.25 µm). Methyl esters were identified by comparisons with a known standard mixture of fatty acids.

Molecular weight profiles of feed ingredients

The molecular weight profiles of potential feed ingredients were performed according to Boza et al. (1994). Firstly, samples were stirred in the phosphate buffer (pH=8; 10

Table 1. The composition of the emulsion used for *Artemia* enrichments.

Composition	*S. PRESSO*
Moisture	58
Crude Protein	3
Crude Lipid	32
Crude Ash	2
Phosphorus	0.5
Vitamin A	110.000 IU/kg
Vitamin D_3	10.000 IU/kg
Vitamin E	5400 mg/kg
Vitamin C	8000 mg/kg
Antioxidants	Ethoxyquin, BHA
HUFA	150 mg/g dry weight
DHA/EPA	9

mg ml^{-1}), centrifuged and the supernatant filtered with 0.22 µm syringe filter and then, analysed HPLC-Gel Filtration Chromatography for determination of the molecular weight profiles. Samples were injected in chromatograph equipment with a TSK-Gel G2000 SWXL column. The eluent was 0.1 mol/sodium sulphate in 0.1 mol L^{-1} phosphate buffer at a flow rate of 1 ml/min, and column effluent was monitored for UV light absorption at 230 nm. Based on the retention time of molecular weight standards, four fractions were defined: 67000 Da≤, 67000-13700 Da, 13700-2532 Da and ≥ 2532 Da. The molecular weight standards (from Sigma) were bovine albumin (67000 Da), ribonuclease A (13700 Da), insulin chain A (2532 Da), val-ala-ala-phe (407 Da), tyr-tyr-tyr (508 Da), tryptophan (204 Da), tyrosine (181 Da) and p-aminobenzoic acid (137 Da).

Statistical methods

In present study, biochemical compositions and the molecular weight profiles of feed ingredients were given as mean ± standard error (SE). The measurements were carried out in triplicates. The experimental data were subjected to one-way (ANOVA) and mean ± standard error (SE) differences by using SPSS 9.0 statistical package (SPSS 1993).

Results

The biochemical compositions (as dry weight) of potential feed ingredients tested in the present study are given in Table 2. The differences observed in the ash, lipid and protein values of potential feed ingredients were statistically significant ($p<0.05$). The lowest and highest ash, lipid and protein contents were found for ash; 5.84 ± 0.05% (ArtN) and 45.18 ± 1.17% (dryD), for lipid 4.71 ± 0.06% (dryD) and 30.19 ± 0.65% (ArtN), and for the protein; 45.45 ± 3.90% (freshD) and 66.74 ± 0.2% (CopM), respectively. Ash value of fish meal except for dryD and freshD were higher than those of other tested ingredients. Lipid values of ArtN, ArtMn, CopM, freshD and *Tubifex* except for dryD were higher than that of FM. Protein value of FM were similar to protein values of ArtN, ArtMn, CopM and *Tubifex*. However, protein values of dryD and freshD were lower than that of FM.

Table 3 shows the differences observed in the fatty acid compositions (as dry weight) of potential feed ingredients tested and the highest palmitic acid level were found in dryD followed by FM, CopM, freshD, ArtN, ArtMn and *Tubifex*. The highest oleic acid levels were determined in ArtMn followed by ArtN, freshD, dryD, FM, *Tubifex* and CopM. The linoleic acid levels were found quite low in tested ingredients except for freshD. Additionally, the linolenic acid levels were not found in the tested ingredients except for *Tubifex* and dryD. The highest DHA (docosahexaenoic acid) level were observed in fish meal followed by CopM but not determined in other feed ingredients. The highest EPA (eicosapentaenoic acid) level were determined in CopM followed by FM, ArtN, *Tubifex*, dryD and freshD but not found in ArtMn. The highest saturated and unsaturated fatty acid levels were determined in fish meal (43.608%) and ArtMn (39.339%). The saturated fatty acid level observed in fish meal were followed by dryD, CopM, ArtN, ArtMn, *Tubifex* and freshD. The unsaturated fatty acid level observed in ArtMn were followed by freshD, ArtN, FM, dryD, *Tubifex* and CopM. The saturated and unsaturated fatty acid values were found as 43.608%-31.397% for FM, 28.2385%-32.346%, ArtN, 24.651%-39.339% ArtMn, 30.4275%-16.872% CopM, 32.064%-29.061% dryD, 16.4815%-33.237% freshD and 16.528%-17.0305% for *Tubifex*, respectively.

Molecular weight profiles of potential feed ingredients analysed in the present study were given in Figure 1.

Table 2. Biochemical compositions of potential feed ingredients used as direct and indirect in aquaculture.

	Ash	***Lipid***	***Protein***
Fish meal	17.64 ± 0.06^{d}	12.18 ± 0.16^{b}	66.52 ± 0.11^{b}
Artemia nauplii	5.84 ± 0.05^{a}	30.19 ± 0.65^{f}	61.55 ± 0.28^{b}
Artemia metanauplii	9.32 ± 0.48^{b}	16.59 ± 0.53^{e}	64.4 ± 0.78^{b}
Copepod meal	12.24 ± 0.05^{c}	12.94 ± 0.06^{bc}	66.74 ± 0.2^{b}
Daphnia sp. (dry)	45.18 ± 1.17^{f}	4.71 ± 0.06^{a}	48.1 ± 1.09^{a}
Daphnia magna (wet)	22.37 ± 0.58^{e}	13.34 ± 0.19^{c}	45.45 ± 3.90^{a}
Tubifex	17.06 ± 0.12^{d}	14.91 ± 0.22^{d}	66.33 ± 0.84^{b}

Different superscripts within a column indicate significant differences ($p<0.05$)

Table 3. Fatty acid levels of potential feed ingredients used as direct and indirect in aquaculture.

	Fish meal	*Artemia nauplii*	*Artemia metanauplii*	*Copepo meal*	*Daphnia sp. (dry)*	*Daphnia magna (wet)*	*Tubifex*
14:0	4.861	0.96	nd	9.1185	2.4455	nd	1.3445
16:0	20.421	8.3415	8.2575	10.977	23.484	8.849	1.4405
16:1	5.413	7.081	7.294	1.9415	6.7955	2.643	1.921
18:0	5.8125	3.337	4.7455	1.3655	4.374	2.2175	1.8625
18:1n-9	11.638	20.7855	27.5085	3.4795	18.1065	18.8675	4.5225
18:2n-6	1.224	2.094	2.94	0.791	2.9495	10.439	3.55
18:3n-3	nd	nd	nd	nd	0.215	nd	2.386
20:0	0.395	0.369	nd	0.695	nd	nd	3.352
20:1n-9	0.154	0.457	nd	nd	0.262	nd	1.585
20:4n-6	1.2335	1.5345	1.5965	nd	0.4185	1.167	2.688
20:5n-3 (EPA)	0.58	0.394	nd	0.8205	0.314	0.1205	0.378
22:0	0.122	7.314	nd	0.223	0.2395	1.4175	0.449
22:6n-3 (DHA)	11.1545	nd	nd	9.8395	nd	nd	nd
23:0	0.068	0.418	nd	0.1285	0.236	nd	0.314
24:0	11.9285	7.499	11.648	7.92	1.285	3.9975	7.7655
Saturated	43.608	28.2385	24.651	30.4275	32.064	16.4815	16.528
Unsaturated	31.397	32.346	39.339	16.872	29.061	33.237	17.0305
Total	75.005	60.5845	63.99	47.2995	61.125	49.7185	33.5585

nd: not detected; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid

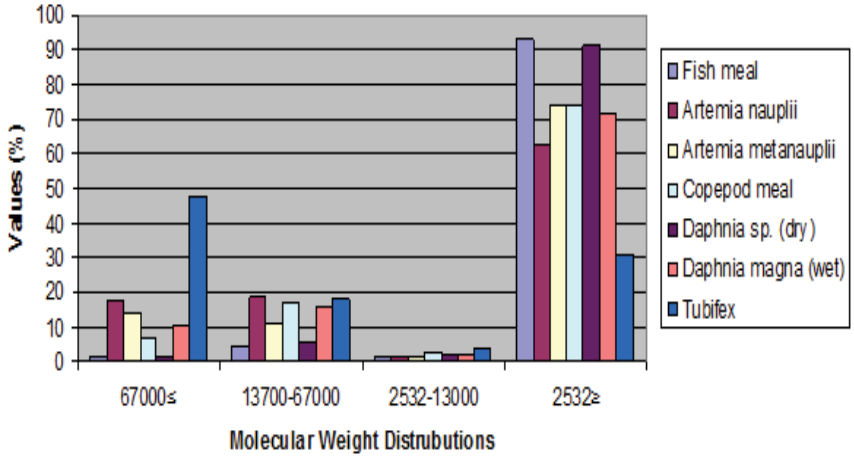

Figure 1. Molecular weight distributions of potential feed ingredients used as direct and indirect in aquaculture.

Based on the retention time of molecular weight standards, four fractions were defined: 67000 Da ≤, 67000 Da-13700 Da, 13700 Da-2532 Da and 2532 Da ≥. The differences between the molecular weight profiles observed of potential feed ingredients such as FM, ArtN, ArtMn, CopM, dryD, freshD and *Tubifex* were found in statistically significant ($p<0.05$). The highest and lowest levels of feed ingredients used were determined in 2532>= Da and 2532-13000 Da, respectively. The highest molecular weight profile belongs to 67000<=Da was observed in *Tubifex*. The molecular weight profiles belong to 67000<=Da and 13700-67000 Da of feed ingredients tested were lower than 20% except for *Tubifex*. The highest and lowest values determined in 2532>= Da was in FM and *Tubifex*, respectively. The other feed ingredients had higher molecular weight profile than ArtN values (62.36%). The molecular weight profiles of

ArtMn (73.63%) and CopM (73.84%) in 2532>= Da group were similar to each other followed by freshD (71.41%).

Discussion

Until now, there is no information about combine analysis of biochemical compositions and the molecular weight profiles for FM, ArtN, ArtMn, CopM, dryD freshD and *Tubifex*. In present study, the molecular weight profiles of potential feed ingredients were revealed by HPLC-Gel filtration chromatography and proximate compositions were determined.

The present results showed that the ash values of dryD and freshD were higher than those of other tested materials. Diken (2017) reported that the ash content of commercial fish meal as 11.83 ± 0.21%. This ash result was found lower than the present study (17.64 ± 0,06%). And Cauchie et al. (1999) found that the ash content of *Daphnia magna* were <10- 32.7%. Also, they indicated that under starvation, the relative ash content of *D. magna* has been found to increase. In present study, the ash value of dryD was higher than that of the reported in Cauchie et al. (1999). Naz (2008) showed that the ash levels of ArtN and ArtMn ranged from 11.52 ± 0.33 to 12.30 ± 0.15. Colombo-Hixon et al. (2013) stated that the ash content of CopM were 7.4%-13.8%. In this study, ash contents of ArtN and ArtMn values were lower than Naz (2008) while the ash content of CopM values was found in similar range with Colombo-Hixon et al. (2013). Mollah and Ahamed (1989), Yanar et al. (2003) and Rech et al. (2013) revealed that the ash contents of *Tubifex* were 7.95%, 9.74% and 4-7%, respectively. The reported values were lower than those of the present study. *Tubifex* ash content was found similar to FM and the ash content of other tested ingredients (except dryD avd freshD) were lower than those of FM results.

The data revealed that lipid values of ArtN, ArtMn, CopM, freshD and *Tubifex* were higher (except dryD) than that of FM lipid content. It is determined that the lipid content of FM (10.43 ± 0.20%) values reported by Diken (2017) was lower than current study FM lipid content. Naz (2008) determined that the lipid contents of ArtN and ArtMn were 20.97 ± 0.15% and 24.80 ± 0.48%. In the present study the lipid contents for ArtN was higher than Naz (2008) while lipid content of ArtMn was lower. The previous studies indicated that the lipid values of *Daphnia* were ranged from 0.91-19.7% (Watanabe et al. 1983; McKee and Knowles 1987; Elendt 1989; Habashy 1998; Cauchie et al. 1999; Kibria et al. 1999; Ghazy et al. 2009). In the current study, the lipid values were detected as 4.71 ± 0.06% for dryD and 13.34 ± 0.19%, for freshD. The differences in lipid values of *Daphnia* can be attributed to the consumed feeds, culture conditions and species differentiation. CopM lipid value was 12.94 ± 0.06% in the present study. This lipid values were found in similar range with Colombo-Hixon et al. (2013) and Evjemo et al. (2003) results. The differences observed may be due to losses during processing. Mollah and Ahamed (1989), Yanar et al. (2003) and Rech et al (2013) found that lipid contents of *Tubifex* sp. was 28.84%, 11.39% and 8-10%. However, current study results revealed that *Tubifex* sp. lipid content was found as 14.91 ± 0.22. This value showed in similar range with tested FM value. The observed values could be attributed to the substrate differences during the growth of *Tubifex*.

Diken (2017) was determined that protein content of FM (74.61 ± 0.46%) was higher than tested FM contents in the present study. Previous studies reported that about the protein content for *Daphnia magna* as 18.6-39.6% (Cauchie et al. 1999), for ArtN and ArtMn as 62.66 ± 0.47% and 49.10 ± 0.32% (Naz 2008), for CopM as 50.5%-64.8% (Colombo-Hixon et al. 2013) and for *Tubifex* sp. as 50-63.32% (Mollah and Ahamed, 1989; Yanar et al. 2003; Rech et al. 2013). Results showed that the protein contents of FM, ArtN, ArtMn, CopM and *Tubifex* were similar to each other and also found higher than freshD and dryD protein levels.

In the present study, even slightly fluctuations were detected for the ash, lipid and protein levels in tested ingredients, differences were also evaluated as sufficient levels for cultured fish. The marine fish larvae demand unsaturated fatty acids (PUFA) in critical larval stages. The previous studies showed that five PUFA are defined as essential fatty acids such as linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, linoleic and arachidonic acid (Von Elert 2002; Kainz et al. 2004; Martin-Creuzburg

et al. 2010)**.** Linoleic acid known as ω-6 acids is less susceptible to oxidation than the essential ω-3 acids. In this study, linoleic acid levels of the tested feed ingredients were found low levels except in freshD. This linoleic acid results were supported with the results belongs to ArtN and ArtMn previously reported by Naz (2008). Therefore, it needs to the combinations of alternative food sources including higher linoleic acid to provide an optimal balance and to produce feed with a higher shelf life that is more suitable as a food supplement. However, oleic acid levels of tested feed ingredients in the study were high.

The highest oleic acid levels were determined in ArtMn followed by ArtN, freshD, dryD, FM, *Tubifex* and CopM. In the study, except for DryD and *Tubifex* the linolenic acid were not found in other tested ingredients. Sener and Yıldız (2003) and Olurin et al. (2004) showed that linoleic or linolenic acids were essential for freshwater fish species. Lavens and Sorgeloos (1996) reported that EPA and DHA levels of *Daphnia sp.* were 10% and 0.2%, respectively. Results showed that EPA levels of freshD and dryD were quite low. The highest EPA level was observed in CopM and it followed by FM, ArtN, *Tubifex*, dryD and freshD but not observed in ArtMn. On the other hand, the highest DHA level was found in FM and it followed by CopM but DHA not determined in other tested ingredients. Yanar et al. (2003) claimed that DHA was not present in *Tubifex* while the authors EPA results were higher than the current EPA level of *Tubifex*. Moreover, present data showed that for the DHA levels of ArtN and ArtMn were lower than Naz (2008) results. The differences observed in DHA levels in ArtN and ArtMn can be attributed to the culture conditions, enrichment techniques and species/varieties differentiation.

The ability of copepods to synthesize some PUFAs is known as limited (Kainz et al. 2004; Burns et al. 2011). Some copepods are able to convert unsaturated fatty acids between fatty acid families. Therefore, copepods can be used to meet the nutritional requirements of fish larvae. In current study, arachidonic acid levels ranged from 2.69% (*Tubifex*) to 0.42% (dryD). Arachidonic acid levels of ArtN and ArtMn reported by Naz (2008) were 0.5 ± 0.01% and 2.0 ± 0.00%, respectively. Herawati et al. (2016) revealed that the total PUFA of *Tubifex tubifex* was 16.79%, the highest linoleic fatty acid was 7.25%, and the lowest was 1.45%. *Tubifex* PUFA and linoleic acid values determined by our study were found similar to those determined by Herawati et al. (2016).

Free amino acids (FAAs) seem to increase the performance of larvae when provided at low levels in feeds, but not a surplus of amino acids (AAs) (Szlaminska et al. 1993; Carvalho et al. 1997; Cahu et al. 1999; Cahu and Zambonino Infante 1995a; Lopez-Alvarado and Kanazawa 1995). The higher absorption of FAAs compared to protein bound, AAs may cause to AA imbalances in larval digestive system and followed lower protein utilization (Ronnestad et al. 2000). Zambonino Infante et al. (1997) revealed that the use of intact protein instead of di-and tripeptides in diets positively affected larval growth. Carvalho et al. (2003) indicated that whole protein are digested into peptides and AAs in the larval digestive system followed by di-and tripeptides are converted for absorption of AAs, a balance among the mentioned peptide groups seems to be important to optimize the protein utilization. Carvalho et al. (2003) suggested that the use of protein hydrolysates providing the mentioned optimum profile should be considered to reach this purpose. In addition, researcher indicated that diets should include a molecular weight profile similar to that determined in live food. A high FAA contents are typically found in the planktonic organisms that constitute the natural prey of marine fish larvae during the switch from endogenous to exogenous feeding (Fyhn et al. 1993; 1995). Moreover, the nauplii of the commonly used live prey *Artemia* contains an appreciable FAA pool (Helland et al. 2000).

In the study, the highest and lowest levels of feed ingredients used were determined in 2532>= Da and 2532-13000 Da. The highest molecular weight profile belongs to 67000<=Da was observed in *Tubifex*. The molecular weight profiles belong to 67000<=Da and 13700-67000 Da of feed ingredients tested were lower than 20% except for *Tubifex*. The highest and lowest values determined in 2532>= Da was in fish meal and *Tubifex*, respectively. The other feed ingredients had the molecular weight profile higher than 62.36% (ArtN). The molecular weight profiles

of ArtMn (73.63%) and CopM (73.84%) in 2532>= Da group were similar to each other followed by FreshD (71.41%). Molecular weights distribution belongs to the 2532>= Da group fish meal reported by Diken (2017) was higher than those of feed ingredients tested. Molecular weight profile results showed that feed ingredients were an exogenous supply of FAA as mentioned by Rønnestad et al. (1999). Also, molecular weight profiles of feed ingredients supported the results reported by Fyhn *et al.* (1993, 1995) and Helland et al. (2000).

In conclusion, accorded to their high protein level, FM, ArtN, ArtMn, CopM, dryD and freshD and due to their high lipid level, except for dryD, can make important contributions to microdiet formulations while dryD and freshD can be provided important contributions with the high ash levels to microdiet formulations because of their protein levels were lower than those of feed ingredients tested. Results revealed that the DHA level of CopM was similar to FM but was not detected in other feed ingredients while the EPA levels of all feed ingredients used were quite low but were not determined in ArtMn. The saturated fatty acid level of FM had the highest level. Except for the molecular weight profiles belong to 2532>= Da and 67000<=Da of *Tubifex*, in general, 2532>= Da levels of feed ingredients were high while 67000<=Da, 13700-67000 Da and 2532-13000 of feed ingredients tested were lower than 20%. Excepted *Tubifex*, the use of all feed ingredients in microdiet formulations may cause the high leaching ratios containing 2532>= Da molecular weight. However, the use of *Tubifex* in rations lead to the high leaching ratios containing 67000<=Da molecular weight. The knowledge obtained from the current study should be taken into account for optimum growth and survival ratio in further studies.

REFERENCES

AOAC. (2000). Official methods of analysis of Association of Analytical Chemist. 15th Edn. Washington DC.

Bligh, E.G, Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37(8), 911-917.

Boza, J.J, Jimenez, J, Martínez O, Suarez MD,Gil A (1994) Nutritional Value and Antigenicity of Two Milk Protein Hydrolysates in Rats and Guinea Pigs. The Journal of Nutritional 124: 1978-1986.

Burns CW, Brett MT, Schallenberg M (2011) A comparison of the trophic transfer of fatty acids in freshwater plankton by cladocerans and calanoid copepods. Freshwater Biology 56: 889–903.

Cahu CL, Zambonino Infante JL (1995a) Effect of the Molecular form of Dietary Nitrogen Supply in Sea Bass Larvae: Response of Pancreatic Enzymes and Intestinal Peptidases. Fish Physiol Biochem 14: 209–214.

Cahu CL, Zambonino Infante JL (1995b) Maturation of the Pancreatic and Intestinal Digestive Functions in Sea Bass (Dicentrarchus labrax): Effect of Weaning with Different Protein Sources. Fish Physiol Biochem 14: 431–437.

Cahu CL, Zambonino Infante JL, Quazuguel P, Le Gass MM (1999) Protein Hydrolysate vs. Fish Meal in Compound Diets for 10–day Old Sea Bass (Dicentrarchus labrax) Larvae. Aquaculture 171: 109–119.

Carvalho AP, Escaffre AM, Oliva-Teles A, Bergot P (1997) First feeding of common carp larvae on diets with high level of protein hydrolysates. Aquac Int 5:361-367.

Carvalho AP, Oliva–Teles A, Bergot P (2003) A preliminary study on the molecular weight profile of soluble protein nitrogen in live food organisms for fish larvae. Aquaculture 225: 445–449.

Cauchie HM, Jaspar-Versali MF, Hoffmann L, Thome JP (1999) Analysis of the seasonal variation in biochemical composition of Daphnia magna Straus (Crustacea : Branchiopoda : Anomopoda) from an aerated wastewater stabilisation pond. Annls Limnol 35 (4): 223-231.

Colombo-Hıxson SM, Olsen RE, Tıbbetıs SM, Lall SP (2013) Evaluation of Calanus finmarchicus copepod meal in practical diets for juvenile Atlantic halibut (Hippoglossus hippoglossus). Aquaculture Nutrition 19: 687-700

Das P, Sagar C, Mandal SC, Bhagabati SK, Akhtar MS, Singh SK (2012) Important live food organisms and their role in aquaculture. In: Frontiers in aquaculture. Sukham M. (ed), Narendra Publishing House, India, pp. 69-86.

Diken G (2017) The use of some animal and vegetable protein sources in the microdiets of meagre (Argyrosomus regius Asso, 1801) larvae. Institute of Science. PhD Thesis, 454 pp, Isparta, Turkey.

Elendt BP (1989) Effects of starvation on growth, reproduction, survival and biochemical composition of Daphnia magna. Arch. Hydrobiol 116:415-433.

Evjemo JO, Reitan KI, Olsen Y (2003) Copepods as live food organisms in the larval rearing of halibut larvae (Hippoglossus hippoglossus L.) with special emphasis on the nutritional value. Aquaculture 227: 191–210.

FAO (2002) The State of the World Fisheries and Aquaculture. FAO, Rome, Italy.

Fyhn HJ (1989) First feeding of marine fish larvae: are free amino acids the source of energy? Aquaculture 80: 111–120.

Fyhn HN, Finn RN, Helland S, Ronnestad I, Lomsland E (1993) Nutritional value of phyto-and zooplankton as live food for marine fish larvae. In: Reinertsen H, Dahle LA, Jorgensen L, Tvinnerheim K (eds) Fish farming technology. Balkema, Rotterdam, pp 121–126.

Garcés R, Mancha M (1993) One–Step Lipid Extraction and Fatty Acid Methyl Esters Preperation from Fresh Plant Tissues. Analytical Biochemistry 211: 139–143.

Ghazy MM, Habashy MM, Kossa FI, Mohammady EY (2009) Effects of salinity on survival, growth and reproduction of the water flea, Daphnia magna. Nature and Science 7(11): 28-41.

Graney RL, Keilty TJ, Giesy JP (1986) Free amino acid pools of five species of freshwater oligochaetes. Canadian Journal of Fisheries and Aquatic Sciences 43(3): 600-607.

Habashy MM (1998) Experimental studies on rearing the freshwater shrimp, Macrobrachium rosenbergii (De Man) by using certain aquatic insects and zooplankton present in the Nile water at El- Qanater El-Khayriya region. PhD thesis, Cairo: Ain Shams University. Faculty of science, Egypt, 210 pp.

Helland S, Triantaphyllidis GV, Fyhn HJ, Evjen MS, Lavens P, Sorgeloos P (2000) Modulation of the free amino acid pool and protein content in populations of the brine shrimp Artemia spp. Mar Biol 137: 1005–1016.

Herawati VE, Nugroho RA, Hutabarat J, Karnaradjasa O (2016) Profile of amino acids, fatty acids, proximate composition and growth performance of Tubifex tubifex culture with different animal wastes and probiotic bacteria. AACL Bioflux 9(3):614-622.

Higgs DA, Dosanjh BS, Prendergast AF, Beams RM, Hardy RW, Riley W, Deacon G (1995) Use of rapeseed/canola protein products in finfish diets. In: D.J. Sessa (Ed.), Nutrition and utilization of technology in aquaculture. AOCS Press, Champaign USA, 130-156.

Hilton JW (1983) Potential of freeze-dried worm meal as a replacement for fishmeal in trout diet formulations. Aquaculture 32:227-283.

Holt GJ (2000) Symposium on recent advances in larval fish nutrition. Aquac Nutr 6:141.

Hossain A, Hasan M, Mollah MFA (2011) Effects of Soyabean Meal and Mustard Oil-cake on the Production of Fish Live Food Tubificid Worms in Bangladesh. W.J. Fish and Marine Sci 3(3):183-189.

Hossain A, Hasan M, Mollah MFA (2012) Ratio optimisation of media ingredients for mass culture of tubificid worms (Oligochaeta, Tubificidae) in Bangladesh. Asian Fisheries Science 25:357-368.

Kainz M, Arts MT, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol. Oceanogr 49:1784-1793.

Kovalenko EE, D'Abramo LR, Ohs CL, Buddington RK (2002) A successful microbound diet for larval culture of freshwater prawn Macrobrachium rosenbergii. Aquaculture 210:385–395.

Kibria G, Nugegoda D, Fairclough R, Lam P, Bradbv A (1999) Utilization of wastewater- grown zooplankton: nutritional quality of zooplankton and performance of silver perch Bidyanus bidyanus fed on wastewater grown zooplankton. Aquaculture Nutrition 5(4):221-227.

Lavens P, Sorgeloos P (1996) Manual on the Production and Use of Live Food for Aquaculture. FAO Fisheries Technical Paper (361), Rome, 305 pp.

Lietz DM (1987) Potential for aquatic oligochaetes as live food in commercial aquaculture. Hydrobiologia 155(1):309-310.

López–Alvarado L, Kanazawa A (1995) Optimum Levels of Crystalline Amino Acids in Diets For Larval Red Sea Bream (Pagrus major). ICES Marine Science Symposia 201:100–105.

Lovell T. (1998) Nutrition and feeding of fish. Kluwer Academic Publishers, Massachusetts

Macedo CF, Pinto-Coelho RM (2001) Nutritional status response of Daphnia laevis and Moina micrura from a tropical reservoir to different algal diets: Scenedesmus quadricauda and Ankistrodesmus gracilis. Brazilian Journal of Biology 61: 555-562.

Marian MP, Pandian TJ (1984) Culture and harvesting techniques for Tubifex tubifex. Aquaculture 42(3-4): 303-31.

Martin-Creuzburg D, Wacker A, Basen T (2010) Interactions between limiting nutrients: consequences for somatic and population growth of Daphnia magna. Limnol Oceanogr 55: 2597–2607.

McKee MJ, Knowles CO (1987) Levels of protein, RNA, DNA, glycogen and lipid during growth and development of Daphnia magna Straus (Crustacea : Cladocera). Freshwat Biol 18:341-351.

McKinnon AD, Duggan S, Nichols PD, Rimmer MA, Semmens G, and Robino B (2003) The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquaculture 223: 89–106.

Memis D, Ercan E, Celikkale MS, Timur M, Zarkua Z (2009) Growth and survival rate of Russian sturgeon (Acipenser gueldenstaedtii) larvae from fertilized eggs to artificial feeding. Turkish Journal of Fisheries and Aquatic Sciences 9:47-52.

Mollah MFA, Ahamed MT (1989) A note on preliminary study of culture of Tubificids Worms. Bang. J. Fish 12: 91-95.

Mollah MFA, Mosharaf K., Mariom Dr. (2012) Selection of suitable media and intervals of media inoculation for culturing tubificid worms. Journal of Bangladesh Agriculture University 10(2):325-330.

Naz M (2008) The changes in the biochemical compositions and enzymatic activities of rotifer (Branchionus plicatilis, Müller) and Artemia during the enrichment and starvation period. Fish Phys and Biochem 34:391-404.

Olurin KB, Akınyemi Y, Obe OY, Olojo EAA (2004) Use of palm oil in the diet of the African mudfish, Clarias gariepinus. African Journal of Biotechnology 3(8): 418-420.

Rech KC, Guereschi RM, de Oliveira Nuner AP, Sticca SC (2013) Density and organic matter influence on egg-laying and hatching of Branchiura sowerbyi (Oligochaeta). Journal of the World Aquaculture Society 44(2):267-272.

Ronnestad I., Thorsen A. and Finn R.N. (1999) Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture, 177:201–216

Ronnestad I, Conceicao LEC, Arago C, Dinis MT (2000) Free amino acids are absorbed faster and assimilated more efficiently than protein in postlarval Senegal sole (Solea senegalensis). J Nutr 130: 2809-2812.

Schipp GP, Bosmans JMP, Marshall AJ (1999) A method for hatchery culture of tropical calanoid copepoda, Acartia spp. Aquaculture 174: 81–88.

Sener E, Yıldız M (2003) Effect of the different oil on growth performance and body composition of rainbow trout (Oncorhynchus mykis W., 1792) juveniles. Turkish Journal of Fisheries and Aquatic Sciences 3:111-116.

SPSS (1993) SPSS for windows base system user's guide, release 8.0.2.Chicago USA.

Støttrup JG (2000) The elusive copepods: their production and suitability in marine aquaculture. Aquaculture Research 31: 703–711.

Szlaminska M, Escaffre AM, Charlon N, Bergot P (1993) Preliminary data on semi synthetic diets for goldfish (Carassius auratus L.) larvae. In:Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practice. INRA, Paris, pp.607-612.Les colloques, 61.

Tacon AGJ (1993) Feed ingredients for warmwater fish: fish meal and other processed feedstuffs. FAO Fisheries Circular No. 856, 64 pp, Rome.

Vineetha A (2001) An evaluation of intertidal oligochaetes Pontodrilus bermudensis Beddard as dietary supplement for stimulation of reproduction in Penaeus semisulcatus De Haan. PhD Thesis, Central Institute of Fisheries Education, Mumbai, India, 156 pp.

Von Elert E (2002) Determination of limiting polyunsaturated fatty acids in Daphnia galeata using a new method to enrich food algae with single fatty acids. Limnol Oceanogr 47: 1764–1773.

Watanabe T, Kitajima C, Fujita S (1983) Nutritional values of life organisms used in Japan for mass propagation of fish: a review. Aquaculture 34:115-143.

Watanabe T, Kiron V (1994) Prospect in larval fish dietetics. Aquaculture 124:223-251.

Yanar M, Yanar Y, Genç MA (2003) Tubifex tubifex Müller,1774 (Annelidae)'in Besin Kompozisyonu. Journal of Fisheries and Aquatic Sciences 20 (1-2): 103-110.

Zambonino Infante JL, Cahu CL, Peres A (1997) Partial substitution of di-and tripeptides for native proteins in sea bass diet improves Dicentrarchus labrax larval development. J Nutr 127: 608-614.