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Effects of Dietary Omega-3 Fatty Acids (DHA/EPA) and Vitamin E on Fatty Acid Profile of Common Carp, *Cyprinus carpio* Ovaries

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Abstract: The current study aimed to clarify the fatty acids profile in the breeding stock ovaries of common carp (*Cyprinus carpio* L.) fed on two food additives. The study was carried out in the Shrimp Hatchery which belonging to the Basrah Agricultural Directorate. Three treatment groups were used, the first treatment (T1) represented as control without any food additives, the second treatment (T2) supplemented with 5 g/kg omega-3 fatty acids, and the third treatment (T3) to which vitamin E was added at a concentration of 200 mg/kg. Fishes were randomly distributed in three replicates for each treatment with six of common carp brood stock per replicate. Nine cages were used which placed in earthen pond. Feeding trail lasted from 29 Oct 2018 to 14 Mar 2019. After feeding fish for 82 days, the oil was extracted from gonads by two methods, the first one by Soxhlet apparatus and the second by Accelerated Solvent Extraction (ASE) method. The fatty acid profiles were analyzed using gas chromatograph by GC/MS (Gas Chromatography Mass spectrometry). Results showed that the content of fatty acids in the ovaries of treated fishes in T1, the presence of saturated and unsaturated omega-3 and omega-6 fatty acids, were monounsaturated fatty acid ($C_{19}H_{36}$:1) scored the highest percentage (52.55%). Fishes in T2 recorded the presence of saturated and unsaturated omega-3 and omega-6 fatty acids were omega-12 ($C_{18}H_{34}$:12) recorded the highest percentage (50.02%), while T3 fishes showed the presence of saturated and unsaturated fatty acids from the group of omega-6 fatty acids and the unsaturated fatty acid omega-9 ($C_{18}H_{34}$:9) record the highest percentage (63.24%).In conclusion this study suggested that the content of fatty acids in fish ovaries reflects the composition of fatty acids in the diet.

Keywords: Reproduction, Gonads, Food additives, Brood stock, Fatty acids, Vitamin

Introduction

Fishes represent about 17% of the animal protein consumed by the world's population. Moreover, fishes provided about 3.2 billion people, approximately 20% of the average per capita share of animal protein. Fishes are a very nutritious source and support the deficiency of nutrients in other sources (FAO, 2018).

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Fishes also provide valuable nutrients, such as Docosahexanoic (DHA) and Eicosapentaenoic (EPA) acids, both of which are long-chain polyunsaturated fatty acids (PUSFA) omega-3. These are important acids to achieve optimal level of neurodevelopment in children and to improve vascular and heart health, there is compelling evidence of beneficial health outcomes for fish consumption in order to reduce the risk of death from coronary artery disease. In addition, fishes are an important source of healthy fatty acids, essential amino acids, vitamins and minerals this unique nutritional composition means that fishes represent a valuable source for healthy dietary diversification, even in relatively small quantities. This is more important for many low-income food-deficit countries (LIFDCs) and least developed countries (LDCs) (FAO, 2020).

Scientific researches indicated that the functions of essential fatty acids of the two groups i.e. omega-3 and omega-6 are fluctuated after knowing that high doses of essential fatty acids from omega-6 have some risks affecting health when the ratio increased from 1:1 during development to 20:1 at present or even higher (Simopoulos, 2016). Increasing in omega-6 intake could induce cancerous tumors, formation of self-thrombosis, and low immunity, so recommendations for increasing n-3 and reducing n-6 consumption have been proposed by nutritionists (Zhang et al., 2020). Health benefits of essential omega-3 fatty acids, especially Docosahexaenic (DHA, C22:6 n-3) and Eicosapenaenic acid (EPA, C20:5 n-3) which found in fish oil and alpha-linolenic acid (ALA, C18:3 n-3) that found in vegetable oils contradict the harmful effects of omega-6 fatty acids (Burdge et al., 2002). Omega-3 was considered an essential fatty acid for humans as final consumer of fishes (Ramezani-Fard et al., 2012).

The need for essential fatty acids is difficult because of its dependence on the quality and source of fat, the ratio of omega-3/omega-6 in food and the fatty acid metabolism within the body (Bezard et al., 1994). Jabeen & Chaudhry (2011) studied the chemical composition of fatty acids in three species of freshwater fishes including common carp from Indus river in Pakistan and found that palmitic acid was found to be more abundant between 32-46%, although these three species of fishes contain a reasonable amount of PUFA omega-3 fatty acids (EPA, DHA, AA).

Vitamin E is one of the most important non-enzymatic soluble antioxidants in fat, as it works to remove the oxygen root and thus is the first line in removing fat oxidation (Puangkaew et al., 2005). Naturally, it consists of from alpha-tocopherol, beta-tocopherol, delta-tocopherol, Kama-tocopherol, as well as alpha-tocotrienol, beta-tocotrienzole, delta-tocotrienol (Sen et al., 2006), researchers indicated the importance of vitamin E in improving the immune response of fishes, as it modifies the physiological changes of rainbow trout *Oncorhynchus mykiss* when fed on diets containing high concentrations of unsaturated fat (Puangkaew et al., 2005). In the study of Sharifzadeh et al. (2015) it was found that improving growth parameters of common carp (*Cyprinus carpio*) with increasing of dietary levels of vit E may be related to the antioxidant role of this vitamin.

The common carp (*C. carpio*) represents one of the most important species of carp family in economic value in Iraqi internal waters. This species of fishes is abundant in various water bodies in the country, especially in the southern and central sections, and it was considered one of the important economic fishes and it is the first cultured fishes in warm water (Al-Daham, 1990). Common carp was the most important species among the productive aquatic species that contribute globally to more than 72% of freshwater production (Kühlwein et al., 2014).

Chacón-Guzmán et al. (2020) revealed that evaluation of fatty acids present in fish eggs and gonads were important to assist in obtaining nutritional information necessary to improve the diets provided in aquaculture production.

The current study aimed to determine the quality of fatty acids in common carp ovaries fed two types of diets containing feed additives, i.e., omega-3 fatty acid and vitamin E.

Materials and Methods

Site of the Study

The research work was implemented for all experiments in the Shrimp Hatchery belonging to the Basrah Agricultural Directorate for the period from 29 October 2018 to 3 March 2019. The study site is located in the north of Basrah Governorate, 22 km from the city center. The hatchery area is one hectare consisting of four earthen ponds, (20 × 80 m) and 1.5 m depth. In addition, it contains a hatching hall with an area of 8 × 12 m.

Experimental Fish

Common carp brood stock (54 fishes) used in all study experiments, were brought from a local floating cages of fish farms in Al-Qurnah city. Fishes were transported to the study site by pickup. Fishes were sterilized by using saline solution (5% NaCl) for 5-10 minutes as soon as they arrived at the work site to get rid of fungi and other organisms that may stuck on the skin or gills. The average weight of fishes were 1476.74±193.58, 1699.17±350.99 and 1723.06±168.15 g for T1, T2 and T3, respectively.

Experimental Design

The experiment was designed with three treatments (control T1, omega-3 T2 and vitamin E T3), each treatment has three replicates, six fishes for each replicate. The fishes were randomly distributed in nine cages made by polypropylene random copolymer (PPR) material for the three treatments (length x width x height 3 × 1.7 × 1.8 m), surrounded by nets with 10×10 mm mesh size, to isolate the three treatments and easy monitoring and feeding of fishes. These cages were placed inside the earthen ponds, which filled with water to one meter height. The brood stock was fed with 3% of body weight two times a day, one in the morning at 8 AM and the second in the afternoon at 2 PM. The amount of feed was adjusted according to the changing of the biomass every two weeks.

Experimental Diets

The diets that used in this experiments were prepared after calculating the percentages of each feed component (homemade fish meal, wheat flour, wheat bran and sunflower oil) without any additives which represent the control treatment (T1). Omega-3 oil, manufactured to IKO-IRAQ-BAGHDAD, was added at a rate of 5 g/kg diet and represent the T2 treatment, vitamin E was added at a rate of 200 mg/kg diet and represent the T3 treatment, and additives were selected according to NRC (2011), as shown in Table 1. Then, the diet was mixed for homogeneity and manufactured in the feed factory in the College of Agriculture, University of Basrah. The chemical analysis of the diets was carried out in the Quality Control Laboratory of the Animal Resources Department of the Ministry of Agriculture.

Table 1: Ingredients and chemical analysis of the experimental diets.

Ingredients	Control (T1)	Omega-3 (T2)	Vitamin E (T3)
Fish meal (g/100g)	30	30	30
Wheat flour (g/100g)	45	45	45
Wheat bran (g/100g)	23	23	23
Sunflower oil (g/100g)	2	2	2
Omega-3 (g/kg)	0	5	0
Vitamin E (mg/kg)	0	0	200
Chemical analysis (%) as dry matter			
Moisture	10.1	9.7	8.8
Crud protein	21.9	23.3	22.8
Crud lipid	4.1	5.5	4.8
Crud fiber	4.6	4.1	3.9
Nitrogen Free Extract (NFE)	59.3	57.4	59.7

Gonad Sampling

After feeding the fishes for 82 days, three fishes were isolated from each treatment, fishes were individually measured (total length (TL); cm)**,** weighed (BW; g), and were dissected to remove the gonads which placed in a plastic container. For each treatment, 200 g from gonad of each treatments was stored in plastic bags and placed in the refrigerator until examination.

Soxhlet Extraction

Fatty acids were determined by extracting fats from the common carp ovaries of the three treatments (control, omega-3 and vitamin E) using the Soxhlet apparatus with the organic hexane solvent at a temperature of 1±68 °C for eight hours (Banat et al., 2013).

Accelerated Solvent Extraction (ASE)

The oil was extracted from common carp ovaries using the modified Bligh & Dyer (1959) organic solvent method after drying the samples with a dryer at a temperature of 30 °C until the moisture was completely removed from the samples.

Methyl Esterification of Fatty Acids

The sample was prepared according to the method described in AOAC (1984) based on the esterification of triglycerides by its interaction with methyl potassium hydroxide standard.

Fatty acids were identified in the oil extracted from the common carp ovaries by Gas chromatography-Mass spectroscopy connected to the SHIMADZU (Ultra QP210 GSMS) mass spectrometer of Japanese origin in the GS-MS Lab, Environmental and Water Department, Environmental Research Center, Baghdad.

Results

Results of the analysis of fatty acids profile in the oil of common carp ovaries which fed control diet (T1) showed the presence of saturated (SF), monounsaturated (MUFA) and polyunsaturated (PUFA) (Table 2). The saturated fatty acids Palmitic recorded 30.43%, while the monounsaturated fatty acid Octadecenoic recorded the highest value 52.55%, the polyunsaturated fatty acids DHA, Arachidonic recorded 2.52% and 6.64%, respectively (Figure 1).

In the ovaries, oil of fishes fed on omega-3 fatty acids (EPA and DHA) showed that the monounsaturated 6-Octadecenoic acid scored 50.02% (Table 1), whereas the percentage of saturated fatty acid (hexadecanoic acid) was 35.55%, omega-6 and omega-3 acids were observed and both EPA and DHA recorded 5.52% (Figure 2).

Omega-3 fatty acids did not appear in the ovaries oil of fishes feeding on the vitamin E diet (Table 1). 10-Octadecenoic acid scored 63.24%, the percentage of saturated Hexadecanoic fatty acid was 28.93%, and omega-6 Arachidonic acid is less saturated with a ratio of 1.96% (Table 2).

The profile for an analysis of fatty acids from the common carp ovaries of the three treatments showed the following pattern MUFA> SFA> PUFA (Figure 2).

The lowest n3/n6 ratio was observed in the ovaries oil of fishes fed vitamin E diet (0.00), followed by control (0.38) while the highest observed in the ovaries oil of fishes fed omega-3 diet (4.92).

Table 2: Fatty acid profile (% of total lipid) of *Cyprinus carpio* females fed three diets.

Fatty acids	Common name	IUPAC name	Chemical Formula	Shortened name	% Total lipid		
					T1	T2	T3
Saturated	Caprylic	Octanoic	$C_8H_{16}O_2$	C8:0	0.03		
	Lauric	Dodecanoic	$C_{12}H_{24}O_2$	C12:0		0.06	
	Pentadecylic	Pentadecanoic	$C_{15}H_{30}O_2$	C15:0			4.49
	Palmitic	Hexadecanoic	$C_{16}H_{32}O_2$	C16:0	30.43	35.55	28.93
	Stearic	Octadecanoic	$C_{18}H_{36}O_2$	C18:0		5.59	
	Tricosylic	Tricosanoic	$C_{23}H_{46}O_2$	C23:0	5.71		
Σ Saturated					36.17	41.2	33.42
Mono-unsaturated	Lauroleic	9-Dodecenoic	$C_{13}H_{24}O_2$	C13:1n-3			0.98
	Isooleic	10-Octadecenoic	$C_{18}H_{43}O_2$	C18:1n-9			63.24
	Petroselinic	6-Octadecenoic	$C_{18}H_{34}O_2$	C18:1n-12		50.02	
	Octadecenoic	methyl (Z)-octadec-14-enoate	$C_{19}H_{36}O_2$	C19:1n-4	52.55		
	Nonadecenoic	methyl nonadec-10-enoate	$C_{20}H_{38}O_2$	C20:1n-9	2.03		
	Erucic	13-Docosenoic	$C_{22}H_{42}O_2$	C22:1n-9		2.83	
Σ Monounsaturated					54.58	52.85	64.22
Poly-unsaturated	Eicosapentaenoic (EPA)	5,8,11,14,17-pentaenoic	$C_{20}H_{30}O_2$	C20:5n-3		2.88	
	Docosahexanoic (DHA)	4,7,10,13,16,19-hexaenoic acid	$C_{22}H_{32}O_2$	C22:6n-3	2.52	2.64	
	Gamolenic	6,9,12-octadecatrienoic	$C_{18}H_{30}O_2$	C18:3n-6		0.37	
	Arachidonic (ARA)	Eicosatetraenoic	$C_{20}H_{30}O_2$	C20:4n-6	6.64		1.96
Σ Polyunsaturated					9.16	5.89	1.96
Σ Omega-3					2.52	5.52	0.00
Σ Omega-6					6.64	0.37	1.96
n-3/n-6					0.38	14.92	0.00

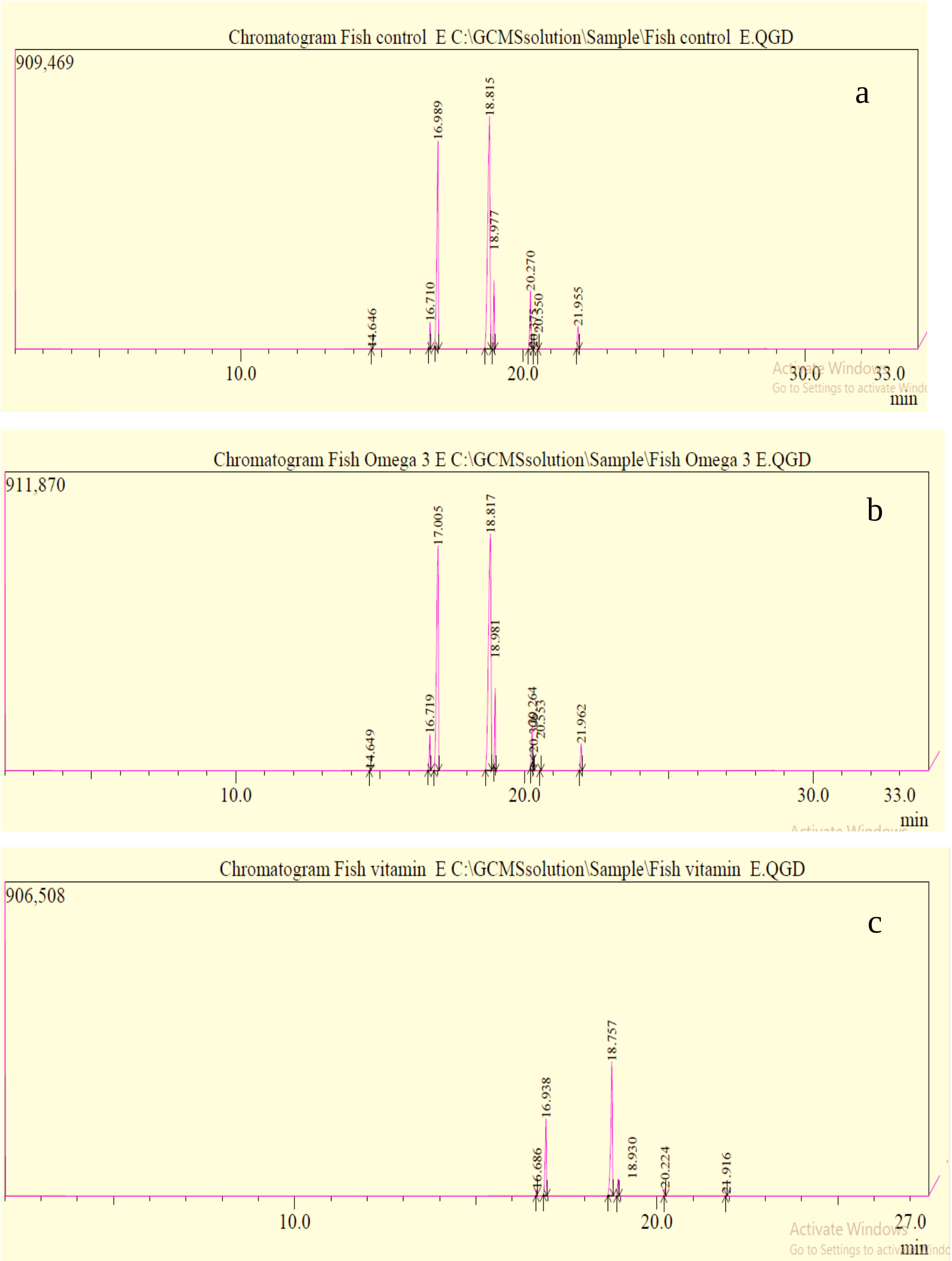

Figure 1: Fatty acids profile of *C. carpio* females fed on control diet (a), omega-3 (b) and vitamin E (c) diet.

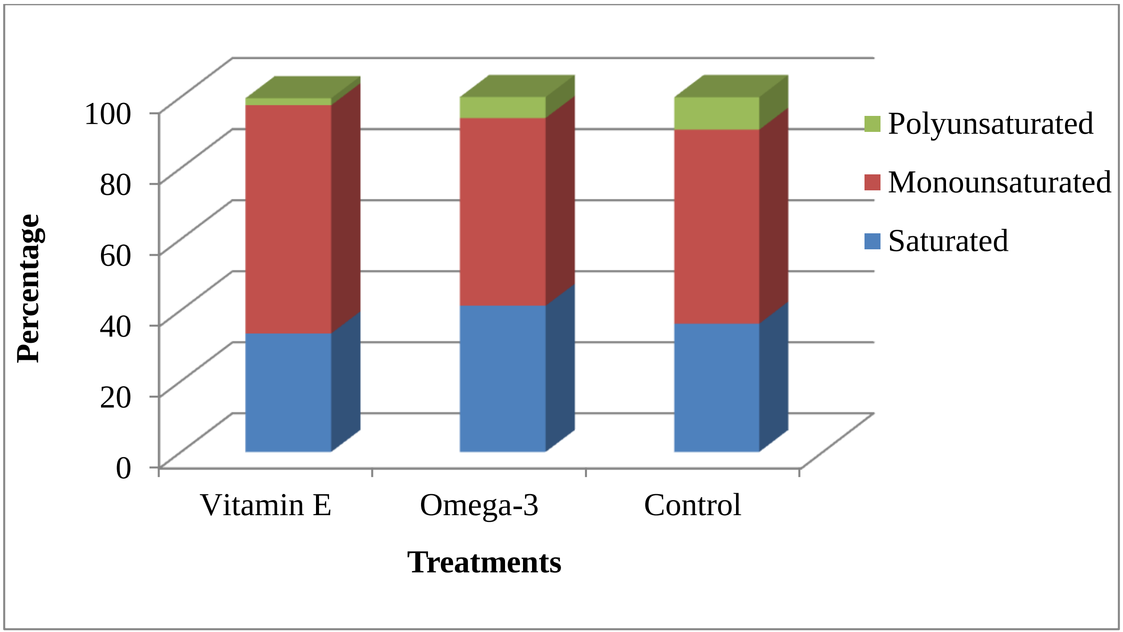

Figure 2: Fatty acids percentage of *Cyprinus carpio* females fed three diets.

Discussion

The emphasis in fish reproduction management has focused about lipid quality due to its importance in tissue formation and as a source of energy in the larval stages of fishes. Results showed that vitamin E diet reflect a decrease in the quality of the fatty acids profile of common carp ovaries. In spite that saturated and unsaturated fatty acids have been recorded, except to omega-6 (ARA), the omega-3 fatty acids (EPA and DHA) have not been recorded compared to the control and omega-3 diets. This indicate the correspondence with the direct effect of dietary fatty acids have on the fatty acid profile of body tissues, this has been confirmed for a number of fishes (Izquierdo et al., 2003; Bell et al., 2004; Turchini et al., 2009; González-Félix et al., 2017). It is clear from the above results that the diet contains omega-3 fatty acids has shown a diversity of fatty acids in the ovaries which greatly affected by changing the composition of fatty acids in the diet. Palmitic acid (16:0) was the most abundant unsaturated fatty acid recorded in all treatments (30.43, 35.55 and 28.93%, respectively). These result resemble the fatty acids profile of wild common carp eggs in India (Mukhopadhyay & Ghosh, 2003). Mourente & Odriozola (1990) indicated that the composition of fatty acids in eggs of *Sparus aurata* had significant differences because of the influence of fatty acid**s** in broodstock diet, our results resemble these indication.

Fish elongate (adding two carbon atoms) to essential fatty acids C: 18 to longer than C: 20 and C: 22 with the ability to desaturation the fatty acids (adding double bonds). Steffens et al. (1995) and Steffens (2009) found that the common carp fed on the natural food showed an increase in the content of omega-3 fatty acids in muscle tissue, and this level decreased when adding wheat grains to the diet. This indicates that the composition of fatty acids in muscle tissue reflects significantly the composition of the diet fatty acids also they recorded percentage of omega-6/ omega-3 fatty acids in common carp muscle tissue ranged between 0.8 and 2.4%.

Bell et al. (1993) indicated that most freshwater fishes achieve the needs of omega-3 fatty acids because of their ability to quickly convert α-Linolenic acid fatty acid (ALA) to a higher methyl 20: 5n-3 (EPA) and 22:6n-3 (DHA). Bou et al. (2017) observed that the brain and heart of Atlantic salmon (*Salmo salar*) retained the levels of PUSFA omega-3 (EPA/DHA) superior than muscles, intestines, skin and liver. Omega-3 PUFA (EPA and DHA) observed in the T2 and only DHA found in control diet, while in vitamin E none of these fatty acids was found. This confirms the conclusion of Tocher et al. (2006) that vertebrates have no ability to synthesize PUFA n-3 internally because of the absence of de-saturating and chain elongation enzymes (Δ^{12} and Δ^{15}), so they get these fatty acids from food such as phytoplankton that are rich in DHA. Böhm et al. (2014) found an increase in the concentration of omega-3 fatty acids in common carp muscles when adding EPA and DHA fatty acids at a concentration of 17 and 20 mg fish/day to their diet compared to fish fed diet containing vegetable oil, and this indicates that common carp retained high efficiency with polyunsaturated fatty acids. Glencross (2009) reported that the needs of common carp from omega-3 fatty acids (EPA and DHA) were low compared to ARA. Garrido et al. (2007) found a relationship between essential fatty acids in the muscle and those in the gonads of sardine (*Sardina pilchardus*).

Spiric et al. (2010) observed that the fatty acids extracted from the muscles of the common carp by Soxhlet method revealed high levels of omega-6 but omega-3 fatty acids were not recorded, while the accelerated solvent extraction (ASE) showed the presence of omega-6 and omega-3 fatty acids in muscle tissue. This is consistent with the results of the present study, as fatty acids were extracted from common carp ovaries by Soxhlet were no omega-3 fatty acid recorded but only saturated, unsaturated and omega-3 and omega-6 recorded by ASE method and this can be attributed to the high temperature which affected the quality of the extracted oil. Emery et al. (2016) indicated that the needs of Atlantic salmon from omega-3 fatty acids are low, and that the acid EPA metabolized to produce energy or transform into a DHA that precipitates in muscle tissue.

Stancheva & Merdzhanova (2011) studied the composition of fatty acids in three species of freshwater fishes and found that the omega-6 fatty acids were the highest compared to omega-3 in all examined fishes. Hadjinikolova (2004) confirmed that common carp fat levels are highly dependent on the type food. Jabeen & Chandhry (2011) observed that there are differences in the pattern of the fatty acid profile in common carp in Indus river in terms of arrangement, as SFA> MUFA> PUFA, which depends on the specificity of the aquatic ecosystem of the region. It is inconsistent with the results of the present study, as the pattern of the fatty acid profile was MUFA> SFA> PUFA. Tilapia needs of vitamin E is affected by the level of dietary fat, especially PUFA and requirement increases almost linearly with the degree of unsaturation (Shiau & Shiau, 2001; Raederstorff et al., 2015). Lim et al. (2009) observed a rise in the total fat content in the body of Nile tilapia fed on a diet containing 100 mg/kg of vitamin E, but decreased when fed on a diet containing 200 mg/kg of vitamin E. This corresponds to the results of the current study. We conclude that feeding common carp on a diet containing omega-3 promoted the diversity of fatty acids in ovaries oil. In addition, fishes can obtain fatty acids from the natural food (algae). This was observed in vitamin E and control treatments.

The level of omega-3 in ovaries of fishes fed on control and vitamin E diets were lower than omega-6. Some studies suggested that the levels of omega-3 must be lower than omega-6 fatty acids in diets of freshwater fishes, but Sargent et al. (2002) observed superior for omega-3 production, especially DHA, than for omega-6 in ovaries. This is in agreement with Coldebella et al. (2013) and Hilbig et al. (2019).

Liang et al. (2014) suggested that optimum dietary ratios of n3/n6 are required to ensure the quality of the eggs and larvae but can be species-dependent. This corresponds with the present results except for ovaries of omega diet group. Kottmann et al. (2020) found that prolonged feeding resulted in higher ARA (n6) and lower EPA (n3) levels in the unfertilized eggs, while DHA (n3) levels did not change. Sargent et al. (1999) recommended 2:1 as the optimal dietary ratio of DHA: EPA and 1:1 of EPA: ARA for sea bass larvae.

Conclusions

The current study showed the importance and role of fatty acids, especially omega-3 in fish diets, and its effect on the fatty acids profile in eggs, which will affect the characteristics and quality of the resulting larvae. Subsequent studies can be conducted on the overlap between fatty acids and vitamin E with different rates of additions.

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