ORIGINAL ARTICLE

Veterinary Research Forum. 2024; 15 (9): 463 - 471
doi: 10.30466/vrf.2024.2013685.4035
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Investigating the effects of different DHA/EPA ratios in rainbow trout (*Oncorhynchus mykiss*) egg composition on foregut development of larvae

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Article Info

Article history:

Received: 16 October 2023
Accepted: 05 June 2024
Available online: 15 September 2024

Keywords:

Digestive tract
Docosahexaenoic acid
Eicosapentaenoic acid
Rainbow trout

Abstract

Lecithotrophic larvae utilize extensive yolk reserves for early development. In this study, the effect of egg docosahexaenoic acid (DHA):eicosapentaenoic acid (EPA) ratios (*i.e.*, 5.92, 10.08, 11.66, and 14.53) on the emerging larvae foregut development of rainbow trout was examined. Larvae samples were taken from day 22 to 36 post-fertilization. Thin whole body longitudinal sections were prepared and stained by Hematoxylin and Eosin and Alcian blue procedure. The sections were examined regarding epithelial layer thickness, intestinal fold height and mucosal layer thickness along with number of enterocytes and goblet cells. Results indicated that maximum thickness of the epithelium was observed on day 36 post fertilization in larvae hatched from eggs with DHA:EPA ratios of 14.53 and 10.08. The highest and lowest intestinal folds height were also observed in larvae hatched from eggs with DHA:EPA ratios of 10.08 and 14.53, respectively. The mucosal-submucosa layer thickness was the highest in larvae hatched from eggs with DHA:EPA ratio of 10.08. Enterocyte's count was the highest in larvae obtained from eggs with DHA:EPA ratio of 10.08 on day 36 post-fertilization. The highest and lowest number of goblet cells were enumerated in larvae obtained from eggs with DHA:EPA ratios of 5.53 and 14.53, respectively. In conclusion, our results revealed that feeding rainbow trout broodstock with diet contained highly unsaturated fatty acids (HUFA):polyunsaturated fatty acids (PUFA) ratio of 0.28 could result in the egg with DHA:EPA ratio of 10.08 which in turn yielded larvae with better foregut development parameters compared to those larvae emerged from the eggs with increased DHA :EPA ratio.

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the mostly grown cold-water fish species in Iran and is economically valuable seafood at the counter. The total worldwide production of rainbow trout is 739.50 kiloton and Iran is producing 167.80 kiloton.[1]

Fish oil (FO) is the most favorable lipid source for fish feed formulation since it contains higher amounts of eicosa-pentanoic (EPA) and docosahexaenoic acid (DHA). These are essential fatty acids required for better physio-logical state and growth performance.[2] Global demand for FO is increasing while its availability is limited so that global FO supplies will not be able to fulfill the increasing FO demand of aquafeeds anymore in near future.[3] There-fore, alternative lipid sources are increasingly included in fish feed to ensure sustainable development of the industry.

Plant oils (POs) are the excellent choice of FO substitute because of their availability and price.[4] Reportedly, fresh-water and salmonids may meet their essential fatty acids requirements using dietary linolenic acid (ALA; 18:3n-3) and linoleic acid (LNA; 18:2n-6).[5] The POs are devoid of EPA and DHA, which are commonly found in different FO sources.[6] Despite salmonids ability to elongate and desaturate fatty acids, bioconversion has been found not to be sufficient to compensate for considerable decrease in dietary n-3 long-chain polyunsaturated fatty acids (LC-PUFA) intake. It has been shown that complete dietary FO replacement by PO would result significant reduction of tissue n-3 LC-PUFA content.[7] It has also been shown that dietary fatty acid contents could affect quality of eggs and larvae of various fish species including rainbow trout.[8] The yolk sac is the source of fatty acids that fish larvae rely exclusively on it during the early developmental period.[9]

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The EPA and DHA are essential long-chain omega-3 fatty acids since they are building blocks of different body organ components, e.g. muscle, brain and eye retinal organogenesis and development.[10] Deficiency of such fatty acids might lead to anemia, increased mortality, and reduced feed efficiency in fish.[11] At the initial stage of fish growth, lower or inappropriate dietary contents of EPA and DHA might result in delayed responsiveness to environmental stimuli and increased abnormal behavior including delay in predator escape behavior and decreased ecological fitness specifically for those species propagated for fish stock enhancement program.[12] In addition, the fatty acids are also important for gilthead seabream (*Sparus aurata*) larvae growth and survival. It has been shown that dietary EPA and DHA contents affect fish response to various environmental stresses. In addition, they are crucial for efficient maturation and reproduction.[13] Moreover, Agh *et al.*[13] and Kottmann *et al.*[8] showed that dietary ratio of DHA:EPA could affect egg quality and fish offspring development. The DHA and EPA are also efficient energy sources during rainbow trout egg development.[14]

The digestive tract is actively involved in feed/ nutrient digestion and absorption.[15] Development of digestive tract could directly affect larval ability to uptake essential nutrients for growth and other physiological events.[16] Shifting from yolk sac nutrition to active/ exogenous feeding requires digestive tract oncogenic development and achieving nutrient digestion and absorption capacity, which affects fish survival, growth, and development.[17] It is fundamental to study the physiology of digestion and absorption of nutrients at different stages of fish ontogeny to determine the main mechanisms that lead fish larvae to successfully pass through the critical fish larval growth stanza for successful aquaculture.[17]

In the present study, the effect of various DHA:EPA ratios of eggs were investigated on the foregut development of rainbow trout larvae from hatching to active feeding.

Material and Methods

Broodstock feeding and egg incubation. The present study was conducted at Artemia and Aquaculture Research Institute, Urmia University, Urmia, Iran. Rainbow trout broodstocks were hand fed experimental diets containing 48.00 ± 0.60% crude protein, 20.00 ± 0.05% crude lipid, and 5.00 ± 0.20% ash for 4 months before final sexual maturation. The diets only differed regarding their dietary lipid sources and therefore their fatty acid profiles (Tables 1 and 2). In present experiment, the diet 1 (D1) contained fish oil as the main dietary lipid source, while the other diets were supplemented with differing contents of plant oils to replace the fish oil. The female and male broodstocks were separated two weeks before final sexual maturation. Semen and ova were manually stripped 3 days after the last meal. After semi-dry fertilization and egg hydration, the fertilized eggs were transferred to incubators. At the same time, two grams of egg samples were taken from each experimental groups to analyze their fatty acid profile (Table 3). All experimental eggs were incubated under similar environmental conditions regarding light intensity, water source and water flow rate throughout the incubation period. Incubation water pH and temperature were 7.10 - 7.40, and 12.00 ± 1.00 ˚C, respectively. The fatty acid profile of eggs was measured right after egg fertilization using auto-sampler gas chromatography (7890 N; Agilent Technologies, Santa Clara, USA) equipped with a flame ionization detector and a cyanopropyl-phenyl capillary column (DB-225MS; 30.00 m × 0.25 mm ID × 0.25-µm film thickness) according to Agh *et al.*[13] DHA:EPA ratios of the eggs were 5.92, 10.08, 11.66, and 14.53 (Table 3). The eggs started hatching on day 21 post-fertilization.

Fish larvae sampling. Six larvae were taken from day 22 to 36 post-fertilization every three days, *i.e.*, six times, from each experimental group. Last sampling day was coincided with considerable yolk sac resorption and beginning of active larval exogenous feeding. The larvae were placed in 5 mL plastic screw cap tubes containing 3 mL Bouin's solution (Sigma, St. Louis, USA), immediately after sampling. Three days after the early fixation step, the samples were transferred to 70.00% ethylic alcohol solution for long-term storage until the preparation and examination of histological sections.

Preparation of tissue sections and microscopic studies Following standard tissue processing procedure, larvae samples were embedded in paraffin blocks, and ultra-thin tissue sections, *i.e.*, 4 µm, were prepared. For this end, six larvae from different experimental egg batches were examined for each sampling time. The sections were stained using Hematoxylin-Eosin (H&E) and Alcian blue (BA) to examine the general structure of foregut tissue and goblet cells, respectively.[18,19] Different morphometric measurements including epithelial layer thickness, intestinal fold height, mucosal layer thickness, number of enterocytes, and goblet cells in the foregut of the *O. mykiss* larvae, were examined.[20,21]

Statistical analysis. Before conducting any statistical analyses, normality of the data distribution and homogeneity of variance were determined using Kolmogorov-Smirnov and levene tests, respectively. The data were analyzed using one-way ANOVA. Tukey's HSD test used for pairwise comparison of various experimental groups. All analyses were performed using SPSS Software (version 21.0; IBM Corp., Armonk, USA), and graphs were prepared by Excel (version 2013; Microsoft Corporation, Redmond, USA).

Table 1. Experimental diets (D_1 - D_4) used in the present study.

Feed ingredients (%)	D_1	D_2	D_3	D_4
Fish meal	20.00	20.00	20.00	20.00
Corn gluten	5.00	5.00	5.00	5.00
Wheat gluten	20.00	20.00	20.00	20.00
Blood meal	1.00	1.00	1.00	1.00
Soybean meal	15.00	15.00	15.00	15.00
Yeast	2.00	2.00	2.00	2.00
Lysine	0.62	0.62	0.62	0.62
Methionine	1.00	1.00	1.00	1.00
Fish oil	12.95	3.24	3.24	-
Canola oil	-	4.05	3.24	4.86
Linseed oil	0.32	0.77	0.12	1.02
Corn oil	2.91	-	0.97	-
Olive oil	-	0.65	5.42	0.89
Sunflower oil	-	3.85	0.24	4.57
Coconut oil	-	3.64	2.95	4.86
Starch	2.00	2.00	2.00	2.00
Wheat middling	10.00	10.00	10.00	10.00
Vitamin [a] and mineral premixes [b]	5.00	5.00	5.00	5.00
Vitamin C	0.06	0.06	0.06	0.06
Vitamin E	0.05	0.05	0.05	0.05
Astaxanthin	0.06	0.06	0.06	0.06
Antioxidant	0.02	0.02	0.02	0.02
Dicalcium phosphate	2.00	2.00	2.00	2.00
Proximate analyses (%)				
Crude protein	42.30	42.30	42.30	42.30
Crude lipid	19.00	19.00	19.00	19.00
Nitrogen free extract	14.53	14.53	14.53	14.53
Crude fiber	22.00	22.00	22.00	22.00
Ash	3.80	3.80	3.80	3.80
Energy (KJ g^{-1})	20.00	20.00	20.00	20.00

[a] Composition of vitamin (vit.) premix (IU or g kg^{-1}): vit. A 8,00,000 IU; vit. D_3 300,000, IU; vit. E 2,500 mg; vit. K 1,000 mg; vit. B1 1,200 mg; vit. B_2 1,200 mg; vit. B_3 2,400 mg; vit. B_5 3,500 mg; vit. B_6 1,300 mg; vit. B_9 600 mg; vit. B_{12} 750 µg; vit. C 35,000 mg; vit. H_2 600 mg.
[b] Mineral premix (g kg^{-1} premix): magnesium 6,400 mg; copper 2,000 mg; ferrous 11,000 mg; zinc 7,000 mg; selenium 100 mg; iodine 300 mg; cobalt 50.00 mg; sodium 5,000 mg.

Table 2. Fatty acid profile of the experimental diets (D_1 - D_4) used in the present study (mg g^{-1} lipid).

Fatty acid	D_1	D_2	D_3	D_4
14:0	29.20	37.40	74.80	39.60
16:0	231.10	144.90	150.70	139.50
18:0	54.80	36.60	39.20	33.30
Saturated fatty acid (SFA) [a]	322.20	224.20	270.00	212.30
18:1n-9	339.60	430.10	362.80	490.30
Monounsaturated fatty acid (MUFA) [b]	416.50	465.00	401.30	514.10
18:2n-6, Linoleic acid (LA)	6.10	157.20	166.30	159.50
20:4n-6, Arachidonic acid (ARA)	4.10	1.20	1.90	0.10
n-6 Polyunsaturated fatty acid (PUFA) [c]	11.10	159.00	168.30	160.30
18:3n-3, α-Linolenic acid (LNA)	28.20	27.70	30.30	29.20
20:5n-3, Eicosapentaenoic acid (EPA)	41.90	16.40	17.60	7.30
22:6n-3, Docosahexaenoic acid (DHA)	129.40	57.40	61.60	26.40
n-3 PUFA [d]	200.10	101.80	110.40	63.10
PUFA [e]	211.20	260.80	278.70	223.40
Highly polyunsaturated fatty acid (HUFA) [f]	175.40	75.00	81.10	33.80
HUFA:SFA	0.54	0.33	0.30	0.16
HUFA:MUFA	0.52	0.16	0.20	0.07
HUFA:PUFA	0.83	0.29	0.29	0.15
ARA:EPA	0.10	0.07	0.11	0.01
DHA:EPA	3.09	3.50	3.50	3.62

[a] includes 20:0 and 22:0; [b] includes 14:1n-5, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9 ; [c] includes 20:2n-6 and 20:3n-6 ; [d] includes 20:3n-3 and 22:5n-3; [e] LA + LNA; [f] 20:2n-6 + 20:3n-6 + ARA + 20:3n-3 + EPA + 22:5n-3 + DHA.

Table 3. Fatty acid profile of the rainbow trout eggs used in the present study (mg g^{-1} lipid).

Fatty acid	D_1	D_2	D_3	D_4
14:0	10.50	13.30	18.10	15.30
16:0	139.80	117.60	136.01	125.60
18:0	62.11	56.40	61.80	60.60
Saturated fatty acid (SFA) [a]	212.50	187.50	216	201.50
18:1n-9	205.20	226.90	271.70	241.40
Monounsaturated fatty acid (MUFA) [b]	272.30	286.10	354.40	304.20
18:2n-6, Linoleic acid (LA)	118.90	166	115.80	178.70
20:4n-6, Arachidonic acid (ARA)	38.06	33.60	35.71	43.20
n-6 Polyunsaturated fatty acid (PUFA) [c]	196.90	240.90	197.60	286.30
18:3n-3, α-Linolenic acid (LNA)	13.30	13.31	10.61	15.41
20:5n-3, Eicosapentaenoic acid (EPA)	40.84	16.93	16.46	10.66
22:6n-3, Docosahexaenoic acid (DHA)	241.01	170.70	191.01	1550
n-3 PUFA [d]	303.10	212.28	221.24	171.02
PUFA [e]	500	453.18	418.84	457.32
Highly polyunsaturated fatty acid (HUFA) [f]	319.91	221.23	243.18	208.86
HUFA:SFA	1.51	1.18	1.13	1.04
HUFA:MUFA	1.17	0.77	0.69	0.69
HUFA:PUFA	0.64	0.49	0.58	0.46
ARA:EPA	0.93	1.98	2.17	4.05
DHA:EPA	5.92	10.08	11.66	14.53

[a] includes 20:0 and 22:0 ; [b] includes 14:1n-5, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9 ; [c] includes 20:2n-6 and 20:3n-6 ; [d] includes 20:3n-3 and 22:5n-3 ; [e] LA + LNA ; [f] 20:2n-6 + 20:3n-6 + ARA + 20:3n-3 + EPA + 22:5n-3 + DHA.

Results

The epithelial layer thickness (Fig. 1) showed that group 4 (larvae emerged from eggs with DHA:EPA ratio of 14.53) had the highest values on days 22 and 25 post-fertilization ($p < 0.05$). On day 28, the thickness of the intestinal epithelium of group 3 (larvae from eggs with DHA:EPA ratio of 11.66) was the highest, while that of the group 1 (larvae from eggs with DHA:EPA ratio of 5.92) was the lowest ($p < 0.05$). On day 31, the thickness of the foregut epithelium was the highest in the group 2 (larvae from eggs with a DHA:EPA ratio of 10.08; $p < 0.05$). However, on day 34, the thickness of the intestinal epithelium of groups 1 and 2 (larvae from eggs with DHA:EPA ratio of 5.92 and 10.08, respectively) was the highest compared to the groups 3 and 4 (larvae from eggs with a DHA:EPA ratio 11.66 and 14.53, respectively, $p < 0.05$). On day 36, at the beginning of active exogenous feeding, groups 2 and 4 (the larvae from eggs with a DHA:EPA ratio of 10.08 and 14.53, respectively) had the highest epithelial layer thickness, which significantly differed from the groups 1 and 3 (larvae from eggs with a DHA:EPA ratio of 5.92 and 11.66, respectively ($p < 0.05$).

The intestinal fold height of the larvae obtained from eggs with different ratios of DHA:EPA (Fig. 2) indicated that on day 22 post-fertilization, the groups 1 and 3 (larvae from eggs with a DHA:EPA ratio of 5.92 and 11.66, respectively) had the highest fold height, which was significantly different from that of the groups 4 and 2 (larvae from eggs with a DHA:EPA ratio of 14.53 and 10.08, respectively ($p < 0.05$). On day 28, group 1 had the highest fold height ($p < 0.05$). In addition, the groups 2 and 4 showed the highest and lowest fold height on day 31respectively ($p < 0.05$). On day 36 post-fertilization, the results showed that the groups 2 and 4 had the highest and lowest fold height values, respectively ($p < 0.05$).

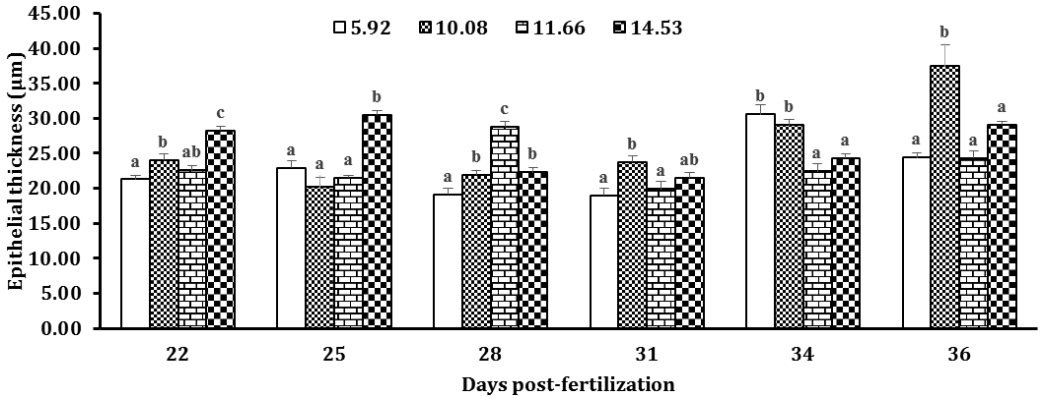

Fig. 1. Intestinal epithelium thickness (mean ± SD, n = 6) of experimental groups on different sampling days. Columns with different letters on each sampling day were significantly different at $p < 0.05$.

The results of the mucosal-submucosa layer thickness of the foregut of larvae obtained from eggs with different ratios of DHA:EPA (Fig. 3) show that on day 22 post-fertilization, the group 3 (larvae from eggs with a DHA:EPA ratio of 11. 66) significantly differed from the groups 1 and 4 (larvae from eggs with a DHA:EPA ratio of 5.92 and 14.53; $p < 0.05$). The group 3 (larvae from eggs with a DHA:EPA ratio of 11.66) had the highest mucosal-submucosa thickness, and the group 1 had the lowest value ($p < 0.05$). On day 25 post-fertilization, the thickness of the mucosal-submucosa layer of the group 2 had the highest value, which was significantly different from the other experimental groups ($p < 0.05$). On day 28 post-fertilization, the group 2 was significantly different from the groups 1, 4, and 3 in this regard. On day 31, the thickness of the mucosal-submucosa layer was the highest in the group 2, which was significantly different from the groups 4 and 3 ($p < 0.05$). On day 34 post-fertilization, the groups 1 and 3 had the highest value ($p < 0.05$).

Meanwhile, on day 36 post-fertilization, coincided with the first exogenous feeding of larvae, the thickness of the mucosa-submucosa layer of the group 2 was the highest and was significantly different from other experimental groups ($p < 0.05$).

The enterocyte count did not significantly differ among various experimental groups on day 22 (Fig. 4A, $p > 0.05$). On day 25, the groups 2 and 3 (larvae from eggs with a DHA:EPA ratio of 10.08 and 11.66, respectively) were significantly different from groups 1 and 2 (larvae from eggs with a DHA:EPA ratio of 5.92 and 14.53, respectively). The group 3 had the most enterocyte number, and the group showed the lowest enterocytes number ($p < 0.05$). On day 34, group 2 had the highest number of enterocytes and group 1 had the lowest number of enterocytes ($p < 0.05$). The results also revealed that on day 36 post-fertilization, the number of enterocytes was the highest in the group 2 ($p < 0.05$). Results of goblet cell count of various experimental groups were depicted Figure 4B. No differences were observed among different groups of larvae on days 22, 25, 28, and 31 ($p > 0.05$). However, group 4 had the highest number of goblet cells and group 2 showed the lowest number of goblet cells on day 34 post-fertilization ($p < 0.05$). On day 36 post-fertilization, the groups 1 and 4 significantly differ from the groups 2 and 3 ($p < 0.05$); group 4 showed the highest number of goblet cells while group 1 had the lowest number of goblet cells. Meanwhile, there were no differences between groups 2 and 3 in this regard ($p > 0.05$).

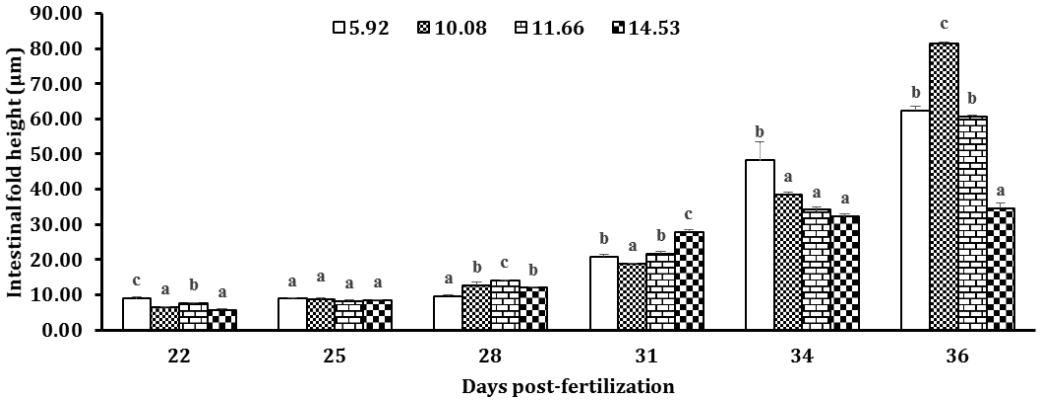

Fig. 2. Height of intestinal folds (mean ± SD, n = 6) of experimental groups on different sampling days. Columns with different letters on each sampling day were significantly different at $p < 0.05$.

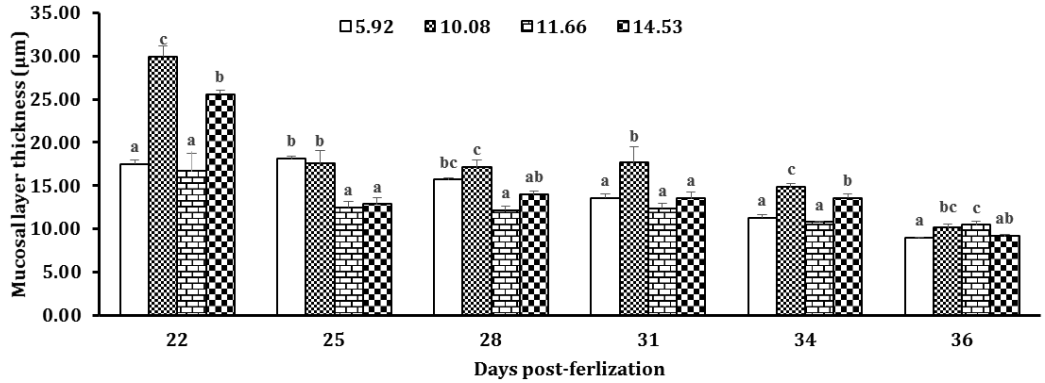

Fig. 3. The thickness of the intestinal mucosal-submucosa layer (mean ± SD, n=6) of experimental groups on different sampling days. Columns with different letters on each sampling day were significantly different at $p < 0.05$.

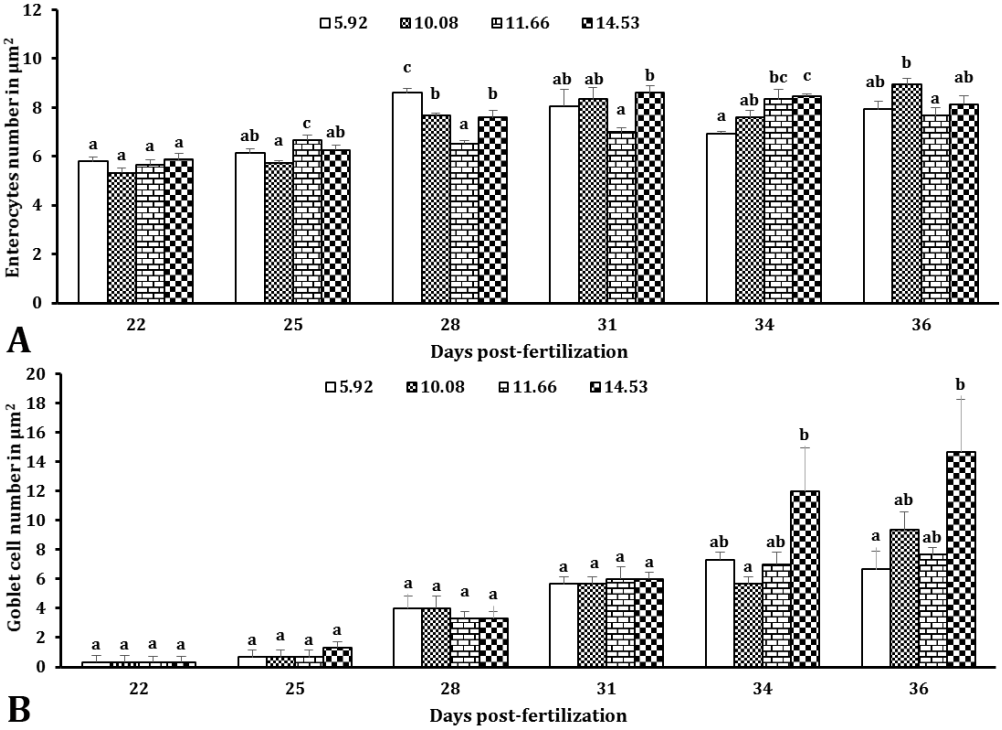

Fig. 4. The number of **A)** intestinal enterocytes and **B)** goblet cells (mean ± SD, n = 6) of experimental groups on different sampling days. Columns with different letters on each sampling day were significantly different at $p < 0.05$.

Discussion

The n-3 series of fatty acids (especially EPA and DHA) play an essential role in growth and development of *O. mykiss* embryos. The EPA and DHA are involved in various physiological functions.[22] The egg quality characteristics including egg fertilization rate, larval hatching rate, and larval survival, are significantly related to n-3-HUFA contents of the broodstock diet.[23] One of the most important indicators of egg quality is the HUFA content.[24] In addition to the total content of dietary HUFA n-3 fatty acids, the ratio of DHA and EPA is also thought to be important since they seem to affect fish embryos and larvae development.[25] It has been discussed that higher dietary contents of EPA and DHA might interfere with the normal development of larval since imbalanced membrane EPA:DHA ratios during larval stage could influence the composition of cell membrane phospholipids and result in decreased larval survival rate.[26] Our results revealed that the thickness of intestinal epithelium layer in larvae obtained from eggs with DHA:EPA ratio of 10.08 reached its highest value on day 36 post-fertilization. The intestinal folds were not observable during early stages of larval development, while they appear as larvae grew. In the present study, the lowest height of intestinal folds was observed in the larvae hatched from eggs with DHA:EPA ratio of 14.53, while those emerged from eggs with DHA:EPA ratio of 10.08 showed the highest intestinal fold height, indicating better digestive tract development and competence for nutrient uptake in the latter group of larvae. On day 36 post-fertilization, the difference was even more apparent in this regard. In *Acipencer stellatus*, intestinal folds were observable four days after larval emerged, and their height increased from day 10 to 37 post-hatch indicating increased intestinal surface for nutrient absortption.[20]

In the present study, the larvae emerged from eggs with DHA:EPA ratio of 10.08 had the highest intestinal mucosal thickness. It has been stated that the intestinal mucosal-submucosa layer involves in blending feed with pancreatic digestive secretions and mucinogen products from goblet cells.[20] The thickness of the intestinal mucosal-submucosa layer in *A. stellatus* increased up to day 47 post hatch.[20] Generally, our results revealed larvae emerged from eggs with DHA:EPA ratio of 10.08 showed better developmental pattern in comparison to other experimental groups, in this regard.

According to the results, the highest enterocyte number was observed in larvae hatched from eggs with DHA:EPA ratio of 5.92 and 11.66 on days 25 and 28. Meanwhile, the number of enterocytes of larvae obtained from eggs with DHA:EPA ratio of 10.08 was the highest on day 38, indicating appropriate intestine development in this group of larvae. Enterocytes are responsible for nutrients uptake from intestinal lumen.[27]As the number of intestinal enterocytes increases; the absorptive competence of the intestine also improves. Therefore, the lower number of intestinal enterocytes in fish larvae might

indicate lower nutrient absorption capacity, reduced growth rate, and consequently, inappropriate nutritional history.[28] Goblet cells and enterocytes are considered very important cell types in fish intestinal epithelium.[29] By secreting mucus, goblet cells facilitate feed passage through the intestinal lumen and protect the intestinal structure from coarse feed particles. They also prevent pathogens translocation and effectively prevent feed-born toxins absorption.[29] In the present research, the batch of eggs with DHA:EPA ratio of 10.08 resulted in larvae with decreased goblet cell counts compared to the group with DHA:EPA ratio of 14.53 at the beginning of active feeding, which might be indicative of better preparation of the digestive tract to uptake nutrients. It has been reported that the number of goblet cells gradually decreases as larvae grow.[20] It has been shown dietary unsaturated fatty acid supplementation without any feed antioxidant inclusion could affect sperm parameters in *Calomys laucha*, or ram.[30] Similarly, a significant reduction of motile sperm counts was observed in male rats following feeding on the highest dietary n-3/n-6 PUFA ratio (2.85) compared to the recommended ratio (*i.e.*, 1.52) mainly due to decreased dietary antioxidant support.[31] Therefore, one might consider dietary antioxidant content when manipulating feed PUFA contents. For instance, in our study all diets were included with the fixed content of 0.80% supplementary antioxidant, while they had increasing ratios of DHA:EPA. As it has been already shown that dietary antioxidant (PROSPERM) supplementation in boars desirably affected biological characteristics of the spermatozoa including intact plasma membrane and osmotic resistance of the acrosomal membrane, which was concomitant with increased contents of total protein and low-molecular antioxidants of the seminal plasma.[32]

In conclusion, our results revealed that feeding rainbow trout broodstock with diet contained HUFA:PUFA ratio of 0.28 could result in the egg with DHA:EPA ratio of 10.08 which in turn yielded larvae with better foregut development parameters compared to those larvae emerged from the eggs with increased DHA:EPA ratio. The finding might be further elucidated regarding any probable involvement of the dietary or even egg antioxidant content on the optimum dietary and/or egg ratio of long chain fatty acids. These fatty acids are the main constituents of fish oil as the essential nutrients in broodstock and larvae nutrition, since the oil is now increasingly being replaced by plant oils mainly due to decreased fish oil production and its increasing prices.

Acknowledgments

Authors are thankful to the Vice Chancellor for Research and Technology of Urmia University, Urmia, Iran, for financial support of this research.

Conflict of interest

The authors declare no conflicts of interest.

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