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Effect of chicken egg lecithin on gut and liver histology in juvenile Binni fish (*Mesopotamichthys sharpeyi*)

Mona Mohammadi[1], Vahid Yavari[1], Hamid Mohammadiazarm[1*], Mohammad Zakeri[1], Solmaz shirali[2]

[1] Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran.
[2] Department of Marine Biology, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran.

*Corresponding Author
E-mail:azarmhamid@gmail.com

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Abstract
This study was conducted to examine the effects of different levels of chicken egg lecithin (EGL) in diets on growth and feeding parameters, lipoprotein fractions and histological changes in gut and liver of binni juveniles (*Mesopotamichthy Sharpeyi*). The experimental fish with initial mean weight of 3.1 ± 0.17 g were fed to satiation 3 times a day with four experimental diets containing different levels of EGL (0, 2%, 4% and 6%) for 8 weeks. Fish were fed EGL4-6% showed significantly higher final growth (FAW), specific growth rate (SGR), improved feed conversation ratio (FCR), protein efficiency (PE) and survival compared with control group. Fish were fed the diet containing EGL 4-6% showed significantly higher high density lipoprotein (HDL) and low density lipoprotein (LDL) than fish fed EGL 2% and control. Fish were fed the diet containing EGL 4% showed higher goblet cells and lower lipid vacuoles in anterior intestine enterocytes than control group. Also, the livers of fish were fed EGL 4% showed normal structure and hepatocyte with clear central nucleus compared with control group, visually. So, it appears phospholipids (PLs) have a specific role for lipid transport and absorption. Therefore, the use of EGL 4% as PLs source is recommended in diet of juvenile binni fish for improvement growth performance.

Keywords: Binni, Chicken egg lecithin, Enterocyte, Growth, Hepatocyte

INTRODUCTION

In recent decades, the aquaculture industry has fastest growing among other industries around the world (FAO, 2018). So, it has become one of the most important parts of food production and human protein requirements. On the other hand, access to high quality fingerling fish is one of the main aspects in aquaculture production (Cahu et al. 2003). One way to improve the quality of the fish is use of suitable dietary ingredients. Lipids are major source of energy for growth, reproduction and movement (including migration) in the diet of fish (Geurden et al. 2006). Studies have shown that neutral plant or animal oils in diet of larval and juvenile fish, lead accumulation of fat in the intestinal entrocytes (Dabrowski et al. 2007) followed by reduced growth (Morais et al. 2007). Because, juvenile fish have not ability for lipoproteins production due to lack of enzyme at these stages (Tocher et al. 2008). It was reported that PLs affect the growth and survival (Cahu et al. 2009) through decreases accumulation of lipid droplets in the intestine (Wold et al. 2007) and increases the absorption of dietary lipids (Hamza et al. 2008). Therefore, PLs induce increase transport of saturated and mono unsaturated fatty acids as an energy source from the intestinal enterocytes toward the blood (Morais et al. 2007). PLs have four main classes containing phosphatidylcholine (PC), phosphatidylinositole (PI), phosphatidylserine (PS) and phosphatidylethanolamine (PE) which PC has major role in lipoprotein structure for dietary lipid transport (Tocher et al., 2008). Two types of usual sources of lecithin are soybean lecithin with four PL classes (PC, PI, PS, PE) and chicken egg lecithin with two classes (PC, PE) predominately with PC (Mohammadiazarm et al. 2013). Therefore, chicken egg lecithin was selected because of high amount of PC.

Mesopotamichthys sharpeyi (Gunther, 1874) is belongs to Cyprinidae family and the Barbus genus (Coad, 1996). It is one of the important economic freshwater fish in Iran. It has great market demand in south of Iran and Iraq and high price due to its tender flesh and good taste in recent years. Full grown specimens may reach a length of half a meter and weigh 800 gram. But, the commercial culture of this species is limited because of taking some time to grow to such good size. One reason is lack of knowledge about their nutritional needs. Therefore, this study was conducted to investigate the effects of different dietary chicken egg lecithin levels as a lipid source on growth and feeding parameters, lipoprotein fractions, foregut and liver histological changes in binni juveniles.

MATERIALS AND METHODS

Diet preparation

Ingredients and proximate composition of the experimental diets are given in Table1. Basal experimental diet was formulated and used as control without EGL and three other diets were prepared to contain 2%, 4% or 6% EGL by replacing soybean oil. All diets were formulated to isonitrogenic and isolipidic. Dry ingredients were weighed and ground (100 µm particle sizes) and then mixed thoroughly. Fish oil, soybean oil, chicken egg lecithin and water were added to the dry ingredients and mixed again until dough was formed. Then prepared dough was pelleted using a pelleting machine which it was dried at room temperature for 24 h and ground into desirable particle sizes. The diets were broken up and sieved into a proper pellet size, packed and stored at -20 ˚C until used.

Experiment fish and feeding conditions

Juveniles of binni were obtained from a local commercial

farm (Maleki Farm, Khozestan Province, Iran). The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Juvenile fish (initial mean weight, 3.1±0.17 g) were allocated randomly into 300 L circular plastic tanks (volume of approximately 250 liters of tap fresh water) with 40 fish per each tank. Also, 25% of water was changed every two days. Three replicate groups of fish were hand fed to apparent satiation three times a day (9:00, 13:00 and 17:00) for 8 weeks. During the experimental period, mean water temperature was 26 ± 1°C, dissolved oxygen was 6.33 ± 0.2 mg L^{-1} and the pH was 7.78± 0.09. The photoperiod was left under natural conditions during the feeding trail. At the end of experiment, fish were starved for 24 h then their weight (FAW) was individually measured at accuracy levels of 0.1 g after anesthetizing with clove flower powder (300 mg L^{-1}). After that, the specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate were calculated using the following equations**:**

SGR (% d^{-1}) = 100 × [Ln (final body weight) - Ln (initial body weight)] / experimental duration (days)

FCR = total dry weight of feed offered / total fish wet weight gained

PER = total fish wet weight gained / total dry weight of feed protein offered

Survival (%) = 100 × (final fish count / initial fish count)

Table 1. Ingredient and proximate composition of experimental diets

	Control	EGL2	EGL4	EGL6
Ingredients diets(g $100g^{-1}$)				
Fish meal[a]	23.00	23.00	23.00	23.00
Corn gluten meal	10.00	10.00	10.00	10.00
Wheat meal	33.00	33.00	33.00	33.00
Wheat bran	15.00	15.00	15.00	15.00
Fish oil [b]	6.00	6.00	6.00	6.00
Soybean oil	6.00	4.00	2.00	0.00
Chicken egg lecithin[c]	0.00	2.00	4.00	6.00
Vitamin premix [d]	3.00	3.00	3.00	3.00
Mineral premix [e]	2.00	2.00	2.00	2.00
Binder[f]	2.00	2.00	2.00	2.00
Proximate composition				
Moisture	8.00	8.80	9.80	8.40
Crude protein (% DM)	32.25	32.28	32.75	32.36
Ash (% DM)	8.42	8.67	8.77	10.40
Crude Lipids (% DM)	12.00	12.31	12.26	12.70
Gross energy[g](kg g^{-1}diet)	19.11	19.00	18.84	18.87

[a]Clopeonella meal, Iran

[b] Kilka oil, Mazandaran Co, Iran

[c]Chicken egg lecithin, Merck, Germany with purity 90% phosphatidylcholine

[d]Vitamin premix (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=2000 mg, B1=6000 mg, B2=8000 mg, B3=12000 mg, B5=40000 mg, B6=4000 mg, B9=2000 mg, B12=8 mg, H2=40 mg, C=60000 mg, Inositol=20000 mg

[e]Mineral premix (composition per 1kg): Iron:6000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:600 mg, $CoCl_2$:6000 mg

[f]Binder: Amet Binder (Component: Crude Protein: 71.98٪, Crude Fiber: 0.9٪, Ash: 17.8٪, Moisture: 9.55٪)

[g]Estimated energy was calculated based on based on 1 g crude protein being 23.6 kJ, 1 g crude fat being 39.5 kJ, and 1 g carbohydrate being 17.2 kJ NRC (1993).

Chemical analyses

Proximate analyses of the diets were determined according to the method of AOAC (2000). Crude protein content was determined by Kjeldahl method using an Auto Kjeldahl System (Kjeltec ™2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105 for 24 h and ash by a furnace muffler (550 for 6 h).

Blood sampling

At the end of experiment, 10 fish from each tank were anesthetized with clove flower powder (300 mg L^{-1}) for bleeding from the caudal vein with un-heparinized syringes. Then, blood specimens were allowed to clot at room temperature. After that, samples were centrifuged (room temperature, 3000 *g*, 10 min), sera were extracted and stored at -80 °C until analysis. Sera biochemical parameters were spectrophotometrically analyzed by means of an auto-analyzer *(*Eurolyser, Austria*)* using commercial kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were conducted for high density lipoprotein (HDL, mg dl^{-1}) and low density lipoprotein (LDL, mg dl^{-1}) according to the procedures of the company

Histological study

For histological study, based on appropriate results obtained from EGL 4%, four fish dissected. Then, samples were taken from the liver and anterior intestine and fixed in 10% formalin. Then the samples were passaged in autotechnicon and blocked in paraffin wax. The 5-6µ sections were made by microtome and were put on microscopic slides and stained by hematoxylin and eosin (H&E). The samples were studied under light microscopy by Dino-lite capture1 software.

Statistical analysis

Data were subjected to one-way ANOVA to test EGL effects on growth and feeding parameters and lipoprotein fractions. When significant differences were found in one-way ANOVA, Duncan's multiple range test was used to rank the groups. All statistical analyses were performed using SPSS version 16 (SPSS, Chicago, IL USA) with a significant level of $p<0.05$. The values presented are mean ± standard error (SE).

RESULTS

The growth and feeding parameters of fish were fed by different experimental diets for 8 weeks are shown in table 2. FAW and SGR of fish were fed EGL 4% or EGL 6% were significantly ($p < 0.05$) higher than fish fed EGL 2% and control group. Fish were fed EGL 4% or EGL 6% showed significantly ($p < 0.05$) better FCR and PE than fish fed EGL 2% and control group. Also, survival rate of fish was significantly higher ($p < 0.05$) by EGL 4% and EGL 6% compared to fish fed EGL 2% and control group.

Table 2. Growth performance of juveniles binni fed the experimental diets for 8 weeks.

	Control	EGL2	EGL4	EGL6
FAW(g)	5.6±0.40[a]	6.2±0.20[a]	9.1±0.60[b]	9.3±0.40[b]
SGR (% day^{-1})	[a] .010±11.	[a] 01.0±.11	1.7±0.10[b]	[b] 060.±8.1
FCR	2.9±0.10[a]	[a] 02.0±72.	[b] 050.±8.1	[b] 03.0±0.2
PE	0.9±0.10[a]	1.1±0.08[a]	1.6±0.08[b]	1.5±0.00[b]
Survival (%)	96.6±2.2[a]	[a] 06.1±.696	[a] 100	[a] 100

(Mean ± SE), n=3 with different letters in each row, indicate the presence of significant differences between the experimental groups ($P<0.05$).

The lipoprotein fractions of fish were fed different experimental diets are shown in table3. Fish were fed different levels of EGL showed significantly ($p < 0.05$) higher HDL and LDL values than control group. So, the highest values of HDL and LDL were observed in fish fed EGL 6% and EGL 4%, respectively.

Table 3. Lipoprotein fractions of juveniles binni fed with different experimental diets for 8 weeks

	Control	EGL2	EGL4	EGL6
LDL(mg dl^{-1})	47.0±0.60[a]	80.0±0.60[b]	109.6±0.90[c]	113.7±1.40[d]
HDL(mg dl^{-1})	35.8±0.40[a]	41.0±0.60[b]	54.7±0.90[c]	61.0±0.60[d]

(Mean ± SE), n=3 with different letters in each row, indicate the presence of significant differences between the experimental groups ($P<0/05$).

The visual study of histological images of intestine showed that enterocytes in anterior portion of intestine in fish EGL 4% had a normal structure with low lipid vacuole and high goblet cells (Fig 2), whereas enterocytes of control had the highest lipid vacuoles and few goblet cells. So, their cytoplasm was completely filled by the lipid vacuoles (Fig 1). Also, histological study of fish liver showed that fish were fed EGL 4%, had hepatocytes with a clear central nucleus whereas in control, esteatosis hepatica and swollen hepatocytes with the large number of lipid vacuoles were observed (Figs 3 and 4).

Figure 1

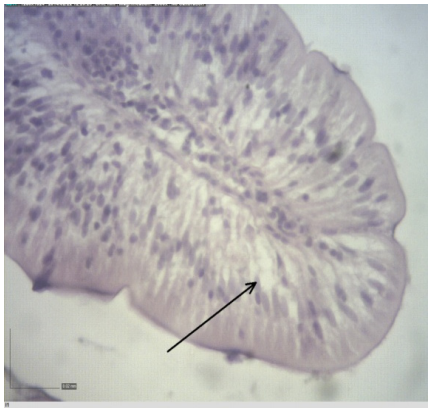

Figure 2

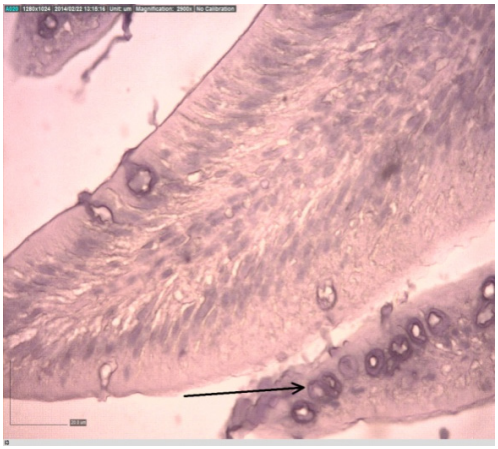

Figure 1 shows lipid vacuole accumulation and few goblet cells in the epithelial layer of intestine in control group. Figure 2 shows high goblet cells and few lipid vacuole in the anterior intestine enterocytes in fish were fed EGL 4% (Scale bar =20 μm).

Figure 3

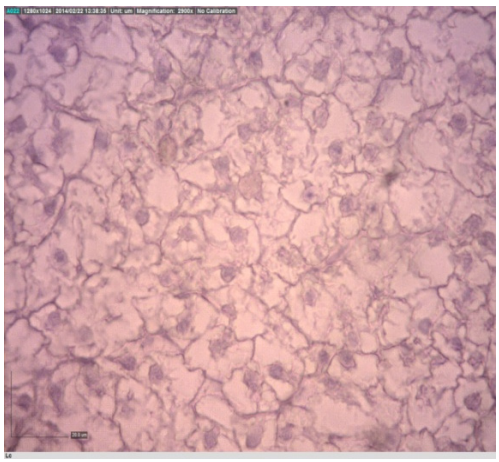

Figure 4

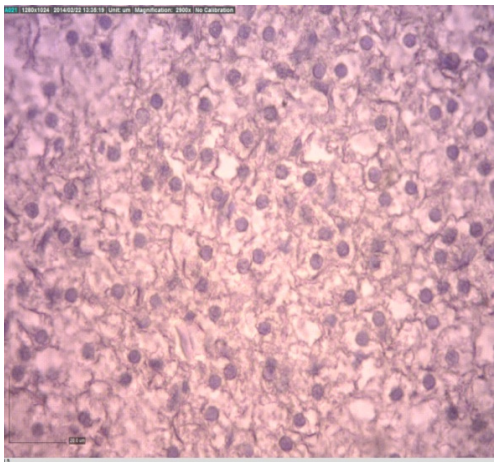

Figure 3 shows the liver of control group with swollen hepatocytes and large number of lipid vacuole and translocation their nuclear of lipid accumulation. Figure 4 shows the liver of fish fed EGL 4% with normal structure and hepatocytes with a clear central nucleus. (Scale bar =20 μm).

DISCUSSION

It was mention that, dietary PLs enhance growth and survival of larval and juvenile stages of marine and fresh water fish species (Tocher et al. 2008). The results of this study showed that PLs have beneficial effect on growth performance of *(M. sharpeyi)*. This is in agreement with result of earlier studies that showed the growth promoting effect of lecithin in a diet of carp larvae (*Cyprinus carpio* L) (Geurden et al. 1995), turbot juvenile (*Scophthalmus maximus* L) (Geurden et al. 1997), ayu (*Plecoglossus altivelus*) (Kanazawa et al. 1981), Caspian brown trout *(Salmo trutta fario)* juvenile (Kenari et al. 2011) and rainbow trout (*Oncorhynchus mykiss*) larvae (Mohammadizarm et al. 2013).

The result of histological morphology of intestine (Figs 1, 2, 3 and4) showed lower lipid accumulation and higher goblet cell in anterior intestine of juveniles were fed EGL 4 % compared to control group. This result is in agreement with result of Olsen et al. (1999) on Arctic charr (*Salvelinus alpines L.*) and Liu et al. (2002) on gilthead sea bream (*Sparus aurata*) that use of lecithin in diets induced lower lipid accumulation in entrocyte of fish and increased lipid transport from intestine to other tissue of fish. Geurden et al. (1998) reported that dietary PLs in diets of turbot (*S. maximus*) induced higher number of goblet cell in intestine of fish that it can be related to higher maturation of intestine. Furthermore, Lu et al. (2008) reported that lower lipid accumulation in entrocyte with higher number of goblet cell in intestine of *Pelteobagrus fulvidraco* can be related to higher maturation of intestine that cause by PLs. On the other hand, liver histology study showed esteatosis hepatica and swollen hepatocytes with the large number of lipid vacuole in liver of fish fed diet without of PLs (Figs 7 and 8). The result is in agreement with earlier studies of Wold et al. (2009) and Salhi et al. (1999) that reported swollen hepatocytes with the large number of lipid vacuole in cod (*Gadus morhua*) and sea bream larvae fed diet without of PLs, respectively. Also, PLs lead secretion of bile acids and modulate fat of liver. Also, it was reported that choline causes prevention of fatty liver in red drum (*Sciaenops ocellatus*) (Craig and Gatlin, 1997).

Furthermore, fish were fed the diet containing EGL 4-6% showed significantly higher HDL and LDL than other groups. In this regards, it was reported dietary PLs contribute to lipoprotein production, thereby it increase the efficiency of lipid transport from the digestive tract to the body s tissues (Salhi et al. 1999). Pervious study reported that *De vono* synthesis of PLs occurs in fish but it seems that this synthesis is not enough for formation of lipoprotein during the rapid growth of early development (Cottuea et al. 1997). Also, it has been reported that fish larvae have a limited ability to de novo synthesize of PLs (Geurden et al. 1995). Lack of dietary PLs resulting in accumulation of lipid droplets in the intestinal mucosa and reduction of growth performance in fish which PtCho has major role in the synthesis and secretion of them (Gisbert et al., 2005). Therefore, exogenous PLs are required to satisfy the demand for lipoprotein synthesis (Fontagne et al. 2000; Mohammadiazarm et al. 2013).

So, the result of the study confirms the role of PLs about decreasing fat accumulation in liver and intestine through lipoprotein synthesis and improvement growth performance of binni juveniles.

CONCLUSION

EGL promoted the growth of juveniles' binni and prevented lipid accumulation in anterior intestine and liver of fish. Therefore, it can be one of the proper nutritional components to increase growth performance and lipoprotein synthesis of fish. So, due to the cost of making feed, inclusion of 4 % chicken egg lecithin in diet of juvenile binni fish is recommended.

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REFERENCE

AOAC, 2000. Official Methods of Analysis of AOAC International, 17th edition. Association of Analytical Communities, Gaithersburg, MD, USA.

Cahu CL, Zambonino Infante JL, & Barbosa V. 2003. Effect of dietary phospholipid level and phospholipid: neutral lipid value on the development of sea bass (*Dicentrarchus labrax*) larvae fed a compound diet. British Journal of Nutrition, 90: 21-28.

Cahu C L, Gisbert E, Villeneuve L A N, Morais S, Hamza N, Wold PAL, Zambonino Infante J. 2009. Influence of dietary phospholipids on early ontogenesis of fish. Aquaculture Research, 40: 989-999.

Coad BW. 1996. Zoogeography of the fishes of the Tigris –Euphrates basin. Zoology in the Middle East, 13: 51-70.

Coutteau P, Geurden I, Camara MR, Bergot P, Sorgeloos P. 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture, 155: 149-164.

Craig SR, Gatlin DM. 1997. Growth and body composition of juvenile red drum *(Sciaenops ocellatus*) fed diets containing lecithin and supplemental choline. Aquaculture, 151: 259-267.

Dabrowski K, Rinchard J, Czesny S. 2007. Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. Aquaculture, 264: 363-371.

Fontagne S, Burtaire L, Corraze G, Bergot P. 2000. Effects of dietary medium-chain triacylglycerols (tricaprylin and tricaproin) and phospholipid supply on survival, growth and lipid metabolism in common carp (*Cyprinus carpio* L.) larvae. Aquaculture, 190: 289–303.

FAO, 2018. The state of world fisheries and aquaculture. Food and Agriculture Organization of the United Nations. FAO, Rome. Available at http://www.fao.org.

Geurden I, Radunz-Neto J, Bergot P. 1995. Essentiality of dietary phospholipids for carp (*Cyprinus carpio*) larvae. Aquaculture, 131: 303-314.

Geurden I, Coutteau P, Sorgeloos P. 1997. Effect of a dietary phospholipid supplementation on growth and fatty acid composition of European sea bass (*Dicentrarchus labrax* L.) and turbot (*Scophthalmus maximus* L.) juveniles from weaning onwards. Fish Physiology and Biochemistry, 16: 259-272.

Geurden I, Bergot P, Schwarz L, Sorgeloos P. 1998. Relationship between dietary phospholipid classes and neutral lipid absorption in newly-weaned turbot, (*Scophthalmus maximus*). Fish Physiology and Biochemistry, 19: 217–228.

Geurden I, Gondouin E, Rimbach M, Koppe W, Kaushik S, Boujard T. 2006. The evaluation of energy intake adjustments and preferences in juvenile rainbow trout fed increasing amounts of lipid. Physiology and behavior, 88: 325-332.

Gisbert E, Villeneuve L, Zambonino Infante JL, Quazuguel P, Cahu CL. 2005. Dietary phospholipids are more efficient than neutral lipids for long chain polyunsaturated fatty acid supply in European sea bass *(Dicentrarchus labrax*) development. Lipids, 40: 1-10.

Hamza N, Mhetli M, Ben Khemis I, Cahu C, Kestemont P. 2008. Effect of dietary phospholipd levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. Aquaculture, 275: 274-282.

Kanazawa A, Teshima S, Inamori S, Iwhashita T, Nagao A. 1981. Effect of phospholipids on survival rate and incidence of malformation in the larval ayu. Memory of Faculty. Fisheri. 30: 301-309.

Kenari AA, Sotoudeh E, Rezaei MH. 2011. Dietary soybean phosphatidylcholine affects growth performance and lipolytic enzyme activity in Caspian brown trout (*Salmo trutta Caspius*) alevin. Aquaculture Research, 42: 655-663.

Lee J M, Lee Y K, Mamrosh JL, Busby SA, Griffin PR. 2011. A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects. Nature, 474: 506-510.

Liu J, Caballero MJ, Izquierdo M, Sayed Ali TE, Hernaedez-Cruz CM,Valencia A, Fernandez-Palacios H. 2002. Necessity of dietary lecithin and eicosapentaenoic acid for growth, survival, stress resistance and lipoprotein formation in gilthead sea bream (*Sparus aurata*). Fisheries Sciences, 68: 1165-1172.

Lu S, Zhao N, Zhao A, He R. 2008. Effect of soybean phospholipid supplementation in formulated microdiets and live food on foregut and liver histological changes of (*Pelteobagrus fulvidraco*) larvae. Aquaculture, 278: 119-127.

Mohammadiazarm H, Kenari A A, Hedayati M. 2013. Effect of dietary phospholipid sources and levels on growth performance, enzymes activity, cholecystokinin and lipoprotein fractions of rainbow trout (*Oncorhynchus mykiss*) fry. Aquaculture Research, 44, 634-644.

Morais S, Conceicao C LEC, Ronnestad I B, Kovenc W, Cahud C, Zambonino Infanted, JL, Dinisa MT. 2007. Dietary neutral lipid level and source in marine fish larvae: effects on digestive physiology and food intake. Aquaculture, 268: 106-122.

NRC (1993) Nutrient Requirements of Fish. Committee on Animal Nutrition, B. O. A. National Research Council. National Academy Press,Washington, DC, USA.

Olsen RE, Myklebust R, Kaino T, Ringo E. 1999. Lipid digestibility and ultrastructural changes in the enterocytes of Arctic char (*Salvelinus alpines L.*) fed linseed oil and soybean lecithin. Fish Physiology and Biochemistry, 21: 35-44.

Salhi M, Hernandez-Cruz CM, Bessonart M, Izquierdo MS, Fernandez-Palacios H. 1999. Effect of different dietary polar lipid levels and different n-3 HUFA content in polar lipids on gut and liver histological structure of gilthead seabream (*Sparus aurata*) larvae. Aquaculture, 179: 253-263.

Tocher DR, Bendiksen EA, Campbell PJ, Bell JG. 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture, 280: 21-34.

Wold P A, Hoehne-Reitan K, Cahu C L, Zambonino Infante JL, Rainuzzo J, Kjrsvik E. 2007. Phospholipids vs. neutral lipids: effects on digestive enzymes in Atlantic cod (*Gadus morhua*) larvae. Aquaculture, 272: 502-513.

Wold PA, Hoehne-reitan K, Cahu C, Infante JZ, Rainuzzo J, Kjorsvik E. 2009. Comparison of dietary phospholipids and neutral lipids: effects on gut, liver and pancreas histology in Atlantic cod (*Gadus morha* L.) larvae. Aquaculture Nutrition, 15: 73-84.