

Lecithin effects on blood biochemical parameters and resistance to thermal stress in juvenile of *Mesopotamichthys sharpeyi* (Cyprinidae family)

Seiedzadeh S.M.¹; Yavari V.¹; Mohammadiazarm H.^{*1}; Mousavi M.¹

Received: July 2015

Accepted: November 2015

Abstract

In this study, the effects of dietary chicken egg lecithin on some blood biochemical factors of *Mesopotamichthys sharpeyi* juveniles and resistance to thermal shock was investigated. Juveniles with initial average weight of 3.10 ± 0.17 were stocked in 12 fiberglass tanks. Four isolipidic and isonitrogenic diets containing 0% (control), 2%, 4% and 6% chicken egg lecithin were used to feed the fish 3 times per day to satiation during 90 days. Total protein, cholesterol, high density lipoprotein (HDL) and albumin levels were significantly higher ($p < 0.05$) in juveniles fed 6% lecithin compared to the control diet. The triglyceride level was also significantly decreased ($p < 0.05$) in juveniles fed 4% and 6% lecithin compared to that of control group. No significant differences ($p > 0.05$) in globulin and the ratio of albumin to globulin were found in dietary treatments. The thermal stress showed that survival rate of juveniles fed different levels of dietary chicken egg lecithin was higher than control group ($p < 0.05$). The results indicated that administration 4 to 6% of chicken egg lecithin in diets of juvenile *M. sharpeyi* have positive effects on promoting health status and resistance to thermal shock.

Keywords: Lecithin, *Mesopotamichthys sharpeyi*, Blood parameters, Thermal stress, Survival

1- Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Khuzestan , Iran.

* Corresponding author's Email: Azarmhamid@gmail.com

Introduction

Lipids are one of the most important parts of fish diets. They are sources of energy and fat soluble vitamins (Sargent *et al.*, 1997). Also, the essential fatty acids of lipids are precursors to the production of hormone like-substances such as prostaglandins and leukotrienes that have regulatory roles and inflammatory reactions (Sotudeh *et al.*, 2011). Now, fish oil, which is supplied from catch is a source of lipids in diet of fish (Pike, 2005). Recent studies have shown that fish oil production through catch will not be able to meet the oil demands of the aquaculture industry in the next few years (Tocher *et al.*, 2008). So, the use of vegetable oil is essential. On the other hand, studies have shown that fish larvae and juveniles have not been able to produce enough lipoprotein such chylomicron for lipid transport from intestine to body tissue. Fish larvae have enzyme restriction for enough phospholipids (PLs) production which is needed for making lipoproteins. Therefore, neutral plant or land animal oils (triglyceride) as a dietary ingredient resulting in fat accumulation in enterocyte (Rinchar *et al.*, 2007), reduces saturated and mono-unsaturated fatty acids absorption in intestinal epithelium as an energy source and retards growth and survival rate of fish (Morais *et al.*, 2007). The beneficial effects of dietary PLs include growth improvement, increased survival and resistance to stress which had been reported previously (Hamza *et al.*, 2008; Tocher *et al.*, 2008; Cahu *et al.*,

2009). Also, other effects are pellet stability in water, nutrient stability due to PLs antioxidant properties, increased diet palatability, food intake, and energy production (Coutteau *et al.*, 1997; Sink, 2014). One of the important indicators for dietary requirements assessment in fish is their health and immune status that is affected by environmental factors, dietary ingredients and life stage of fish (Ghaderi Ramazi *et al.*, 2013).

Binni fish with the scientific name *Mesopotamichthys sharpeyi* (Gunther, 1874) belongs to the Cyprinidae family and the genus *Barbus* (Coad, 1996). It is a native fish of southern Iran, Syria, Iraq and Turkey. Binni is one of the most economically native fish species in Khuzestan area (in southern Iran). Attempts to increase stocking this fish in natural habitats and the development of its industrial rearing requires appropriate diets which are formulated according to the nutritional needs and life stage of the fish. Therefore, this study was conducted to investigate the effects of different levels of chicken egg lecithin as a lipid source on blood biochemical parameters and resistance to heat stress in juvenile of *M. sharpeyi*.

Table 1: Ingredient and proximate composition of experimental diets.

Treatment	Control	EGL2	EGL4	EGL6
Ingredients diets(g 100g⁻¹)				
Fish meal ^a	22.71	22.71	22.71	22.71
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
Egg lecithin ^c	0	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
Antioxidant ^g	0.02	0.02	0.02	0.02
Lysine	0.27	0.27	0.27	0.27
Proximate analysis				
Moisture	8.00	8.80	9.80	8.40
Crude protein (% DM)	32.25	32.28	32.75	32.36
Crude lipid (% DM)	12.00	12.31	12.26	12.70
Ash (% DM)	8.42	8.67	8.77	10.40
Lysine (% DM)	2.00	2.00	2.00	2.00

^a Clupeonella meal, Iran.

^b Clupeonella 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, CoCl₂:6000 mg.

^f Binder: Amet Binder (Component: Crude Protein: 71.98%, Crude Fiber: 0.9%, Ash: 17.8%, Moisture: 9.55%)

^g Antioxidant: Butylated hydroxytoluene (BHT).

DM, dry matter.

Diets prepare

All diets were formulated to be 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 a dough was formed. Then the prepared dough was pelleted using a pelleting machine and pellets were 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

The experiment was done in the wet lab of Khorramshar University of Marine Science and Technology, Khorramshar, Iran. Juveniles of binni were obtained from a local farm (Maleki Farm, Khuzestan 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 with 40 fish per each tank for the feeding trial after being collectively weighed. Three replicate groups of fish were hand-fed to apparent satiation three times a day (9:00, 13:00 and 17:00) for 90 days. During the experimental period (90 days), water temperature was 26 ± 1°C, dissolved oxygen was 6.33 ± 0.073 mg L⁻¹ and the pH was about 7. The photoperiod was left under natural conditions during the feeding trail.

Chemical analyses

Proximate analyses of the diets were determined according to the method of AOAC (1995). Crude protein content was determined using the Kjeldahl method using an Auto Kjeldahl System (Kjeltec™2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system (D-63450, Heraeus, Hanau, Germany), moisture content using a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105°C for 24 h and ash using a furnace muffler (550°C for 4 h).

Sample preparation

Blood sampling from juveniles was scheduled after 90 days of the start of feeding with lecithin. For sampling, nine fish from each treatment (three from each replicate) after starvation for 24 h were captured and anaesthetized with 2-phenoxyethanol 2%. Blood was sampled immediately after capture by puncturing the caudal vessels with a heparinized syringe. Plasma was separated by centrifugation at 4500 g for 10 min and stored at -80°C until analysis (Velisek *et al.*, 2005).

Blood biochemical assessment

Triglyceride, cholesterol, albumin and HDL were measured based on enzyme-colorimetric method by using commercial kits (Pars Azmon, Tehran, Iran). Also, total protein was assayed based on biuret method and globulin was calculated with regards to albumin and total protein.

Thermal stress

At the end of the experiment, 15 fish from each replicate per treatments and also from the control group were maintained at 12°C for 24 hours as a thermal stress. Then, the fish were returned to their normal temperature (28°C) and after 48 h they were assessed for the extent of the losses.

Statistical analysis

Data were expressed as mean \pm SE. Data were tested for normality by Shapiro–Wilk test and homogeneity of variances ANOVA was employed to reveal significant differences using SPSS 18, and where significant differences were found, means were tested using Duncan post hoc to compare the means of treated groups against that of the corresponding control. $p < 0.05$ was the accepted significance level.

Results

The effect of dietary chicken egg lecithin on blood biochemical parameters of juveniles at the end of the trial is presented in Table 2. Blood biochemical parameters including cholesterol and HDL contents in all groups fed egg lecithin were significantly higher ($p < 0.05$) than control group. Juvenile fish fed 6% lecithin showed the highest plasma cholesterol and HDL. The triglyceride values of plasma in fish fed 4% and 6% lecithin was significantly lower ($p < 0.05$) than fish fed 2% egg lecithin and control diet. There was no

significant difference in triglyceride values ($p > 0.05$) between 2% egg lecithin group and the control. The results of some immune parameters and resistance of juvenile binni to thermal shock are presented in Table 3. Total protein and albumin in fish fed 6% egg lecithin were significantly higher ($p < 0.05$) than control, but there were no significant differences ($p > 0.05$) in other groups compared to control. There was no significant difference ($p > 0.05$) in globulin values and ratio of albumin to globulin among different dietary groups of trial.

The challenge test with temperature showed that mortality significantly reduced in groups that received egg lecithin compared to control (Table 3).

Discussion

Generally, in fish like other animals, hematological and biochemical parameters of blood are indicators of fish physiological status that can be affected by consumed diets (Najafi *et al.*, 2009), systematics, fish feeding, health status and stress (McCarthy *et al.*, 1973). Najafi *et al.* (2010) reported that 5 to 7.5% soybean lecithin in diets of *Acipenser baerii* improved growth and hematological factors. In the present study, total protein levels of plasma increased significantly with dietary lecithin. Total protein is an important indicator of health and nutrition status of fish (Patriche *et al.*, 2011) and is used to evaluate the liver condition in fish (Hernandez *et al.*, 2007).

Table 2: The result of blood plasma biochemical parameters in juveniles of binni (*Mesopotamichthys sharpeyi*) fed with different experimental diets for 90 days

Treatment	Control	2%lecithin	4%lecithin	6%lecithin
Cholesterol(mg dL ⁻¹)	260.50±0.50 ^a	268.50±0.50 ^b	269.50±0.50 ^b	295.00±1.00 ^c
HDL(mmol L ⁻¹)	35.8 ±0.4 ^a	41±0.6 ^b	54.7±0.9 ^c	61±0.6 ^d
Triglyceride(mg dL ⁻¹)	401.00±0.57 ^c	379.00±12.00 ^c	303.50±1.50 ^b	260.00±13.00 ^a

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

Table 3: The result of immune parameters and resistance to thermal shock in juveniles of binni (*Mesopotamichthys sharpeyi*) fed different experimental diets for 90 days

Treatment	Control	2%lecithin	4%lecithin	6%lecithin
Total Protein(g dL ⁻¹)	2.79±0.00 ^a	3.05±0.005 ^{ab}	3.08±0.01 ^{ab}	3.28±0.18 ^b
Albumin (g dL ⁻¹)	1.44±0.00 ^a	1.57±0.02 ^{ab}	1.65±0.00 ^{ab}	1.81±0.13 ^b
Globulin (g dL ⁻¹)	1.35±0.01	1.48±0.03	1.52±0.09	1.52±0.09
Albumin/Globulin ratio	1.07±0.01	1.06±0.03	1.09±0.06	1.18±0.01
Survival	80.00±11.54 ^a	93.33±6.67 ^b	100.00±0.00 ^b	100.00±0.00 ^b

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

Also, albumin, globulin and ratio of albumin to globulin increased with dietary egg lecithin. However, amount of globulin and the ratio of albumin to globulin were not significantly different compared to those of control group. Albumin is one of important blood proteins that is formed in the liver. It acts like an antioxidant and protects tissues and cells from damage of free radicals. It also transfers vitamins, mineral materials and hormones in blood (Burtis and Ashwood, 1994; Svetina *et al.*, 2002). Other plasma proteins are globulin with important roles in immunological actions (Bnaei *et al.*, 2009). High levels of protein and immunoglobulin are signs of healthy fish that result in irritation of leukocytes (Nayak *et al.*, 2004).

The results of study showed that dietary PLs increased blood cholesterol, HDL levels and decreased triglyceride values in plasma of juvenile fish. This result is in agreement with study of Niu *et al.* (2008) on cobia (*Rachycentron canadom*) larvae that dietary PLs increased cholesterol and reduced triglyceride levels. Also, he reported that increased plasma cholesterol is in accordance with higher HDL levels that indicate the internal transfer of dietary fat in response to dietary PLs. Dietary PLs help to produce lipoproteins (Koven *et al.*, 2001), which transport lipids from intestinal cells to blood (Salhi *et al.*, 1999). It was reported that dietary PLs contribute to chylomicron (one type of lipoproteins) production, thereby increasing the efficiency of lipid transport from the digestive tract

to the body tissues (Salhi *et al.*, 1999). Pervious studies reported that *denovosynthesis* of PLs occurs in fish but it seems that this synthesis is not enough for formation of chylomicron during the rapid growth of early development (Coutteau *et al.*, 1997). Also, it has been reported that fish larvae have a limited ability in *denovo* synthesis 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 (phosphatidyl choline, PtCho) has a 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; Azarm *et al.*, 2013).

Dietary chicken egg lecithin increased resistance of juvenile to thermal shock. It was mentioned that chicken egg lecithin induced higher survival in ayu (*Plecoglossus altivelis*), carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) larvae (Kanazawa *et al.*, 1981; Geurden *et al.*, 1995; Azarm *et al.*, 2013). The possible explanation may be related to n-3 and n-6 highly unsaturated fatty acids (HUFA) content of chicken egg lecithin (Azarm *et al.*, 2013). PC or PI containing a HUFA moiety in the sn-2 position showed a high efficacy as the PL sources in fish, but the mechanism of action is still not clear. Recently, to clarify the mechanism of PtCho in lipid metabolism, several studies were

conducted using chemically synthesized PtCho with different fatty acids. Tago *et al.* (1999) synthesized the PtCho with a Docosahexaenoic acid (DHA) or Eicosapentaenoic acid (EPA) moiety (DHA-PC, EPA-PC) and determined the growth and tolerance to environmental change stress in flat fish. They found that DHA-PtCho was more effective than EPA-PtCho and triglyceride containing DHA in increasing tolerance to low salinity, low dissolved oxygen levels and raised water temperature. Samples *et al.* (1999) examined the influence of adding fatty acids to a medium on the expression of heat shock protein 70 (HSP70) mRNA in white blood cells of rainbow trout. They demonstrated that expression of HSP70 mRNA was increased with the addition of fatty acids after stress. The expression of HSP70 mRNA with HUFA supplementation such as docosahexaenoic acid or arachidonic acid was higher than oleic acid. During temperature stress phospholipase A₂ influenced PLs in the cell membrane and released DHA from the PLs. The supposed hypothesis which released DHA performed as a signal transmistor of HSP70 that was used for the restoration of the membrane proteins (Samples *et al.* 1999). So, it seems that dietary chicken egg lecithin could have the positive effects on promoting health status and resistance to heat shock.

Conclusion

Generally, the present study showed that health status of juveniles' binni could be affected by different dietary chicken egg lecithin levels. So, fish fed at 4 to 6% chicken egg lecithin showed better health status and resistance to thermal stress.

Acknowledgements

The author is thankful to the authorities of Khorramshar University of Marine Science and Technology for providing the laboratory facilities and fund during MSc project.

References

- AOAC., 1995.** Official methods of analysis of the association of official analytical chemistry. 16th ed. AOAC International, Washington, USA. 1141P.
- Azarm, H.M., Kenari, A.A. and 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.
- Banaei, M., Mirvaghefi, E., Rafiei, Gh. and Sordagomil, A., 2009.** Effect of oral administration of silymarin on biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Fishery Journal. Natural Resources Journal*, 4, 271-286
- Burtis, C.A. and Ashwood, E.R., 1994.** Tietz textbook of clinical chemistry (2nd ed.), pp.1275-1512.
- Cahu, C.L., Gisbert, E., Villeneuve, L.A.N., Morais, S., Hamza, N., Wold. P.A.L. and Zambonino Infante, J., 2009.** Influence of dietary phospholipids on early ontogenesis of fish. *Aquaculture Research*, 40, 989-999.
- Coad, B.W., 1996.** Zoogeography of the fishes of the Tigris –Euphrates basin. *Zoology in the Middle East*, 13,51-70.
- Coutteau, P., Geurden, I., Camara, M.R., Bergot, P. and Sorgeloos, P., 1997.** Review on the dietary effects of phospholipids in fish and crustacean larviculture. *Aquaculture*, 155, 149-164.
- Fontagne, S., Burtaire, L., Corraze, G. and 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.
- Geurden, I., Radünz-Neto, J., Bergot, P., 1995.** Essentiality of dietary phospholipids for carp (*Cyprinus carpio* L.) larvae. *Aquaculture*, 131, 303-314.
- Ghaderi Ramazi, F., Jahanbakhshi, A. and Soudagar, M., 2013.** Hematological and biochemical changes in Common carp (*Cyprinus carpio*) fed with corn gluten. *Fisheries Science and Technology*, 1, 53-62.

- Gisbert, E., Villeneuve, L., Zambonino Infante, J.L., Quazuguel, P. and Cahu, C.L., 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. and Kestemont, P., 2008.** Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. *Aquaculture*, 275, 274-282.
- Hernandez, L.H.H., Teshima, S., Koshio, S., Ishikawa, M., Tanaka, Y. and Alam, M., 2007.** Effects of vitamin A on growth, serum anti-bacterial activity and transaminase activities in the juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 262, 444-450.
- Kanazawa, A., Teshima, S., Inamori, S., Iwhashita, T. and Nagao, A., 1981.** Effect of phospholipids on survival rate and incidence of malformation in the larval ayu. *Memory of Faculty of Fisheries, Kagoshima University*, 30, 301-309.
- Koven, W., Kolkovski, S., Hadas, E., Gamsiz, K. and Tandler, A., 2001.** Advances in the development of microdiets for gilthead seabream (*Sparus aurata*) a review. *Aquaculture*, 194, 107-121.
- McCarthy, D.H., Stevensom, J.P. and Roberts, M.S., 1973.** Some blood parameters of the rainbow trout (*Salmo gairdneri* Richardson). I. The kamloops variety. *Journal of Fish Biology*, 1, 5-8.
- Morais, S., Conceição, L., Rønnestad, I., Koven, W., Cahu, C., Zambonino Infante, J. and Dinis, M., 2007.** Dietary neutral lipid level and source in marine fish larvae: effects on digestive physiology and food intake. *Aquaculture*, 268, 106-122.
- Najafi, A., Falahatkar, B. and Kalbasi, M., 2009.** Effect of lecithin on growth, hematological and biochemical of blood of *Acipenser baeri*. *Iran Fishery Science Journal*, 3, 143-154.
- Nayak, A., Das, B., Kohli, M. and Mukherjee, S., 2004.** The immunosuppressive effect of α permethrin on Indian major carp, rohu (*Labeo rohita* Ham.). *Fish & shellfish immunology*, 16, 41-50.
- Niu, J., Liu, Y., Tian, L., Mai, K., Yang, H., Ye, C. and Zhu, Y., 2008.** Effects of dietary phospholipid level in cobia (*Rachycentron canadum*) larvae: growth, survival, plasma lipids and enzymes of lipid metabolism. *Fish physiology and biochemistry*, 34, 9-17.
- Patriche, T., Patriche, N. and Bocioc, E., 2011.** Determination of some normal serum parameters in juvenile sevruga sturgeons *Acipenser stellatus* (Pallas, 1771). *Archiva Zootechnica*, 14, 49-54.
- Pike, I., 2005.** Eco-efficiency in aquaculture: global catch of wild fish

- used in aquaculture. *Internat. Aquafeed*, 8, 38–40.
- Rinchar, J., Czesny, S. and Dabrowski, K., 2007.** Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. *Aquaculture*, 264, 363–371.
- Salhi, M., Hernandez-Cruz, C.M., Bessonart, M., Izquierdo, M.S. and 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*). *Aquaculture*, 179, 253–263.
- Samples, B.L., Pool, G.L. and Lumb, R.H., 1999.** Polyunsaturated fatty acids enhance the heat induced stress response in rainbow trout (*Oncorhynchus mykiss*) leukocytes. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 123, 389–397.
- Sargent, J., McEvoy, L. and Bell, J., 1997.** Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture*, 155, 117–127.
- Sink, T.D. and Lochmann, R.T., 2014.** The effects of soybean lecithin supplementation to a practical diet formulation on juvenile channel catfish, *Ictalurus punctatus*: growth, survival, hematology, innate immune activity, and lipid biochemistry. *Journal of the World Aquaculture Society*, 45, 163–172.
- Sotoudeh, E., Kenari, A.A. and Rezaei, M.H., 2011.** Growth response, body composition and fatty acid profile of Caspian brown trout (*Salmo trutta Caspius*) juvenile fed diets containing different levels of soybean phosphatidylcholine. *Aquaculture International*, 19, 611–623.
- Svetina, A., Matašin, Ž. and Tofant, A., 2002.** Haematology and some blood chemical parameters of young carp till the age of three years. *Acta Veterinaria Hungarica*, 50, 459–467.
- Tago, A., Yamamoto, Y., Teshima, S. and Kanazawa, A., 1999.** Effects of 1,2-di-20:5 phosphatidylcholine (PC) and 1,2-di-22:6-PC on growth and stress tolerance of Japanese flounder (*Paralichthys olivaceus*) larvae. *Aquaculture*, 179, 1–4.
- Tocher, D.R., Bendiksen, E.Å., Campbell, P.J. and Gordon Bell, J., 2008.** The role of phospholipids in nutrition and metabolism of teleost fish, *Aquaculture*, 280, 21–34.
- Velisek, J., Svobodova, Z., Piackova, V., Groch, L. and Nepejchalova, L., 2005.** Effects of clove oil anaesthesia on common carp (*Cyprinus carpio*). *Veterinaria Medicina*, 6, 269–275.