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Original Article

Evaluation effect of dietary egg lecithin on digestive enzymes and body composition of juvenile binni (*Mesopotamichthys sharpeyi* Gunther, 1874)

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Abstract: In this study, the effects of dietary egg lecithin on digestive enzymes and body biochemical composition of juveniles *Mesopotamichthys sharpeyi* was evaluated. Four experimental diets including control diet (with 0% egg lecithin) and three diets containing 2%, 4% and 6% egg lecithin were used. At the end of the experiment, digestive enzymes activity (lipase, amylase and alkaline phosphatase) and body biochemical compositions were assessed. The results showed no significant differences between experimental treatments in moisture and ash content. Maximum content of the crude protein and crude lipid were recorded in 4% lecithin treatment and it had significant differences with control group. The digestive enzymes activity (lipase, amylase and alkaline phosphatase) showed significant differences between control and experimental groups. An increasing trend was observed in the digestive enzymes activity among treatments. Based on the results, it was concluded that 4% to 6% dietary egg lecithin in the diet, can promote growth and survival rate of juvenile binni.

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Introduction

In the recent decade, the aquaculture industry had a fast growth (Heydarnejad, 2012). The factors such as availability, stability, appropriate quantity, price and nutritional value of the diet are crucial in development of this industry (Nasopoulou and Zabetkis, 2012). Lipids are the most important ingredient of fish diet. Because, it acts as an energy source, contains essential fatty acids and fat-soluble vitamins (Sargent et al., 1997). Fish oil is a source of lipids in fish diets; that supply through catch (Pike, 2005). Estimates show that the amount of fish oil production will not be able to supply required fish oil through catch in next years (Tocher et al., 2008). The productions of fish diets are based on soybean oils and fish oil, but researches have shown that use of animals and plant neutral oils in the diets of fish, especially during larvae stage leads the accumulation of fat in the intestinal enterocyte (Rinchard et al., 2007). This topic also causes the reduction in uptake of saturated and mono-unsaturated fatty acids as energy sources in the intestine and subsequently, decreases growth and survival of fish larvae (Moraise et al., 2007). In addition, fish larvae have limited enzymatic ability for producing the lipoproteins; therefore, adding the phospholipids in the diet can provide larval requirements to phospholipids helping to produce lipoproteins (Tocher, 2008). Industrial source of phospholipids is lecithin, which produced during the purification process of crude oils such as soybean crude oil, sunflower, canola, turnip and chicken egg.

Mesopotamichthys sharpeyi is a cyprinid species (Nikpey, 1996), known as Binii or Bunii. This species is native freshwater fishes of Tigris basin found in Iran, Iraq, Turkey and Syria. Binii has been recently considered as a proper candidate for aquaculture in Iran due to its high resistance to a wide range of the environmental conditions, having proper features for rearing and marketability

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Table 1. Ingredient and proximate composition of experimental diets.

Treatment	Control	EGL2	EGL4	EGL6
Ingredients diets(g $100g^{-1}$)				
Fish meal[a]	23.0	23.0	23.0	23.0
Corn gluten meal	10.0	10.0	10.0	10.0
Wheat meal	33.1	33.1	33.1	33.1
Wheat bran	15.0	15.0	15.0	15.0
Fish oil[b]	6.0	6.0	6.0	6.0
Soybean oil	6.0	4.0	2.0	0
Egg lecithin[c]	0	2.0	4.0	6.0
Vitamin premix[d]	3.0	3.0	3.0	3.0
Mineral premix[e]	2.0	2.0	2.0	2.0
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 composition (%DM)				
Moisture	8	8.8	9.8	8.4
Crude protein	32.25	32.28	32.75	32.36
Crude fat	12	12.31	12.26	12.7
Ash	8.42	8.67	8.77	10.40
Lysine	2.00	2.00	2.00	2.00

[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]Antioxidant: Butylated hydroxytoluene (BHT).
DM, dry matter.

(Hamidian, 2003). It is also cultivated for stocking in its natural habitats. There is little information available about its physiological features, especially during larval development and growth performance. In addition, the appropriate diet needs to be formulated for this species based on its requirements and life stage. Therefore, this study was carried out to investigate the effect of dietary egg lecithin on the digestive enzymes and body composition of juvenile Binni.

Materials and methods

Diet preparation: The ingredients and composition of the experimental diets are given in Table 1. Four experimental diets containing chicken egg lecithin were prepared. All diets were formulated to isonitrogenic and isolipidic. To determine the effect of different levels of the lecithin on the growth performance; four levels of lecithin (0%, 2%, 4% and 6%) were used in reduction of soybean oil. 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, which was dried at room temperature for 24 hrs and grounded into desirable particle sizes. The diets were broken up and sieved into a proper pellet size, packed, and stored at -20°C.

Experiment fish and feeding conditions: The experiment was conducted from September until November 2013 in wet-lab of Khorramshar University of Marine Science and Thechnology. Juveniles of *M. sharpeyi* were obtained from a local farm (Maleki Farm, Khozestan, Iran). The fish were

Table 2. Analysis of the digestive enzymes activity of juveniles *Mesopotamichthys sharpeyi* fed different experimental diets for 56 days.

Treatment	control	2% lecithin	4% lecithin	6% lecithin
AM(U/mgprotein)	878.97 ± 82.76[a]	1089.05 ± 0.05[b]	1094.69 ± 52.88[b]	1085.09 ± 0.09[b]
ALP(U/mgprotein)	231.24 ± 16.77[a]	312.28 ± 0.28[b]	300.25 ± 1.09[b]	376.92 ± 10.17[c]
LIP(U/mgprotein)	0.85 ± 0.07[a]	1.08 ± 0.05[b]	1.21 ± 0.01[b]	1.42± 0.03[c]

AM: Amylase, ALP: Alkaline phosphatase, LIP: Lipase

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

acclimated to the laboratory condition for two weeks prior experiment. The fish (with initial mean weight of 3.1 ± 0.17 g) were introduced randomly into 300-L circular plastic tanks with 40 fish per 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 8 weeks. During the experimental period, water temperature, DO and pH were 26 ± 1°C, 6.33 ± 0.073 mg L^{-1} and about 7, respectively. The photoperiod was natural conditions during experiment. At the end of experiment, the juvenile fish of each tank were collectively weighed after anesthetizing with a clove powder solution with a concentration of 30 mg L^{-1} after starvation for 24 hrs. In addition, at the end of experiment, fifteen specimens from each tank were transferred to tubes for assessment of digestive enzymes activity and body biochemical composition.

Chemical analyses: Proximate analyses of the diets and fish were determined according to the AOAC (1995). Crude protein content was measured using the Kjeldahl method by an Auto Kjeldahl system (Kjeltec TM2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by an oven (D-63450, Heraeus, Hanau, Germany) drying at 105°C for 24 hrs and ash by a furnace muffler (550°C for 4 hrs).

Digestive enzymes activity: For assessment of enzyme activities, the intestine samples were homogenized in 100 mM Tris-HCl, 0.1 mM EDTA and 0.1% Triton X-100 (pH 7.8) with a homogenizer (IKA®TI8 basic, Germany), and centrifuged (12000 g for 30 min at 4°C). We used 100 mg tissue mL^{-1} buffer for homogenization and then the extracted supernatants were kept frozen in -80°C to determine biochemical Features. The activity of α-amylase, lipase and alkaline phosphatase were measured by the enzymatic photometric method using amylase, lipase and alkaline phosphatase pharmaceutical kits (Pars Azmoon, Tehran, Iran), respectively. Protein was also determined by enzymatic colorimetric method using biuret kit (Pars Azmoon, Tehran, Iran). Enzyme activity expressed as a specific activity of U mg^{-1} protein.

Statistical analysis: Data were subjected to one-way ANOVA to test the effect of sources and levels of lecithin on growth performance 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 Deviation (SD).

Results

The results of digestive enzymes activity are shown in Table 2. The activity of lipase indicated a significant difference between treatments and control groups. The activity of lipase increased with increasing dietary egg lecithin in diet (P<0.05). The activity of alkaline phosphates in brush border of intestinal epithelium was significantly increased with increasing the dietary egg lecithin compared to that of control group (P<0.05). Activity of alkaline phosphates in juvenile fed the control diet was 231.24 ± 16.77 U mg^{-1} protein and 376.92 ± 10.17 Umg^{-1} protein in juvenile fed 6% egg lecithin. Also, the activity of amylase significantly increased as dietary egg lecithin was increased (878.97 ± 82.76 to 1085.09 ± 0.09 Umg^{-1} protein) (P<0.05).

Table 3. Proximate composition (%) of the whole body of juveniles *Mesopotamichthys sharpeyi* fed the experimental diets for 56 days (wet weight %).

Treatment	control	2% lecithin	4% lecithin	6% lecithin
ash	2.39 ±0.29[a]	2.33 ± 0.12[a]	2.19 ± 0.35[a]	2.50 ± 0.41[a]
moisture	69.59 ± 1.31[a]	69.64 ± 0.83[a]	67.30 ± 0.18[a]	67.91 ± 0.4[a]
lipid	9.51 ± 0.08[a]	11.67 ± 1.36[ab]	13.21 ± 0.84[b]	12.23 ± 0.47[ab]
protein	14.92 ± 0.47[a]	15.94 ± 0.60[ab]	16.69 ± 0.07[b]	16.32 ± 0.15[ab]

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

The result of the body biochemical composition is shown in Table 3. The crude protein and crude lipid compositions of juvenile increased with increase amount of dietary egg lecithin (P<0.05). The highest crude protein content was observed in juvenile fed diets containing 4% egg lecithin. The crude lipid of juvenile fed 4% lecithin was the highest. Others body biochemical composition such as moisture and ash showed no significantly different compared with control group (P>0.05).

Discussion

The results showed that crude protein increase by increasing the levels of dietary egg lecithin. This result is in agreement with the results of Zhao et al. (2013) on large yellow croaker (*Larmichthys crocea*), Hung et al. (1997) on juvenile of Atlantic salmon (*Salmo salar*), Azarm et al. (2013) on Rainbow trout (*Onchorynchus mykiss*) and Sink (2014) on Channel catfish (*Ictalurus punctatus*). Generally, many factors are attributed to the body biochemical composition such as species, primary size and duration of experiment. But in the case of lecithin, it was reported that metabolism of many proteins is changed by phosphatidylcholine. Therefore, high growth in treatments with high level of phospholipids is due to increasing demand for amino acids that induces increasing protein production (Sotudeh et al., 2011).

The higher level of egg lecithin in the diet showed an increase in the amount of lipid in the body of fish, that it was significantly higher in treatment with 4% egg lecithin. This result is similar to those of large yellow croaker (*L. crocea*) (Zhao et al., 2013), Caspian brown trout (*Salmo trutta caspius*) (Sotudeh et al., 2011) and Atlantic salmon (*S. salar*) (Hung, 1997). The lecithin induces the lipoprotein production and increases efficiency of nutrient absorption from the digestive tract via intestinal epithelium and then improves transferring of nutrients to the body tissues and consequently, increases consumption of fatty acids (Morais et al., 2007). Many studies showed that phospholipids has important role in lipid transfer inducing an increase in growth performance and access to energy and consequently, increase in the whole lipid of fish body (Niu et al., 2008; Sink, 2014). Many works showed that phospholipid deficiency in larvae and juvenile diets is led to lipid accumulation in enterocyte. Hence, the phospholipids are essential for transfer of lipids from enterocytes to blood and lymph of fish (Teshima et al., 1986; Tocher et al., 2008). Different levels of egg lecithin had no significant effect on ash and moisture of fish. This result corresponds with those stated on juvenile flounder (*Paralichthys olivaceus*) (Kim et al., 2006), Caspian brown trout (*S. t. caspius*) (Sotudeh et al., 2011) and Channel catfish (*I. punctatus*) (Sink, 2014).

Higher levels of egg lecithin in diet showed significantly higher amylase, alkaline phosphates and lipase activity. The specific activity of amylase was significantly higher in egg lecithin treatments compared to that of control group that can be related to lysophospholipids which acts as an emulsifier in intestine (Azarm et al., 2013). Also, specific activity of amylase can be different due to feeding habits (Hidalgo et al., 1999), sex (Chakrabarti et al., 1995), amount of diet carbohydrate (Kuzmina, 1996) and ion concentrations (Munilla-Moran and Saborido-Rey, 1996).

Alkaline phosphates is a metal enzyme that mainly presents in the brush border epithelial cells of the intestine and its activity related significantly lipid, glucose, calcium and inorganic phosphorus absorption (Zhao et al., 2013). Thus, alkaline phosphates is used for absorption of nutrients by larvae intestinal of vertebrates (Zhao et al., 2013). In the present study, the specific activity of alkaline phosphates increased significantly with increase of dietary egg lecithin. This result is similar to those of *Sander lucioperca* (Zambonino Infante and Cahu, 2001) and European sea bass (*D. labrax*) larvae (Cahu et al., 2003) that application of the phospholipids in the diet had been led to higher alkaline phosphates activity, which reflects rapid maturation of the intestine. Also, it was reported the ratio between alkaline phosphates and cytosole enzyme (leucine aminopeptidase) is a maturation index of enterocytes (Wold et al., 2007; Hamza et al., 2008).

The activity of lipase increased significantly by higher level of the dietary egg lecithin which is consistent with the findings of Cahu et al. (2003) on sea bass (*D. labrax*) larvae. Cahu et al. (2003) pointed out that regulation of lipase and phospholipase enzymes activity is controlled by genetic (mRNA amount) and hormonal factors. Increased levels of phospholipids and triglycerides in the diet induce increasing the rate of mRNA translation of relevant enzymes and the other hormonal factors such as cholecystokinin in the regulation of enzymes activity. This result is similar to that of Azarm et al. (2013) on rainbow trout (*O. mikyss*) which reported increased chylomicron production by phosphatidylcholine is led to increase in cholecystokinin concentration and activity of pancreatic enzymes, which ultimately improves digestion and absorption and as a result growth performance of fish.

As a conclusion, the present study showed that digestive enzyme activity and protein content of juvenile binni could be influenced by different chicken egg lecithin. Therefore, 4% up to 6% chicken egg lecithin induces the higher growth performance in binni fish.

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