

Effects of dietary phospholipid levels and sources on growth performance, fatty acid composition and oxidative responsiveness of juvenile Malaysian mahseer, *Tor tambroides*

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Abstract. A 60-day long feeding trial was conducted to investigate the effect of various levels and sources of dietary phospholipids (PL) on growth, survival, feed utilization, fatty acid composition and oxidative status of juvenile *Tor tambroides*. Seven diets were formulated to contain one control group (PL0), three levels of soybean lecithin (PL2%, PL4%, PL6%) and two sources of soybean lecithin viz. granular and liquid, but remaining isolipiditic and isonitrogenous. Diets were named as PL0 (control), LPL2 (D2), LPL4 (D3), LPL 6 (D4), GPL2 (D5), GPL4 (D6) and GPL6 (D7). Triplicate groups of juvenile with an initial weight of 0.51 ± 0.1 g (mean \pm SE) were stocked in 21 (50 L each) glass aquaria connected with a closed system. The respective test diets were fed two times a day 8:00 hrs and 16:00 hrs to visually near satiation. Results attributed highest final weight (FW), weight gain (WG), specific growth rate (SGR) and condition factor (CF) were 1.85 ± 0.02 , 256 ± 4 , 2.11 ± 0.02 and 1.18 ± 0.01 , respectively and obtained in fish group fed GPL4 followed by GPL6 and GPL2; while, lowest in control group (PL0). The concentration of Σ PUFA in muscle increased as PL supplementation increased. The n-3/n-6 ratio found higher in control diet and had a decreasing tendency accompanying the PL level increased. Significant differences were not found in feed intake and feed conversion ratio among treatments. There was an increasing trend in hepatosomatic indices with the increasing levels of PL in diets. This was caused due to the fatty liver of the fish. Superoxide dismutase activity did not varied significantly among treatments. Based on growth performance parameters, this study suggested that 4% granular PL supplemented diet could satisfy the requirement of phospholipid for juvenile *T. tambroides*.

Key Words: polar lipid, soybean lecithin, feed utilization, superoxide dismutase, phosphatidylcholine.

Introduction. Phospholipids (PL) are beneficial for the survival, growth and development of freshwater fishes (Geurden et al 1998; Geurden et al 2008; Fontagne et al 1998; Fontagne et al 2000; Olsen et al 2003) as well as marine fishes (Cahu et al 2003a; Tocher 2003; Morais et al 2004; Gisbert et al 2005; Villeneuve et al 2005). Lecithin from a number of sources has frequently been used for dietary supplementation of PL. Phosphatidylcholine, which contains the vitamin choline, is the predominant phospholipid in lecithin (Craig & Gatllin III 1997). Soybean lecithin, recurrently used as PL source, contains around 62% total PL (including 45% phosphatidylcholine, 20% phosphatidylethanolamine, 16% phosphatidylinositol), 5% triacylglycerol and 15% cholesterol. Contrary to fish oil, it does not contain highly unsaturated fatty acids (HUFA), namely EPA (eicosapentaenoic acid, 20:5(n-3)) and DHA (docosahexaenoic acid, 22:6(n-3)). Different lecithin sources attributed different effects on growth performance of fish due to their fatty acid composition (Azarm et al 2013). Most commonly, the levels of phospholipid requirement can vary depending upon species and developmental stage (larvae or juveniles) from around 2% up to much higher levels of 12–14% of diet (Cahu et al 2003a; Rinchar et al 2007). Tocher et al (2008) reviewed that juvenile fishes require 2-4% phospholipid of the diet.

Phospholipid plays a vital role in maintaining the structure and function of cellular membranes (Kanazawa 1985; Tocher 2003) by increasing the efficiency of absorption and transport of dietary lipids (Teshima et al 1986; Kanazawa 1991; Fontagne et al 2000; Izquierdo et al 2000; Hadas et al 2003; Morais et al 2004) through lipoprotein synthesis (Cahu et al 2003b, 2009; Tocher et al 2008). Feeding trials with PL-enriched diets attributed an earlier maturation of the digestive tract in fish larvae (Cahu et al 2003a; Gisbert et al 2005; Hamza et al 2008). Therefore, it is predominantly imperative to determine the indispensable requirements of PL and FA in early stages of fish life (Koven et al 1993). Tocher et al (2008) reviewed the optimal levels of dietary PL for growth as 3% for ayu seewtfish, *Plecoglossus altivelis altivelis*; 4% for rainbow trout, *Oncorhynchus mykiss*; and 6% for Atlantic salmon, *Salmo salar*.

The Malaysian mahseer, *Tor tambroides* (Bleeker), is a highly valued fish and an exemplary candidate species for aquaculture (Ng et al 2008). This cyprinid fish is widely distributed throughout the trans-Himalayan and South-east Asian regions (Ambak et al 2007). Recently this fish is getting attention to study their biology, culture and conservation (Siraj et al 2007) after successful artificial breeding done by Ingram et al (2005). Recent studies concerning the nutritional requirements of *T. tambroides* focused on juvenile stage (Ng et al 2008; Ng & Andin 2011; Misieng et al 2011; Ramezani-Fard et al 2012). In these studies, requirements of lipids and crude protein for *T. tambroides* juvenile were estimated at 5% (Ng & Andin 2011; Ramezani-Fard et al 2012) and about 50% (Ng et al 2008), respectively. *T. tambroides* requires relatively low dietary lipid levels with a preference for lipid sources with high n-6 PUFA, high monounsaturated fatty acids and very low n-3 PUFA content. Ramezani-Fard et al (2012) emphasized that the precise lipid source with an appropriate fatty acid composition is decisive to safeguard optimal growth of *T. tambroides*. However, being the most important nutritional component, phospholipid requirements is still poorly studied for this fish. Published information on the phospholipid requirements of *T. tambroides* is scanty. The aim of this study was to determine the effects of dietary PL sources and levels on the *T. tambroides* juveniles' growth performances, survival rate, body composition and fatty acid profile.

Material and Method

Feed formulation and preparation of experimental diets. The formulation and proximate composition of experimental diets are shown in Table 1. All the dietary ingredients were obtained from commercial sources. Seven diets were formulated to contain one control group (PLO), three levels of soybean lecithin (PL2%, PL4%, PL6%) and two sources of soybean lecithin viz. granular and liquid, but remaining isolipidic and isonitrogenous. Diets were named as PLO (control), LPL2 (D2), LPL4 (D3), LPL 6 (D4), GPL2 (D5), GPL4 (D6) and GPL6 (D7). All the dietary ingredients were ground through a sieve (500- μ m mesh). The dietary ingredients were thoroughly blended with the lipid sources and water in a food mixture. Then the diets were pelleted with a pelletizer and were oven-dried for 4-5 h at 60°C, bagged and stored at -20°C until use.

Rearing of fish and commencement of feeding trial. Juvenile *T. tambroides* was collected from commercial traders at Ampang, Selangor, Malaysia and reared in the Kelah Hatchery, School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Malaysia. Prior to the initial stocking, the fish were acclimatized for 10 days in the laboratory condition by feeding with a commercial pelleted diet (crude protein 43%, crude fat 3%, crude fiber 3%, ash 16% and moisture 11%). The feeding trial was conducted in 50 L glass aquaria with a closed water system. All the aquaria were covered with black plastic nets to prevent fish from jumping out. Each aquarium was equipped with continuous aeration. The aquaria were maintained under natural light and dark regime. After acclimatization, the homogenous sized 20 juveniles with an initial weight of 0.51 ± 0.1 g (mean \pm SE) were placed in previously prepared 21 tanks in triplicates for each dietary treatment. The fish were hand fed with the respective test diets at visually near satiation, twice a day at 8.00 am and 16.00 and seven days per week for 60 days. All the fish were weighted in bulk at every two weeks interval to determine growth and

health condition. The water quality such as water temperature, pH, and dissolve oxygen (DO) was measured and recorded every day during feeding trials. During the feeding trial, the temperature, pH and DO were varied between 28.8 to 29.9°C, 6.9 to 9.1 and 5.6 to 7.2 mg L⁻¹, respectively.

Table 1

Ingredients and proximate composition of experimental diets

Ingredients (%)	(PL 0%) Control	Soybean lecithin (liquid)			Soybean lecithin (granular)		
		LPL2% (D2)	LPL4% (D3)	LPL6% (D4)	GPL2% (D5)	GPL4% (D6)	GPL6% (D7)
Fishmeal ^a	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Shrimp meal ^b	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Wheat flour ^c	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Cod liver oil ^a	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palm oil ^c	6.00	4.00	2.00	0.00	4.00	2.00	0.00
Soybean lecithin ^a	0.00	2.00	4.00	6.00	2.00	4.00	6.00
Vitamin mixture ^d	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mixture ^e	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Stay-C ^a	0.20	0.20	0.20	0.20	0.20	0.20	0.20
CMC ^f	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose	0.80	0.80	0.80	0.80	0.80	0.80	0.80
<i>Proximate composition (% dry matter basis)</i>							
Crude protein	48.94	49.91	51.77	49.27	49.12	50.27	50.99
Lipid	14.51	14.60	14.62	14.64	14.48	14.60	14.63
Ash	9.7	9.6	9.8	9.7	9.5	9.5	9.6

^a Sri Purta Trading, Alor Setar, Kedah.

^b Raw materials collected from local market, oven dried and made shrimp meal in laboratory.

^c Collected from the local market in Terengganu.

^d Rovithai, DSM Nutritional Products Ltd. Scotland; composition (IU/g/mg per kg): vitamin A 50 IU, vitamin D3 10 IU; vitamin E130 g, vitamin B1 10 g, vitamin B2 25 g, vitamin B6 16 g, vitamin B12 100 mg, biotin 500 mg, pantothenic acid 56 g, folic acid 8 g, niacin 200 g, anticake 20 g, antioxidant 0.2 g and vitamin K3 10 g.

^e Rovithai, DSM Nutritional Products Ltd. Scotland; composition (g per kg): copper 7.50 g, iron 125.0 g, manganese 25.0 g, zinc 125.0 g, cobalt 0.50 g, iodine 0.175 g, selenium 0.300 g and anticake 10.0 g.

^f Carboxymethyl cellulose.

Collection of samples. At the beginning, a pooled sample of 10 fish from the stock was stored at -20°C for fatty acid (FA) analysis. At the end of the feeding trial, all fish were fasted for 24 hours prior to final sampling. All the fish were anaesthetized by following method described by Ng & Andin (2011). The total number, individual body weight and length of fish from each aquarium were measured accordingly. A pooled sample of four fish from each replicate tank was randomly collected and stored at -20°C for final fatty acid analysis. Fish were dissected out to collect liver from three fish in each replicate tank and weighted to calculate hepatosomatic index.

Proximate composition. The proximate compositions of the feed ingredients and experimental diets samples were analysed using standard methods (AOAC 1997). The samples were dried to a constant weight at 105°C to determine the moisture content. The crude protein contents were determined by measuring nitrogen (N×6.25) using the Kjeldahl method (2300-Auto-analyzer, FOSS, Denmark), crude lipid content by ether extraction using Soxhlet method (36680-analyser, BUCHI, Switzerland), ash content by combustion at 550°C for 12 h.

Fatty acid analysis. The freeze dried samples of muscles of *T. tambroides* was analyzed for FA composition. Samples (200–300 mg) were taken and the one-step method of FA analysis was carried out by combining the extraction and esterification processes using a

single tube following the method described by Abdulkadir & Tsuchiya (2008). The fatty acid methyl esters (FAMES) were separated and quantified by gas chromatography equipped with flame ionization detection (GC-FID-QP2010 Ultra). Quantitatively (as a percentage), composition in terms of individual FAs was calculated by comparing the peak area of each FA with the total peak area of all FAs in the samples.

Oxidative responsiveness. The superoxide dismutase activity (SOD) of muscle samples were analyzed by using the Stresspress Superoxide Dismutase Kit (StressMarq Biosciences INC.) following standard protocol.

Calculation of growth indices. The following variables were evaluated:

$$\text{Weight gain (WG, \%)} = (\text{final weight} - \text{initial weight}) \times 100 / \text{initial weight}$$

$$\text{Specific growth rate (SGR \% , day}^{-1}\text{)} = \{ \text{Ln (final weight)} - \text{Ln (initial weight)} / \text{duration in days} \} \times 100$$

$$\text{Survival (\%)} = 100 \times (\text{final no. of fish} / \text{initial no. of fish})$$

$$\text{Feed intake (g fish}^{-1}\text{ 70 days}^{-1}\text{)} = (\text{dry diet given} - \text{dry remaining diet recovered}) / \text{no. of fish}$$

$$\text{Feed conversion ratio (FCR)} = \text{total dry feed fed (g)} / \text{wet weight gain (g)}$$

$$\text{Condition factor (CF, \%)} = \text{weight of fish} / (\text{length of fish})^3 \times 100$$

$$\text{Hepatosomatic index (HSI, \%)} = \text{weight of liver} / \text{weight of fish} \times 100$$

Statistical analysis. All the data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The statistical analyses were performed in SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). A significance level of 5% ($P < 0.05$) was used for all comparisons.

Results and Discussion. The current study was designed to determine the consequences of dietary lecithin as phospholipids on *T. tambroides* juveniles. Experimental diets were isolipidic and isoenergetic except the levels of phospholipids (Table 1). The dietary fatty acid compositions were influenced with the PL supplementation. PUFA content of the phospholipid supplemented diets were significantly higher than the control diet (Table 2). Moreover, granular phospholipid supplemented diets had slightly higher content of PUFA over liquid phospholipid. The *T. tambroides* juveniles readily accepted the experimental diets from the very beginning of the trial and maintained standard behavior throughout the growth trial. The mean final weight FW, WG and SGR values were higher in groups fed phospholipid treated diets than the control diet (Table 3). Moreover, granular phospholipid treated groups showed higher growth related parameters than the liquid phospholipid treated groups. The survival of juveniles in different treatment groups ranged from 77% to 100% and LPL2 attributed no mortality at all. However, this variation was not due to the effects of feed since some juveniles died due to accidental errors.

The results of the current study exhibited advantageous effects of PLs on growth performance. Moreover, different sources of lecithin have different effects on the growth performance of juvenile *T. tambroides*. It was observed that 4% and 6% granular phospholipid led to a significant increase in juveniles' final weight, specific growth rate and weight gain compared to control group. Similarly, Tocher et al (2008) suggested that, phospholipid supplemented diets lead to increase growth and survival of larval and juvenile stages of marine and fresh water fish species. Moreover, dietary supplementation with different phospholipid composition from various sources like soy lecithin and krill (e.g. PC, PE and PI) differentially influenced growth in *S. salar* (De Santis et al 2015).

Highest growth was attributed in *S. salar* fed 2.6% krill phospholipid and 3.6% soybean phospholipid (De Santis et al 2015). Poston (1990) demonstrated that supplemental soy lecithin and choline each augmented growth, survival, and deposition of body fat in early-feeding rainbow trout fry. Feeding a combined supplement of 4% soybean lecithin and 0.3% choline, or 8% soybean lecithin with or without choline resulted in greater growth in rainbow trout larvae than that of fish fed the ether-extracted herring meal supplemented with smaller amounts of lecithin (Poston 1990). *S. salar* and *O. mykiss* yielded inferior growth, enzymatic activity and chylomicron concentration while fed soybean lecithin containing equal amounts of PC, PE and PI (Palacios & Wang 2005). On the other hand, PC rich egg-yolk lecithin or krill oil resulted in better growth (Azarm et al 2013). Furthermore, phosphatidylcholine was identified as growth promoting component in soy lecithin and recommended level was 1.5% PC for Nile tilapia, *Oreochromis niloticus* (Kasper & Brown 2003).

Table 2

Fatty acid composition of experimental diets (% of total fatty acids)

Fatty acids	Control (D1)	Liquid soybean lecithin			Granular soybean lecithin		
		LPL2% (D2)	LPL4% (D3)	LPL6% (D4)	GPL2% (D5)	GPL4% (D6)	GPL6% (D7)
C16:0	26.81	22.53	18.64	16.03	19.05	18.19	17.11
C16:1n-7	3.60	2.90	2.80	2.99	2.40	2.23	2.76
C18:1n-9	23.37	19.36	13.51	11.57	12.34	11.00	10.76
C18:2n-6	8.19	12.40	19.33	20.22	16.98	23.77	26.45
C18:3n-3	1.63	2.38	3.50	3.88	2.64	3.53	4.27
C20:0	1.85	1.57	1.47	1.59	1.28	1.57	1.54
C20:1n-9	4.56	3.94	3.55	3.87	2.78	2.65	3.74
C20:5n-3	9.42	6.71	7.38	7.35	7.02	6.50	6.53
C22:1n-9	5.04	4.73	4.06	4.49	2.92	2.91	4.63
C22:6n-3	17.15	11.42	13.28	13.06	13.14	12.13	11.13
ΣSFA	26.83	22.55	18.66	16.05	19.07	18.21	17.12
ΣMUFA	36.57	31.63	22.17	22.92	21.4	18.79	21.89
ΣPUFA	8.19	14.78	22.83	24.12	19.62	27.30	30.72
ΣHUFA	26.57	18.13	20.66	20.41	20.16	18.63	17.66
Σn-3/Σn-6	3.24	1.65	1.25	1.20	1.34	0.93	0.83

In the present study, FW of juveniles fed diets with 4% and 6% PLs were higher than those of juveniles fed control diet. On the contrary, SGR was significantly higher in fish group fed diet D6 and D7. Juveniles fed control diet had significantly ($P < 0.05$) lower WG, SGR and final weight (FW) compared to those of other groups. The SGR values of 4% granular PL supplemented group was higher ($P < 0.05$) than those of 2% PL supplemented groups and the control group. It is well established that PL is a good source of nutrient in the early life stages of fish and crustaceans from both marine and freshwater (Coutteau et al 1997).

Several studies exhibited that PL supplemented diets enhanced the efficiency of lipid utilization and supply phosphatidylcholine for good growth (Sánchez et al 2012; Tocher et al 2008). PL supplemented groups showed higher growth and was attributed to the stimulatory effect of PL on intestinal lipoprotein secretion accordingly (Fontagne et al 1998; Geurden et al 1998). The transport of dietary lipids was thus improved (Teshima et al 1986; Fontagne et al 2000; Hadas et al 2003) as well as FA absorption in intestine (Geurden et al 1998; Geurden et al 2008). Likewise, current study revealed that WG significantly increased with 4% PL supplementation ($P < 0.05$), but there was a slight decreasing trend at higher PL supplementation. Earlier studies testified the deleterious effects of excessive dietary PL supplementation (Coutteau et al 1996; Teshima et al 1986). Moreover, higher supplementation of PL exhibited poorer growth performance in crab (Li et al 2014).

Table 3

Growth performances of *Tor tambroides* fed diets supplemented with different levels and sources of phospholipids after 60 days feeding trial

Parameters	Control (D1)	Liquid soybean lecithin			Granular soybean lecithin		
		LPL2% (D2)	LPL4% (D3)	LPL6% (D4)	GPL2% (D5)	GPL4% (D6)	GPL6% (D7)
Fn wt ¹	1.53±0.01 ^a	1.62±0.01 ^{ab}	1.72±0.01 ^{cd}	1.57±0.03 ^{ab}	1.66±0.04 ^{bc}	1.85±0.02 ^{ce}	1.78±0.02 ^{de}
WG ²	200±2.2 ^a	208±1.9 ^b	242±5.2 ^{bc}	209±9.4 ^a	233±8 ^b	256±4 ^c	235±5 ^b
SGR ³	1.83±0.01 ^a	1.87±0.01 ^b	2.05±0.02 ^{bc}	1.88±0.05 ^a	2±0.04 ^b	2.11±0.02 ^c	2.01±0.02 ^b
Survival	82±7 ^a	100±0 ^b	77±1 ^a	87±3 ^a	90±7 ^a	82±7 ^a	80±0 ^a
FI ⁴	2.5±0.10 ^a	2.4±0.08 ^a	2.6±0.05 ^a	2.5±0.02 ^a	2.4±0.11 ^a	2.5±0.09 ^a	2.5±0.02 ^a
FCR ⁵	1.7±0.16 ^b	1.39±0.01 ^a	1.64±0.02 ^a	1.58±0.08 ^a	1.41±0.09 ^a	1.46±0.11 ^a	1.56±0.02 ^a
CF ⁶	1.01±0.00 ^a	1.05±0.01 ^{ab}	1.12±0.01 ^{cd}	1.06±0.00 ^{ab}	1.15±0.01 ^{bc}	1.18±0.01 ^{ce}	1.11±0.00 ^{de}

Values are means ± S.E.M. Within a row, means with the same letters are not significantly different (P>0.05).

¹Final weight (g).

²Weight gain (%) = (final weight – initial weight) × 100 / initial weight.

³Specific growth rate (% day⁻¹) = {Ln (final weight) – Ln (initial weight) / duration in days} × 100.

Survival (%) = 100 × (final no. of fish / initial no. of fish)

⁴Feed intake (g fish⁻¹ 60 days⁻¹) = (dry diet given – dry remaining diet recovered) / no. of fish.

⁵Feed conversion ratio = total dry feed fed (g) / wet weight gain (g).

⁶Condition factor (%) = weight of fish / (length of fish)³ × 100.

Being the most abundant PL class on the surface of lipoprotein, PC can promote lipid transport from liver to perihepatic tissues by enhancing the formation of very low density lipoprotein (Yao & Vance 1988). Thus, dietary PLs might also reduce lipid content of the liver in juvenile *T. tambroides* by optimizing lipid metabolism. Previous studies demonstrated that PLs could facilitate lipid digestion and absorption in the intestine of the fish larvae via improved emulsification (Hung et al 1997; Koven et al 1993). However, the exact mechanisms need further investigation.

In the present study, FW and SGR increased with the increasing levels of dietary PLs from 2% to 6% and thereafter declined which is similar to the findings of Zhao et al (2013) in case of large yellow croaker. Fish oil was used to keep the experimental diets isolipidic in that study, which led to decreasing levels of n-3 HUFA from 2.51% to 1.17% dry diet with increment of dietary PLs (Zhao et al 2013). It was observed that, feeding trials with isolipidic diets, a replacement of fish oil by soybean lecithin persuaded a change in diet fatty acid composition, and mainly EPA and DHA decreased when soybean lecithin level increased in the diet (Cahu et al 2009). Relatively lower content of n-3 HUFA in the highest level of PLs group might inhibit the growth performance of larvae. On the contrary, the present study used palm oil to keep n-3 HUFA content of the experimental diets constant and SGR was not compromised by the highest level of dietary PLs. These results emphasized the importance to maintain constant n-3 HUFA content of the experimental diets in order to better understand the role of intact PLs. In addition, it should be noticed that even though some fatty acids levels (C16:0, C18:1, C18:2n-6 and C18:3n-3) seemed different between diets supplemented with 4% and 6% GPLs, no significant differences in survival and growth performance were observed in these two treatments, indicating that these fatty acids might play less important role in *T. tambroides* juveniles comparing with n-3 HUFA (Feng et al 2017).

The FA composition of muscle of *T. tambroides* is presented in Table 4. The C20:1n-9 content of the phospholipid treated diets were significantly ($P < 0.05$) higher than the control diet. The concentration of Σ PUFA in muscle increased as PL supplementation increased. The C18:1n-9 was significantly ($P < 0.05$) higher than C20:5n-3 (EPA) content in muscle of juvenile *T. tambroides*. Σ HUFA ranged from 17.90 ± 1.50 to 21.01 ± 1.33 , and was slightly higher in control diet. The n-3/n-6 ratio found higher in control diet and had a decreasing tendency accompanying the PL level increased.

Cahu et al (2003a) examined that negligible amounts of EPA and DHA lead to improved growth and survival over feeding fish oil containing HUFA. Furthermore, PL diets supplemented with a moderate level of n-3 HUFA, DHA or EPA contributed to a good growth for European seabass (*Dicentrarchus labrax*) larvae (Gisbert et al 2005), Atlantic cod (*Gadus morhua*) larvae (Wold et al 2007), Pike-perch (*Sander lucioperca*) larvae (Hamza et al 2012). Villeneuve et al (2005) disclosed that higher incorporation of EPA+DHA as PL, could not improve further growth. *D. labrax* and pike-perch (*Sander lucioperca*) responded positively to dietary PL concentration and showed higher growth and survival while negatively to EPA + DHA (Cahu et al 2003b; Hamza et al 2008). Almost similar trend was reported in the current study.

Both n-6 and n-3 polyunsaturated fatty acids (PUFA) were reported to be very essential for fish from both marine and freshwater (Tocher 2003). It has been reported that some freshwater fish species may have a higher requirement for n-6 PUFA over n-3 PUFA in the diet, particularly those species capable of synthesizing long chain-PUFAs de novo from 18-carbon precursors such as 18:2n-6 (Mishra & Samantaray 2004; Zuraini et al 2006). Likewise, carps have need of greater amounts of n-6 fatty acids than n-3 fatty acids for proper growth and development. Turchini et al (2009) and NRC (2011) suggested the recommended levels of 1% 18:2n-6 and 0.5–1.0% of 18:3n-3 maintaining a n-3:n-6 ratios of 0.5 to 1.0. But our finding for this study perceived that n-3:n-6 ratio was slight higher which gave better growth (0.99 to 1.41). Diet GPL4% and GPL6% gave the required ratio for this fish. On the contrary, Ng & Andin (2011) witnessed better growth in *T. tambroides* by supplying a mixed lipid source with a n-3:n-6 fatty acids ratios of 0.3. It is well established that the fatty acid composition of the tissue lipids in fish is diligently related to dietary fatty acid composition (Turchini et al 2009).

Table 4

Fatty acid composition (% total fatty acids) of muscle lipids of *Tor tambroides* fed diets supplemented with different levels and sources of phospholipids after 60 days feeding trial

Fatty acids (%)	Control	Liquid soybean lecithin			Granular soybean lecithin		
		LPL2% (D2)	LPL4% (D3)	LPL6% (D4)	GPL2% (D5)	GPL4% (D6)	GPL6% (D7)
C16:0	27.09±0.3 ^a	25.33±0.3 ^{ab}	23.20±0.2 ^{bc}	22.60±0.1 ^{bc}	25.89±0.3 ^{ab}	24.24±0.3 ^{bc}	23.18±0.2 ^{bc}
C16:1-7	3.45±0.01 ^b	3.71±0.01 ^c	3.28±0.01 ^a	4.80±0.02 ^d	3.67±0.01 ^c	3.77±0.01 ^c	3.99±0.03 ^e
C18:1n-9	10.08±0.04 ^e	9.49±0.04 ^d	7.37±0.03 ^b	5.80±0.02 ^a	9.39±0.04 ^d	7.95±0.02 ^c	7.26±0.04 ^b
C18:2n-6	7.62±0.1 ^a	11.32±0.1 ^b	12.01±0.1 ^b	16.32±0.3 ^c	11.00±0.1 ^b	15.29±0.1 ^c	18.01±0.0 ^d
C18:3n-3	1.31±0.01 ^a	2.00±0.0 ^b	3.96±0.01 ^e	2.90±0.01 ^c	1.79±0.0 ^b	2.50±0.02 ^d	2.89±0.03 ^c
C20:1n-9	2.17±0.1 ^a	5.78±0.3 ^b	4.09±0.2 ^c	5.28±0.7 ^b	4.6±0.5 ^c	5.06±0.1 ^b	5.48±0.2 ^b
C20:5n-3	4.65±0.3 ^b	5.04±0.0 ^c	4.22±0.1 ^a	4.76±0.5 ^{bc}	4.73±0.0 ^b	4.90±0.2 ^{bc}	4.86±0.2 ^{bc}
C22:6n-3	16.36±0.7 ^c	15.85±0.6 ^{bc}	13.67±0.1 ^a	13.15±0.1 ^{ab}	14.93±0.0 ^{abc}	14.13±0.1 ^{abc}	13.85±0.7 ^a
ΣSFA	27.12 ^a	25.36 ^{ab}	23.23 ^{ab}	23.74 ^{ab}	25.92 ^{ab}	24.26 ^{ab}	23.21 ^b
ΣMUFA	15.70 ^a	18.98 ^b	14.74 ^a	15.34 ^a	17.66 ^b	16.78 ^b	16.73 ^{ab}
ΣPUFA	8.93 ^a	13.32 ^b	15.97 ^{bc}	19.22 ^c	12.79 ^b	17.79 ^{bc}	20.90 ^c
ΣHUFA	21.01 ^a	20.89 ^a	17.90 ^a	17.91 ^a	19.66 ^a	19.03 ^a	18.71 ^a
Σn-3/Σn-6	2.93 ^a	2.02 ^b	0.99 ^c	1.28 ^c	1.95 ^c	1.41 ^c	1.20 ^c

Values are means ± S.E.M. Within a row, means with the same letters are not significantly different (P>0.05).

Wide ranges of SFA content was estimated in the muscle lipid of *T. tambroides* than that found in the experimental diets, while Ng & Andin (2011) reported the opposite trend in case of SFA content. It was established that like other freshwater fish *T. tambroides* are able to bio-convert 18:3(n-3) and 18:2(n-6) to metabolically more active long chain PUFA Du et al (2008).

It was observed that, FCR value was significantly higher in control diet, while differences were not evident among the PL supplemented groups. HSI showed no significant differences ($P>0.05$) among treatments. Condition factor (CF) attributed the same trend followed by the final weight in fish. Our results showed that higher dietary PL led to significantly higher HSI. Dietary PLs increased whole-body (Coutteau et al 1997; Liu et al 2002) and liver lipid concentrations (Craig & Gatlin III 1997; Liu et al 2002) in several species. Fontagne' et al (1998) showed that dietary soybean phosphatidylcholine, a significant portion of soybean lecithin, prevented intestinal steatosis and resulted in a larger liver volume and a larger hepatocyte volume.

SOD is the primary enzyme for radicals scavenging, which are involved in protective mechanisms within tissue injury following oxidative processes and phagocytosis (Fontagne-Dicharry et al 2014). This is one of the activities commonly used as antioxidant indicators to evaluate antioxidant defense system of fish (Sun et al 2011). Usually, higher levels of SOD, activities indicated an increased antioxidant defense in fish (Yang et al 2010). In this study, tissue SOD increased with incremental dietary PL level up to 4%, but the activities of this enzyme reduced in fish fed GPL6 diet (Figure 1). This result indicated that appropriate supplementation dietary PL could enhance stress resistance and induce antioxidant responses to protect an organ against oxidative damage (Hamza et al 2008; Zhao et al 2013; Gao et al 2014).

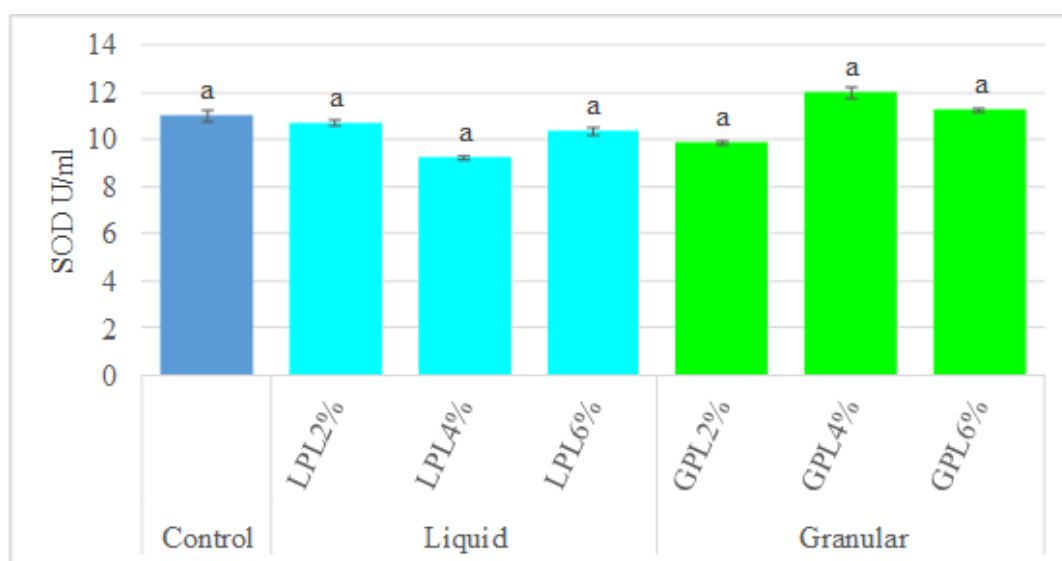


Figure 1. Superoxide dismutase activity (units/mg protein) of muscle protein of *Tor tambroides* fed diets supplemented with different levels and sources of phospholipids after 60 days feeding trial.

Conclusions. In conclusion, there are beneficial effects of dietary PLs on survival and growth performance of juvenile *T. tambroides*. The present study showed that the growth performance of *T. tambroides* could be enhanced by the addition of dietary soybean lecithin. Based on the SGR of *T. tambroides*, a diet with 4.0% PL is recommended for maximum growth under the experimental conditions used in this study. With regard to growth performance, as a phospholipid source, granular soybean lecithin is suggested for the better growth of *T. tambroides* juveniles.

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