



Dietary Lecithin Modulates Growth, Selected Hemato-immunological Indices, and Glutathione Peroxidase Activity of Hoven's Carp (*Leptobarbus hoevenii*)

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Abstract: Beneficial effects of dietary phospholipids on growth performance, hemato-immunological parameters, and antioxidant status in Hoven's carp (*Leptobarbus hoevenii*) are reported in this study. Experimental fish were fed four isoproteic (40%) and isolipidic (13%) experimental diets supplemented with lecithin at 0, 1, 3, and 5% for 90 days. The results showed that at 30, 60, and 90 days into the feeding trial, fish fed dietary supplementation containing 3% or 5% lecithin showed significantly improved total length, mean body weight, feed conversion ratio, and specific growth rate compared to the control group ($p < 0.05$). No significant differences in condition factor and survival rate were observed between fish receiving dietary lecithin at any tested concentration and the control group ($p > 0.05$). A study on hemato-immunological parameters of fish fed the experimental diets for 90 days revealed the highest red blood cell count in those receiving the 1% lecithin diet ($p < 0.05$), whereas the white blood cell count was significantly higher in fish fed 3% and 5% lecithin diets ($p < 0.05$). In addition, fish fed a 3% lecithin diet showed significantly higher glutathione peroxidase activity than those fed with the control diet or the 1% lecithin diet ($p < 0.05$). These findings suggest that dietary supplementation with lecithin modulates growth and some hematological and immunological parameters, as well as antioxidant activity, in this fish species. Based on the findings, dietary supplementation with lecithin at 3-5% could promote growth, stimulate the immune response, and enhance antioxidant activity in Hoven's carp.

Keywords: Hoven's carp; lecithin; growth; immunity; antioxidant activity

1. Introduction

Hoven's carp (*Leptobarbus hoevenii*), also known as Sultan fish, Jelawat [1], and mad carp [2], is a native fish found in rivers and lakes in Malaysia, Cambodia, Indonesia, Laos, Vietnam, and Thailand [3]. This species is a commercially important freshwater fish with high potential for aquaculture production as a food and ornamental fish in Southeast Asian countries [3-4]. Despite this fish being a valuable aquatic species, only a few studies have reported on its nutritional management. Previously, Sunarno [4] reported that the maximum growth and high feed conversion efficiency of this fish were observed at about 40% protein level. Furthermore, Farahiyah *et al.* [5] reported that the optimal protein level for this fish's growth was 38%. In addition,

nutritional management of Hoven's carp broodstock, larvae, and juveniles in culture systems has been reviewed recently [3].

Phospholipids, defined as lipids containing phosphorus, are well-known important precursors of biologically active mediators involved in physiology and metabolism [6]. However, fish may have a limited capacity to synthesize phospholipids, insufficient to fully meet their requirements at larval and early juvenile stages [6]. Regarding this concern, lecithin has been used as a ubiquitous phospholipid source in aquafeed [7]. Lecithin is a glycerol phospholipid with varying amounts of sphingosyl phospholipids, glycolipids, triglycerides, and fatty acids [7], and lecithin can be found in both plants and animals, especially in soybean, sunflower, rapeseed, corn, camelina seed, canola, rice bran, egg yolk, dairy products, and marine products [8]. Previous studies have reported beneficial effects of dietary lecithin on growth performance, survival rate, immunity, and antioxidant capacity in aquaculture species. Incorporating lecithin at an optimum dose of 3.56% in the diet enhances survival, overall growth performance, and improves carcass composition of early juvenile milkfish (*Chanos chanos*) [9]. Furthermore, the optimum dose of lecithin required for better productivity and health performances of striped catfish (*Pangasianodon hypophthalmus*) is 3.65-4.42% [10]. Moreover, 4% dietary lecithin could not only improve growth, hematological indices, antioxidant capabilities, immune responses, and intestinal functions, but also alleviate inflammatory responses in largemouth bass (*Micropterus salmoides*) juveniles [11]. Therefore, the present study aimed to investigate the effects of dietary lecithin on growth performance, blood parameters, and antioxidant activity of Hoven's carp. The results of this study can serve as guidelines for developing Hoven's carp aquaculture in Southeast Asia.

2. Materials and Methods

2.1 Ethics statement

This study was carried out in accordance with the "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes", National Research Council, Thailand, and has been approved by the Institutional Animal Care and Use Committee, Prince of Songkla University, under permission number Ref. AQ069/2022.

2.2 Experimental fish

Healthy Hoven's carp with an average weight of 0.1 g obtained from Songkhla Inland Aquaculture Research and Development Center were used in this study. The fish were reared in 3,000 L fiberglass tanks containing 2,000 L of dechlorinated tap water with aeration until reaching approximately 2.0 g. During the cultivation period, water exchange was performed every 3 days at 80%. Experimental fish were fed to satiation with commercial feed twice daily at 9:00 AM and 4:00 PM.

2.3 Experimental diets

Sunflower lecithin powder (NOW Foods, IL, USA) was used in the present study. Four experimental diets were formulated to be isoproteic at 40% and isolipidic at 13% (Table 1). The experimental diets were designed to contain lecithin at 0%, 1%, 3%, and 5%. The raw materials were ground to a fine particle size, passed through an 80-mesh sieve, mixed, and pelleted using the HOBART machine (LEGACY®, OH, USA). The pellet-form fish feed was then dried in a hot-air oven at 60 °C for 3 h to obtain a moisture content not exceeding 10%. The experimental diets were analyzed for moisture, crude protein, crude lipid, crude fiber, and ash content, according to AOAC [12].

2.4 Experimental design

The experiment was carried out with 600 fish stocked in fiberglass tanks (120×50×40 cm) each containing 180 liters of dechlorinated tap water and equipped with an aeration system. Each tank was stocked with thirty fish. The experiment was conducted in a completely randomized design with 4 treatments and 5 replications: T1 (Control; 0% lecithin), T2 (1% lecithin), T3 (3% lecithin), and T4 (5% lecithin). During the rearing period, fish were fed to satiation twice daily at 9:00 AM and 4:00 PM for 90 days. Uneaten feed was siphoned out daily. Water exchange was performed every day at 30%. The water quality parameters, temperature, and dissolved oxygen, were measured using a DO meter (Pro20, YSI); pH was determined using a pH meter (Starter 3100-F, gibthai); and total ammonia-nitrogen, nitrite-nitrogen, and alkalinity were analyzed by standard methods following Boyd and Tucker [13] every 7 days.

Table 1. Ingredients and proximate chemical compositions of the experimental diets.

Ingredient	Dietary lecithin supplementation (%)			
	0	1	3	5
Fish meal	32.00	32.00	32.00	32.00
Defatted-soybean meal	17.00	17.00	17.00	17.00
Rice bran	9.00	9.00	9.00	9.00
Corn meal	5.00	5.00	5.00	5.00
Poultry meal	15.00	15.00	15.00	15.00
Crude palm oil	9.00	8.20	6.60	5.00
Tapioca starch	9.00	8.80	8.40	8.00
NaCl	1.00	1.00	1.00	1.00
Vitamin premix ¹	0.50	0.50	0.50	0.50
Mineral premix ²	0.50	0.50	0.50	0.50
Carboxymethyl cellulose	2.00	2.00	2.00	2.00
Sunflower lecithin	0.00	1.00	3.00	5.00
Total	100.00	100.00	100.00	100.00
Proximate composition (% as fed basis)³				
Moisture	9.38 ± 0.10	7.32 ± 0.13	7.78 ± 0.02	7.10 ± 0.16
Crude protein	40.21 ± 0.12	40.34 ± 0.10	40.48 ± 0.19	40.88 ± 0.15
Crude lipid	12.66 ± 0.22	12.84 ± 0.09	12.97 ± 0.42	12.83 ± 0.86
Crude fiber	1.80 ± 0.02	1.79 ± 0.02	1.79 ± 0.01	1.78 ± 0.02
Ash	10.17 ± 0.58	9.96 ± 0.07	10.11 ± 0.03	10.26 ± 0.07

¹ Vitamin premix delivers the following per 100g of diet: Retinal (A) 250 IU; Cholecalciferol (D3) 50 IU; Tocopherol (E) 2.5 mg; Menadione sodium bisulfite (K) 1 mg; Thiamine (B1) 1.25 mg; Riboflavin (B2) 0.5 mg; Pyridoxine (B6) 0.5 mg; Cobalamin (B12) 0.005 mg; Ascorbic acid (C) 5 mg; Niacin 1.5 mg; Calcium pantothenate 1.5 mg; Folic acid 0.15 mg; Inositol (B8) 0.5 mg; and Biotin 0.005 mg.

² Mineral premix delivers the following per 100g of diet: Ca 50 mg; P 40 mg; Cu 1.25 mg; Fe 0.6 mg; Mn 0.6 mg; Zn 0.77 mg; K 0.13 mg; I 0.37 mg; Mg 1.08 mg; Se 0.005 mg; and Co 0.12 mg

³ Proximate compositions are expressed as mean ± SD of triplicates.

2.5 Growth performance and survival rate

At 30, 60, and 90 days into the feeding trial, all experimental Hoven's carp were fasted for 24 h before being counted, weighed, and measured for body length. Total length was measured according to the method of Upasit *et al.* [14]. Condition factor (CF) was calculated according to the method of Kliangetapong *et al.* [15]. Feed conversion ratio (FCR), specific growth rate (SGR), and survival rate were calculated according to the method of Kamnerddee *et al.* [16]. The growth performance and survival rate were calculated as follows.

$$CF = [\text{body weight (g)} / \text{total length (cm)}^3] \times 100$$

$$FCR = \text{total feed consumed (g)} / \text{weight gained (g)}$$

$$SGR (\%/day) = \{[\ln \text{ final weight} - \ln \text{ initial weight}] / \text{time interval (day)}\} \times 100$$

$$\text{Survival rate} = [\text{final number of fish} / \text{initial number of fish}] \times 100$$

2.6 Proximate compositions in muscle tissue of Hoven's carp

At the end of the feeding trial, 2 fish from each replication (10 fish/treatment) were randomly selected and analyzed for proximate composition. Moisture, crude protein, crude lipid, and ash were analyzed for muscle tissue using the methods of AOAC [12].

2.7 Hemato-immunological parameter analysis

At the end of the experiment, two fish from each replication were randomly collected (10 fish/experiment) for blood parameter analyses. Fish were anesthetized with clove oil at a concentration of 40 mg/L [17]. Blood was withdrawn from the caudal vein/artery for the study of blood parameters, namely red blood cell and

white blood cell counts, hematocrit, and hemoglobin, measured using the method of Suwannasang *et al.* [18]. Wintrobe erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) were measured according to the method of Malawa *et al.* [19]. Serum protein was measured using the Lowry *et al.* [20] method. The nitroblue tetrazolium reduction was assayed using the modified method of Stasiak and Baumann [21]. Lysozyme activity was measured according to the method of Demers and Bayne [22]. Glucose content was measured according to the method of Hyvärinen and Nikkilä [23]. Alkaline phosphatase in blood serum was determined using a Cobas 8000 analyzer (Roche Diagnostics).

2.8 Antioxidant activity and thiobarbituric acid reactive substances in liver

At the end of the experiment, liver samples from two fish from each replication (10 fish/treatment) were collected for antioxidant activity analysis. Tissue samples were preserved in liquid nitrogen before enzyme extraction, as described by Ávila-Villa *et al.* [24]. The antioxidant activities determined were catalase (CAT) activity, analyzed according to the method of Trasviña-Arenas *et al.* [25], and Glutathione peroxidase (GPx) activity, determined using the Glutathione Peroxide Assay Kit No. 703102 (Cayman, USA). Thiobarbituric acid reactive substances (TBARs) were determined according to the method of Senphan and Benjakul [26].

2.9 Statistical analysis

Data are expressed as mean $\pm$ standard deviation. Data that are percentages were subjected to arcsine transformation before analysis of variance. Significant differences were analyzed using one-way analysis of variance. Differences between treatments were then confirmed using Duncan's multiple range test, with $p < 0.05$ required for statistical significance.

3. Results and Discussion

3.1 Growth performance and survival rate

In aquafeed, phospholipids are an essential component as they provide energy for metabolic activities and promote the digestion and absorption of other dietary lipids [7]. Accordingly, soybean lecithin has been widely used as a phospholipid source in aquafeed [11, 27-30]. However, growing genetically modified organisms (GMOs) and environmental concerns have led to a demand for developing alternative sources of vegetable lecithin, but research on these alternatives remains limited compared to extensive studies on soybean lecithin [31]. This study documents the growth performance, hematological and immunological parameters, and antioxidant status of Hoven's carp fed a diet supplemented with sunflower lecithin. In this study, the experimental diets were prepared to contain 40% protein and 13% fat, consistent with a study by Sunarno [4], who reported that a feed containing 40% protein and 14% fat is optimal for this fish species, resulting in maximum growth. Growth performance and survival rate of the experimental fish receiving dietary supplementation with lecithin at various concentrations are shown in Table 2. At 30, 60, and 90 days of the feeding trial, fish fed with dietary supplementation of lecithin at the concentrations 3% or 5% showed significantly improved total length, average body weight, FCR, and SGR, in comparison to the control group ($p < 0.05$). In addition, no significant differences in CF or survival rate were observed between fish receiving dietary supplementation with lecithin at any tested concentration and the control group ($p > 0.05$). Water quality parameters in all the experimental tanks during the experimental period were within the acceptable ranges for aquaculture [32-33]: temperature was 26.49 ± 0.35 °C, pH was 7.06 ± 0.04 , dissolved oxygen was 5.33 ± 0.44 mg/L, total ammonia-nitrogen was 0.74 ± 0.07 mg/L, nitrite-nitrogen was 0.08 ± 0.01 mg/L, and alkalinity was 20.05 ± 2.6 mg/L.

Vegetable lecithins, containing primarily phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid, are typically produced from soybean, rapeseed, or sunflower [34-36]. According to van Nieuwenhuyzen and Tomás [35], the distribution of the main phospholipid components of sunflower lecithin is considerably similar to that of soybean lecithin. Additionally, sunflower lecithin is considered an attractive alternative to soybean lecithin due to its similar composition [35] and its advantage of being derived from a non-GMO source [36]. The phospholipid composition in sunflower lecithin used in this study includes 25% phosphatidylcholine, 18% phosphatidylinositol, and 11% phosphatidylethanolamine.

Phospholipids are vital precursors for a range of highly biologically active mediators of metabolism and physiology [6]. The improved growth of aquaculture species induced by dietary lecithin has been previously investigated. Batista *et al.* [29] reported that Nile tilapia (*Oreochromis niloticus*) fed a diet supplemented with soybean lecithin at 42-50 g/kg showed better growth, lipid digestibility, and body composition. Moreover, Amer *et al.* [10] reported that the final weight, weight gain, SGR, feed intake, and protein efficiency ratio were markedly higher in striped catfish treated with 2-4% soybean lecithin than in the control treatment group (0% soybean lecithin). In addition, Sink and Lochmann [27] reported that dietary supplementation with soybean lecithin at concentrations of 0-4% did not affect the survival rate of juvenile channel catfish (*Ictalurus punctatus*). In this study, however, no significant difference in growth performance was observed between Hoven's carp fed a diet supplemented with 1% soybean lecithin and the control group ($p > 0.05$). This is consistent with Ciji *et al.* [37], who reported that dietary supplementation with 0-2% lecithin did not show significant differences in growth performance, measured as percentage weight gain, SGR, and survival rate, in golden mahseer (*Tor putitora*). In contrast, Butler *et al.* [38] reported that increasing the lipid content from 10% to 15%, combined with 1% sunflower lecithin inclusion, enhanced the weight gain of juvenile giant grouper (*Epinephelus lanceolatus*) by 12.14%. The differences in our findings compared to prior studies may be attributed to effects from fish species, the size of experimental subjects, and culture conditions.

Table 2. Growth performance of Hoven's carp fed with dietary supplementation with lecithin at various concentrations for 90 days.

Treatment	Dietary lecithin supplementation (%)				p-value
	0	1	3	5	
Average total length (cm)					
Initial total length	5.86 ± 0.03	5.88 ± 0.03	5.85 ± 0.02	5.85 ± 0.02	0.244
Day 30	8.20 ± 0.20 ^a	8.40 ± 0.20 ^a	8.65 ± 0.12 ^b	8.63 ± 0.13 ^b	0.002
Day 60	9.91 ± 0.16 ^a	10.11 ± 0.43 ^{ab}	10.56 ± 0.26 ^b	10.54 ± 0.35 ^b	0.017
Day 90	11.24 ± 0.17 ^a	11.49 ± 0.27 ^{ab}	11.68 ± 0.27 ^b	11.80 ± 0.32 ^b	0.023
Average body weight (g)					
Initial body weight	2.25 ± 0.01	2.26 ± 0.01	2.24 ± 0.01	2.25 ± 0.02	0.388
Day 30	6.94 ± 0.07 ^a	7.21 ± 0.40 ^a	7.82 ± 0.26 ^b	7.86 ± 0.42 ^b	0.001
Day 60	13.96 ± 0.55 ^a	14.52 ± 1.10 ^a	15.74 ± 0.45 ^b	16.76 ± 0.63 ^b	<0.001
Day 90	22.06 ± 1.13 ^a	22.41 ± 1.87 ^a	25.08 ± 1.03 ^b	26.67 ± 1.02 ^b	<0.001
Condition factor					
Day 30	1.26 ± 0.10	1.21 ± 0.04	1.21 ± 0.05	1.22 ± 0.06	0.620
Day 60	1.44 ± 0.11	1.41 ± 0.13	1.34 ± 0.09	1.44 ± 0.14	0.631
Day 90	1.56 ± 0.12	1.47 ± 0.06	1.58 ± 0.06	1.63 ± 0.11	0.099
Feed conversion ratio					
Day 0-30	2.13 ± 0.03 ^b	2.06 ± 0.18 ^b	1.77 ± 0.07 ^a	1.79 ± 0.15 ^a	<0.001
Day 0-60	2.02 ± 0.10 ^b	1.90 ± 0.16 ^b	1.68 ± 0.05 ^a	1.59 ± 0.08 ^a	<0.001
Day 0-90	1.77 ± 0.11 ^b	1.75 ± 0.15 ^b	1.52 ± 0.07 ^a	1.45 ± 0.06 ^a	<0.001
Specific growth rate (%/day)					
Day 0-30	3.75 ± 0.03 ^a	3.86 ± 0.20 ^a	4.16 ± 0.11 ^b	4.17 ± 0.18 ^b	0.001
Day 0-60	3.04 ± 0.07 ^a	3.10 ± 0.14 ^a	3.25 ± 0.05 ^b	3.35 ± 0.06 ^b	<0.001
Day 0-90	2.53 ± 0.06 ^a	2.54 ± 0.09 ^a	2.68 ± 0.05 ^b	2.75 ± 0.04 ^b	<0.001
Survival rate (%)					
Day 0-90	100.00 ± 0.00	99.33 ± 1.49	100.00 ± 0.00	100.00 ± 0.00	0.454

Values within the same row with different superscripts are significantly different ($p < 0.05$).

3.2 Proximate compositions in muscle tissue of Hoven's carp

Proximate composition of muscle tissue was significantly affected by dietary supplementation with lecithin (Table 3). The fish fed with 1-5% lecithin had significantly higher crude lipid than those in the control

group ($p < 0.05$). There were no statistical differences in moisture, crude protein, or ash content of the muscles of fish examined in this study ($p > 0.05$). The enhanced lipid digestion and absorption of fish in response to lecithin supplementation is associated with the emulsifying property of lecithin [9, 39]. Balito-Liboon *et al.* [9] reported that the highest tissue protein and lipid contents were observed in early juvenile milkfish fed a diet with 3% soybean lecithin, and declines in tissue protein and lipid contents were observed beyond this dose level. The lipid composition in the muscle of the fish consisted of non-phospholipids and phospholipids. Major lipid component of the muscle from red sea bream (*Pagrus major*), amberjack (*Seriola dumerili*), and striped jack (*Caranx delicatissimus*) was triglyceride. At the same time, phospholipids, including phosphatidylcholine and phosphatidylethanolamine, were the main lipid components in the muscle of puffer (*Takifugu rubripes*) and flounder (*Paralichthys olivaceus*) [40]. Generally, freshwater fish are an important source of n-3 fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid [41–42]. Several studies have shown that consuming freshwater fish has beneficial effects on human health [41]. The deposition of fatty acids in fish tissue is influenced by the fatty acid profiles of the diets [43]. In addition, lipid distribution patterns are related to flesh quality and the yield rate of aquaculture products. A preferable lipid distribution pattern, characterized by small lipid depots evenly distributed in the edible part of muscle, positively impacts consumers' taste perception [44]. This study, however, did not analyze lipid class and fatty acid composition, as well as lipid distribution, in the fillet of Hoven's carp. Further research on dietary lecithin and its effects on flesh quality should be conducted to promote this fish species as a valuable source of nutrition.

Table 3. Proximate compositions (% wet weight basis) of muscle tissue of fish fed experimental diets supplemented with various concentrations of lecithin for 90 days.

Treatment	Dietary lecithin supplementation (%)				p-value
	0	1	3	5	
Moisture	75.44 $\pm$ 0.28	74.88 $\pm$ 0.59	75.12 $\pm$ 0.93	75.59 $\pm$ 1.14	0.512
Crude protein	19.45 $\pm$ 0.41	19.55 $\pm$ 0.49	19.61 $\pm$ 1.32	19.36 $\pm$ 0.83	0.968
Crude lipid	1.95 $\pm$ 0.04 ^a	2.84 $\pm$ 0.04 ^b	2.83 $\pm$ 0.12 ^b	2.73 $\pm$ 0.10 ^b	<0.001
Ash	1.30 $\pm$ 0.17	1.25 $\pm$ 0.08	1.36 $\pm$ 0.07	1.40 $\pm$ 0.14	0.232

Values within the same row with different superscripts are significantly different ($p < 0.05$).

3.3 Hemato-immunological parameters

Hemato-immunological parameters have been used to monitor fish growth and health status [45–46]. Red blood cells transport oxygen [46]. In the present study, significantly higher red blood cell count was observed in fish fed with dietary supplementation of 1% lecithin in comparison to the other treatments ($p < 0.05$) (Table 4), suggesting that 1% lecithin provides an optimal supply of phospholipids necessary to support membrane fluidity and stability potentially involved in enhancing erythropoiesis and oxygen-carrying capacity in Hoven's carp. In addition, the fish fed a diet supplemented with 1% lecithin showed lower mean corpuscular volume and mean corpuscular hemoglobin values than other groups, although the difference was not statistically significant ($p > 0.05$). This could be the result of variation in cell size and hemoglobin content, which may have had physiological consequences for the fish [47]. The results from the present study are consistent with the results for striped catfish fed with dietary soy lecithin, which showed significantly higher red blood cells at 2% compared to the control group, with the highest level observed at 6% supplementation, followed by a reduction when fed beyond this level ($p < 0.05$) [48]. Similarly, significantly increased red blood cell counts have also been reported in female shark catfish (*Pangasius nasutus*), where the red blood cell count increased with phospholipid supplementation, with the highest value recorded in the 2.0–2.5% group [49]. In this study, fish fed diets supplemented with 3–5% lecithin showed significantly higher white blood cell counts than those in the other treatments ($p < 0.05$) (Table 4). The results from the present study resemble those in a previous study on juvenile largemouth bass fed diets supplemented with 2–4% soybean lecithin [11], and on stellate sturgeon (*Acipenser stellatus*) fed diets supplemented with 4–8% soybean lecithin [28]: these had significantly higher white blood cell counts in comparison to the control group ($p < 0.05$). While white blood cells are involved in both acquired and innate immunity [46], increasing white blood cell counts of fish fed

with experimental diet supplementation with lecithin may be related to phospholipids and their unsaturated fatty acids, which can maintain fluidity and permeability of cell membranes and enhance fish immunity [28, 50]. In this study, however, no significant differences were observed in hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, serum protein, lysozyme, nitroblue tetrazolium reduction, glucose, or alkaline phosphatase between the dietary treatment groups ($p>0.05$). This finding is consistent with a study by Sink and Lochmann [27], who reported that channel catfish fed dietary supplementation of 0-4% soybean lecithin showed no significant differences in hematocrit, hemoglobin, mean corpuscular hemoglobin concentration, or lysozyme activity. Additionally, Pagheh *et al.* [51] reported that silvery-black porgy (*Sparidentex hasta*) fed with dietary supplementation of 0-9% soybean lecithin had no significant differences in red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, or white blood cell count ($p>0.05$).

Table 4. Hemato-immunological parameters of fish fed experimental diets supplemented with various concentrations of lecithin for 90 days.

Parameter	Dietary lecithin supplementation (%)				p-value
	0	1	3	5	
RBC ($\times 10^9$ cell/mL)	2.63 ± 0.18^a	3.17 ± 0.41^b	2.66 ± 0.28^a	2.42 ± 0.57^a	0.005
WBC ($\times 10^7$ cell/mL)	2.12 ± 0.21^a	2.51 ± 0.87^a	3.74 ± 0.85^b	3.85 ± 1.03^b	<0.001
Hct (%)	22.51 ± 5.78	24.30 ± 2.55	23.19 ± 1.81	21.82 ± 1.28	0.409
Hb (g/dL)	5.49 ± 0.96	5.65 ± 0.44	5.71 ± 0.97	5.11 ± 0.32	0.274
MCV (femtoliter)	89.94 ± 10.55	79.69 ± 16.87	85.78 ± 6.37	83.06 ± 11.70	0.429
MCH (picogram)	20.97 ± 3.87	17.71 ± 1.59	21.79 ± 4.03	22.55 ± 7.04	0.221
MCHC (g/dL)	25.32 ± 5.52	23.59 ± 3.78	24.63 ± 3.72	23.44 ± 1.08	0.664
Serum protein (mg/mL)	33.16 ± 0.59	32.31 ± 2.35	33.03 ± 1.26	32.98 ± 1.66	0.728
Lysozyme (μ g/mL)	22.04 ± 8.86	21.09 ± 7.36	24.42 ± 6.18	32.81 ± 12.40	0.235
NBT reduction	2.90 ± 0.89	3.13 ± 0.65	3.01 ± 0.62	2.85 ± 0.44	0.824
Blood glucose (mg %)	27.15 ± 2.97	28.66 ± 4.30	28.39 ± 3.73	28.68 ± 3.89	0.771
ALP (U/L)	24.80 ± 3.35	21.40 ± 1.67	25.00 ± 5.92	23.80 ± 4.44	0.515

RBC; red blood cell, WBC; white blood cell, Hct; hematocrit, Hb; hemoglobin, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration, NBT; nitroblue tetrazolium, ALP; Alkaline phosphatase

Values within the same row with different superscripts are significantly different ($p<0.05$).

3.4 Antioxidant status

It is widely recognized that reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, can be generated in all cells by mitochondrial and enzymatic sources. These ROS can cause oxidative damage to DNA, proteins, and membrane lipids [52]. GPx and CAT are intracellular enzymatic antioxidants that are involved in the detoxification of hydrogen peroxide; GPx catalyzes the reduction of hydrogen peroxide into water [52], whereas the CAT catalyzes the decomposition of hydrogen peroxide into water and oxygen [53], thereby protecting tissue against oxidative damage. In this study, fish fed a diet supplemented with 3% lecithin showed significantly higher GPx activity compared to those that received dietary lecithin at 0 or 1% ($p<0.05$) (Fig. 1b), indicating that lecithin may provide protective effects against oxidative damage. This result is similar to those reported for largemouth bass juveniles fed with dietary soybean lecithin at 4% [11]. In this current study, however, no significant differences were found in CAT activity among the treatments at 90 days of the feeding trial ($p>0.05$) (Fig. 1a). Several studies on the antioxidative status in fish have reported that antioxidative enzyme activities vary among different growth phases [54], organs [55], and feeding and starvation conditions [56]. This is consistent with a previous study, which found that fish receiving dietary soybean lecithin did not show differences in CAT [51]. Basically, malondialdehyde, a TBAR, is an important indicator of lipid peroxidation. It is endogenously formed during intracellular oxidative stress and its reaction

with DNA, leading to cellular damage [57]. This study observed a decrease, though not statistically significant, in TBAR levels in fish fed 1-5% lecithin compared to the control group, indicating that lecithin may reduce oxidative stress in fish.

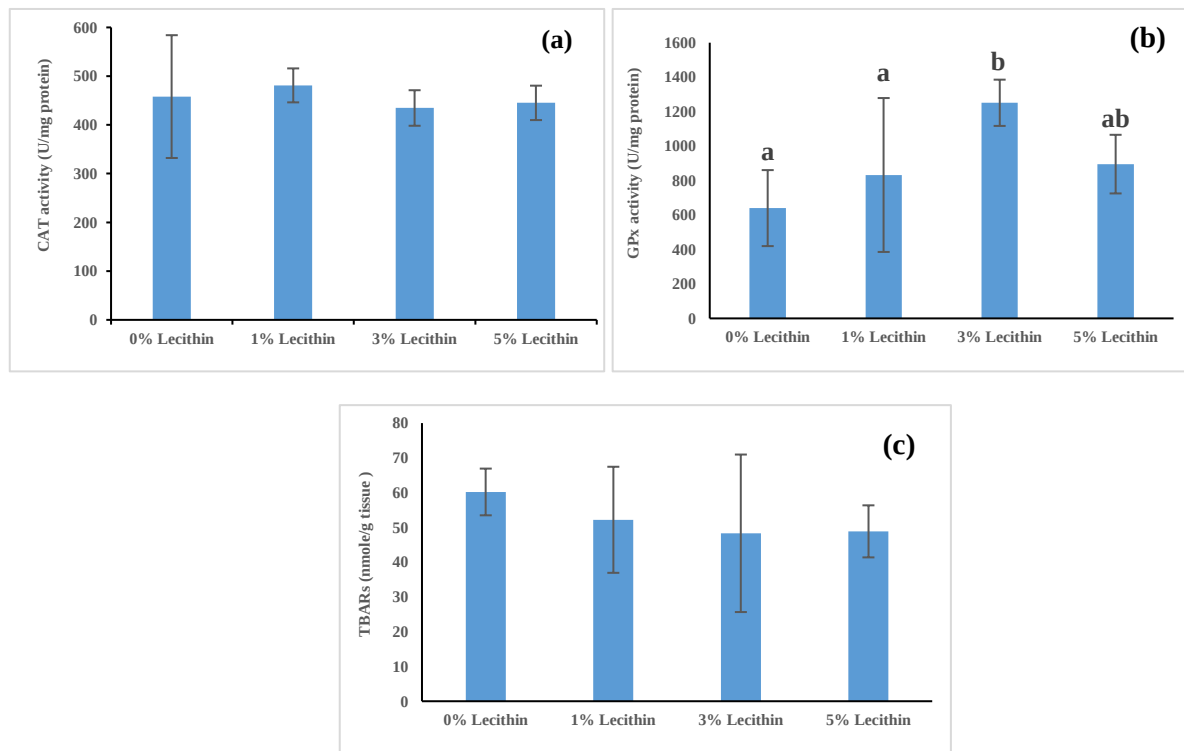


Figure 1. CAT activity (a), GPx activity (b), and TBARs (c) of Hoven's carp fed experimental diets supplemented with lecithin at various concentrations for 90 days.

4. Conclusions

Our findings from the present study indicate that dietary 3-5% lecithin modulates growth, hemato-immunological indices, and antioxidant activity in Hoven's carp. However, this study was conducted in small-sized experimental fish under laboratory conditions. Further investigation into a long-term field-based study may provide additional insights and improve the practical applicability of the findings. In addition, studies on the effects of lecithin on lipid class and fatty acid composition, as well as lipid distribution, in the fillet of Hoven's carp could provide more valuable information.

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