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Efficacy of dietary lysophospholipids (Lipidol™) on growth performance, serum immuno-biochemical parameters, and the expression of immune and antioxidant-related genes in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

A 56-day feeding experiment was conducted to evaluate the dietary Lipidol™ (functional ingredient: lysophospholipids) on growth performance, biochemical and immunological parameters, and selected immune and antioxidant-relevant genes expression in rainbow trout. Fish (N = 840; 70.4 ± 1.5 g) were randomly distributed in 12 circular fiberglass tanks and nominated to four treatments: 0 (control), 1, 2, and 3 g Lipidol™ per kg diet. Results indicated that growth performance and feed intake were significantly higher and feed conversion ratio was lower in fish fed with 2 g kg^{-1} Lipidol™ ($p < .05$). The lowest albumin, glucose, and high-density lipoprotein, and the highest low-density lipoprotein were observed in fish fed with 2 g kg^{-1} Lipidol™ ($p < .05$). Similarly, the most activity of IgM and lysozyme were observed in 2 g kg^{-1} Lipidol™ and complement proteins were higher in fish fed with 1 and 2 g kg^{-1} Lipidol™ than other groups ($p < .05$). The lowest hepatic enzymes activity and the highest villus height were observed in fish fed with 2 g kg^{-1} Lipidol™ ($p < .05$). Fish fed with 2 g kg^{-1} Lipidol™ had significantly higher expression of tumor necrosis factor-α, interleukin-1β and interleukin-8 compared to others ($p < .05$). Also, the expression of liver genes including superoxide dismutase, catalase, and glutathione peroxidase were significantly up-regulated in 2 g kg^{-1} Lipidol™ ($p < .05$). Taken together, supplemental Lipidol™ at 2 g kg^{-1} in diet could promote the growth performance, serum biochemical parameters, and immune-related genes expression of rainbow trout.

1. Introduction

Over the past three decades, it has been confirmed that adding phospholipids (PLs) supplements in aqua-feeds can be used at about 2–4% in the diets to boost up the growth performance in some marine and freshwater fish species (Tocher et al., 2008; Saleh et al., 2013;). Moreover, many investigators showed the positive effect of PLs as a growth promoter (Tocher et al., 2008; Daprà et al., 2011; Azarm et al., 2013; Zhang et al., 2019), prevention of malformations (Kanazawa et al., 1981; Tocher et al., 2008; Kenari et al., 2011), stress resistance (Tocher et al., 2008; Zhao et al., 2013; Kumar et al., 2014; Adel et al., 2017), and increase in survival ratio in fish and shellfish (Niu et al., 2008; Saleh et al., 2015; Noordin et al., 2018). In fact, PLs are considered as a group of polar lipids in aquatic animals diet that may promote emulsification of lipid and assistance in the digestion and absorption of lipids in the intestine of fish (Tocher et al., 2008), shrimp (Kanazawa et al., 1985; Kumaraguru Vasagam et al., 2005), and crayfish (Thompson et al., 2003). Despite the fact that PLs will be prioritized as a pathway for various substances across the cell membranes, they also act as a feed attractant in fish diet (Adel et al., 2017; La et al., 2018; Noordin et al., 2018) and lead to reduce leaching of water-soluble nutrients (Hu et al., 2011). Although previous investigations described that de novo synthesis of PLs happens in fish (Kanazawa et al., 1985; Tocher, 1995; Tocher et al., 2008), it seems that the rate of this synthesis usually occurs insufficiently to confront their metabolic demands (Kenari et al., 2011; Azarm et al., 2013; Adel et al., 2017; Noordin et al., 2018).

Naturally, lysophospholipids (LPLs) are produced by the degradation of PLs with pancreatic phospholipase A_2 and act as an emulsifier in order to increase other lipid digestibility (Tocher et al., 2008). The capability for stabilizing water-in-oil emulsion allows fatty acids to form into the micelles; therefore, it enhances lipid metabolism, nutrient digestibility, and growth performance of animals (Zampiga et al., 2016; Zhao et al., 2017; Mohammadigheisar et al., 2018;). In general,

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synthetic LPLs are commercially produced from hydrolyzing lecithin from soybean, sunflower or egg yolk for animal nutrition (Olsen et al., 2003; Tocher et al., 2008; Saleh et al., 2013). The produced LPLs can accelerate the absorption of some nutrients with their freely entering through the cell's membrane. However, there are no researches on the effects of supplementing fish diet with LPLs, even though some investigations have been reported on the influences of dietary LPLs in broilers, lactating sows, and weanling pigs.

The immune system of fish like other vertebrates is a complex network of molecules, signaling pathways, receptors, and cells to make a group of defense responses. In this regard, cytokines are signaling group of molecular which are excreted by different immune cells in order to modulate and regulate immunity system (Secombes et al., 2001). Since PLs along with LPLs are presented in many biological membranes like immune cells, it was not unexpected to exhibit an immunomodulatory effect. For instance, they can be found in the intestinal epithelial cells which act in the mucosal immune responses, express several receptors, and inflammatory mediators (Ehehalt et al., 2004; Treede et al., 2007). Moreover, the unsaturation degree of fatty acyl chain in polar lipids such as PLs and LPLs is greatly associated with the antioxidant capacity, immunological function, and other biological activates (Sugino et al., 1997; Treede et al., 2009). Hence, numerous studies have been demonstrated that dietary fatty acids, especially polyunsaturated fatty acids, induced the innate immunity (Xu et al., 2010; Sun et al., 2011), specific immunity (Montero et al., 2010; Zuo et al., 2012; Li et al., 2013) and the expression level of inflammatory (Tan et al., 2017) and anti-inflammatory cytokines (An et al., 2020) in fish. To our best knowledge, there is no study focus on the effects of dietary PLs and especially LPLs on the mRNA level of cytokines in fish, which is worthy to research.

Rainbow trout (*Oncorhynchus mykiss*) is one of the important cultivable Salmonidae fish species in the world and also it is the most important cultivated inland fish species in the cold-water aquaculture industry of Iran (Hosseini Shekarabi et al., 2020). Since nutrition plays a vital role in fish growth performance and immunity system as well as production cost, researchers seek to improve the fish diet using dietary supplements in line with sustainable development. Therefore, this study aimed to appraise the effects of dietary levels of a commercial product (Lipidol™) as an exogenous source of LPLs on some growth performance, hematology and serum biochemical parameters, immunity, intestinal morphology, and antioxidant responses of rainbow trout.

2. Material and methods

2.1. Experimental feeding trials and husbandry

The present study was conducted in Ghadi farm in the Haraz road (Mazandaran province, Iran). Rainbow trout, *O. mykiss*, (n = 840) with an initial weight of 70.4 ± 1.5 g was randomly distributed into 12 circular fiberglass tanks (2000*L*) at a stocking density of 70 fish per tank and was equipped with air stones. After the arrival, the fish were fed with the control diet for 14 days for acclimatization. During the investigation, fish were fed manually three times a day (8:00, 12:00, and 16:00) to ad libitum for 56 days. Each trial diet included three replicates. In order to prevent contamination of the tanks, daily feces and leftover feed from the bottom of the tanks were siphoned out, and one-third of the tank water was replaced to maintain the water quality. The physicochemical parameters were monitored daily. Water temperature, pH, dissolved oxygen, unionized ammonia, and nitrite were at 12 ± 3 °C, 7 ± 1.4, 7 ± 1.4 mg L^{-1}, < 0.02 mg L^{-1}, < 0.01 mg L^{-1}, respectively during the trial. In addition, the fish were held under natural photoperiod conditions throughout the feeding trial.

2.2. Experimental diets

Diets were formulated based on the protocols reported by Gatlin (2010) using UFFDA software (UFFDA, 1992, Athens, GA) according to the different levels of Lipidol™ (functional ingredient: LPLs). Lipidol™ is derived from soy lecithin and it was prepared from Easy Bio (Seoul, South Korea). Information, which was obtained by the manufacturer reported that Lipidol™ contains 12.18% total PLs, 4.06% LPLs, and 27.21% lecithin. Furthermore, it contains 22.76% saturated fatty acids (SFA), 18% 18:1 and 52.95% 18:2n6 as the percentage of total fatty acids. Diets were prepared using different levels of Lipidol™ including 0 (control), 1, 2, and 3 g kg^{-1} (Table 1). The measured phospholipid contents for four treatments were 2.83, 3.1, 3.26, and 3.89 g kg^{-1}, respectively. Total PLs of the experimental diets were determined by the colorimetric determination method of phosphorus as molybdenum blue, according to Li et al. (2005) method. Primarily, all the ingredients are finely ground (< 500 μm), weighed, and mixed (vertical mixer, Khazar Electric, Iran) to obtain a homogeneous mixture. All ingredients were thoroughly mixed in a mixer until a homogenous consistency was given. Water and molasses were gradually added to the blended ingredients and mixed for 15 min to make a stiff dough. This stiff dough mixture was extruded by a 1.5-mm diameter noodle-like die using a twin-screw extruder (Muyang, China) with temperature ranging 105–110 °C for 90 s. All diets were dried at 25 °C for 30 h until the

Table 1
Ingredients and proximate composition of the experimental diets (g kg^{-1}, dry matter).

Ingredients (g kg^{-1})	Lipidol™ in the experimental diets			
	0 (control)	1	2	3
Fishmeal[a]	280	280	280	280
Meat meal (cattle slaughter waste)	150	150	150	150
Wheat flour	140	140	140	140
Soybean meal[b]	250	250	250	250
Sunflower oil	85	85	85	85
Yeast	30	30	30	30
Molasses	40	40	40	40
L-Lysine[c]	2.5	2.5	2.5	2.5
DL-Methionine[d]	1.5	1.5	1.5	1.5
Vitamin mixture[e]	10	10	10	10
Mineral mixture[f]	5	5	5	5
Sand[g]	6	5	4	3
Lysophospholipids[h]	0	1	2	3
Total	1000	1000	1000	1000
Proximate analysis (g kg^{-1})				
Crude protein	420	425	421	420
Crude lipid	176	180	181	179
Crude fiber	19.6	20	20.6	19.8
NFE[i]	251.4	233	237.4	242.2
Crude ash	71	72	69	71
Moisture	62	70	71	68
Gross energy (MJ/kg)	21.9	21.6	22	22.2

[a] Fishmeal was produced from black sea sprat (*Clupeonella cultriventris*) (Momtazdaneh Company, Iran) and included 701 g kg^{-1} protein, 96 g kg^{-1} fat and 93 g kg^{-1} ash.
[b] The proximate composition of roasted full-fat soy flakes was 36.0% protein, 21.46% lipid, 4.7% ash, and 3.5% fiber (Shayan Energy and Protein Company, Tehran, Iran).
[c] Aras Taban Pharmaceutical Factory (Amol, Mazandaran Province, Iran).
[d] Aras Taban Pharmaceutical Factory (Amol, Mazandaran Province, Iran).
[e] Vitamin premix was prepared from Science Laboratories Company (Qazvin, Iran) and the composition per 1000 g was: 160,000 IU vitamin A, 400,000 IU Vitamin D3, 40 mg vitamin E, 2 g vitamin K3, 6 g vitamin B1, 8 g vitamin B2, 12 g Pantothenate calcium, 40 g vitamin B3, 4 g vitamin B6, 2 g vitamin B9, 8 g vitamin B12, 0.24 g H 2, 60 g vitamin C, and 20 mg Inositol.
[f] Mineral premix (2600 mg Man, 600 mg Cu, 6000 mg Fe, 4600 mg Zn, 100 mg Se, 100 mg I, and 50 mg Co).
[g] Afrandtooska mine company (Semnan, Iran) which was sieved to 2 mm in size (66.5% SiO_2 and 11.81% Al_2O_3).
[h] Lipidol™ (active ingredient: lysophospholipids) obtained from Easy Bio Inc. (Seoul, South Korea).
[i] Nitrogen-free extract (NFE) = dry matter – (crude protein + crude lipid + ash + fiber).

moisture content of the diets reached less than 10%. Afterward, they were sealed in the plastic bags and preserved at −20 °C until usage.

2.3. Growth performance

At the end of the feeding test, the fish were starved for 24 h and anesthetized by 150 mg L^{-1} of clove oil (Hushangi and Hosseini Shekarabi, 2018). All fish were individually weighed (with an accuracy of 0.01 g) to determine weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), body weight increase (BWI), protein efficiency ratio (PER), condition factor (CF), feed intake (FI), and survival rate (SR). The calculation of the mentioned biological indices was done in the following formulas:

WG (g) = final weight (g) − initial weight (g).

FCR = [dry feed intake (g)]/[wet weight gain (g)].

SGR (% day^{-1}) = 100 × [Ln (final body weight (g)) –Ln (initial body weight (g))]/ days of culture.

BWI (%) = 100 × [final weight (g) - initial weight (g)]/initial weight (g).

PER (%) = wet weight gain (g)/ total protein intake (g).

CF (g cm^{-3}) = final weight (g)/final body length (cm).

FI (g day^{-1}) = total feed intake per fish/day.

SR (%) = 100 × (final fish number)/(initial fish number).

2.4. Chemical composition of diets and fish

The proximate analysis of the diets and whole-body was performed according to the standard methods (AOAC, 2000). Three fish per tank (nine fish per treatment) were randomly caught and anesthetized by an overdose of clove powder (1000 mg L^{-1}). The fish were eviscerated and the flesh was stored at −20 °C until use. The moisture content was detected by drying in the oven (ED 53, Binder, Germany) at 105 °C to steady weight. Crude protein and lipid contents were measured by the Kjeldahl apparatus (V40, Bakhshi, Tehran, Iran) and extraction by the Soxhlet system (Bakhshi, Tehran, Iran), respectively. The ash content was determined by incineration samples in a muffle furnace at 550 °C for 6 h.

2.5. Serum biochemical and immunological analysis

At the end of the research, fish feeding was ceased for one day, and nine fish were randomly captured from each treatment (three fish for each replicate) and euthanized in clove powder (300 mg L^{-1}). Blood samples were taken from the caudal vine using 1 mL syringes and pooled into non-heparin tubes and centrifuged (10 min at 836 ×*g*) at 4 °C. Afterward, serum specimens were stored at −80 °C until used to measure the serum biochemical and immunological indices (Hushangi and Hosseini Shekarabi, 2018).

Fish serum biochemistry parameters including Cholesterol, Albumin, Glucose low-density lipoprotein (LDL), high-density lipoprotein (HDL), and hepatic indices of serum such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured by an autoanalyzer instrument (Pristage Biomedical Ltd., Tokyo, Japan) use of commercial kit (Pars-Azmoon, Karaj, Iran) based on the manufacturer's protocol.

Also, some immune parameters including complement C3, complement C4, and immunoglobulin (IgM) content determined by a Pristage Biomedical Autoanalyzer using assay kits purchased from Pars-Azmoon Company (Iran). Lysozyme level was estimated by a turbidimetric assay based on the method described by Ellis (1990).

2.6. Intestinal morphology

For the mid intestinal histological analyses of the fish, samples were primarily fixed in 10% and then dehydrated in a graded series by ethanol, dealcoholized in butanol. The fixed samples were embedded in paraffin, sectioned into 4 μm, and stained using hematoxylin-eosin (Merck, Darmstadt, Germany). The structure of the intestinal tissue morphology was recognized using an optical imaging microscope (ECLIPSE 80i, Nikon, Japan) equipped with image analysis software (ImageJ 1.32j; National Institute of Health, USA). The villus height was assayed from the apex of the villus to the depths of villus trenches. The crypt depth was assayed from the base of the submucosa to the villus (Qu et al., 2019).

2.7. Gene expression studies

At the end of the experimental feeding period, five were randomly caught from each tank fish (n = 15 fish per treatment). Anesthetized fish were rapidly dissected on ice to collect the liver and kidney tissue samples. The tissues were immediately moved into liquid nitrogen (−196 °C) to prevent any RNA degradation and preserved at −80 °C before RNA extraction.

2.7.1. RNA extraction and cDNA synthesis

Total RNA extraction was performed using Biozol commercial kit (Bioflux-Bioer, China) according to the manufacturer's guidelines. In addition, a secondary DNA-removal step was carried out on 1 μg of RNA with the DNase I (Invitrogen, USA) to prevent any genomic DNA contamination. The quantity of the isolated RNA was measured by a nanodrop spectrophotometer (Eppendorf, Hamburg, Germany) at 260/280 nm and the quality also checked by stained gel-electrophoresis (1.5% agarose gel). The first-strand of cDNA was synthesized from the extracted RNA samples using commercial cDNA synthesis kit (GeNet BIO Inc., Daejeon, South Korea) based on the manufacturer's protocol.

2.7.2. Primer design

The qPCR specific-primers for catalase (*CAT*), superoxide dismutase (*SOD*), glutathione peroxidase (*GPX*), interleukin-1β (*IL-1β*), interleukin-8 (*IL-8*) and tumor necrosis factor-a (*TNF-α*) were designed according to the conserved regions of *O. mykiss* Gene Bank using Oligo 7 software (Table 2). Also, the β-actine was used as the reference gene.

2.7.3. Quantitative real-time PCR

The expression of the selected genes was measured with an iCycler iQ real-time PCR (Bio-RAD, USA) using the Wizpure qPCR master mix (Wizibiosolutions, South Korea) as an SYBR green fluorescent dye and gene-specific primers. Also, 5 serial dilutions of the synthesized cDNA were prepared to estimate the efficiency of primers according to the equation reported by et al. (Radonić et al., 2004). The fold chain of mRNA expression of selected genes was calculated by the DDCt ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen, 2001). The iQ5 optical system software version 2.0 (Bio-Rad) was used to analyze the achieve data.

2.8. Statistical analysis

The quantitative analyses of data were expressed as men ± standard deviation (*SD*). The normality of data was assessed with Kolmogorov-Simonov test. Differences between treatments were identified using one-way ANOVA (Duncan's test). The entire procedures were conducted by SPSS software no 19.0 (SPSS, Chicago IL, USA). The relation between the different levels and the SGR were subjected to second-degree polynomial regression analysis by Excel software 2016. Differences were examined significant at $P < .05$ for all analyses.

3. Results

3.1. Growth performance

No mortalities were observed in any group throughout the feeding experiments. The growth performance and feed utilization of rainbow trout fed with supplemented diets, including several levels of Lipidol™ are shown in Table 3. Overall, rainbow trout fed with 2 g kg^{-1} Lipidol™ supplemental diet revealed improved factors SGR, PER, FCR, FI, and

Table 2
Primers sequences of selected immune and antioxidant-related genes used for the real-time PCR assay in rainbow trout.

Gene	Sequences (Forward/reverse)	Target tissue	Application	Amplicon	Efficiency (%)	Accession no.
CAT	F: GAGGGCAACTGGGACCTTACT R: GGACGAAGGACGGGAACAG	Liver	Antioxidant	79	96	BE669040.1
SOD	F:GTAGTCGTGGCTCAATGGTAAG R:GCTTTATATTCTGCGGGTCATT	Liver	Antioxidant	201	94	NM_001160614.1
GPX	F:AAATTGCCATTCCCCTCCGA R: TCCATCAGGACTGACCAGGA	Liver	Antioxidant	126	98	100,136,264
IL-1β	F: ACATTGCCAACCTCATCATCG R: TTGAGCAGGTCCTTGTCCTTG	Kidney	Immune	91	96	AJ223954
IL-8	F: AGAATGTCAGCCAGCCTTGT R: TCTCAGACTCATCCCCTCAGT	Kidney	Immune	69	99	AJ279069
TNF-α	F: TGGAGGGGTATGCGATGACACCTG R:TGAGGCCTTTCTCTCAGCGACAGC	Kidney	Immune	116	99	AJ249755.1
β- actin	F: TCACCCACACTGTGCCCATCTACGA R: CAGCGGAACCGCTCATTGCCAATGG	Liver and kidney	Reference	210	96	AC006483.3

Notes. *CAT*: catalase; *SOD:* superoxide dismutase; *GPX:* glutathione peroxidase; *IL-1β:* interleukin-1β; *IL-8:* interleukin-8; *TNF-α*, tumor necrosis factor-a.

BWI compared to other treatments. Final weight, PER, and SGR revealed a decreasing trend and FCR showed an increasing trend with an increase of Lipidol™ more than 2 g kg^{-1} in the diets. SGR exhibited a second-degree polynomial regression model (Fig. 1). The regression equation was as follows: $y = -0.3983 \times ^{2} + 1.2863\times + 1.7097$; $R^2 = 0.9334$, x = dietary PLPs levels and y = SGR.

3.2. Whole-body proximate chemical analysis

According to the results of Table 4, the crude protein contents of fish fed with 1 and 2 g kg^{-1} Lipidol™ diets were significantly lower than those fed with 3 g kg^{-1} Lipidol™ and control diets. In contrast, the whole-body lipid content in fish fed with 2 and 3 g kg^{-1} Lipidol™ was significantly higher than those fed with the control treatment ($p < .05$) but did not significantly differ with 1 g kg^{-1} Lipidol™ ($p > .05$). The lowest ash content was measured in fish fed with 3 g kg^{-1} Lipidol™ diet. Furthermore, the highest and lowest levels of moisture were observed in 2 and 3 g kg^{-1} Lipidol™ treatments, respectively.

3.3. Serum biochemical and immunological indices

All serum biochemical parameters were influenced by various levels of Lipidol™ in rainbow trout diets ($p < .05$; Table 5). The albumin and HDL values increased with a decrease in dietary Lipidol™ up to 2 g kg^{-1} ($p < .05$); however, these values were decreased in 3 g kg^{-1} Lipidol™ ($p > .05$). Cholesterol and glucose levels did not show a regular trend with increasing Lipidol™ inclusion, but the highest cholesterol level and lowest glucose levels were observed in 3 g kg^{-1} Lipidol™ treatment. Serum LDL level was at the lowest value in 2 g kg^{-1} Lipidol™, while the highest value was measured in the control group.

The complements C3 and C4 activities were highest in the fish fed diets 1 and 2 g kg^{-1} Lipidol™; however, these levels were significantly different from control and 3 g kg^{-1} Lipidol™ groups ($P < .05$; Table 6). The highest levels of IgM and lysozyme were observed in the fish fed with 2 g kg^{-1} Lipidol™ diet and the lowest values were seen in 3 g kg^{-1} Lipidol™ diet.

3.4. Hepatic enzymes activity

As shown in Table 7, hepatic enzymes activity are markedly affected by dietary levels of Lipidol™. The highest levels of AST, ALT, and ALP were observed in the fish fed diet containing 3 g kg^{-1} Lipidol™ followed by control and 1 g kg^{-1} Lipidol™, while the lowest hepatic enzymes were observed in the 2 g kg^{-1} Lipidol™ group ($p < .05$).

3.5. Intestine morphological changes

The effects of dietary Lipidol™ on the mid intestinal morphology of rainbow trout are presented in Table 8. The fish fed with 2 g kg^{-1} Lipidol™ diet displayed superior villus height than those in other groups ($p < .05$). There were no significant differences among control and 3 g kg^{-1} Lipidol™ diets. The depth of crypt revealed a decreasing trend with increasing dietary levels of Lipidol™ inclusions up to 2 g kg^{-1}, but afterward, the trend declined.

3.6. Immune-related genes expression

The expression of three genes from the kidney tissues of rainbow trout fed with graded levels of Lipidol™ was shown in Fig. 2. As the results revealed, dietary 2 g kg^{-1} Lipidol™ considerably increased the expression of *TNF-α* compared to the other groups ($p < .05$). *IL-1β* and *IL-8* expression were upregulated in 2 g kg^{-1} Lipidol™ diet.

Table 3
Effect of different dietary levels of Lipidol™ on growth performance and feed utilization of rainbow trout for 56 days.

Index	Lipidol™ in the experimental diets (g kg^{-1})			
	0 (control)	1	2	3
Initial weight (g)	71.17 ± 0.28	72.00 ± 1.00	72.23 ± 0.23	69.53 ± 0.50
Final weight (g)	134.23 ± 0.68^{c}	175.80 ± 2.75^{b}	200.30 ± 1.57^{a}	139.43 ± 0.51^{c}
PER (%)	1.28 ± 0.04^{d}	1.73 ± 0.02^{b}	2.05 ± 0.06^{a}	1.47 ± 0.02^{c}
CF (g cm^{-3})	1.15 ± 0.02^{b}	1.90 ± 0.05^{a}	1.19 ± 0.04^{b}	1.15 ± 0.04^{b}
FCR	2.04 ± 0.06^{a}	1.37 ± 0.03^{c}	1.28 ± 0.90^{c}	1.78 ± 0.04^{b}
SGR (% day^{-1})	1.75 ± 0.01^{a}	2.45 ± 0.05^{b}	2.83 ± 0.03^{c}	1.93 ± 0.00^{d}
FI (g day^{-1})	2.30 ± 0.04^{a}	2.55 ± 0.03^{b}	2.92 ± 0.05^{c}	2.24 ± 0.02^{a}
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Notes. PER: protein efficiency ratio; CF: condition factor; FCR: feed conversion ratio; SGR: specific growth rate; FI: feed intake; SR: survival rate. Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$). Absence of letters shows no significant difference between treatments.

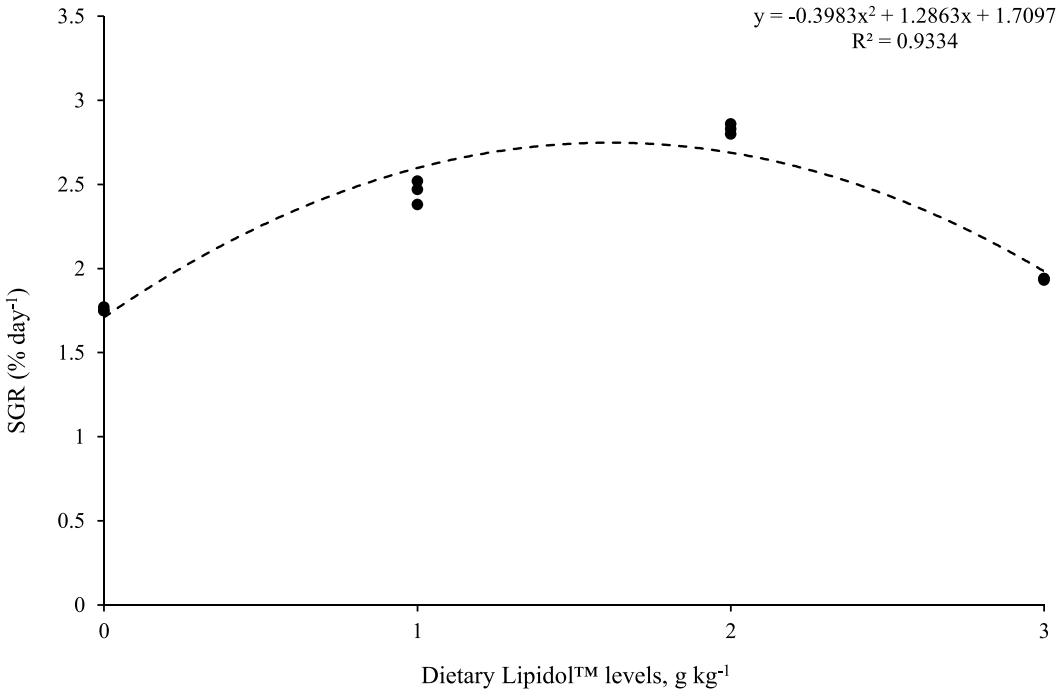

Fig. 1. Relationship among dietary Lipidol™ levels and specific growth rate (SGR) of rainbow trout fed with experimental diets for 56 days.

Table 4
Effect of different dietary levels of Lipidol™ on whole-body proximate composition (%, wet weight) in rainbow trout for 56 days.

Composition (%)	Lipidol™ in the experimental diets (g kg^{-1})			
	0 (control)	1	2	3
Protein	17.02 ± 0.12^{a}	17.55 ± 0.11^{a}	16.15 ± 0.05^{b}	16.15 ± 0.05^{b}
Crude lipid	6.77 ± 0.25^{b}	7.55 ± 0.27^{ab}	8.33 ± 0.23^{a}	8.27 ± 0.13^{a}
Ash	1.77 ± 0.04^{ab}	2.16 ± 0.33^{a}	2.31 ± 0.47^{a}	1.48 ± 0.53^{b}
Moisture	74.31 ± 0.53^{b}	77.45 ± 0.86^{a}	73.20 ± 0.71^{b}	71.60 ± 0.37^{c}

Notes. Data represented as means ± *SD* ($n = 3$). Values in the same row with various superscripts denote a significant difference ($p < .05$).

Table 5
Serum biochemical parameters of rainbow trout fed with different dietary levels of Lipidol™ for 56 days.

Parameter	Lipidol™ in the experimental diets (g kg^{-1})			
	0 (control)	1	2	3
Cholesterol (mgdL^{-1})	281.50 ± 0.70^{b}	266.00 ± 2.82^{c}	282.00 ± 1.41^{b}	322.50 ± 2.12^{a}
Albumin (g dL^{-1})	5.90 ± 0.14^{bc}	6.40 ± 0.14^{b}	7.25 ± 0.07^{a}	5.65 ± 0.21^{c}
Glucose (mg dL^{-1})	45.48 ± 0.83^{a}	42.92 ± 1.52^{b}	46.57 ± 1.32^{a}	32.28 ± 0.01^{c}
LDL (mg dL^{-1})	102.00 ± 1.41^{a}	91.50 ± 0.70^{b}	80.50 ± 0.70^{c}	93.50 ± 0.70^{b}
HDL (mg dL^{-1})	155.00 ± 2.82^{b}	158.50 ± 0.70^{b}	162.25 ± 0.35^{a}	158.50 ± 2.12^{b}

Notes. LDL: low-density lipoprotein; HDL: high-density lipoprotein. Values in the same row with various superscripts denote a significant difference ($p < .05$). Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$).

3.7. Antioxidant related genes expression

Antioxidant genes expression from the liver tissues of rainbow trout fed with different levels of Lipidol™ are represented in Fig. 3. Rainbow trout fed with 2 g kg^{-1} Lipidol™ in the diet rather than other groups displayed notably higher expression gene for *GPx* and *SOD* ($p < .05$). No significant difference was observed in *GPx* gene expression between control, 1 and 3 g kg^{-1} Lipidol™ treatments ($p > .05$). The same outcomes were seen in *CAT* gene expression which the highest value belonged to the rainbow trout fed with 2 g kg^{-1} Lipidol™ and control diets ($p < .05$).

4. Discussion

Based on the data of this study, rainbow trout which was fed with a diet containing 2 g kg^{-1} Lipidol™, showed the best growth performance (final weight, CF, and SGR) and feed utilization (PER, FCR, and FI). Chen et al. (2019) and Hosseini et al. (2018) claimed that improved growth efficiency with LPLs supplementation could be due to their effect on cell membrane enterocytes, promoting the flux rate of macromolecules across the cell membrane and modified permeability membrane whereby leading to bioavailability performance. Furthermore, improved growth performance and feed utilization by lysolecithin (A removed fatty acid molecule from phospholipid) supplements were

Table 6
The immune factors value of rainbow trout fed with different dietary levels of Lipidol™ for 56 days.

Parameter	Lipidol™ in the experimental diets (g kg^{-1})			
	0 (control)	1	2	3
C3 (mg L^{-1})	32.25 ± 1.34^{b}	34.85 ± 1.20^{a}	35.47 ± 0.82^{a}	31.50 ± 0.70^{b}
C4 (mg L^{-1})	18.50 ± 0.70^{b}	22.50 ± 0.70^{a}	23.00 ± 1.41^{a}	19.25 ± 1.06^{b}
IgM (mg L^{-1})	42.84 ± 1.64^{b}	38.46 ± 1.11^{c}	50.67 ± 2.34^{a}	34.48 ± 3.79^{d}
Lysozyme (μg mL^{-1} min)	10.74 ± 0.87^{b}	11.87 ± 0.54^{b}	13.62 ± 0.86^{a}	9.00 ± 1.41^{c}

Notes. C3: Complement 3; C4: Complement 4; IgM: Immunoglobulin M. Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$). Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$).

Table 7
Hepatic enzymes activity of rainbow trout fed with different dietary levels of Lipidol™ for 56 days.

Parameter	Lipidol™ in the experimental diets (g kg^{-1})			
	0 (control)	1	2	3
AST (IU L^{-1})	140.50 ± 1.41^{b}	121.50 ± 2.12^{c}	110.00 ± 1.41^{d}	206.50 ± 1.41^{a}
ALT (IU L^{-1})	64.08 ± 0.70^{b}	53.93 ± 0.70^{c}	51.04 ± 0.70^{d}	67.96 ± 1.41^{a}
ALP (IU L^{-1})	410.14 ± 0.70^{b}	383.13 ± 0.70^{c}	352.09 ± 0.70^{d}	452.35 ± 0.70^{a}

Notes. AST: Aspartate transaminase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase. Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$). Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$).

Table 8
Villus height and crypt depth in the mid-gut of rainbow trout fed with different dietary levels of Lipidol™ for 56 days.

Parameter	Lipidol™ in the experimental diets (g kg^{-1})			
	0 (control)	1	2	3
Villous height (μm)	336.62 ± 0.63^{c}	409.61 ± 5.68^{b}	442.87 ± 8.89^{a}	340.03 ± 7.05^{c}
Crypt depth (μm)	98.23 ± 0.30^{b}	112.58 ± 6.47^{a}	114.37 ± 5.25^{a}	92.84 ± 1.41^{c}
VH/CD	3.42 ± 0.01^{a}	3.57 ± 0.1^{a}	3.94 ± 0.25^{b}	3.71 ± 0.14^{ab}

Notes. VH/CD = villous height to crypt depth ratio. Data represented as means ± *SD* (n = 3). Values in the same row with various superscripts denote a significant difference ($p < .05$). Data represented as means ± SD (n = 3). Values in the same row with various superscripts denote a significant difference ($P < .05$).

observed in broiler chickens, turbot (*Scophthalmus maximus*), and channel catfish (*Ictalurus punctatus*) (Brautigan et al., 2017; Li et al., 2019; Liu et al., 2019). Liu et al. (2019) pointed out that one of the reasons for the improvement of food utilization by dietary lysolecithin supplementation (375 mg kg^{-1}) was an increase in intestinal lipase enzymes (> 100%) and alkaline phosphatase activity (> 15%) compared to the control group in the channel catfish. These researchers postulated that the emulsifying property of lysolecithin leads to enhance the active surface of lipids and transforms them to form micelles which ultimately augment the nutrient bioavailability.

It has been shown that dietary Lipidol™ supplementation up to 2 g kg^{-1} induced the feed utilization of rainbow trout. Similarly, some investigators reported that PLs (especially LPLs) in supplemented diets can act as a food attractant and lead to high feed palatability in juvenile amberjack (*Seriola dumerili*) (Uyan et al., 2009), common carp fingerling (Adel et al., 2017), and weanling pigs (Zhao et al., 2015). Lipidol™ has about 27% soy lecithin which is a significant source of PLs (i.e. phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) and it may increase in transferring of neutral lipids from enteric enterocytes into the bloodstream and boost up the somatic growth (Seiliez et al., 2006; Morais et al., 2007). Since lecithin is supplied from soybean, sunflower or rapeseed oils in the fish diet, higher content of lecithin carrying with Lipidol™ can lead to decrease in the amount of feed consumption due to the earlier satiety of the fish (Gawlicka et al., 2002) and have a negative impact on the growth performance of rainbow trout fed with 3 g kg^{-1} Lipidol™.

Crude lipid content of rainbow trout whole-body augmented with increasing in Lipidol™. This may be related to the emulsifying properties of LPLs, which could improve the absorption of lipids (Zhao et al., 2013; Taylor et al., 2015). A similar result was shown in cobia (*Rachycentron canadum*) larvae (Niu et al., 2008) and juvenile drum (*Sciuenops ocellatus*) (Craig and Gatlin, 1997). Based on the result of the current study, a significant decrease in crude protein was seen in fish fed with higher levels of Lipidol™ (2 and 3 g kg^{-1}), which corroborated with Niu et al. (2008). In contrast with our result, Uyan et al. (2009), Zhao et al. (2013), and Li et al. (2015) addressed that PLs notably augmented crude protein of the whole-body in blunt snout bream (*Megalobrama amblycephala*) fingerling, yellow croaker (*Larmichthys crocea*) larvae and juvenile amberjack, respectively. In addition, Liu et al. (2019) concluded that dietary lysolecithin supplementation could reduce whole-body lipid deposition in channel catfish (14.77 ± 0.45) for 10 weeks. This apparent conflicting results between fry and adults may be related to a different formulation of diet, variations in the amount of secreted digestive enzymes, developmental stages, and fish species.

LPLs exacerbates glucose uptake, which is generally utilized as a source of energy and a metabolic intermediate in the serum (Boontiam et al., 2017). Boontiam et al. (2017) reported that broiler chickens, which were with 1.5 g kg^{-1} LPLs in the diet showed higher levels of glucose in the serum. In the present study; however, glucose level in the rainbow trout fed with dietary of 1 and 2 g kg^{-1} Lipidol™ was increased and then decreased. Circulating proteins like albumin, reflecting the condition of the organism and playing an influential role in the nutritional and immune status of fish (Acar et al., 2018). In our study, adding Lipidol™ more than 2 g kg^{-1} in rainbow trout diet caused a decrease in

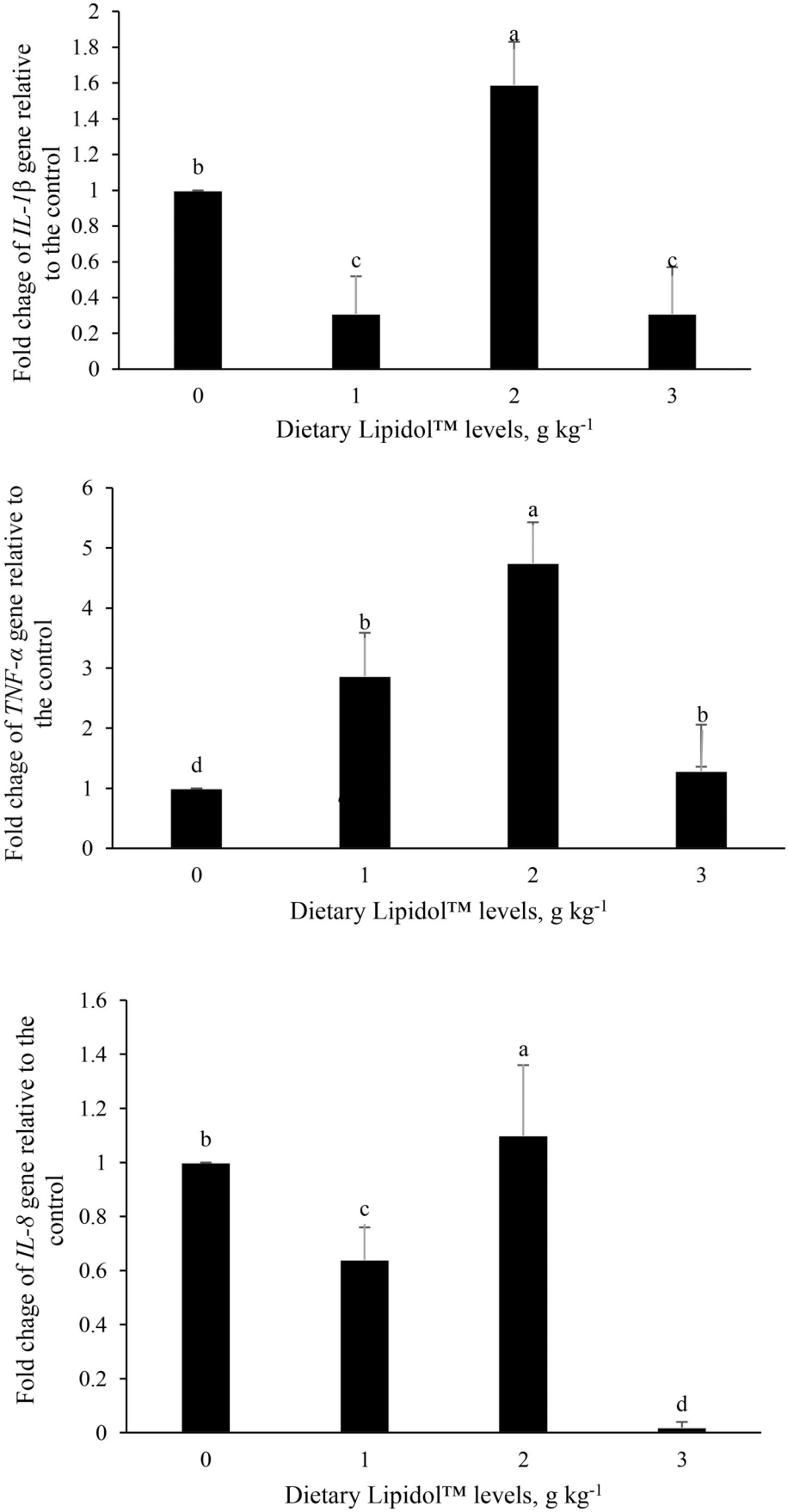

Fig. 2. Effect of different dietary levels of Lipidol™ on the mRNA expression of *TNF-α, IL-1β* and *IL-8* genes in rainbow trout at day 56 of the experiment. The bars with different letters showed statistical difference between the treatments ($n = 5$, $P < .05$).

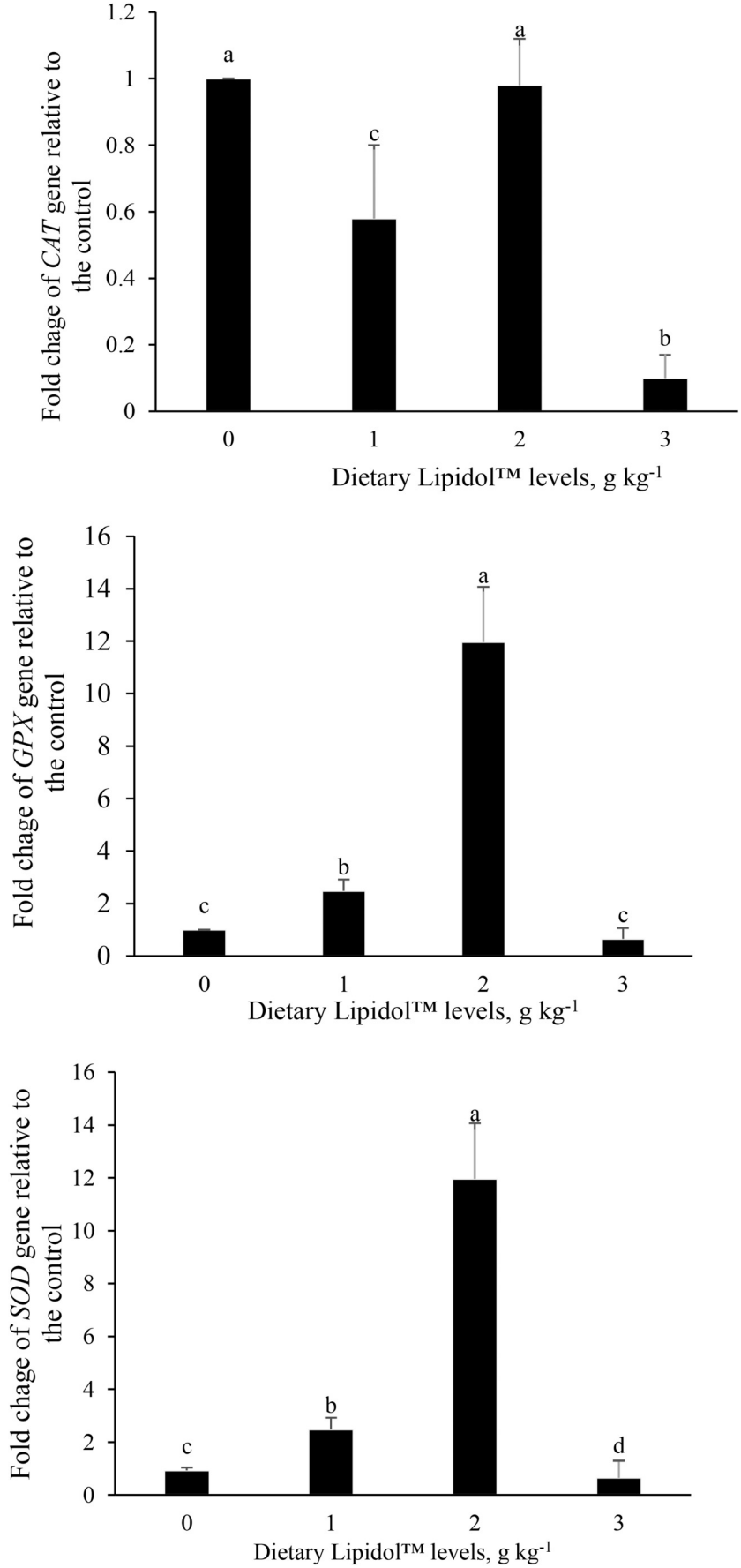

Fig. 3. Effect of different dietary levels of Lipidol™ on the mRNA expression of *CAT, GPx,* and *SOD* genes in rainbow trout at day 56 of the experiment. The bars with different letters showed statistical difference between the treatments (n = 5, $P < .05$).

serum albumin values in comparison with the 1 and 2 g kg^{-1} Lipidol™ groups. That may be related to a disorder of protein metabolism and suppressed immunity. Cholesterol value rose linearly with increasing Lipidol™ in the diet of rainbow trout. One of the reasons could be that LPLs in Lipidol™ functioned as an emulsifier and reduced the secretion of bile salt, thereby prevented from cholesterol to be excreted in the body. These findings are inconsistent with the results of Hosseini et al. (2018). They postulated that cholesterol level was reduced by LPLs supplementation in broilers. Furthermore, our results showed that the lowest and highest levels of LDL and HDL were observed in the serum of fish fed with dietary Lipidol™ at 2 g kg^{-1}. According to the presence of lecithin and other PLs in the supplemented diets with Lipidol™, the absorption of lipids from the intestinal cells was enhanced and therefore a higher rate of lipids was transported and metabolized in the liver leading to elevate HDL content (Fontagne et al., 1998; Tocher et al., 2008). In the same way, previous studies showed that PLs could upregulate the expression of fatty acids oxidation-related genes and lead to reduce lipid accumulation in the liver (Liu et al., 2013; Lin et al., 2018).

Lysozyme contains lytic activity versus Gram-positive and Gram-negative bacteria and represents an influential indicator of innate immune function in fish, which inhibits biofilm formation by the colonization of microorganisms (Adel et al., 2017; Xiao et al., 2019). As observed, non-specific (lysozyme, C3, and C4) and specific immune (IgM) parameters were also increased in fish fed with 2 g kg^{-1} Lipidol™ diet, thus, this positive effect may be attributed the increase in the number of blood lymphocytes, ultimately promoting the immune function (Corripio-Miyar et al., 2007).

The three enzymes AST, ALT, and ALP are the main indicator for showing liver function. Under normal liver function, these enzymes stay low, and their increase is a sign of disruption in normal liver conditions (Zhang et al., 2019). The present study confirmed that AST, ALT, and ALP activities in the 2 g kg^{-1} Lipidol™ group were significantly lower than 1 and 3 g kg^{-1} Lipidol™ and control group, which implied that beneficial influences on the liver of rainbow trout. Although studies of the effects of LPLs on hepatic enzymes were rarely published, some researchers have pointed out that AST and ALT did not show a significant difference in the hepatopancreas of white shrimp, which was fed with different levels of phospholipid (Zhang et al., 2019).

Fish intestinal morphology is a sign of digestive health and nutrients absorption ability. Our finding demonstrated that both villus height and VH/CD were markedly increased in rainbow trout fed with dietary supplementation 2 g kg^{-1} Lipidol™ group. It was proposed that improving growth performance and feed utilization might be attributed to an increased surface area between nutrients and intestinal villi. In agreement with the results of our study, Hosseini et al. (2018) and Boontiam et al. (2017) reported that LPLs have an increasing effect on villus height and VH/CD in broiler chicken. In general, feeding fish with inadequate PLs can cause the accumulation of fats in their intestinal tissues (Fontagne et al., 1998; Olsen et al., 2003). However, LPLs and lecithin of Lipidol™ have a key role in the cell membrane synthesis and improving digestion and absorption of feed ingredients due to their emulsifying effects and therefore they have been suggested as an attractive ingredient in fish diets (Orthoefer et al., 1995; Tocher et al., 2008).

Cytokines or glycoproteins are water-soluble proteins, which play an important role in the regulation of immune responses and inflammatory reactions. Several studies showed that supplementing of aquatic organisms diet with immunostimulants can elevate key inflammatory cytokines gene expression to enhance the immunity status of fish (Awad et al., 2011; Guardiola et al., 2016; Adel et al., 2019). Inflammatory cytokines include *IL-1β* that play a vital role in the host defense responses to infections, tissue necrosis, and other pro-inflammatory responses and are made mainly by leukocytes (Corripio-Miyar et al., 2007). *IL-8* is also involved in initiating and enhancing inflammatory responses, respectively (He et al., 2014). Besides, *TNF-α* is a potent element during early inflammatory reactions. Various cell types synthesize *TNF-α* upon stimulation with endotoxin and inflammatory mediators (Corripio-Miyar et al., 2007; Nootash et al., 2013). Our findings showed that *TNF-α, IL-1β,* and *IL-8* were elevated in rainbow trout fed with 2 g kg^{-1} Lipidol™ diet, while a downward trend was observed in higher levels of Lipidol™. This down-regulation may be attributed to oxidative damage in the high concentration of LPLs. Hartmann et al. (2009) demonstrated that bio-surfactant phospholipids play an essential role in the immune system of rats. The positive functions of PLs in innate immunity and inflammation of cytokines were recognized by Yun et al. (2005). Since no information has been published about evaluating the effects of dietary LPLs levels on mRNA expression of immune-related genes in fish, further studies are recommended particularly after challenging the treated fish with some infectious agents.

Oxidative status in fish physiology plays a significant role in monitoring health status (Mirghaed et al., 2019). The expression of *SOD, CAT,* and *GPx* genes were upregulated in rainbow trout fed with 2 g kg^{-1} Lipidol™ diet, and this results may show that the appropriate level of Lipidol™ can enhance the antioxidative ability and also interfere with the immunity to neutralize the oxidative destruction of rainbow trout. Inconsistent with our results, an earlier study reported that oral administration of polar lipids in larval gilthead sea bream (*Sparus aurata*) enhanced *SOD*, *CAT*, and *GPx* genes expression as well as the serum antioxidant enzyme activities (Saleh et al., 2015). In another study, Li et al. (2015) showed that the expression levels of peroxisome proliferator-activated receptors (*PPAR-a* and *PPAR-c*) and the activities of CAT, SOD, and GPx enzymes were increased with dietary PLs up to 6% in blunt snout bream (*Megalobrama amblycephala*). Adding exogenous LPLs in the fish diet probably has the same effect as PLs. According to the upregulation of antioxidant-related genes expression in rainbow trout fed with 2 g kg^{-1} Lipidol™ supplementation, the fish can probably maintain general health status by increasing their radical scavenging activities and protecting them against oxidation-induced lesions (Li et al., 2012). In this study, the expression of antioxidant genes in fish fed with 3 g kg^{-1} Lipidol™ was down-regulated. This could be attributed to the β-oxidation-related gene (Li et al., 2015) and inhabitation of the liver antioxidant-related genes expression at a high dose of LPLs supplementation.

In conclusion, the outcomes of this study indicate that supplementation of rainbow trout diet with 2 g kg^{-1} Lipidol™ is suggested due to the enhancement of growth performance, feed efficiency, immunological parameters, and antioxidant activities.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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