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Changes in growth performance, haematological parameters, hepatopancreas histopathology and antioxidant status of pacific white shrimp (*Litopenaeus vannamei*) fed oxidized fish oil: Regulation by dietary *myo*-inositol

Shijun Chen[a,1], Zhenxiao Zhuang[a,1], Peng Yin[a], Xu Chen[b], Yanmei Zhang[a], Lixia Tian[a,*], Jin Niu[a,**], Yongjian Liu[a]

[a] Guangdong Provincial Key Laboratory of Improved Variety Reproduction in Aquatic Economic Animals, Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-Sen University, Guangzhou, 510275, PR China
[b] South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, 510300, PR China

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ABSTRACT

A 58-day feeding trial was conducted to evaluate the effects of dietary *myo*-inositol (MI) supplementation on growth performance, haematological parameters, hepatopancreas histopathology and antioxidant status of *Litopenaeus vannamei* fed with oxidized fish oil (OFO). Control diet contained fresh fish oil (FFO) without MI supplementation. The other four diets contained two oxidation levels of OFO (peroxide value: 133.2 and 268.7 meq kg^{-1}) with or without 200 mg MI kg^{-1} diets (MI0 + L, MI0 + H, MI200 + L and MI200 + H). Results showed that OFO-supplemented groups (without MI supplementation) showed better growth performance and lower whole-body inositol content when opposed to control group. MI supplementation significantly improved whole-body inositol content in high-oxidized fish oil (HOFO) groups, and also reduced whole-body lipid in low-oxidized fish oil (LOFO) groups. Moreover, Supplementation of OFO and MI markedly hit the fatty acid profile of muscle. HOFO caused severe histopathological changes in hepatopancreas of shrimp, which slightly alleviated by MI supplementation. MI supplementation also grew the total protein (TP) content and alkaline phosphatase (AKP) activity and decreased the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of serum in OFO-supplemented groups. Ingestion of OFO increased levels of lipid peroxidation and protein oxidation in serum or hepatopancreas, which partly ameliorated by MI supplementation. Activities of antioxidant enzymes exhibited different expression patterns because of OFO and MI. In addition, HOFO markedly increased mRNA expression levels of antioxidant genes including ferritin (FT), thioredoxin (Trx), GPX, glutathione S-transferase (GST) and catalase (CAT) and decreased peroxiredoxin (Prx) expression, in which expression of GPX and Prx were increased owing to MI supplementation. Therefore, it suggested that dietary OFO stimulated growth performance, but also induced oxidative stress and caused impairment to hepatopancreas in *L. vannamei*. The negative impact brought about by OFO was partially mitigated by dietary MI supplementation.

1. Introduction

Lipids, as the essential nutrients, play a critical role in regulating health, growth, reproduction and body functions of aquatic animals [1]. Generally, certain essential fatty acids, especially polyunsaturated fatty acids (PUFAs), are required for preparation of aquatic feeds to support optimal health and growth for aquatic animals [2]. Fish oil, being wildly used in aquatic feeds, is extremely susceptible to lipid peroxidation because of high content of PUFAs [3,4]. Fish oil oxidation usually occurs during storage and preparation of feed, which could form hydroperoxides as primary products of oxidation and secondary oxidation products including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids and epoxy compounds [5–7]. These compounds have a negative impact on the nutritive value of the diet,

* Corresponding author.
** Corresponding author.
E-mail addresses: lixiatian2002@163.com (L. Tian), gzniujin2003@163.com (J. Niu).
[1] These authors contributed equally to this study and share the first authorship.

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which may finally impair the health status of fish [8,9]. The adverse effects of oxidized lipids (including OFO) administration, such as loss of appetite, slow growth, reduced feed efficiency, decreased survival, skeletal deformity, muscular dystrophy, anaemia, depletion of tissue vitamin C and E and degradation of meat quality, were reported in previous studies of fish [10–13]. In contrast, only few studies have been conducted to investigate the harmful effects of oxidized oils on crustacean [14], and research on alleviating its influence through diet management is limited [6].

OFO may also induce oxidative stress and produce excessive reactive oxygen species (ROS) [15], leading to negative effects on normal physiological function of fish and shrimp [16,17]. Actually, organisms have developed antioxidant system, including CAT, superoxide dismutase (SOD), GPX, GST and glutathione reductase (GR), to counteract the detrimental effects of oxidative stress under normal physiological condition [7,18]. Besides, exogenous antioxidants, such as vitamin C [19] and vitamin E [20,21], have also been employed into aquatic feeds to maintain health and prevent oxidative damage caused by oxidized oils.

L. vannamei is a widely cultured shrimp species in China because of its outstanding characters including rapid growth, euryhalinity and high economic value [22]. As an important component of the diet in shrimp, fish oil has also faced the adverse impact resulting from lipid peroxidation. Inositol has been experiencing as a vital vitamin for animals [23]. Except for several aquatic species, such as channel catfish (*Ictalurus punctatus*) [24] and sunshine bass (*Morone chrysops* female × *M. saxatilis* male) [25], most of the aquatic animals including shrimp required inositol supplementation to support normal growth and development [26,27]. Recent studies have reported that MI could enhance growth performance, non-specific immunity and specific immune responses in fish [27,28]. MI supplementation also could alleviate oxidative stress induced by copper in Jian carp (*Cyprinus carpio* var. Jian) [29]. In addition, our recent research has demonstrated that supplementation of 200 mg MI kg^{-1} diet increased the survival rate and antioxidant enzyme activities and reduced levels of lipid peroxidation and protein oxidation in *L. vannamei* after 3 h hypoxia stress [30]. However, as far as we all know, the relationship between MI and OFO in the diet of *L. vannamei* has remained unclear. Therefore, the present research aimed to evaluate the effects of MI and OFO supplementation on the growth performance, antioxidation status, haematological parameters and hepatopancreas histopathology in *L. vannamei*.

2. Materials and methods

2.1. Experimental diets

The formulation and composition analysis of the experimental diets is shown in Table 1. The practical basal diet was formulated to contain approximately 43.9% crude protein and 7.3% crude lipid, which have been shown to be sufficient to support normal growth of *L. vannamei* [30]. Five experimental diets were prepared for the shrimp: (1) fresh fish oil, non-MI supplemented diet (MI0, control group), (2) low-oxidized fish oil, non-MI supplemented diet (MI0 + L), (3) high-oxidized fish oil, non-MI supplemented diet (MI0 + H), (4) low-oxidized fish oil, MI-supplemented diet (added 200 mg MI kg^{-1} diet, MI200 + L), (5) high-oxidized fish oil, MI-supplemented diet (added 200 mg MI kg^{-1} diet, MI200 + H). The diets were manufactured by using the methods described by Chen et al. [31], and saved at −20 °C until used. Before adding into diets, oils were measured for oxidative state including POV and MDA according to the State Standard of the People's Republic of China (GB/T 5009.37–2003 and GB5009.181–2016). Fresh fish oil was oxidized until the values of POV reached 133.2 and 268.7 meq kg^{-1} according to the methods reported by Chen et al. [5]. Experimental fish oils were then saved at −20 °C prior to diet preparation. Besides, *myo*-inositol was obtained from Guangzhou Chengyi Company Ltd (Guangzhou, China) and dietary inositol and whole-body inositol content were analysed by gas chromatograph assay (Agricultural industry standard of the People's Republic of China, NY/T 1345–2007; the State Standard of the People's Republic of China, GB/T 5009.196–2003).

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients	Groups				
	MI0	MI0 + L	MI0 + H	MI200 + L	MI200 + H
Fish meal	20	20	20	20	20
Dehulled soybean meal	25	25	25	25	25
Peanut bran	12	12	12	12	12
Wheat flour	24.3	24.3	24.3	24.28	24.28
Shrimp meal	5	5	5	5	5
Beer yeast	3	3	3	3	3
Soy protein concentrate	4	4	4	4	4
Fresh fish oil (FFO)	2	0	0	0	0
Low-oxidized fish oil (LOFO)	0	2	0	2	0
High-oxidized fish oil (HOFO)	0	0	2	0	2
Soy oil	2	2	2	2	2
Monocalcium phosphate	1	1	1	1	1
Choline chloride (50%)	0.2	0.2	0.2	0.2	0.2
Mineral mixture[a]	0.5	0.5	0.5	0.5	0.5
Vitamin mixture[b]	1	1	1	1	1
Myo-inositol (97%)	0	0	0	0.02	0.02
Proximate composition (% dry matter)					
Moisture	8.38	10.54	10.64	10.48	10.44
Crude protein	43.88	43.93	43.58	43.75	43.42
Crude lipid	7.25	7.22	7.20	7.23	7.25
Ash	9.11	9.47	9.47	9.44	9.68
Inositol (mg kg^{-1} diet)	293.04	317.97	331.99	529.95	507.1

[a] Mineral mixture (mg kg^{-1} of diet): $FeSO_4 \cdot H_2O$, 30.41; $CuSO_4 \cdot H_2O$, 41.91; $ZnSO_4 \cdot H_2O$, 274.34; $MgSO_4 \cdot H_2O$, 284.47; $Ca(IO_3)_2$, 6.14; Na_2SeO_3, 0.44; $CoSO_4$, 2.89.

[b] Vitamin mixture (kg^{-1} of diet): vitamin A, 12000 IU; riboflavin, 40 mg; cyanocobalamin, 0.02 mg; thiamin, 50 mg; menadione, 40 mg; vitamin C, 250 mg; folic acid, 10 mg; calcium pantothenate, 100 mg; nicotinic acid, 120 mg; biotin, 1 mg; vitamin D_3, 2000 IU; a-tocopherol, 120 mg; pyridoxine HCl, 60 mg.

2.2. Experimental procedure

L. vannamei postlarvae (mean weight about 2–3 mg), obtained from a commercial hatchery, were reared in the Hainan Tropical Aquatic Research and Development Centre (South China Sea Fisheries Research Institute) and fed with the commercial feeds for 30 days (Zhanjiang Yuehua Aquatic Feed Co., Ltd., China), and then acclimated to laboratory condition for two weeks.

After acclimation, healthy shrimp were graded to a similar size (mean initial body weight approximately 1.12 g) and randomly stocked into 15 cylindrical fiberglass tanks (500 L, height 80 cm, base diameter 90 cm) at 40 shrimp per tank. For the next 58 days, shrimp in triplicate were fed the respective diets to apparent satiation three times a day at 07:30, 15:00 and 22:00, and feed consumption was accounted for daily. Uneaten feeds and faecal waste were removed by siphoning 2 h after feeding. Water temperature, pH, salinity and dissolved oxygen were monitored daily and total ammonia nitrogen and sulfur compounds were measured weekly. Throughout the experimental period, water temperature ranged from 22.8 to 28.0 °C. pH, salinity and sulfur compounds was kept at 7.6–7.8, 35–38‰ and lower than 0.05 mg L^{-1}, respectively. Total ammonia nitrogen was less than 0.05 mg L^{-1}, and dissolved oxygen was kept above 7.0 mg L^{-1}. Natural light-dark cycle was employed during the experiment, and the photoperiod was 12-h light/12-h dark.

2.3. Sample collection and chemical analysis

About 200 shrimp were collected at the beginning of the feeding trial for analysis of initial proximate composition. At the end of the feeding experiment, shrimp were fasted for 24 h, and then the shrimp were weighed and counted for analysis of growth performance and feed utilization. Four shrimp from each tank were randomly collected for the analysis of the whole-body composition. Another four shrimp per tank were randomly selected for CF and whole-body inositol content. Besides, hepatopancreas of four shrimp in each tank were obtained and immediately placed into RNAlater (Thermo Fisher Scientific, USA) to test the relative expression levels of antioxidant genes. Another four shrimp from each tank were used to collect hepatopancreas to determine the antioxidant parameters. Ice-cold normal saline was added into tubes containing hepatopancreas, and then using an automatic sample rapid grinding instrument (Jingxin, China) to prepare 10% (w:v) homogenates. Homogenates were centrifuged at 3,550 g for 20 min at 4 °C. Supernatants were immediately stored at −80 °C until used. Moreover, the rest of the muscle was used to analyze the muscle composition. Haemolymph was individually collected from the ventral sinus of seven shrimp from each tank and stored at 4 °C for 12 h, and then centrifuged at 4 °C, 7,100 g for 10 min. The supernatant was gathered and kept at −80 °C until analysed for haematological parameters and antioxidant indexes.

The analysis of proximate composition on experimental diets, muscle and whole-body was performed by the standard methods of AOAC (1997) [32]. Moisture, crude protein, crude lipid and ash of samples needed to be measured according to the methods described by Chen et al. [31]. The fatty acid composition of muscle was determined by China National Analytical Centre (Guangzhou, China) using gas chromatograph assay (meat and meat products-determination of fatty acids, GB/T9695.2–2008, the State Standard of the People's Republic of China).

2.4. Pathology of hepatopancreas

According to Xu et al. [33], after 8-week feeding trial, hepatopancreas from three shrimp were randomly collected from each tank and fixed in 10% neutral formalin, and then dehydrated in ascending concentrations of alcohol and cleared in xylol, finally, embedded in paraffin. The embedded hepatopancreas was sectioned with a rotary microtome at 5 μm. The tissue slices were stained with hematoxylin and eosin (H&E) and observed under the microscope camera DS-Ri2 (Nikon, Japan).

2.5. Enzyme activity assays

The total protein (TP) was measured using an automatic biochemical analyser Hitachi 7170 (DAICHI, Japan). Lipid Peroxidation MDA Assay Kit (Beyotime Institute of Biotechnology, China) was selected to determine the MDA content in serum and hepatopancreas. The content of PC and activities of AST, ALT, AKP, acid phosphatase (ACP), T-AOC, GST, total superoxide dismutase (T-SOD), anti-superoxide anion radicals capacity (ASARC) and GPX were tested by using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Absorbance of indicators mentioned above (except TP) was measured by a microplate spectrophotometer Epoch (BioTek, USA).

2.6. Total RNA extraction and real-time quantitative polymerase chain reaction (qRT-PCR)

Total RNA extraction and qRT-PCR analysis were carried out according to our previously established methods [30]. Briefly, total RNA was extracted from hepatopancreas using TRIzol reagent (Invitrogen, USA) and then dissolved in DEPC treated water. The quantity and quality of isolated RNA were evaluated by using spectrophotometric (OD260/280) analysis and 1% agarose gel electrophoresis, respectively. Primers (shown in Table 2) for β-actin and antioxidant genes were designed using software (primer premier 5.0) based on the published mRNA sequences of *L. vannamei*. The cDNA was generated by utilizing PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan) according to the manufacturer's protocol. qRT-PCR assays were carried out in an ABI 7900 real-time fluorescence quantitative PCR System (Applied Biosystems, USA) with 10 μL reaction volumes containing 5 μL of SYBR® Green Realtime PCR Master Mix (TOYOBO, Japan), 0.5 μL of cDNA, 0.2 μL of each primer (10 mM) and 4.1 μL of RNase Free dH_2O. The PCR program was 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 58 °C for 15 s and 72 °C for 20 s. After that, the threshold cycle (Ct) values were collected from each sample and then used to calculate the relative expression levels of the genes by using $2^{-\Delta\Delta\ Ct}$ method reported by Livak and Schmittgen [34].

Table 2
Primers used for quantitative real-time PCR.

Gene name	Sequence of primer (5′-3′)	Product (bp)
Ferritin (FT)	F:CCACAGAATTTGGATGGAAG R:ATGATTACCAAGCTGAAGCG	293
Thioredoxin (Trx)	F:TTCATGAAGAATGGCCAGAA R:TCCATCTTTCATGCTCTTGG	128
Peroxiredoxin (Prx)	F: GAAGAGCAATGCCATACGTT R: CTTGAGCTCACGGAACTCTC	159
Glutathione peroxidase (GPX)	F:CCAAAGTGCATCATTTGGAC R:CAGCAAGTTTGCGATTTCAT	150
Cu/Zn superoxide dismutase (Cu/ZnSOD)	F:TTAGTGGGACCTCGTACGGT R:CTCAAGCGTGACCTATGACC	145
Glutathione S-transferase (GST)	F:TAAGGCAGGCCAAACTGTAG R:AGCTGAGGAGACCCATTCTT	171
Catalase (CAT)	F:AGAGGGTTGTGCATGCTAAG R:CAGCTGATCCACTCTCACCT	159
β-actin	F:CCACGAGACCACCTACAAC R:AGCGAGGGCAGTGATTTC	142

2.7. Statistical analysis

Data are presented as means ± standard error (standard error of the mean, SEM). All data were firstly detected for homogeneity of variance by using SPSS Statistic 20.0 software (IBM, USA). When homogeneity of variance appeared, one-way analysis of variance (ANOVA) followed by Tukey test was selected to test significant differences among all treatments. The Kruskal-Wallis ANOVA test (non-parametric test) was used when the data had non-homogeneity of variance, followed by pairwise comparisons choosing Kruskal-Wallis. To evaluate the effects of MI and OFO supplementation and their interaction, two-way ANOVA was utilized to analyze the differences between experimental groups (except for the control group). If interaction occurred, method of simple effect analysis was selected to analyze the data. Pearson model was implemented to analyze the correlation of POV and MDA in fish oil. Probability value of $P < 0.05$ and $P < 0.01$ was deemed to be statistically significant and extremely significant, respectively.

3. Results

3.1. Characteristics of the experimental fish oils

Fish oils were detected for oxidative state before inclusion in the diets. The oil oxidation, measured as POV and MDA, is presented in Table 3. The oxidation was clearly higher in oxidized fish oil than that in fresh fish oil. MDA significantly increased with increasing POV value of fish oil ($P < 0.05$). Based on Pearson correlation model, extremely significant positive correlation was found between MDA and POV ($P < 0.05$).

Table 3
Oxidative status of fish oil.

Group	Parameter	
	POV (meq kg^{-1})	MDA (mg kg^{-1})
FFO	17.0 ± 0.14[a]	0.97 ± 0.04[a]
LOFO	133.2 ± 1.38[b]	45.8 ± 0.13[b]
HOFO	268.7 ± 3.97[c]	67.2 ± 0.91[c]
R[1]	1.00	0.97**

Results are presented as the mean ± SEM (n = 3). Values with different letters in the same row indicate significant differences ($P < 0.05$).
[1]R indicated the correlation index using Pearson correlation model between MDA and POV of fish oil. ** indicated $P < 0.01$. POV, peroxide value; MDA, malondialdehyde.

3.2. Growth performance

As shown in Table 4, compared with control group, ingestion of OFO caused a significant increase in FBW, WG, SGR, PER and PPV while decrease in FCR of shrimp ($P < 0.05$). MI supplementation lowered FBW, WG, SGR and increased survival rate in OFO-supplemented groups ($P < 0.05$). However, FI and CF did not vary considerably among experimental groups ($P > 0.05$).

3.3. Body composition and fatty acid profile

Whole-body and muscle composition are given in Table 5. MI supplementation reduced the whole-body lipid content in LOFO groups ($P < 0.05$), and supplementation of HOFO increased whole-body lipid content in MI-supplemented groups ($P < 0.05$). Control group had lower muscle ash content when compared with other experimental groups ($P < 0.05$). However, no significant difference was found in other parameters among treatments ($P > 0.05$).

The fatty acid composition of the muscle was assayed, as shown in Table 6. Shrimp fed OFO diets without extra MI supplementation had higher eicosadienoic acid (C20:2n-6) and eicosapentaenoic acid (EPA, C20:5n-3) and lower epoxyeicosatrienoic acid (C20:3n-3) percentages when compared with control group ($P < 0.05$). In OFO-supplemented groups, increased oxidation degree of OFO raised linoenic acid (C18:3n-3, ALA) and EPA percentages and decreased lignoceric acid (C24:0), monounsaturated fatty acid (MUFA), epoxyeicosatrienoic acid (C20:3n-3) and arachidonic acid (C20:4n-6, ARA) percentages in shrimp ($P < 0.05$). MI supplementation caused a significant reduction in the eicosanoic acid (C20:0), docosanoic acid (C22:0) and eicosaenoic acid (C20:1n-9) percentages of shrimp ($P < 0.05$). In addition, due to MI supplementation, higher ARA and lower C24:0, C18:1n-9, MUFA and C20:3n-3 percentages was observed in HOFO groups and LOFO groups, respectively ($P < 0.05$).

3.4. Hepatopancreas histology

The hepatopancreatic tissue sections of *L. vannamei* fed the experimental diets are shown in Fig. 1. Shrimp hepatopancreas are made up of numerous hepatopancreas tubules [35]. Each tubule has four kinds of cells, namely B-cell (blasenzellen), F-cell (fibrillazellen), R-cell (restzellen) and E-cell (embryonalzellen). The hepatopancreas of the control group showed the well-organized glandular tubular structure normally seen in *L. vannamei* (Fig. 1a). The tubules were closely arranged. The lumen were star-shaped, and there were abundant B-cells and R-cells. Although some B-cells in the MI0 + L group decreased in size, other tissue structures were generally normal (Fig. 1b). Nevertheless, shrimp given the diet with HOFO showed severe histopathological changes including abnormal lumen, reduced B-cells and R-cells, ruptured epithelial cells, melanization of cells and degeneration of tubules (Fig. 1c). Shrimp fed LOFO diet with MI supplementation basically exhibited no histological changes, except that number of R-cell was reduced (Fig. 1d). Compared with shrimp fed with HOFO diet, those in the MI200 + H groups had less histopathological damage (Fig. 1e).

3.5. Whole-body inositol content

As shown in Fig. 2, compared with control group, shrimp feeding on OFO diets without MI supplementation showed reduced whole-body inositol content ($P < 0.05$), and as the oxidation degree of fish oil increased, its content showed a downward trend. MI supplementation increased whole-body inositol content in HOFO groups ($P < 0.05$), and increment of oxidation degree in OFO raised inositol content in MI-supplemented groups ($P < 0.05$).

3.6. Serum biochemical parameters

As shown in Table 7, shrimp fed OFO diets without MI supplementation had obviously lower AKP activity and TP content than those of shrimp fed control diet ($P < 0.05$). In OFO-supplemented groups, increased oxidation degree of OFO enhanced activities of AKP, ACP, ALT and AST and TP content ($P < 0.05$). In addition, MI supplementation reduced activities of AST, ALT and AKP and increased TP content ($P < 0.05$).

3.7. Antioxidant-related parameters in serum and hepatopancreas

Changes in antioxidant-related parameters of serum are presented in Fig. 3. In shrimp fed diets without MI supplementation, MDA content increased with the increment of oxidative degree of fish oil, and shrimp fed MI0 + H diet had markedly higher MDA content than those of shrimp fed control and MI0 + L diets ($P < 0.05$). MI supplementation significantly reduced MDA content in HOFO groups ($P < 0.05$). The highest GST activity was observed in MI200 + H group. Besides, Increment of oxidation degree in OFO raised GST activity in MI-supplemented groups and GPX activity in OFO-supplemented groups, respectively ($P < 0.05$). MI supplementation obviously increased GST and GPX activities in OFO-supplemented groups. Shrimp fed MI200 + H diet had the highest GPX activity, and the lowest in shrimp fed MI0 + L diet ($P < 0.05$). However, ASARC and T-SOD showed no significant differences among all groups ($P > 0.05$).

The results of antioxidant-related parameters in hepatopancreas are shown in Fig. 4. Ingestion of OFO significantly increased GST activity and decreased GPX activity relative to the control ($P < 0.05$). Despite the fact that MI supplementation enhanced the GPX activity in OFO-supplemented groups, there were no significant difference among OFO-supplemented groups. MI supplementation increased T-AOC activity and PC content in LOFO groups ($P < 0.05$), which ran counter to the trend of MDA content. MI supplementation also reduced GST activity in HOFO groups ($P < 0.05$). PC content increased with the increment of oxidation degree of fish oil, and a significant difference was found in MI0 + H group when compared with control group ($P < 0.05$). Increment of oxidation degree of OFO reduced T-AOC activity and increased MDA content in MI-supplemented groups, on the contrary, it raised activities of T-AOC and GST and PC content and decreased MDA content in MI-unsupplemented groups.

3.8. Relative expression of antioxidant-related genes in hepatopancreas

Relative expression of antioxidant-related genes in hepatopancreas for shrimp is shown in Fig. 5. Significantly higher expression of CAT, GPX and FT was obtained in MI0 + H and MI200 + H groups than the control group ($P < 0.05$). The expression of Prx markedly decreased with increasing oxidative degree of fish oil ($P < 0.05$), which was contrary to the trend in GST expression. In shrimp fed OFO diets, increased oxidation degree of OFO enhanced expression of CAT, GPX, Trx, FT, Prx and GST and decreased expression of Cu/ZnSOD

Table 4
Effect of MI and OFO supplementation on growth performance of *L. vannamei* fed experimental diets.

Groups	Parameters									
	IBW	FBW	WG	SGR	FCR	FI	Survival	CF	PER	PPV
MI0	1.12 ± 0.01	10.11 ± 0.12^{a}	802.8 ± 6.9^{a}	3.79 ± 0.01^{a}	1.29 ± 0.02^{b}	11.64 ± 0.05	99.17 ± 0.83	1.14 ± 0.02	1.76 ± 0.03^{a}	34.52 ± 0.31^{a}
MI0 + L	1.12 ± 0.00	11.33 ± 0.26^{b}	914.1 ± 20.4^{b}	3.99 ± 0.03^{b}	1.14 ± 0.03^{a}	11.68 ± 0.21	98.33 ± 0.83	1.22 ± 0.02	1.99 ± 0.05^{b}	38.37 ± 1.10^{b}
MI0 + H	1.11 ± 0.00	11.22 ± 0.21^{b}	914.6 ± 18.7^{b}	3.99 ± 0.03^{b}	1.13 ± 0.01^{a}	11.43 ± 0.16	97.50 ± 0.00	1.18 ± 0.02	2.02 ± 0.02^{b}	39.21 ± 0.32^{b}
MI200 + L	1.11 ± 0.01	10.39 ± 0.33^{ab}	835.9 ± 22.6^{a}	3.85 ± 0.04^{a}	1.18 ± 0.01^{a}	10.90 ± 0.34	100.00 ± 0.00	1.16 ± 0.02	1.94 ± 0.01^{b}	37.05 ± 0.30^{ab}
MI200 + H	1.13 ± 0.00	11.01 ± 0.11^{ab}	876.2 ± 8.9^{ab}	3.93 ± 0.02^{ab}	1.16 ± 0.00^{a}	11.48 ± 0.10	99.17 ± 0.83	1.19 ± 0.02	1.98 ± 0.00^{b}	38.06 ± 0.70^{b}
Two-way ANOVA (*P* value)										
MI	ns	< 0.05	< 0.05	< 0.05	ns	ns	< 0.05	ns	ns	ns
OFO	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
MI × OFO	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

IBW, initial mean body weight (g shrimp^{-1}); FBW, final mean body weight (g shrimp^{-1}).
WG, weight gain (%) = 100 × (FBW − IBW)/IBW.
SGR, specific growth ratio (% day^{-1}) = 100 × [Ln (FBW) - Ln (IBW)]/days.
FCR, feed conversion rate = feed consumed (g, dry weight)/weight gain (g, wet weight).
FI, Feed intake (g shrimp^{-1}) = total feed intake (g, dry weight)/number of shrimp.
Survival (%) = 100 × (final number of shrimps)/(initial number of shrimps).
CF, condition factor (g cm^{-3}) = 100 × body weight (g)/body length (cm) ^{3}.
PER, protein efficiency ratio = weight gain(g, wet weight)/protein intake (g, dry weight).
PPV, protein productive value (%) = 100 × protein gain (g, wet weight)/protein intake (g, dry weight).
Results are presented as the mean ± SEM (n = 3). The values with different letters in the same column indicate significant differences (P < 0.05). < 0.05 means significant difference; ns means not significant difference ($P > 0.05$).

Table 5
Effect of MI and OFO supplementation on body composition of *L. vannamei* fed experimental diets.

Parameter	Groups					Two-way ANOVA (*P* value)		
	MI0	MI0 + L	MI0 + H	MI200 + L	MI200 + H	MI	OFO	MI × OFO
Whole body (% wet weight)								
Moisture	73.1 ± 0.5	73.9 ± 0.3	73.4 ± 0.3	73.8 ± 0.1	73.4 ± 0.3	ns	ns	ns
Crude Protein	18.9 ± 0.1	18.7 ± 0.1	18.8 ± 0.1	18.4 ± 0.2	18.6 ± 0.3	ns	ns	ns
Crude lipid	2.00 ± 0.10^{b}	2.09 ± 0.07^{b}	1.96 ± 0.01^{ab}	1.71 ± 0.03^{a}	1.91 ± 0.05^{ab}	< 0.01	ns	< 0.05
Ash	3.37 ± 0.18	2.79 ± 0.07	3.13 ± 0.21	3.28 ± 0.14	3.27 ± 0.12	ns	ns	ns
Muscle (% wet weight)								
Moisture	74.7 ± 0.2	74.2 ± 0.2	74.2 ± 0.4	74.6 ± 0.1	74.0 ± 0.1	ns	ns	ns
Crude Protein	21.2 ± 0.1	21.7 ± 0.1	21.7 ± 0.3	21.4 ± 0.2	21.7 ± 0.1	ns	ns	ns
Crude lipid	0.81 ± 0.02	0.86 ± 0.03	0.84 ± 0.03	0.79 ± 0.03	0.81 ± 0.03	ns	ns	ns
Ash	1.48 ± 0.02^{a}	1.57 ± 0.01^{b}	1.60 ± 0.01^{b}	1.56 ± 0.01^{b}	1.58 ± 0.02^{b}	ns	ns	ns

Results are presented as the mean ± SEM (n = 3). The values with different letters in the same line indicate significant differences ($P < 0.05$). < 0.05 means significant difference; < 0.01 means extremely significant difference; ns means not significant difference ($P > 0.05$).

($P < 0.05$). Due to MI supplementation, reduced expression of Cu/ZnSOD and increased expression of GPX and Prx was observed in LOFO and HOFO groups, respectively ($P < 0.05$).

4. Discussion

4.1. Effect of MI and OFO supplementation on growth performance and tissue responses

In the present study, no deleterious effect of OFO was noted on growth performance of shrimp. In contrast, the present study showed that the growth performance of *L. vannamei* was significantly stimulated by dietary OFO. Similar result has been reported in largemouth bass (*M. salmoides*) [5]. However, this was not in agreement with previous studies on black sea bream (*Acanthopagrus schlegeli*) [11], channel catfish (*I. punctatus*) [9], sea cucumber (*Apostichopus japonicas*) [19], *L. vannamei* [15] and blunt snout brean (*Megalobrama amblycephala*) [17] of which all reported that dietary OFO had detrimental effects on growth performance. The present results were also contrary to the reports in European sea bass (*Dicentrarchus labrax*) [36], gilthead sea bream (*Sparus aurata*) [2], Atlantic halibut (*Hippoglossus hippoglossus*) [12], Chinese longsnout catfish (*Leiocassis longirostris*) [13], sturgeon hybrid (*Huso huso*♂ × *Acipenser ruthenus*♀) [3] and rainbow trout (*Oncorhynchus mykiss*) [37], in which increased dietary lipid oxidation did not influence the growth performance of fish. OFO (POV: 45.48 meq kg^{-1}) could reduce weight gain and survival rate in black sea bream (*A. schlegeli*) [11]. However, POV value as high as 192 meq kg^{-1} has no significant difference on growth in Chinese longsnout catfish (*L.*

Table 6
Fatty acid composition (% total fatty acids) of the muscle in *L. vannamei* fed the experimental diets.

Fatty acid	Groups					Two-way ANOVA (*P* value)		
	MI0	MI0 + L	MI0 + H	MI200 + L	MI200 + H	MI	OFO	MI × OFO
C14:0	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	ns	ns	ns
C16:0	4.07 ± 0.00	4.16 ± 0.00	4.13 ± 0.05	4.10 ± 0.07	4.17 ± 0.02	ns	ns	ns
C18:0	3.03 ± 0.02	3.11 ± 0.01	3.09 ± 0.03	3.10 ± 0.07	3.19 ± 0.03	ns	ns	ns
C20:0	0.09 ± 0.00^{b}	0.09 ± 0.00^{ab}	0.09 ± 0.00^{ab}	0.08 ± 0.00^{a}	0.08 ± 0.00^{a}	< 0.05	ns	ns
C22:0	0.04 ± 0.00^{b}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{a}	0.03 ± 0.00^{ab}	< 0.01	ns	ns
C24:0	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	ns	ns	< 0.05
ΣSFA[1]	7.32 ± 0.03	7.51 ± 0.02	7.42 ± 0.04	7.39 ± 0.13	7.56 ± 0.05	ns	ns	ns
C16:1n-7	0.30 ± 0.01	0.31 ± 0.02	0.28 ± 0.00	0.27 ± 0.01	0.28 ± 0.00	ns	ns	ns
C18:1n-9	5.00 ± 0.08^{b}	4.95 ± 0.04^{ab}	4.80 ± 0.06^{ab}	4.72 ± 0.05^{a}	4.81 ± 0.04^{ab}	ns	ns	< 0.05
C20:1n-9	0.24 ± 0.00^{b}	0.25 ± 0.00^{b}	0.25 ± 0.00^{b}	0.23 ± 0.01^{ab}	0.22 ± 0.00^{a}	< 0.01	ns	ns
ΣMUFA[2]	5.55 ± 0.08^{b}	5.51 ± 0.04^{b}	5.33 ± 0.07^{ab}	5.22 ± 0.06^{a}	5.31 ± 0.04^{ab}	< 0.05	ns	< 0.05
C18:3n-3	0.20 ± 0.01	0.20 ± 0.00	0.22 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	ns	< 0.05	ns
C20:5n-3	2.59 ± 0.06^{a}	2.73 ± 0.03^{ab}	2.90 ± 0.08^{b}	2.83 ± 0.05^{ab}	2.90 ± 0.03^{b}	ns	< 0.05	ns
C22:5n-3	0.35 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.36 ± 0.01	ns	ns	ns
C22:6n-3	3.51 ± 0.06	3.65 ± 0.06	3.69 ± 0.14	3.69 ± 0.08	3.80 ± 0.05	ns	ns	ns
Σn-3 PUFA[3]	6.65 ± 0.13	6.92 ± 0.06	7.12 ± 0.22	7.05 ± 0.13	7.26 ± 0.03	ns	ns	ns
C18:2n-6	3.61 ± 0.08	3.67 ± 0.01	3.75 ± 0.06	3.66 ± 0.02	3.70 ± 0.03	ns	ns	ns
C20:2n-6	0.56 ± 0.01^{a}	0.60 ± 0.01^{b}	0.62 ± 0.01^{b}	0.59 ± 0.02^{ab}	0.61 ± 0.01^{b}	ns	ns	ns
C20:3n-3	0.06 ± 0.00^{b}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	ns	ns	< 0.05
C20:4n-6	0.62 ± 0.01	0.65 ± 0.01	0.62 ± 0.01	0.64 ± 0.01	0.66 ± 0.01	ns	ns	< 0.05
Σn-6 PUFA[4]	4.85 ± 0.09	4.98 ± 0.01	5.04 ± 0.07	4.94 ± 0.01	5.02 ± 0.04	ns	ns	ns

Results are presented as the mean ± SEM (n = 3). The values with different letters in the same line indicate significant differences ($P < 0.05$). < 0.05 means significant difference; < 0.01 means extremely significant difference; ns means not significant difference ($P > 0.05$).
C14:0, Myristic acid; C16:0, Palmitic acid; C18:0, Stearic acid; C20:0, Eicosanoic acid; C22:0, Docosanoic acid; C24:0, Lignoceric acid.
C16:1n-7, Palmitoleic acid; C18:1n-9, Oleic acid; C20:1n-9, Eicosaenoic acid.
C18:3n-3, Linoenic acid, ALA; C20:5n-3, Eicosapentaenoic acid, EPA; C22:5n-3, Docosapentaenoic acid, DPA; C22:6n-3, Docosahexaenoic acid, DHA.
C18:2n-6, Linoleic acid, LNA; C20:2n-6, Eicosadienoic acid; C20:3n-3, Epoxyeicosatrienoic acid; C20:4n-6, Arachidonic acid, ARA.
[1]SFA (Saturated fatty acid): C14:0, C16:0, C18:0, C20:0, C22:0, C24:0.
[2]MUFA (Monounsaturated fatty acid): C16:1n-7, C18:1n-9, C20:1n-9.
[3]Σn-3 PUFA (n-3 polyunsaturated fatty acid): C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3.
[4]Σn-6 PUFA (n-6 polyunsaturated fatty acid): C18:2n-6, C20:2n-6, C20:3n-3, C20:4n-6.

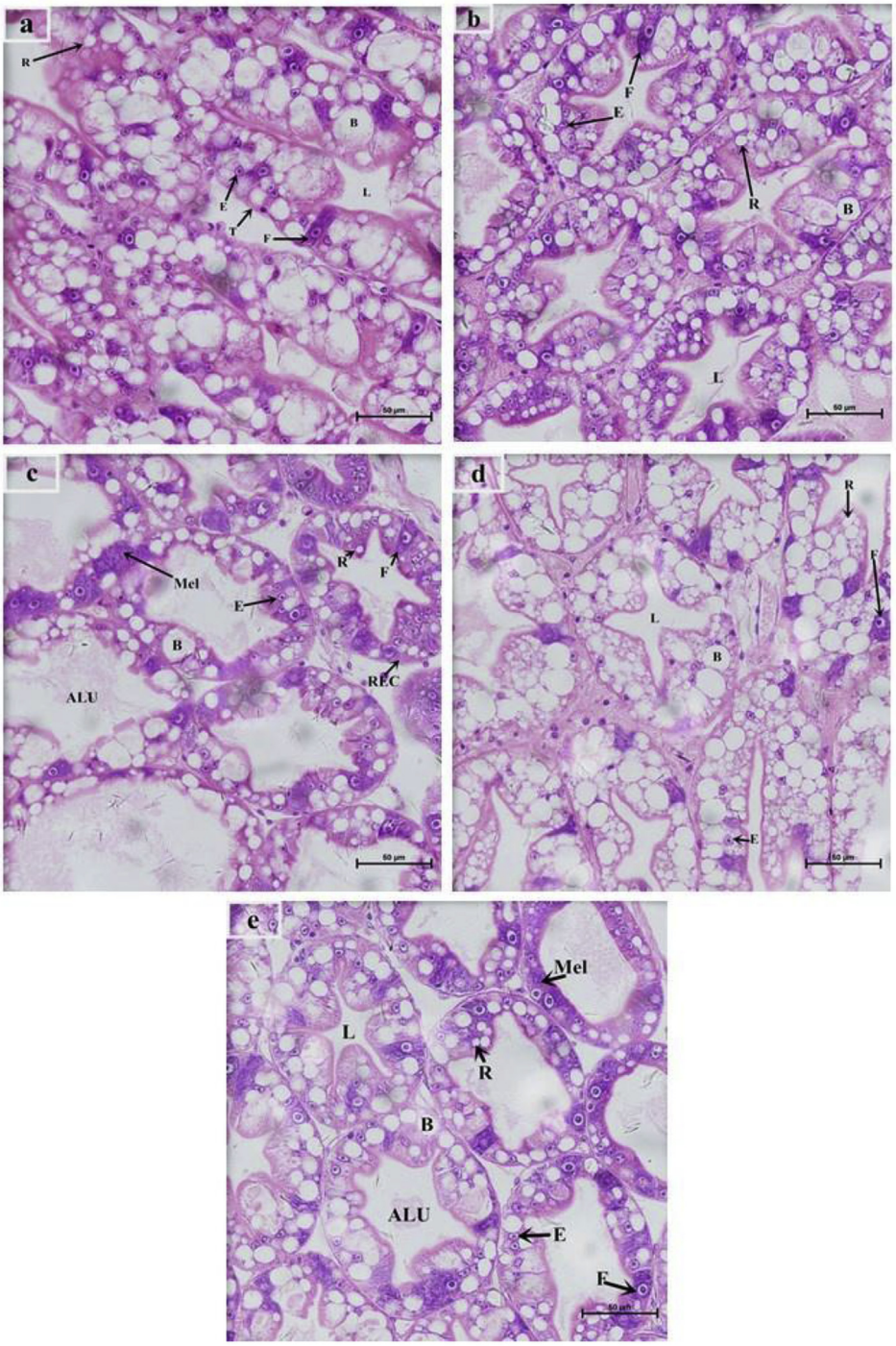

Fig. 1. Transverse section of the hepatopancreas of the shrimp fed with MI0 (a), MI0 + L (b), MI0 + H (c), MI200 + L (d) and MI200 + H (e) diets. H&E staining, Magnification 400 × , scale bar = 50 μm. The letters in the figure indicated that: T (hepatopancreas tubule), B (B-cell, Blasenzellen), F (F-cell, Fibrillazellen), R (R-cell, Restzellen), E (E-cell, Embryonalzellen), L (star shape-like lumen), ALU (abnormal lumen), Mel (melanization of cells) and REC (ruptured epithelial cells).

longirostris) [13]. Different aquatic animals may have different sensitivity to dietary oxidized lipids [6,38]. These discrepancies on growth performance between results may be linked to the differences of animal size, animal species, experimental period, the intake of oxidized oils, oxidation degree of oils and experimental formulation [5,9,37]. Furthermore, OFO promoted growth may be related to the hormesis, which was a dose-response phenomenon characterized as a low dose stimulation, high dose inhibition [39]. Sometimes, low doses of toxic substances stimulated growth of shrimp [40]. Szczerbik et al. [41] reported that high dose of cadmium inhibited growth and a lower dose of cadmium stimulated growth in goldfish (*Carassius auratus gibelio*). However, further research is still required to explore the mechanism. Besides, MI supplementation reduced the WG in OFO-supplemented groups, which might be associated with decreased whole-body lipid content.

Present research showed that shrimp fed OFO-supplemented diet did not exhibit a significant decrease of feed intake compared with shrimp fed control diet. The result was in general agreement with those reports observed in red sea bream (*Pagrus major*) [42], and disagreement with research reported in Japanese sea bass (*Lateolabrax*

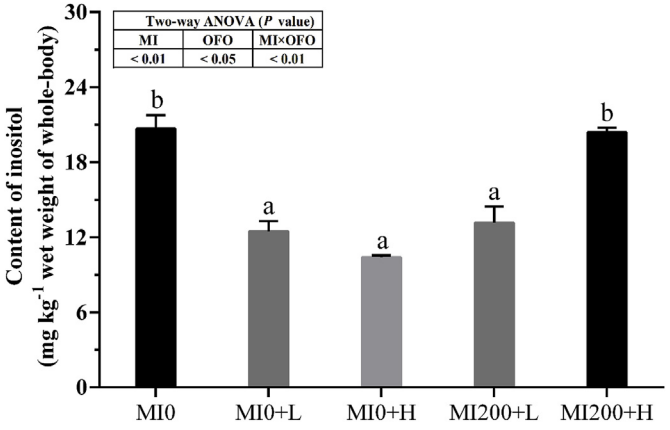

Fig. 2. Effect of MI and OFO supplementation on whole-body inositol content in *L. vannamei*. Results are presented as the mean ± SEM (n = 3). The values with different letters in the column indicate significant differences ($P < 0.05$).

japonicas) [43]. It may be due to the fact that shrimp did not distinguish against diet containing this level of oxidized oil [44].

Previous studies have demonstrated that consumption of diets containing oxidized oils led to a reduction of α-tocopherol and vitamin C in tissues of fish [5,45], which may be due to the reaction with free radicals resulting from lipid peroxidation [46]. Similarly, reduced whole-body inositol content owing to ingestion of OFO was observed in the present study, while MI supplementation enhanced whole-body inositol content. Jiang et al. [23] reported that MI could inhibit generation of superoxide radicals ($O_2^{\bullet-}$) and hydroxy radical ($OH^{\bullet}$) and enhance enzymatic antioxidant capacity in Jian carp (*C. carpio*). Reduction of whole-body inositol content might be related to its participation in protecting shrimp from oxidative stress caused by dietary OFO.

4.2. Effect of MI and OFO supplementation on body composition and muscle fatty acid composition

Dong et al. [13] reported that dietary OFO did not influence body composition of *L. longirostris*. Similar result was noted in the present study. However, reduction of body protein content due to OFO was observed in black sea bream (*A. schlegeli*) and channel catfish (*I. punctatus*) [11,47]. Dietary OFO also decreased whole-body lipid and ash content in largemouth bass (*M. salmoide*) [48]. In addition, ingestion of oxidized oil reduced muscle lipid content, which was not obtained from our study [48]. The discrepancies in body composition between these results might be linked to the difference between aquatic species or the different response to various oxidation degrees of dietary oxidized oils. Moreover, MI supplementation reduced whole-body lipid content in LOFO groups in this study, which may be related to its role in lipid metabolism. Previous research has shown that MI reduced fat mass accretion in mice [49]. Furthermore, the fact that MI supplementation did not cause the decrease of whole-body lipid content in HOFO groups may be explained by the damage caused by HOFO to the shrimp, which affected the lipid metabolism. However, further studies are needed in order to decipher the mechanisms underlying the effect of MI.

In Atlantic cod (*Gadus morhua*), level of PUFA in muscle was decreased by dietary oxidized oil [50], which was in agreement with the study conducted by Gao et al. [20]. However, OFO increased EPA content of muscle in this study, which did not agree with the result observed in sea cucumber (*A. japonicas*) [45]. Dietary OFO also enhanced C20:2n-6% and reduced C20:3n-3%. Previous studies have shown that a selective pathway might exist for fatty acid oxidation to obtain energy in shrimp [51,52], which caused different retention rates of different fatty acids [53]. The mechanism behind OFO supplementation effect on muscle fatty acid composition of shrimp needs further research.

4.3. Effect of MI and OFO supplementation on serum biochemical indexes and histopathology of hepatopancreas

Haematological indexes including TP, AKP, ACP, AST and ALT have often been applied to reflect the health status of fish [54,55]. Serum AST and ALT activities have often been regarded as biomarkers for liver function [56]. Its activities may reflect the degree of liver injury [57]. Toxic substances including cadmium increased the activities of AST and ALT in serum [58]. Dietary OFO has also been confirmed to cause a significant increment in serum AST and ALT activities [3]. Changes in activities of AKP and ACP have been taken into account as indicators of growth and illness of fish [59]. TP including albumin and globulin has been declared to be related to non-specific immune response [60]. Higher TP content may mean higher stronger innate immunity response [61]. Moreover, fish treated with immunostimulants, such as vitamin C, vitamin E and β-glucan, have lower serum activities of AST and ALT and higher TP content and AKP activity than untreated group [12,62–64]. After exposed to HOFO, AKP activity and TP content decreased in serum, and activities of AST and ALT substantially increased in serum compared with the control group in the present study, suggesting that HOFO may have a negative impact on hepatopancreas's health and innate immunity response. However, the parameters mentioned above showed opposite trends because of MI supplementation, which indicated that MI supplementation may be beneficial for improving health status of *L. vannamei*.

Noxious substances, such as endosulfan [65] and aflatoxin B1 [66], caused damage to tissue structure in shrimp, which ultimately influenced the physiological functions of shrimp. The hepatopancreas is composed of many tubules, which consisting of different epithelial cell types, namely E-cell, R-cell, F-cell and B-cell [67]. Histological analysis of the hepatopancreas has been reckoned as one of the important means for reflecting health status in shrimp [68,69]. The B-cell in hepatopancreas has the function of ingesting and secreting [70]. In the present

Table 7
Effect of MI and OFO supplementation on serum biochemical parameters in *L. vannamei*.

Parameter	Group					Two-way ANOVA (*P* value)		
	MI0	MI0 + L	MI0 + H	MI200 + L	MI200 + H	MI	OFO	MI × OFO
AST	13.8 ± 0.6^{ab}	14.4 ± 1.0^{b}	15.8 ± 1.1^{b}	10.7 ± 0.2^{a}	14.4 ± 0.4^{b}	< 0.05	< 0.05	ns
ALT	34.5 ± 1.1^{b}	31.5 ± 2.9^{ab}	36.8 ± 2.6^{b}	23.1 ± 1.5^{a}	29.9 ± 1.6^{ab}	< 0.01	< 0.05	ns
TP	114.7 ± 2.2^{c}	72.7 ± 1.7^{a}	77.3 ± 1.1^{ab}	77.5 ± 1.7^{ab}	82.2 ± 0.9^{b}	< 0.01	< 0.05	ns
AKP	7.01 ± 0.28^{b}	5.32 ± 0.21^{a}	5.21 ± 0.02^{a}	5.27 ± 0.18^{a}	6.53 ± 0.29^{b}	< 0.05	< 0.05	< 0.01
ACP	11.32 ± 0.12^{ab}	10.77 ± 0.53^{a}	12.12 ± 0.19^{b}	11.32 ± 0.05^{ab}	11.51 ± 0.15^{ab}	ns	< 0.05	ns

Results are presented as the mean ± SEM (n = 3). The values with different letters in the same line indicate significant differences ($P < 0.05$). < 0.05 means significant difference; < 0.01 means extremely significant difference; ns means not significant difference ($P > 0.05$). TP (total protein, g L^{-1}), AST (aspartate aminotransferase, IU L^{-1}), ALT (alanine aminotransferase, IU L^{-1}), AKP (alkaline phosphatase, serum, King unit 100 mL^{-1}) and ACP (acid phosphatase, serum U 100 mL^{-1}).

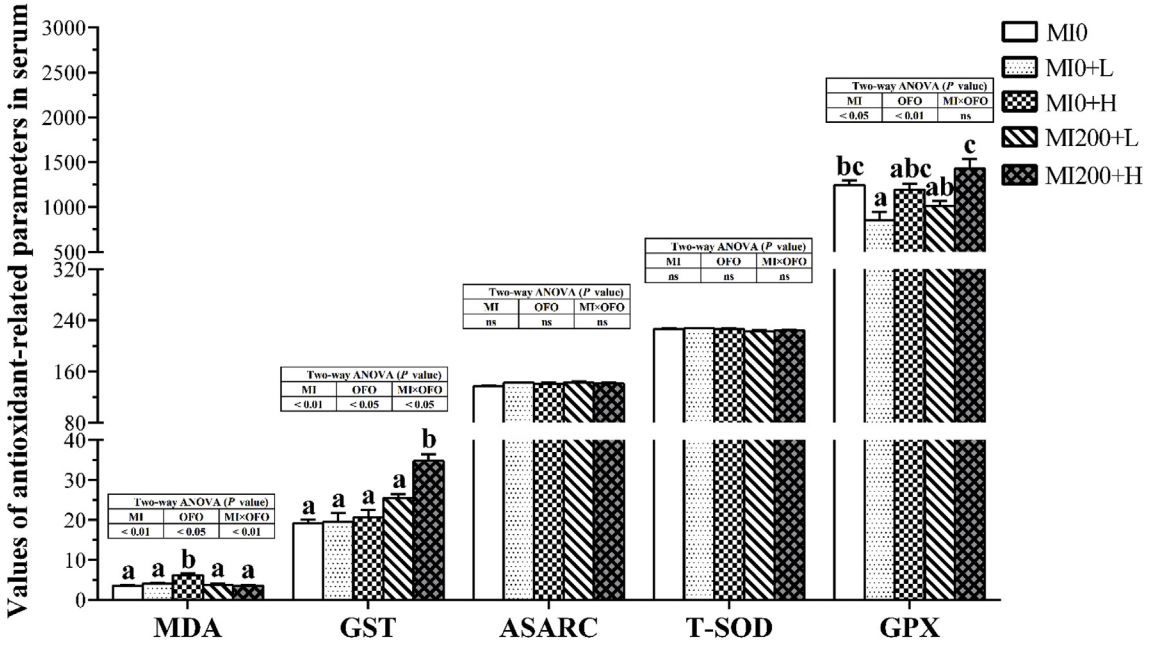

Fig. 3. Effect of MI and OFO supplementation on antioxidant-related parameters of serum in *L. vannamei*. Results are presented as the mean ± SEM (n = 3). The columns with different letters indicate significant differences ($P < 0.05$). MDA (malondialdehyde, nmol mL^{-1}), GST (glutathione-S-transferase, U mL^{-1}), ASARC (anti-superoxide anion radicals capacity, U L^{-1}), T-SOD (total superoxide dismutase, U mL^{-1}), GPX (glutathione peroxidase, U).

study, except for volume reduction of some B-cells, other structures of hepatopancreas in shrimp fed LOFO diet were normal like the control group, suggesting that low oxidation degree of OFO did not cause serious damage to the hepatopancreas. MI supplementation could improve above condition while also reducing the amount of R-cells. The R-cells in hepatopancreas are known as the principal site for storage of lipid droplet [71]. Xu et al. [33] have demonstrated that high levels of dietary lipid caused an increase in the number of R-cells, which finally induced lipid deposition in hepatopancreas of *L. vannamei*. These results reported that the reduction of whole-body lipid content caused by MI supplementation may be related to the decrease in the number of R-cells. In addition, severe degeneration of hepatopancreatic tubules was observed in shrimp fed HOFO diet, and tending to normal structural changes, such as increased in the number of B-cell and R-cell and less ruptured epithelial cells, was found in hepatopancreas due to MI supplementation. Hepatopancreas, as a vital organ of crustacean, has been reported to adjust nutrient metabolism, detoxification of xenobiotcs, antioxidant and immune responses [33,72]. The present study suggested that high oxidation degree of OFO (POV: 268.7 meq kg^{-1}) led to severe damage to hepatopancreas, which could be slightly mitigated by MI supplementation.

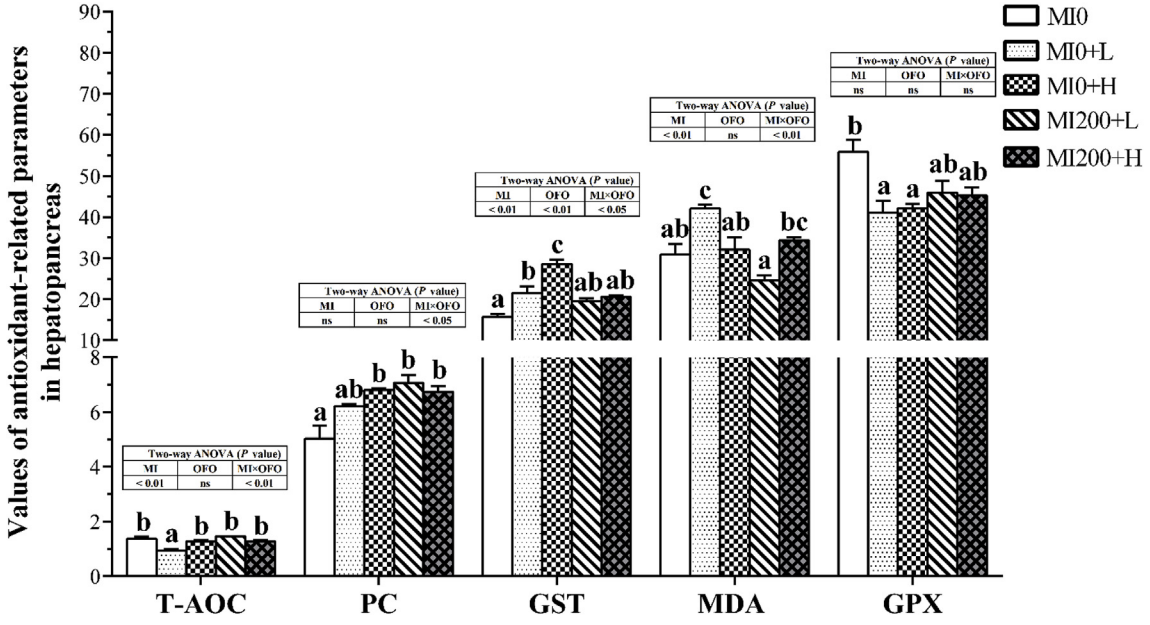

Fig. 4. Effect of MI and OFO supplementation on antioxidant-related parameters of hepatopancreas in *L. vannamei*. Results are presented as the mean ± SEM (n = 3). The columns with different letters indicate significant differences ($P < 0.05$). T-AOC (total antioxidant capacity, U mg $protein^{-1}$), PC (protein carbonyl, nmol mg $protein^{-1}$), GST (glutathione-S-transferase, U mg $protein^{-1}$), MDA (malondialdehyde, nmol g $tissue^{-1}$), GPX (U).

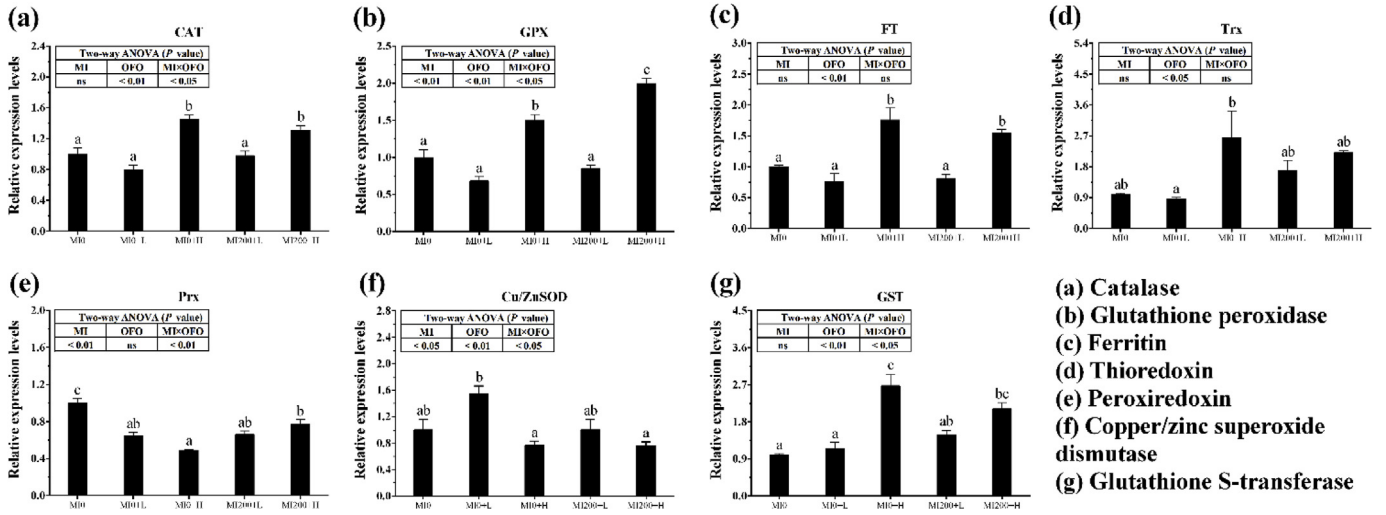

Fig. 5. Relative expression of antioxidant-related genes in hepatopancreas of *L. vannamei* fed experimental diets. Results are presented as the mean ± SEM (n = 3). The column with different letters indicate significant differences ($P < 0.05$).

4.4. Effect of MI and OFO supplementation on antioxidant parameters in serum and hepatopancreas

The imbalance between the production of free radicals and their elimination through antioxidant defence system led to oxidative stress. The metabolites derived from oxidation of fish oil may induce oxidative stress, resulting in damage to biomolecules in organisms [13]. Therefore, antioxidant enzymes belonging to the antioxidant system, such as CAT, SOD, GPX and GST, have been often used as indicators for evaluating antioxidant status [73]. GST has been confirmed to be related to conjugation and elimination of xenobiotics [74]. In turbot (*Scophthalmus maximus*), activities of GST in the liver were increased by dietary oxidized oil and reduced by dietary vitamin E [38]. Similarly, GST activity in hepatopancreas was enhanced by HOFO and decreased by MI supplementation. Nuclear factor erythroid-derived 2-like 2 (Nrf2) pathway, as the most important pathway to prevent oxidative damage for cells, could be activated by moderately oxidized oil, leading to some Nrf2 target genes (including SOD and GPX) increased in liver of pigs [75]. However, SOD activity was not significantly affected by addition of OFO and MI, which were opposite to the results observed in *M. amblycephala* and *M. salmoide* [17,48]. Previous study showed that liver GPX activity in rats was not affected by oxidized oil [76]. Similar result was obtained in *O. mykiss* [37]. Nevertheless, reduction of GPX was found in *L. vannamei* due to dietary OFO, which was in agreement with the results observed in *E. sinensis* [6]. T-AOC is utilized to reflect total antioxidant capacity of organisms [77]. OFO could reduce the T-AOC activity in shrimp [15], which was in accordance with the present study of which reported that dietary LOFO reduced T-AOC activity in hepatopancreas. But MI supplementation increased T-AOC activity in LOFO groups. The expression of genes CAT, GPX, FT, Trx, Cu/ZnSOD, Prx and GST have been confirmed to be associated with antioxidant status [78–81], and an intense induction of these genes may alleviate DNA damage and eliminate reactive radicals induced by environmental stress [82]. In the present study, HOFO significantly increased the expression of CAT, GPX, FT, Trx and GST while lowering Prx expression. It might indicate that the antioxidant system was intensely stimulated by HOFO. Prx has been declared to share the same function with CAT and GPX [83,84]. The decrease in the expression of Prx may be related to the main role of CAT and GPX in the removal of hydroperoxide. In addition, MI supplementation increased expression of GPX and Prx in shrimp. These results suggested that MI supplementation may be beneficial for improving antioxidant status in *L. vannamei* under the stimulation of dietary OFO.

Generally, MDA has been chosen to assess the condition of lipid peroxidation in tissue [85]. Consumption of dietary oxidized oils have often resulted in a significant increment in MDA content in serum or tissue [7,15], which was alleviated by adding antioxidants including α-lipoic acid, vitamin C and vitamin E [8,85]. Addition of OFO resulted in elevated serum and hepatopancreas MDA production in the present study, suggesting that OFO induced oxidative stress to *L. vannamei*. Such effect was counteracted by administration of MI, indicating that lipid peroxidation brought about by OFO could be alleviated by MI supplementation. PC is another parameter for assessing the damage of oxidative stress [86]. Dietary oxidized oil could enhance the protein oxidation in muscle of broilers [87]. PC content in hepatopancreas markedly increased with increasing oxidation degree of fish oil, implying that OFO induced protein oxidation in hepatopancreas. However, MI supplementation had no effect on lowering protein oxidation caused by OFO.

In summary, OFO used in the present study stimulated the growth performance, and *L. vannamei* could tolerate the diets with low-oxidized fish oil (POV: 133.2 meq kg^{-1}). However, dietary OFO enhanced levels of lipid peroxidation and protein oxidation and had a detrimental effect on health status of shrimp. High-oxidized fish oil (POV: 268.7 meq kg^{-1}) also caused serious damage to hepatopancreas. MI supplementation might be beneficial for improving adverse condition mentioned above in *L. vannamei*.

Acknowledgments

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