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Original Research Article

Effect of dietary supplementation of selenium-L-methionine on growth, antioxidant capacity and resistance to nitrite stress of spotted seabass (*Lateolabrax maculatus*) under two rearing water temperatures

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ABSTRACT

A 10-week feeding trial, followed by 24-h nitrite stress, was performed to evaluate the effects of dietary selenium-L-methionine (Se-Met) on growth, Se accumulation, antioxidant capacity, transcripts of selenoproteins and histological changes of muscle as well as resistance to nitrite stress in spotted seabass (*Lateolabrax maculatus*) reared at optimal (27 °C) and high (33 °C) temperatures. Five experimental diets were formulated to contain 0, 0.9, 1.8, 3.5, and 7.0 mg Se-Met/kg. Each diet was fed to fish (2.60 ± 0.2 g) in two parallel treatments at 27 or 33 °C. The results showed that elevated temperature (33 °C) induced thermal stress in fish, and fish under thermal stress exhibited lower weight gain and hepatosomatic index but a higher condition factor compared to those reared at 27 °C. However, the growth and feed utilisation were promoted in *L. maculatus* with 0.9 to 3.5 mg/kg Se-Met treatments. The protein and lipid content in the muscle increased with the dietary Se-Met level, and the total Se level in the whole body and muscle showed a linear increase with dietary Se-Met supplementation. Thermal stress changed the histology of the muscle, leading to raised levels of malondialdehyde (MDA), reduced antioxidant parameters in the serum and liver, and a decrease in the transcripts of selenoprotein genes in the muscle. Meanwhile, increased antioxidant capacity of serum and liver and up-regulated transcripts of selenoprotein of muscle were observed in *L. maculatus* reaching a maximum with 3.5 mg Se-Met/kg treatment. After 24 h of nitrite stress, thermal stress exacerbated oxidative damage caused by nitrite stress in *L. maculatus*. In contrast, dietary Se-Met enhanced the resistance to nitrite stress of *L. maculatus* fed with Se-Met enriched diets containing 0.9 to 1.8 mg Se-Met/kg. Based on the effects of dietary Se-Met on the growth, antioxidant capacity and resistance to nitrite stress of *L. maculatus*, this study suggests that the optimal range of Se-Met supplementation in *L. maculatus* diets is 1.80 to 2.39 mg Se-Met/kg of diet at 27 °C and 1.80 to 4.46 mg Se-Met/kg of diet at 33 °C.

1. Introduction

Water temperature is one of the most pervasive and influential environmental factors in intensive aquaculture, which directly impacts nearly all aspects of fish physiology (Buentello et al., 2000; Zak and Manzon, 2019). Maintaining an optimal water temperature range is crucial for ensuring the normal growth, feed utilization, physiological metabolism, and survival of fish (Bansemer et al., 2018; Buentello et al., 2000). However, water temperatures in outdoor aquaculture fluctuate with seasons, often deviating from the optimal range for cultured fish (Zeng et al., 2021). In summer, the impact of elevated temperatures on aquaculture fish cannot be avoided. In a short-term high-temperature environment, fish maintain relative body temperature balance mainly through regulating various hormones (i.e., thyroxine) (Zak and Manzon, 2019). However, chronically elevated water temperature-induced thermal stress can have various negative effects on fish growth, physiology

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and survival (Kim et al., 2016; Matthews and Berg, 1997; Vinagre et al., 2012), with oxidative stress caused by excess reactive oxygen species (ROS) being one of the main reasons for these adverse responses in fish (Cheng et al., 2018; Kim et al., 2017). In general, adding an appropriate proportion of antioxidants into fish feed is a practical and effective way to prevent or mitigate oxidative stress caused by thermal temperature (Li et al., 2023; Yuan et al., 2023). Thus, it is important to explore the interactions between fish nutritional requirements for antioxidants and water temperature to achieve maximum growth potential.

In aquatic organisms, selenium (Se) is an effective exogenous antioxidant which plays a role in the removal of reactive oxygen species and preventing oxidative stress caused by changes in environmental factors (Li et al., 2022; Wang et al., 2019, 2021a). Meanwhile, Se plays a crucial role in maintaining normal growth and physiological functions in fish (Gatlin and Wilson, 1984; Schrauzer, 2000) and is toxic in excess (Janz et al., 2010). In general, Se forms supplemented in feed mainly include sodium selenite and organic Se, i.e., selenium-L-methionine (Se-Met) and Se-yeast (Ghaniem et al., 2022; Liu et al., 2017). Early studies on dietary Se mainly focused on evaluating the optimal requirement of inorganic Se (Gatlin and Wilson, 1984). However, Se-Met is the dominant organic Se form (>80%) found in organisms, with higher bioavailability than inorganic Se and is more readily assimilated and accumulated in fish (Wang and Lovell, 1997). Some studies have demonstrated that several fish species exhibit greater weight gain and feed utilisation when fed diets supplemented with organic Se as opposed to inorganic Se (when total Se levels are comparable) (Lin, 2014; Liu et al., 2017; Wang et al., 2007). Thus, a lower amount of dietary Se supplementation is expected to achieve similar effects on growth with the replacement of inorganic Se with Se-Met (Liu et al., 2017; Wang and Lovell, 1997). Overall, it is necessary to revise or evaluate the optimal level of supplementation of Se for fish species commonly used in aquaculture when Se is added to fish diets in the form of Se-Met.

Se exerts its diverse biological functions by participating in the synthesis of 41 selenoproteins in the teleost (Mariotti et al., 2012). These selenoprotein families involved in thermal stress regulation primarily include regulation of the redox homeostasis by glutathione peroxidase (GPx) and thioredoxin reductase (TXNRD), as well as synthesis and metabolism of thyroxine by iodothyronine deiodinase (DIO) in organisms (Drigo et al., 2013; Weekley and Harris, 2013). Although the functions of Se-Met in improving growth, Se accumulation, feed conversion, antioxidant capacity, immunity and flesh quality in fish have been investigated (Chen et al., 2020; Lin, 2014; Liu et al., 2017; Mechlaoui et al., 2019; Rider et al., 2009; Wang et al., 2021b), there is limited research on the beneficial effects of Se-Met in mitigating thermal stress and resisting nitrite stress in fish.

Spotted seabass (*Lateolabrax maculatus*) has become a major fish cultured in southern China due to its high nutritional and economic value (Cai et al., 2020). Its total production reached approximately 218,000 metric tons in 2022 (MARA, 2023). The optimal water temperature range for *L. maculatus* is from 16 to 28 °C (Wen et al., 2019). In summer, the water temperature in the main farming areas for *L. maculatus* often reaches 33 °C or higher along the southeast coast of China, leading to thermal stress that affects the growth, feed efficiency and immunity of *L. maculatus* (Li et al., 2023; Yuan et al., 2023). Providing an appropriate amount of Se via dietary Se-Met to *L. maculatus* is one such strategy to counter the oxidative stress caused by elevated water temperature. A complete literature search revealed that the dietary Se requirement of *L. maculatus* is yet to be reported. Thus, this study aimed to evaluate the interactive effects of different water temperatures (27 and 33 °C) and dietary Se-Met levels on the growth, Se accumulation, antioxidant capacity, transcripts of selenoproteins and histological changes of muscle as well as resistance to nitrite stress in *L. maculatus*. The results will help us to understand the effects of Se-Met supplementation in *L. maculatus* at different temperatures and provide baseline information for the optimisation of feed for *L. maculatus* culture during different seasons.

2. Materials and methods

2.1. Animal ethics statement

In this study, we followed the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) to ensure the transparency and quality of our animal experiments. All procedures in this study were conducted following the guidelines of the Ethics Committee of Care and Use for Laboratory Animals of Jimei University (JMU202303004).

2.2. Preparation of Se-Met enriched diets

In this study, the formulation and proximate composition of the Se-amended diet for juvenile *L. maculatus* are shown in Table 1. The measured total Se levels in the control diets were 1.40 mg/kg Se dry weight (DW). Seleno-L-methionine (purity > 98%, Adisseo Life Science Co., Ltd., Shanghai, China) of 0.9, 1.8, 3.5 and 7.0 mg/kg DW (the nominal levels) was supplemented in the control diet to achieve the designated levels of total Se of 1.80, 2.45, 3.39 and 4.94 mg/kg, respectively. Feed pellets of approximately 2.5 mm diameter were prepared according to our previous research with modifications (Yuan et al., 2023). The feed was dried at 50 °C for 10 h, then stored at −20 °C until use.

Table 1
Formulation and proximate composition of the experimental diets (g/kg, dry matter basis).

Item	Se-Met supplementation, mg/kg diet				
	0 (Con)	0.9	1.8	3.5	7.0
Ingredients					
Deboned fishmeal [1]	150.00	150.00	150.00	150.00	150.00
Shrimp paste	30.00	30.00	30.00	30.00	30.00
Casein	250.00	250.00	250.00	250.00	250.00
Wheat gluten	50.00	50.00	50.00	50.00	50.00
Corn dextrin	350.00	350.00	350.00	350.00	350.00
Fish oil	50.00	50.00	50.00	50.00	50.00
Soybean oil	30.00	30.00	30.00	30.00	30.00
Lecithin	20.00	20.00	20.00	20.00	20.00
L-ascorbate-2-phosphate	1.00	1.00	1.00	1.00	1.00
Premix [2]	10.00	10.00	10.00	10.00	10.00
Choline chloride	5.00	5.00	5.00	5.00	5.00
KH_2PO_4	15.00	15.00	15.00	15.00	15.00
Microcrystalline cellulose	39.00	39.00	39.00	39.00	39.00
Se-Met [3], mg/kg		0.88	1.75	3.50	7.00
Proximate composition					
Crude protein	446.10	445.20	446.20	442.80	444.00
Crude lipid	112.00	112.00	112.00	115.30	115.90
Crude ash	37.70	40.50	41.40	39.10	37.00
Total Se levels, mg/kg	1.40	1.80	2.45	3.39	4.94

[1] Deboned fish meal (crude protein: 91.78%, crude lipid: 7.36%) obtained from Fengziya food Co., Ltd., Zhangzhou, China.

[2] Vitamin premix provided the following per kilogram diet: thiamin, 10 mg; riboflavin, 8 mg; pyridoxine HCl, 10 mg; vitamin B_{12}, 0.2 mg; vitamin K_3, 10 mg; inositol, 100 mg; pantothenic acid, 20 mg; niacin acid, 50 mg; folic acid, 2 mg; biotin, 2 mg; retinol acetate, 400 mg; cholecalciferol, 5 mg; alpha-tocopherol, 100 mg; ethoxyquin, 150 mg; wheat middling, 1132.8 mg. Mineral premix provided the following per kilogram diet: NaF, 2 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 25 mg; $MgSO_4 \cdot 7H_2O$, 200 mg; zeolite, 4582 mg.

[3] Seleno-L-methionine (Se-Met; purity > 98%) obtained from Adisseo Life Science Co., Ltd, Shanghai, China.

2.3. Experimental design and feeding trial

Two thousand juvenile *L. maculatus* were obtained from a commercial fish breeding farm (Zhaoan, Fujian). To acclimate to the experimental aquaculture conditions, 2000 fish were evenly distributed across two aquariums (each with a volume of 1000 L per tank) set at pre-determined temperatures of 27 and 33 °C, respectively. They were fed the same control diets twice daily for 2 weeks to acclimate to the experimental environment. After acclimation, 450 fish (1.09 ± 0.02 g) adapted to a water temperature of 27 °C, and they were randomly grouped into fifteen 150 L tanks (30 fish per tank) of recirculating aquaculture systems, with a pre-set temperature of 27 °C (optimal temperature). Another 450 fish of the same size acclimated to 33 °C were transferred to another circulating water system consisting of 15 tanks with a pre-set temperature of 33 °C. Out of the 15 tanks, three tanks (replicates) were allocated to each dietary Se-Met treatment. During the feeding trial for 10 weeks, fish were fed the Se-enriched diet at approximately 3% of their body weight twice daily at 08:00 and 17:00. Uneaten feed and faeces were removed after 30 min of feeding. The dechlorinated tap water was renewed, and feed intake was recorded daily. The conditions of the culture were as follows: water temperature at 27.0 ± 0.5 °C and 33 ± 0.5 °C, 12 h/12 h light/dark photoperiod, total ammonia–nitrogen < 0.3 mg/L, dissolved oxygen ≥ 6 mg/L, and pH 7.0 to 7.5.

2.4. Sample collection

After the end of the feeding experiment, the experimental fish were starved for 24 h to purge the gut content, followed by euthanasia with eugenol (1:10,000; Sigma–Aldrich, USA). All fish in each tank were counted and weighed individually to calculate survival rate (SR) and weight gain. Blood samples from 9 fish per tank (with three fish pooled as one sample, $n = 3$) were randomly collected from the caudal vein and stored at 4 °C. Blood was centrifuged at 850 × *g* at 4 °C for 10 min to obtain the serum samples. For measuring the total Se body burden and whole-body composition, two fish per replicate ($n = 6$) were selected and stored at −20 °C. The livers of 2 fish per tank ($n = 6$) were collected for the antioxidant capacity measurement. Dorsal muscles of 2 fish per tank were collected for measuring the total Se levels and transcripts of genes related to selenoproteins and antioxidants ($n = 6$). The dorsal muscle of 2 fish per tank was fixed in Bouin's solution for histological examination ($n = 6$). Finally, the individual body and dissected liver were weighed to calculate the morphological parameters, i.e., hepatosomatic index and condition factor (Yu et al., 2023).

2.5. Proximate composition of diets, whole fish, and muscle

The proximate compositions of diets, whole-body and muscle samples were measured according to standard procedure (AOAC, 1995). Crude protein content was measured according to method 981.10 using a 2300 Auto-analyzer Kjeldahl System (FOSS, Hillerød, Denmark). Crude lipid content was analysed using Soxhlet's extraction, following method 960.39. For the determination of moisture content, samples were dried to a constant weight at 105 °C, method 950.46. Crude ash content was measured by combustion using a muffle furnace at 550 °C for 8 h, method 942.05.

2.6. Determination of total Se content

The total Se content of the diets, whole body, and dorsal muscle was determined according to our previous method with slight modifications (Li et al., 2021). Briefly, approximately 0.2 g oven-dried diet samples, whole fish and muscle ($n = 6$) were digested with 10 mL of HNO_3 (analysis grade, 68%) and 2 mL of HCl (analysis grade, 37%) (Aladdin, Shanghai, China) using a microwave digestion system at 170 °C for 45 min (MARS 6, CEM Co., Ltd, USA). After digestion, total Se concentrations in the diets and fish tissues were measured on an atomic fluorescence spectrometer (AFS-8300, Titan Co., Ltd, Beijing, China). The instrumental detection limit was 0.1 to 0.2 μg/L. For quality assurance and quality control (QA/QC), the recovery for total Se in the standard reference material (GBW10024, National Research Center for Certified Reference Materials, Beijing, China) was 98% to 105% of the certified value. Other QA/QC included spiked samples and acid blanks. One blank was carried out after every 5 samples.

2.7. Histological analysis in dorsal muscle

Histological analysis of the dorsal muscle samples was performed following a previous method with minor modifications (Liu et al., 2021). In brief, paraformaldehyde-fixed muscles were cut into pieces of approximately 0.3 cm^3, followed by dehydration in graded alcohols and clearance in xylene. Afterwards, the muscle samples were embedded in paraffin wax, sectioned at 6 μm thickness, and stained with hematoxylin and eosin. Six randomly chosen fields of the muscle from each treatment were observed under a microscope (ECLIPSE Ni-U, Nikon, Japan). The diameter and density of muscle fibre per 1-mm^2 field of view were determined using Image J software connected to the microscope ($n = 6$).

2.8. Antioxidant capacity analysis of serum and liver

To assess the antioxidant capacity of experimental fish, activities of glutathione peroxidase (GPx) and glutathione reductase (GR), levels of glutathione (GSH), oxidized glutathione (GSSG) and malondialdehyde (MDA) in the serum and liver samples were determined following the protocols of commercial assay kits from Jiancheng Bioengineering Institute (Nanjing, China).

2.9. RNA extraction and quantitative real-time PCR (qRT-PCR) assay

The total RNA extraction, RNA quantity and quality, synthesis of cDNA and qRT-PCR procedures were conducted following a previously described method with modifications (Li et al., 2021). Total RNA from the liver and muscle tissues were isolated using Trizol reagent (Vazyme Biotech Co., Ltd., Nanjing, China). RNA was quantified on a NanoDrop 2000 spectrophotometer (Wilmington, USA). The quality of RNA was assessed by 1.5% agarose gel electrophoresis.

The expression levels of 6 genes related to selenoproteins in the muscle and 6 genes related to antioxidants in the liver were determined using qRT-PCR. The sequences of the primers are shown in Table 2. In brief, the RT-qPCR program was run at 95 °C for 30 s, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. After the PCR reaction, melting curve analysis was performed to confirm the specificity of the genes. The expression of genes was normalized to β-actin and *18S* using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

2.10. Acute nitrite stress test

Prior to the acute nitrite stress, the lethal concentration for 50% mortality in 24 h (LC_{50}-24 h) of *L. maculatus* reared at 27 and 33 °C was determined to be 73 and 57 mg/L, respectively. Based on the results of the acute toxicity test, the nominal concentrations of nitrite (sodium nitrite, AR, Macklin Biochemical Technology Co., Ltd., China) were set at 29.2 mg/L (27 °C) and 22.8 mg/L (33 °C)

Table 2
Sequences of the qRT-PCR primers of *L. maculatus* used in this study.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	AT, °C	AE
txnrd1	tgacggacatgaacggaaggaag	gggtgagacagggagaggagatc	60	1.27
dio2	agtcgccaccctcatagtttcttc	ctcacaccgccgaacacctg	60	1.18
gpx4a	ggcggactctttggagatggc	cgttcaacaacattcccgtctctg	60	1.31
selt	tggcgggcgagcagatatttc	gcacgcagggaataataccaagc	60	0.95
sep15	acacaactcggccttgatagatgg	cgtctgggctctgtgagtatgtc	60	0.94
selp	aaagcactggcccgttactgag	cggcattcatggtagttactgctg	60	1.35
nrf2	aagcgctctaatgtgcgtct	Gttctgggcagctaccttgt	60	1.02
ho-1	acgtcagggcagaaaacaca	agccgggaagtaaatgggtg	60	1.11
keap1	agccgccttcgccataatgaac	actccatgccgcactccttca	60	0.99
NF-κβ	tgtggtgtacgtaccgcttc	Ttctcaacggctggactac	60	1.03
ap-1	cctttctgcctctgtgtcgt	ccatgcagagctgggtgtaa	60	1.06
hsp70	actacgaccgtctccacaga	cctggtcgttggcgatgatt	60	1.09
β-Actin	caactgggatgacatggagaag	Ttggctttggggttcagg	60	1.08
18S	Gggtccgaagcgtttact	Tcacctctagcggcacaa	60	0.94

AT = annealing temperature; AE = amplification efficiency; *txnrd1* = thioredoxin reductase 1; *dio2* = iodothyronine deiodinase 2; *gpx4a* = glutathione peroxidase 4a; *selt* = selenoprotein T; *sep15* = 15-kDa selenoprotein; *selp* = selenoprotein P; *nrf2* = nuclear factor erythroid-2 related factor; *ho-1* = heme oxygenase-1; *keap1* = Kelch-like ECH-associated protein 1; *NF-κβ* = nuclear factor kappa-β; *ap-1* = activator protein-1; *hsp70* = heat shock protein 70; *18S* = 18S ribosomal RNA gene.

under different water temperatures. At the end of the 10-week feeding trial of dietary Se-Met, 12 fish per tank were selected and exposed to a predefined nitrite solution (120 L/tank) for 24 h to perform a nitrite stress test. During the 24-h exposure period, surviving individuals were recorded from each tank. In order to determine the changes in antioxidant parameters before and after nitrite stress, serum samples of 2 fish per replicate (two fish were pooled as one sample, $n = 3$) were collected at 0 and 24 h, respectively, then stored at −80 °C.

2.11. Statistical analyses

All data are presented as mean ± standard error unless otherwise stated. Kolmogorov–Smirnov and Levene's tests were conducted to check the assumptions of normality and homogeneity of variance, respectively. Two-way analyses of variance (ANOVA) were used to check the differences in dietary Se treatments, water temperatures or their interaction with the measured parameters, followed by Tukey's test for multiple comparisons. All statistical analyses were performed using SPSS 26.0 software, and $P < 0.05$ was regarded as a significant difference. A multiple linear regression analysis was employed to determine the relationship between the dietary Se-Met level at which the maximum weight gain was obtained under two water temperatures.

3. Results

3.1. Growth performance and feed utilization

The growth performance and feed utilization of *L. maculatus* were significantly affected by water temperature and dietary Se-Met (Table 3). However, the interaction between water temperature and dietary Se-Met did not show a significant effect. Overall, the final body weight (FBW) and weight gain of *L. maculatus* reared at 33 °C were significantly lower ($P < 0.001$) than those of fish reared at 27 °C. The FBW and weight gain of fish increased ($P < 0.001$) with dietary Se-Met levels from 0.9 to 3.5 mg/kg but decreased at 7.0 mg/kg, with the largest values obtained at 3.5 mg/kg. In contrast, FCR showed an opposite trend to FBW and weight gain ($P < 0.001$). Meanwhile, the cubic regression model analysis based on weight gain suggested that the optimal dietary Se-Met supplementation of *L. maculatus* was 2.39 and 4.46 mg/kg diet at 27 and 33 °C, respectively (Fig. 1). In addition, fish survival was not significantly different among all treatments.

The morphological parameters of *L. maculatus* were influenced by temperature, but not by Se-Met or the interaction term of Se-Met × temperature (Table 3). The hepatosomatic index of *L. maculatus* reared at 33 °C treatment was significantly lower than at 27 °C treatment ($P = 0.003$), while condition factor showed an opposite result ($P = 0.018$).

3.2. Proximate composition analysis of the whole fish and muscle tissue

Proximate composition analysis of the whole fish and muscle tissue are reported in Table 4. The crude ash content of the whole fish, and the intramuscular crude lipid, and crude ash of *L. maculatus* reared at 33 °C were significantly higher ($P < 0.001$) than those of fish reared at 27 °C. In contrast, the opposite was observed in the moisture of muscle ($P < 0.001$). The crude lipid content in the whole fish ($P = 0.005$) and muscle ($P = 0.024$) as well as intramuscular crude ash content ($P < 0.001$) was affected by temperature × Se-Met. Meanwhile, crude protein content in the whole fish and muscle was increased ($P < 0.001$) from 0.9 to 3.5 mg Se-Met/kg but decreased at 7.0 mg Se-Met/kg, with the highest values observed at 1.8 mg Se-Met/kg treatment. The crude lipid content in the whole fish was increased at 0.9 mg Se-Met/kg but decreased with higher Se-Met levels ($P = 0.046$), whereas crude lipid content of muscle was increased with increasing Se-Met levels ($P < 0.001$). The crude ash content in the whole fish was decreased at 1.8 mg Se-Met/kg then increased with higher Se-Met levels ($P < 0.001$), whereas intramuscular crude ash content was increased with increasing Se-Met levels ($P = 0.037$).

Dietary Se-Met × water temperature interaction significantly influenced the total Se content of muscle but not that of the whole fish (Fig. 2). Meanwhile, the total Se content of whole fish and muscle showed a dose-dependent increase with dietary Se-Met ($P < 0.001$). However, water temperatures had no significant effects on the total Se content of whole fish ($P = 0.493$) and muscle ($P = 0.063$).

3.3. Histological alteration in the muscle

The histological analysis showed that the fish muscle from both control and 7.0 mg Se-Met/kg treatments exhibited a typical pathological feature of fiber disintegration (Fig. 3A and B). Meanwhile, the diameter and density of muscle fiber were significantly affected by Se-Met and temperature (Fig. 3C and D). Overall, the diameter of the muscle fiber of *L. maculatus* reared at 33 °C was significantly larger ($P = 0.007$) than that of fish reared at 27 °C, whereas muscle fiber density was reduced ($P = 0.004$). The diameter of muscle fiber was decreased ($P = 0.001$) with Se-Met levels from 3.5 to 7.0 mg/kg. The muscle fiber density was elevated ($P = 0.001$) at 0.9 mg Se-Met/kg but decreased from 1.8 to 7.0 mg Se-Met/kg, with the maximum density observed at 0.9 mg Se-Met/kg.

3.4. Antioxidant capacity in the serum and liver

Antioxidant parameters in the serum and liver of *L. maculatus* were all significantly affected by Se-Met and temperature, respectively (Tables 5 and 6). Overall, malondialdehyde (MDA) levels in the serum and liver of *L. maculatus* reared at 33 °C were significantly higher ($P < 0.001$) than those of fish reared at 27 °C. The activity of GPx in the serum and activity of GR in the liver from the 33 °C treatments were lower ($P < 0.001$) than those from the 27 °C treatments; however, GPx activity in the liver followed the opposite

Table 3
Effect of dietary Se-Met levels on growth, feed utilization, and morphometric parameters of *L. maculatus* reared at two temperatures for 10 weeks.

Temp, °C	Se-Met, mg/kg	FBW, g	Weight gain, %	FCR	Hepatosomatic index, %	Condition factor, g/cm^3	Survival, %
27	0	56.29 ± 2.10	5102.95 ± 60.34	1.26 ± 0.03	1.48 ± 0.05	1.88 ± 0.06	98.89 ± 1.92
	0.9	62.04 ± 1.91	5737.90 ± 241.47	1.22 ± 0.10	1.63 ± 0.01	1.98 ± 0.08	88.89 ± 8.39
	1.8	70.40 ± 0.84	6324.76 ± 114.81	1.15 ± 0.01	1.52 ± 0.14	1.92 ± 0.11	96.67 ± 3.34
	3.5	67.92 ± 0.92	6112.70 ± 113.08	1.20 ± 0.02	1.55 ± 0.13	2.04 ± 0.06	94.44 ± 5.09
	7.0	62.00 ± 5.41	5573.61 ± 442.75	1.24 ± 0.03	1.51 ± 0.11	1.97 ± 0.12	97.78 ± 1.92
33	0	52.06 ± 1.67	4748.86 ± 111.42	1.27 ± 0.02	1.40 ± 0.08	2.04 ± 0.03	98.89 ± 1.92
	0.9	57.47 ± 1.21	5150.51 ± 194.19	1.17 ± 0.02	1.48 ± 0.02	2.10 ± 0.06	97.78 ± 3.85
	1.8	59.01 ± 3.71	5275.13 ± 420.25	1.18 ± 0.02	1.44 ± 0.07	2.03 ± 0.17	98.89 ± 1.92
	3.5	65.32 ± 0.85	5901.86 ± 200.45	1.11 ± 0.03	1.42 ± 0.15	2.05 ± 0.09	100.00 ± 0.00
	7.0	55.15 ± 1.42	4973.20 ± 24.20	1.20 ± 0.01	1.32 ± 0.15	2.00 ± 0.03	96.67 ± 5.77
Temp, °C							
27		63.73^{Y}	5770.38^{Y}	1.22	1.54^{Y}	1.96^{X}	95.33
33		57.80^{X}	5209.91^{X}	1.19	1.41^{X}	2.04^{Y}	98.44
Se-Met, mg/kg							
0		54.18^{A}	4925.90^{A}	1.26^{B}	1.44	1.96	98.89
0.9		59.76^{B}	5444.21^{BC}	1.20^{A}	1.55	2.04	93.33
1.8		64.70^{C}	5799.94^{CD}	1.17^{A}	1.48	1.98	97.78
3.5		66.62^{C}	6007.28^{D}	1.16^{A}	1.48	2.04	97.22
7.0		58.58^{B}	5273.41^{AB}	1.22^{AB}	1.42	1.99	97.22
P-values							
Temp		<0.001	<0.001	0.050	0.003	0.018	0.052
Se-Met		<0.001	<0.001	<0.001	0.228	0.392	0.225
Interaction		0.052	0.056	0.066	0.899	0.612	0.243

Se-Met = seleno-L-methionine; Temp = temperature; FBW = final body weight; FCR = feed conversion rate.
Values are mean ± SE (n = 3 for each treatment).
$^{A–D}$ Values in the same column with different superscripts represent significant differences among Se-Met treatments ($P < 0.05$).
^{X,Y} Values in the same column with different superscripts represent significant differences among temperature treatments ($P < 0.05$).

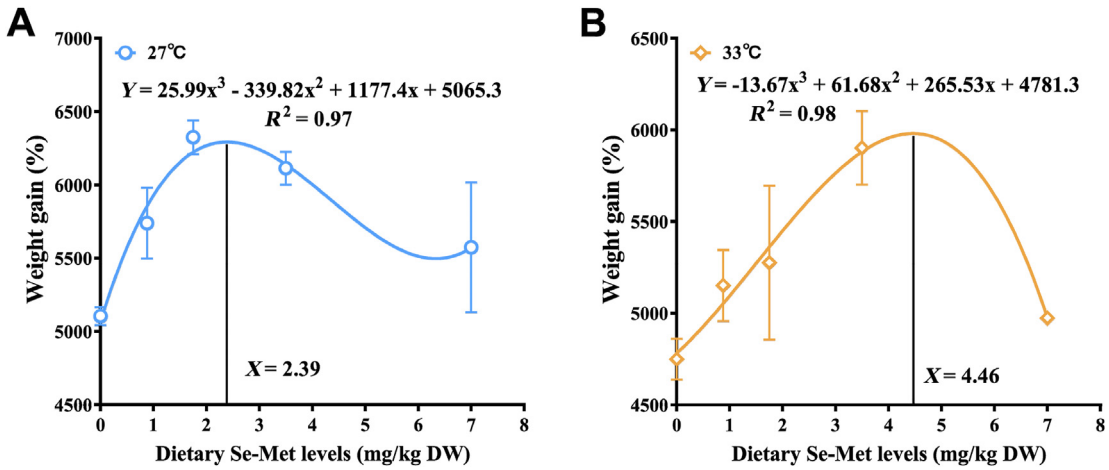

Fig. 1. Cubic regression analyses of weight gain to dietary Se-Met levels in spotted seabass (*L. maculatus*) reared at 27 °C (A) and 33 °C (B). Se-Met = seleno-L-methionine; DW = dry weight.

trend (P = 0.036). Meanwhile, the activities of GPx, GR, and the levels of GSH in the serum (P = 0.012; P = 0.012; P = 0.003) and liver ($P < 0.001$; $P < 0.001$; $P < 0.001$) were enhanced when fed low levels of Se-Met but decreased ($P < 0.05$) afterwards with increased dietary Se-Met levels. In contrast, the opposite trend was witnessed in MDA levels from the serum ($P < 0.001$) and liver ($P < 0.001$).

3.5. Transcripts of genes related to antioxidants in the liver

Dietary Se-Met × water temperature interaction significantly influenced the transcripts of *keap1*, *ap-1*, and *hsp70* ($P < 0.001$) but not those of *nrf2* ($P = 0.701$), *ho-1* ($P = 0.547$), and *NF-kβ* ($P = 0.105$) (Fig. 4). The transcriptional expression of *keap1*($P < 0.001$) and *NF-kβ* (P = 0.001) in the liver from the 33 °C treatments was down-regulated ($P < 0.01$) compared to the 27 °C treatments. In contrast, the opposite was shown for *ap-1* ($P < 0.001$) and *hsp70* ($P < 0.001$). The transcriptional expression of *ho-1* ($P = 0.003$), *ap-1* ($P < 0.001$), and *hsp70* ($P < 0.001$) was up-regulated in the liver by Se-Met from the 3.5 and 7.0 mg/kg treatments. The transcriptional expression of *keap1* ($P < 0.001$) was up-regulated in treatments with 1.8 to 7.0 mg Se-Met/kg. In contrast, the transcript of *NF-kβ* was down-regulated ($P = 0.037$) in the 7.0 mg Se-Met/kg treatment.

3.6. Transcriptional expressions of selenoprotein genes in the muscle

There was a significant interaction effect between dietary Se-Met and water temperature on the transcripts of selenoprotein-related genes: *txnrd1* ($P < 0.001$), *dio2* ($P < 0.001$), *gpx4a* ($P = 0.034$), *selt* ($P < 0.001$), *sep15* ($P = 0.012$), and *selp* ($P < 0.001$) in the muscle (Fig. 5). Overall, the transcripts of *txnrd1* ($P < 0.001$), *dio2* ($P < 0.001$), *gpx4a* ($P < 0.001$), *selt* ($P < 0.001$), *sep15*

Table 4
Effect of dietary Se-Met levels on the proximate composition (%, wet weight) of whole fish and muscle of *L. maculatus* reared at two temperatures for 10 weeks.

Temp, °C	Se-Met,	Whole body				Muscle			
	mg/kg	Moisture	Crude protein	Crude lipid	Crude ash	Moisture	Crude protein	Crude lipid	Crude ash
27	0	67.01 ± 0.70	16.69 ± 0.10	12.25 ± 0.45^{bc}	3.55 ± 0.08	77.31 ± 0.06	19.67 ± 0.04	0.59 ± 0.05^{a}	1.72 ± 0.12^{abc}
	0.9	67.47 ± 0.56	16.87 ± 0.14	11.60 ± 0.72^{abc}	3.54 ± 0.09	77.06 ± 0.07	20.06 ± 0.06	0.63 ± 0.04^{ab}	1.65 ± 0.04^{a}
	1.8	67.23 ± 0.41	17.12 ± 0.15	11.85 ± 0.60^{abc}	3.52 ± 0.14	77.36 ± 0.39	20.21 ± 0.05	0.76 ± 0.04^{bc}	1.74 ± 0.07^{abc}
	3.5	67.45 ± 0.14	16.90 ± 0.04	11.61 ± 0.22^{abc}	3.63 ± 0.07	77.17 ± 0.06	20.14 ± 0.04	0.84 ± 0.13^{cd}	1.74 ± 0.02^{abc}
	7.0	67.39 ± 0.80	16.61 ± 0.12	11.52 ± 0.69^{abc}	3.63 ± 0.10	76.83 ± 0.37	19.68 ± 0.24	0.96 ± 0.07^{d}	1.68 ± 0.05^{ab}
33	0	67.19 ± 0.53	16.71 ± 0.20	11.50 ± 0.30^{abc}	3.89 ± 0.12	76.45 ± 0.51	19.67 ± 0.25	0.90 ± 0.09^{cd}	1.68 ± 0.06^{ab}
	0.9	66.69 ± 0.16	16.72 ± 0.14	12.35 ± 0.20^{c}	3.85 ± 0.06	76.53 ± 0.14	19.99 ± 0.09	1.17 ± 0.18^{ef}	1.79 ± 0.02^{bc}
	1.8	67.73 ± 0.65	16.85 ± 0.11	11.33 ± 0.43^{ab}	3.72 ± 0.16	76.28 ± 0.12	20.09 ± 0.13	1.15 ± 0.04^{e}	1.76 ± 0.05^{bc}
	3.5	66.76 ± 1.06	16.81 ± 0.06	11.83 ± 0.79^{abc}	3.96 ± 0.12	76.46 ± 0.07	19.88 ± 0.07	1.32 ± 0.05^{fg}	1.80 ± 0.04^{c}
	7.0	66.91 ± 0.69	16.66 ± 0.06	11.22 ± 0.17^{a}	3.97 ± 0.08	76.56 ± 0.26	19.70 ± 0.08	1.44 ± 0.06^{g}	1.82 ± 0.03^{c}
Temp, °C									
27		67.31	16.84	11.77	3.58^{X}	77.14^{Y}	19.95	0.76^{X}	1.70^{X}
33		67.06	16.75	11.65	3.88^{Y}	76.46^{X}	19.87	1.20^{Y}	1.77^{Y}
Se-Met, mg/kg									
0		67.10	16.70^{AB}	11.87^{AB}	3.72^{AB}	76.88	19.67^{A}	0.75^{A}	1.70^{A}
0.9		67.08	16.80^{ABC}	11.97^{B}	3.70^{AB}	76.79	20.02^{B}	0.90^{B}	1.72^{AB}
1.8		67.48	16.99^{C}	11.59^{AB}	3.62^{A}	76.82	20.15^{B}	0.96^{B}	1.75^{AB}
3.5		67.10	16.85^{BC}	11.72^{AB}	3.80^{B}	76.82	20.01^{B}	1.08^{C}	1.77^{B}
7.0		67.15	16.63^{A}	11.37^{A}	3.80^{B}	76.69	19.69^{A}	1.20^{D}	1.75^{AB}
P-values									
Temp		0.281	0.055	0.363	<0.001	<0.001	0.075	<0.001	<0.001
Se-Met		0.786	<0.001	0.046	<0.001	0.798	<0.001	<0.001	0.037
Interaction		0.344	0.168	0.005	0.442	0.111	0.412	0.024	<0.001

Se-Met = seleno-L-methionine; Temp = temperature. Values are mean ± SE (n = 6 for each treatment).
[a-g] Values in the same column with different superscripts represent significant differences (P < 0.05).
[A–D] Values in the same column with different superscripts represent significant differences among Se-Met treatments (P < 0.05).
[X,Y] Values in the same column with different superscripts represent significant differences among temperature treatments (P < 0.05).

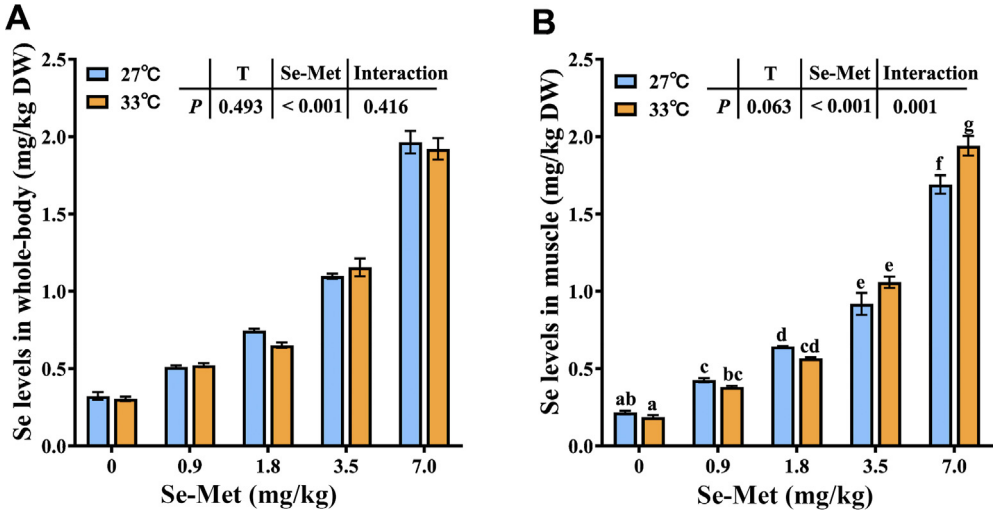

Fig. 2. Effect of dietary Se-Met levels on the level of total Se in the whole body (A) and muscle (B) of *L. maculatus* reared at two temperatures for 10 weeks. Values are mean ± SE (n = 6). Bars with different letters represent significant differences (P < 0.05). Se-Met = seleno-L-methionine; DW = dry weight; T = temperature.

(P = 0.038), and *selp* (P < 0.001) were up-regulated in the muscle by dietary Se-Met supplementation ranging from 1.8 to 7.0 mg/kg. The transcripts of *txnrd1*, *selt*, and *selp* in the muscle from the 33 °C treatments were down-regulated (P < 0.001) compared to those from the 27 °C treatments, whereas an opposite trend (P < 0.001) was found for *gpx4a*. However, the transcripts of *dio2* (P = 0.578) and *sep15* (P = 0.761) in the muscle of *L. maculatus* were not affected by water temperature.

3.7. Changes in serum antioxidant capacity after acute nitrite stress

After nitrite exposure for 24 h, the survival rate of *L. maculatus* cultured at 27 and 33 °C was 92% and 86%, respectively. Serum MDA levels, GSH/GSSH ratio, and GPx activity of *L. maculatus* cultured at 27 and 33 °C were significantly affected by dietary Se-Met and dissolved nitrite, respectively (Fig. 6). Relative to the control exposed for 0 h, serum MDA levels (P = 0.005; P < 0.001) and activity of GPx (P < 0.001; P = 0.006) in *L. maculatus* reared at both 27 and 33 °C were elevated after 24 h of exposure (Fig. 6A–D). In contrast, serum GSH/GSSG ratio (P < 0.001; P = 0.036) decreased in the 24 h of exposure treatments for all fish (Fig. 6E and F). Meanwhile, serum GPx activity (P = 0.042; P = 0.012) from two temperatures were affected by the interaction between dietary Se-Met and dissolved nitrite. Furthermore, the alterations in antioxidant physiology due to nitrite stress were consistent with the changes induced by varying levels of Se-Met.

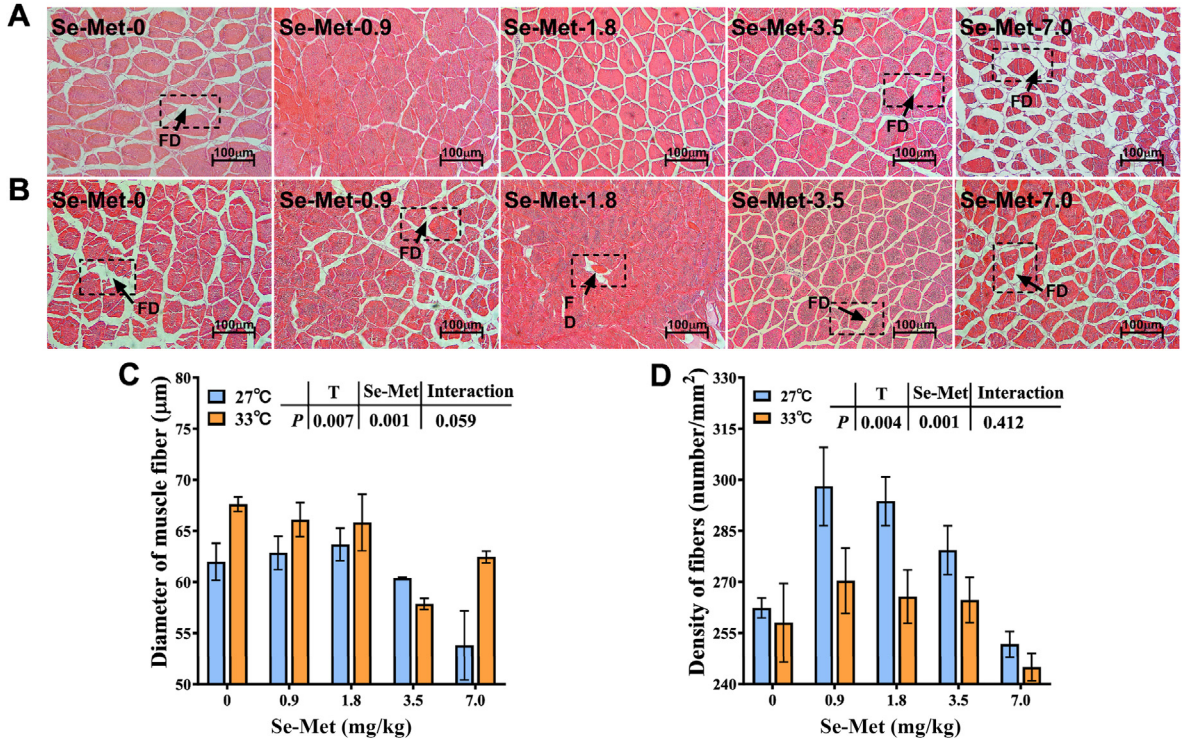

Fig. 3. Effect of dietary Se-Met level on muscle fiber histology, diameter, and density in *L. maculatus* reared at two temperatures for 10 weeks. (A and B) Representative histological images (hematoxylin and eosin staining, scale bar = 100 μm) of muscle for *L. maculatus* reared at 27 °C ($n = 6$) and at 33 °C ($n = 6$), respectively. (C and D) The changes in diameter and density of muscle fiber, respectively ($n = 6$). Se-Met = seleno-L-methionine; FD = fiber disintegration; T = temperature.

Table 5
Effect of dietary Se-Met levels on serum antioxidant physiology in *L. maculatus* reared at two temperatures for 10 weeks.

Temp, °C	Se-Met, mg/kg	GPx, U/mL	GSH, μmol/L	GR, U/L	MDA, nmol/mL
27	0	583.99 ± 67.04	10.36 ± 0.38	11.79 ± 1.85	10.76 ± 0.74
	0.9	630.43 ± 8.79	19.79 ± 0.20	15.01 ± 1.86	9.33 ± 1.14
	1.8	628.17 ± 15.24	18.82 ± 2.32	16.08 ± 3.22	9.28 ± 0.33
	3.5	682.03 ± 44.09	15.68 ± 2.75	16.08 ± 3.22	10.66 ± 0.94
	7.0	573.04 ± 20.14	11.90 ± 3.07	15.01 ± 1.86	11.95 ± 1.48
33	0	508.44 ± 55.51	15.58 ± 2.22	11.79 ± 1.85	14.07 ± 0.79
	0.9	518.22 ± 57.47	16.81 ± 5.96	17.15 ± 1.85	12.84 ± 0.56
	1.8	603.54 ± 50.75	17.19 ± 3.13	18.22 ± 1.85	11.96 ± 0.19
	3.5	588.06 ± 36.18	15.37 ± 4.25	17.15 ± 1.85	15.47 ± 0.96
	7.0	573.99 ± 23.64	12.49 ± 4.53	13.93 ± 1.86	16.71 ± 1.35
Temp, °C					
27		619.53^{Y}	15.31	14.79	10.40^{X}
33		558.45^{X}	15.49	15.65	14.21^{Y}
Se-Met, mg/kg					
0		546.22^{A}	12.97^{AB}	11.79^{A}	12.42^{BC}
0.9		574.33^{AB}	18.30^{B}	16.08^{B}	11.09^{AB}
1.8		615.86^{AB}	18.01^{B}	17.15^{B}	10.62^{A}
3.5		635.05^{B}	15.53^{AB}	16.61^{B}	13.07^{CD}
7.0		573.51^{AB}	12.20^{A}	14.47^{AB}	14.33^{D}
P-values					
Temp		<0.001	0.884	0.298	<0.001
Se-Met		0.012	0.012	0.003	<0.001
Interaction		0.151	0.298	0.662	0.239

Se-Met = seleno-L-methionine; Temp = temperature; GPx = glutathione peroxidase; GSH = glutathione; GR = glutathione reductase; MDA = malondialdehyde.
Values are mean ± SE ($n = 6$ for each treatment).
$^{A–D}$ Values in the same column with different superscripts represent significant differences among Se-Met treatments ($P < 0.05$).
^{X,Y} Values in the same column with different superscripts represent significant differences among temperature treatments ($P < 0.05$).

4. Discussion

Se-Met is the dominant organic Se form in organisms, including fish (Sele et al., 2018; Stewart et al., 2004). The dietary route is predominant for the accumulation of Se in fish (Lemly, 2002). It has been shown that the level of Se-Met in the whole fish and tissues is positively correlated with dietary intake (Liu et al., 2017; Rider et al., 2009; Wang et al., 2018). In this study, there was a linear increase in total Se contents in the whole fish and muscle with increasing Se levels in the diet, regardless of the two culture

Table 6
Effect of dietary Se-Met levels on antioxidant physiology in the liver of *L. maculatus* reared at two temperatures for 10 weeks.

Temp, °C	Se-Met, mg/kg	GPx, U/mg prot	GSH, nmol/mg prot	GR, U/g prot	MDA, nmol/mg prot
27	0	66.61 ± 2.95	11.01 ± 0.41	12.17 ± 0.49	0.72 ± 0.03
	0.9	74.68 ± 5.08	12.16 ± 0.61	14.15 ± 0.79	0.69 ± 0.02
	1.8	78.07 ± 0.96	16.62 ± 2.98	13.32 ± 0.87	0.63 ± 0.03
	3.5	75.66 ± 12.37	14.23 ± 1.95	11.86 ± 0.40	0.61 ± 0.02
	7.0	62.28 ± 0.91	8.92 ± 1.64	10.86 ± 0.83	0.71 ± 0.01
33	0	68.48 ± 3.83	8.19 ± 1.61	10.34 ± 0.30	0.82 ± 0.02
	0.9	74.21 ± 1.07	10.37 ± 1.93	11.76 ± 0.19	0.79 ± 0.01
	1.8	77.92 ± 3.91	13.94 ± 3.97	12.65 ± 0.17	0.75 ± 0.03
	3.5	82.36 ± 1.46	17.48 ± 3.53	10.94 ± 0.19	0.73 ± 0.01
	7.0	73.74 ± 1.07	10.43 ± 2.01	9.83 ± 0.29	0.87 ± 0.01
Temp, °C					
27		71.46^{X}	12.59	12.47^{Y}	0.67^{X}
33		75.34^{Y}	12.08	11.10^{X}	0.79^{Y}
Se-Met, mg/kg					
0		67.54^{A}	9.60^{A}	11.26^{B}	0.77^{BC}
0.9		74.45^{AB}	11.26^{AB}	12.96^{C}	0.74^{B}
1.8		78.00^{B}	15.28^{BC}	12.98^{C}	0.69^{A}
3.5		79.01^{B}	15.85^{C}	11.40^{B}	0.67^{A}
7.0		68.01^{A}	9.68^{A}	10.35^{A}	0.79^{C}
P-values					
Temp		0.036	0.560	<0.001	<0.001
Se-Met		<0.001	<0.001	<0.001	<0.001
Interaction		0.178	0.124	0.052	0.055

Se-Met = seleno-L-methionine; Temp = temperature; GPx = glutathione peroxidase; GSH = glutathione; GR = glutathione reductase; MDA = malondialdehyde.
Values are mean ± SE ($n = 6$ for each treatment).
$^{A–C}$ Values in the same column with different superscripts represent significant differences among Se-Met treatments ($P < 0.05$).
^{X,Y} Values in the same column with different superscripts represent significant differences among temperature treatments ($P < 0.05$).

temperatures. Similarly, Atlantic salmon (*Salmo salar*) fed dietary Se-Met accumulated Se mainly in the form of Se-Met in the whole fish, liver, and muscles (Lorentzen et al., 1994). Other similar results regarding Se accumulation have been reported in the hybrid striped bass (*Morone saxatilis*) and rainbow trout (*Oncorhynchus mykiss*) fed a Se-Met enriched diet over 8 weeks (Cotter et al., 2008; Wang et al., 2018). The fact that Se-Met accumulates in fish muscle strongly indicates its indispensability for fish, probably more specifically associated with the synthesis of selenoproteins (see discussion below) (Li et al., 2021; Wang et al., 2018).

As an essential micronutrient, Se plays an important role in growth promotion in fish, but has a narrow demarcation between beneficial and toxic effects (Janz et al., 2010). It is important to determine the optimal level of dietary Se-Met for cultured fish. In this study, diets supplemented with 0.9 to 3.5 mg Se-Met/kg increased the growth performance of *L. maculatus*. Growth performance decreased at 7.0 mg Se-Met/kg, indicating that optimal dietary Se-Met could improve the growth and feed utilization of *L. maculatus* and that excess Se-Met led to deleterious effects on fish growth. It has been shown that organic Se supplementation of 0.4 mg Se/kg could improve the growth and feed efficiency of blunt snout bream (*Megalobrama amblycephala*), while 0.8 mg Se/kg causes negative effects (Liu et al., 2017). Other similar results have been obtained in grouper (*Epinephelus malabaricus*) (Lin, 2014), Atlantic salmon (Berntssen et al., 2018), and Nile tilapia (*Oreochromis niloticus*) (Chen et al., 2020).

In the present study, water temperature also affected growth and feed utilization of *L. maculatus* independent of Se-Met level, with the inhibition of growth and feed utilization under high temperature conditions. This is consistent with the results of our previous research on the effects of elevated temperature (33 °C) for *L. maculatus* (Cai et al., 2020; Li et al., 2023; Yuan et al., 2023). Early studies have demonstrated that the sub-optimal and supra-optimal water temperatures have a negative impact on growth (Xie et al., 2011) and feed utilization in tilapia (Likongwe et al., 1996). On the one hand, high-temperature induced thermal stress probably inhibits digestive capacity in *L. maculatus* (Yuan et al., 2023). On the other hand, fish reared at higher water temperatures need to allocate more resources and energy to maintain their basic metabolic functions rather than allocating it towards growth (Xie et al., 2011; Zeng et al., 2021). Furthermore, in this study, the optimal supplementation of 4.46 mg/kg for dietary Se-Met of *L. maculatus* reared at 33 °C was higher than that of 2.39 mg/kg of fish reared at 27 °C. This indirectly implies that elevated water temperature induces thermal stress in fish, which in turn triggers a mechanism of selenium depletion to restore and maintain cellular homeostasis. Therefore, the nutrient requirements of Se-Met to achieve a maximal increase in weight gain of *L. maculatus* increase in high-temperature environments.

Previous data have shown that optimal levels of dietary Se-Met can improve the protein deposition in whole fish and tissues (Wang et al., 2021b), while excess Se accumulation will lead to adverse effects in fish (Lemly, 2002; Li et al., 2021; Liu et al., 2021). In this study, the content of crude protein was increased in the whole fish by supplementation of Se-Met at 1.8 mg/kg and was also increased in the muscle by Se-Met at 0.9 to 3.5 mg/kg. These findings are consistent with the result that dietary Se-yeast (containing >80% of Se-Met) improves the protein content in the muscle of *O. mykiss*, implying that optimal Se-Met may promote protein deposition by stimulating postprandial protein synthesis (Wang et al., 2021b). It is believed that both Se forms (organic and inorganic) can serve as an active substrate for various selenoproteins in the cells, whereas only Se-Met can be incorporated into proteins via non-specific Se-sulfur substitution without restriction (Janz et al., 2010; Schrauzer, 2000). This could account for the consisted results observed between the highest protein deposition and maximum WG in *L. maculatus* with increasing dietary Se-Met. However, the content of crude protein was lower in *L. maculatus* fed the diet containing 7.0 mg Se-Met/kg for 10 weeks, indicating that excess Se accumulation is likely to induce protein degradation for additional energy expenditure to counteract the adverse effects of excess Se (De et al., 2014). In this study, the crude lipid content was decreased in whole fish but increased in the muscle of *L. maculatus* as the dietary Se-Met level increased. Similarly,

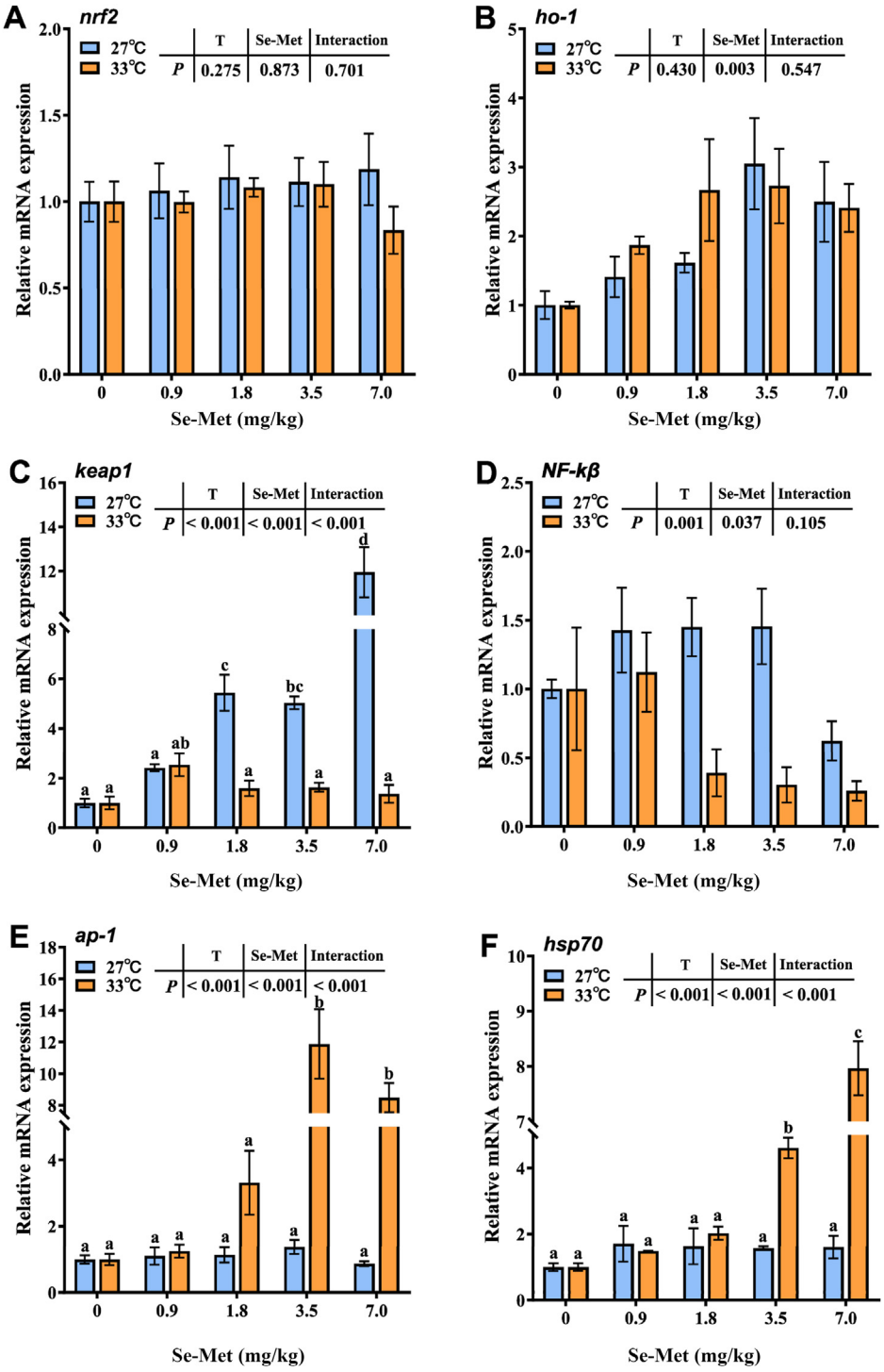

Fig. 4. Effect of dietary Se-Met level on transcripts of genes related to antioxidants (A-E) and heat shock protein 70 (F) in the liver of *L. maculatus* reared at two temperatures for 10 weeks. Values are mean ± SE ($n = 6$). Bars with different letters represent significant differences ($P < 0.05$). Se-Met = seleno-L-methionine; *nrf2* = nuclear factor erythroid-2 related factor; *ho-1* = heme oxygenase 1; *keap1* = Kelch-like ECH-associated protein 1; *NF-κβ* = nuclear factor kβ; *ap-1* = activator protein- 1; *hsp70* = heat shock protein 70; T = temperature.

decreased lipid content in whole fish was witnessed in white sturgeon (*Acipenser transmontanus*) (Tashjian et al., 2006) and juvenile green sturgeon (*A. medirostris*) fed a Se-Met enriched diet (De et al., 2014). It has been found that Se causes dysregulation of lipid metabolism in the intestine of yellow catfish *Pelteobagrus fulvidraco* (Zhang et al., 2021). However, lipid deposition in muscle induced by Se has not been reported in fish species; its specific causes remain to be explored. Furthermore, in the present study, the muscular proximate composition of *L. maculatus* was altered by elevated water temperature, suggesting that the redistribution of energy in fish was possibly further triggered by thermal stress under elevated water temperature (Zhao et al., 2022). The effects of Se-Met on the proximate composition of muscle remain inconclusive, and further research is warranted.

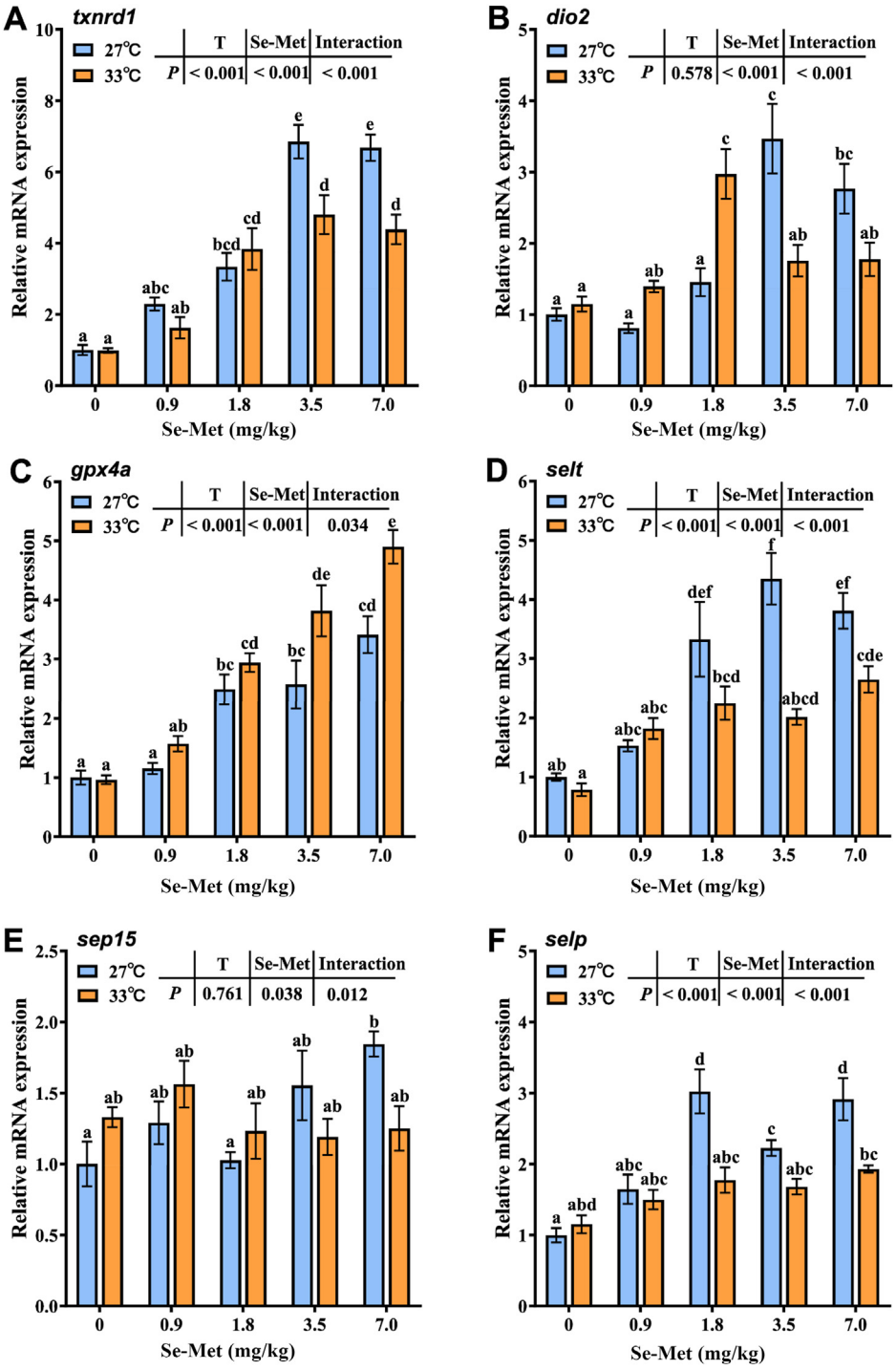

Fig. 5. Effect of dietary Se-Met levels on transcripts of genes related to selenoproteins in the muscle of *L. maculatus* reared at two temperatures for 10 weeks. Values are mean ± SE ($n = 6$). Bars with different letters represent significant differences ($P < 0.05$). Se-Met = seleno-L-methionine; *txnrd1* = thioredoxin reductase 1; *dio2* = iodothyronine deiodinase 2; *gpx4a* = glutathione peroxidase 4a; *selt* = selenoprotein T; *sep15* = 15-kDa selenoprotein; *selp* = selenoprotein P; T = temperature.

Myofiber properties are crucial in determining muscle quality (Listrat et al., 2016). It has been reported that the proximate composition (i.e., moisture, lipid and collagen protein) of muscle is positively correlated with myofiber morphology (Dunajski, 1980; Periago et al., 2005). In this study, dietary Se-Met supplementation of 0.9 mg Se-Met/kg increased the density of muscular fibers. However, deficient or excess Se-Met decreased the diameter and density of muscular fibers, leading to further muscle fiber disintegration. Muscle degradation was also visually evident in the muscular histology from the control and 7.0 mg Se-Met/kg diets. Similarly, the optimal level of Se-Met added to feed may stimulate intermuscular binding ability and maintain the integrity of muscle fibers in fish (Liu et al., 2017). Meanwhile, compared to the fish from the 27 °C treatment, fish from the 33 °C treatment exhibited muscle fibers with a larger diameter, lower density, and more noticeable disintegration. Extremely

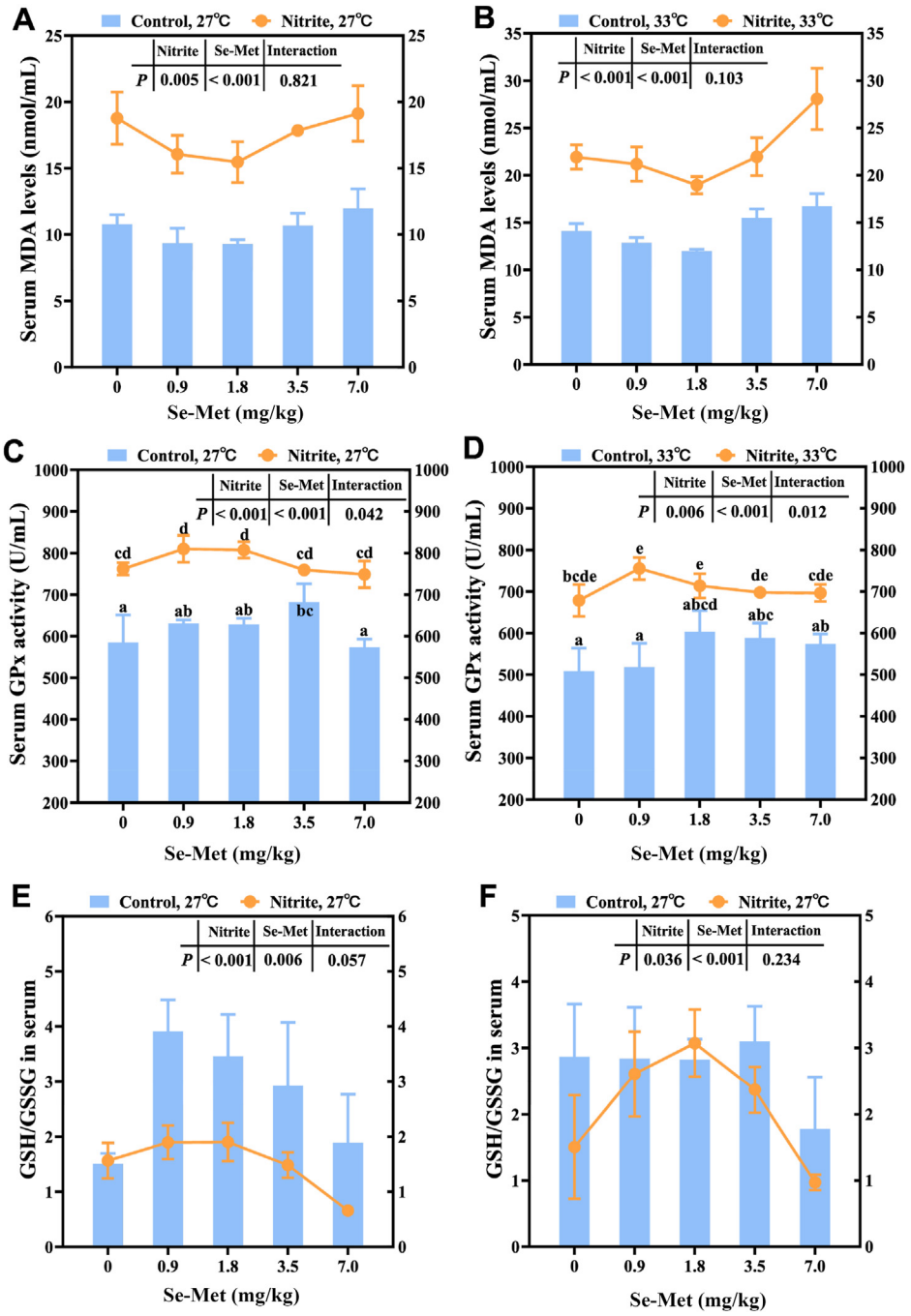

Fig. 6. Alteration of serum antioxidant physiology for *L. maculatus* fed diets with different Se-Met levels for 10 weeks after exposure to nitrite for 24 h (A, C and E) The serum MDA level, GPx activity, and the ratio of GSH/GSSG for *L. maculatus* reared at 27 °C ($n = 6$), and the same parameters (B, D and F) in the serum of *L. maculatus* reared at 33 °C. Bars with different letters represent significant differences ($P < 0.05$). The bars in blue color represent the results of nitrite stress at 0 h. The orange lines represent the results of nitrite stress at 24 h. Se-Met = seleno-L-methionine; MDA = malondialdehyde; GPx = glutathione peroxidase; GSH = glutathione; GSSG = oxidized glutathione.

high or low rearing water temperatures alter arrangement and density of muscle fibers in zebrafish (Johnston et al., 2009), which could prove that extreme temperature-stress alters the structure of muscle fibers in *L. maculatus*. Overall, thermal stress negatively impacts the muscle fibers of *L. maculatus*. However, the accumulation of appropriate Se-Met may help mitigate muscle disintegration caused by thermal stress, which may be attributed to the neutralization of excessive ROS by Se (Banh et al., 2016; Nakano et al., 2014). It is worth noting that excessive Se-Met may have the opposite effect.

In the intensive aquaculture industry, fish are often subjected to oxidative stress caused by various environmental factors (e.g., temperature) (Kim et al., 2016; Matthews and Berg, 1997). In general, oxidative stress occurs when there is an imbalance between the formation and removal of ROS in organisms (Torun et al., 2009). The accumulation of un-detoxified ROS can cause lipid peroxidative damage in the cell membrane (Su et al., 2019). Se is well known for its role in neutralizing ROS and maintaining redox balance in organisms (Weekley and Harris, 2013). In the present study, the MDA level in the serum and liver of *L. maculatus* decreased with dietary

Se up to 1.8 mg Se-Met/kg and increased with higher dietary Se-Met level and thermal stress. Similarly, there was a negative correlation between the alteration of antioxidant-related indicators (i.e., GPx, GSH, and GR) and MDA levels of *L. maculatus* fed with Se-Met enriched diets. This result, together with those of previous studies (Ashouri et al., 2015; Ghaniem et al., 2022; Wang et al., 2018), confirms that optimal dietary Se-Met helps to prevent lipid peroxidation and maintain normal oxidative/antioxidant physiological homeostasis in fish via the rapid clearance of excessive ROS (Zhu et al., 2012). However, as our results have shown, elevated levels of Se-Met produce ROS beyond the scavenging capacity of antioxidants present in the organism, which can subsequently cause severe oxidative damage and possibly secondary decreased growth (Li et al., 2021; Liu et al., 2021; Palace et al., 2004; Tashjian et al., 2006). In addition, adaptive changes in GPx activity were observed in the serum and liver of *L. maculatus* reared at 33 °C under thermal stress, which further confirms the necessity of optimal dietary supplementation of Se-Met for maintaining normal antioxidant capacity in *L. maculatus* cultured at high temperatures.

Nuclear factor erythroid-2 related factor (*nrf2*) is a crucial regulator of the antioxidant response in organisms, and Kelch-like ECH-associated protein 1 (*keap1*) has been proven to interact with *nrf2* (Ma, 2013; Wang et al., 2008). Nuclear factor kβ (*NF-κβ*) and heme oxygenase (*ho-1*) can be induced in response to cellular stress caused by ROS (Ferrándiz and Devesa, 2008). It has been shown that cell antioxidation is related to the upregulation of *ho-1*, *NF-κβ* and *ap-1* (Li et al., 2021; Zhao et al., 2021). In the present study, transcripts of *ho-1*, *keap1*, *NF-κβ* and *ap-1* in the liver were increased with dietary Se-Met (except for a decreased transcript of *NF-κβ* in the liver of fish fed with 7 mg Se-Met/kg diet). These findings could imply that different Se-Met levels induce the up-regulation of *Nrf2/NF-κβ*, leading to the transcriptional activation of downstream antioxidant genes, including *ho-1* and *ap-1* (Zhao et al., 2021). HSP70 is a commonly used biomarker for stress in fish, which can inhibit intracellular protein denaturation and misfolding in fish exposed to stressful conditions (Iwama et al., 1999). In this study, the transcripts of *hsp70* in the liver were up-regulated by dietary Se-Met supplementation of 3.5 to 7.0 mg Se-Met/kg under the thermal stress conditions. This is consistent with previous research that dietary nano-Se supplementation of 5 mg Se/kg induces the transcripts of *hsp70* and *hsp90* in the liver of *O. mykiss* under thermal stress (Li et al., 2022).

Selenoproteins perform a pleiotropic role in regulating physiological processes in fish (Tsuji et al., 2015). The beneficial effects of Se against oxidation are often attributed to the removal of various ROS by multiple families of selenoproteins (e.g., TXNRD, GPx, SEP15 and SelP) (Björnstedt et al., 1995; Weekley and Harris, 2013). GPx can catalyse the reduction of ROS to water or alcohol at the expense of consuming GSH (Weekley and Harris, 2013). In contrast, TXNRD plays a role in maintaining the intracellular redox status by utilizing NADPH to reduce thioredoxin (Arnér and Holmgren, 2000). SEP15 is a thiol-oxidoreductase-like selenoprotein involved in forming disulfide bonds to neutralize ROS (Tsuji et al., 2015). In the present study, transcriptional expression of *txnrd*, *gpx4a*, and *selp* in the muscle was enhanced with increased dietary Se-Met levels and an increased expression of *sep15* by 7.00 mg Se-Met/kg diet at 27 °C. DIO plays a crucial role in regulating thyroid hormones, growth, development, and immunity in fish (Saffari et al., 2018). In agreement with these findings, up-regulated transcripts of *gpx4b*, *txnrd*, *selp* have been observed in the muscle and liver of *O. mykiss* fed an optimal level of dietary Se-Met at 2 to 6 mg Se/kg diet (Wang et al., 2018). The present study also evidenced that optimal Se-Met up-regulated the transcripts of *dio2* in the muscle of *L. maculatus*. These results suggest that an optimal level of Se-Met in fish feed could promote Se accumulation in the muscle, which could stimulate the expression of antioxidant-related selenoproteins. Meanwhile, DIO may resist thermal stress by regulating the synthesis and metabolism of thyroid hormone, whereas disruption of *dio2* leads to a state of localized hypothyroidism (Drigo et al., 2013). In the present study, when fish were fed Se-Met enriched diets of 3.5 to 7.0 mg/kg, the transcripts of *dio2* in fish reared at 33 °C were significantly lower ($P < 0.05$) than those reared at 27 °C. This implies that thermal stress affects the production and metabolism of thyroxine in fish and interferes with the production of triiodothyronine (T3), which may also be one of the reasons for the decline in fish growth under thermal stress.

Furthermore, thermal stress inhibited the transcripts of these selenoproteins in muscle, which is consistent with the fact that high temperatures result in the disruption of enzymes and denaturation of proteins in fish (Dalvi et al., 2009). Previous research also suggests that the transcripts of *gpx1a* and *txnrd* in the liver of *O. mykiss* are induced by acute thermal stress but are inhibited by chronic heat stress (Li et al., 2022). These findings may indicate that increased expression levels of *gpx1a* and *txnrd* are helpful in reducing oxidative damage in organisms. However, the activity of these selenoproteins is insufficient to effectively counteract chronic oxidative stress, leading to concurrent transcriptional inhibition of selenoproteins (Li et al., 2022).

Nitrite (NO_2^-) is produced as an intermediate product during bacterial nitrification and denitrification processes (Jensen, 2003). It can be accumulated in intensive aquaculture systems. Once accumulated in excess, it has toxic effects on aquatic organisms (Wang et al., 2019). In the present study, compared with the control fish, increased MDA levels and GPx activity, as well as decreased GSH/GSSG, were observed in fish cultured at different temperatures after 24 h of nitrite exposure. Meanwhile, relative to fish reared at 27 °C, nitrite exposure in fish cultured at 33 °C exacerbated oxidative stress even further, resulting in higher MDA levels in various Se-Met feeding treatments. These findings suggest that exposure to nitrite leads to an increase in ROS levels in organisms, which is further amplified by thermal stress. However, the antioxidant defense system activated by optimal dietary Se-Met can partially mitigate the oxidative stress induced by those two environmental factors (Li et al., 2022), as evidenced by the increase in GPX activity and the GSH/GSSG ratio observed in the 0.9 to 1.8 mg/kg Se-Met treatments. Similar studies have reported that different aquatic animal species fed with organic Se-enriched diets decreased levels of MDA in tissues and effectively enhanced resistance to nitrite stress (Long et al., 2017; Wang et al., 2019; Wang et al., 2020).

5. Conclusion

In summary, this study demonstrates that thermal stress induced by elevated water temperature (33 °C) results in reduced growth and antioxidant capacity of tissues, alters the proximate composition and histological structure of muscle, inhibits the transcripts of muscle selenoproteins, and exacerbates oxidative damage caused by nitrite stress in *L. maculatus*. However, dietary supplementation of Se-Met enhances the growth, antioxidant capacity, transcripts of selenoproteins, improves the histology structure of muscle, and enhances the resistance to nitrite stress of *L. maculatus* fed on Se-Met enriched diets at a maximum of 3.5 mg Set-Met/kg for 10-weeks. Based on cubic regression analyses of weight gain, the optimal dietary supplementation of Se-Met for *L. maculatus* to achieve maximal growth is 2.39 and 4.46 mg Se-Met/kg reared at 27 and 33 °C, respectively. Meanwhile, based on the results of serum antioxidant capacity after nitrite stress, it can be concluded that dietary Se-Met supplementation of 0.9 to 1.8 mg Se-Met/kg can achieve the optimal antioxidant capacity of

L. maculatus at both temperatures. Overall, considering the effects of dietary Se-Met on the growth, antioxidant capacity and resistance to nitrite stress of *L. maculatus*, this study suggests that the range of Se-Met supplementation in diets of *L. maculatus* is 1.80 to 2.39 mg Se-Met/kg of the diet at 27 °C and 1.80 to 4.46 mg Se-Met/kg of the diet at 33 °C.

Credit author statement

Xiao Li: Investigation, Formal analysis, Data curation, Writing – review & editing. **Jing Li:** Resources, Investigation, Writing – review & editing. **Kangle Lu:** Resources, Investigation. **Xueshan Li:** Visualization, Resources, Investigation. **Kai Song:** Resources, Methodology. **Ling Wang:** Conceptualization, Methodology, Funding acquisition, Methodology, Supervision. **Chunxiao Zhang:** Investigation, Data curation, Software.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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