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ORIGINAL ARTICLE

Aquaculture

Effects of organic selenium-containing diets on *Penaeus vannamei* molecular response under induced heat stress challenge

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Abstract

Organic selenium is a major antioxidant that can protect against oxidative stresses by stimulating the antioxidant cascades. We have evaluated the capacity of dietary selenium-enriched yeast (Se-Y) to alleviate thermal stress in juvenile *Penaeus vannamei* shrimp. The Se-Y level in the control group (Cont) was 0 mg Se kg^{-1} ration and 0.4 mg Se kg^{-1} in the AlkoSel and SelPlex groups (commercial Se-Y products). The shrimp were initially provided the three experimental diets while being maintained in their thermal comfort zone (28 °C) for 30 days, following which time the temperature was increased up to 33 °C for 1 and 6 h of induced heat stress challenge (IHSC). Variations in the levels of malondialdehyde (MDA), glutathione, and several transcriptional factors encoding antioxidant variables and stress-responsive proteins were analyzed in the gill, muscle, and hepatopancreas. The results revealed that the MDA and glutathione content of the shrimp were significantly impacted by Se-Y supplementation both before and after the IHSC. Prior to the IHSC, in all evaluated tissues, Se-Y supplementation enhanced the antioxidant variables (glutathione peroxidase, glutathione-S-transferase, and superoxide dismutase) and heat shock protein (HSP) biosensors HSP70 and HSP90 while depressing the immune stress-related indices (gamma-interferon inducible lysosomal thiol reductase-like and aspartate aminotransferase cytoplasmic-like (AST). After IHSC, in all evaluated tissues, the AST gene was significantly downregulated in all Se-Y-fed groups while the other genes were upregulated. These results imply an important role of organic selenium in preventing organ dysfunctions after exposure to IHSC. Taken together, these findings on gene regulation after Se-Y pretreatment will be useful for monitoring organ dysfunction and preparing therapeutic agents for thermal endurance in *P. vannamei*.

Keywords Selenium-enriched yeast · Thermal stress · Antioxidant variables · Heat-responsive genes

Introduction

Whiteleg shrimp *Penaeus vannamei*, the primary marine shrimp species cultivated worldwide, has favorable inherent biological features for commercial farming at the global level (FAO 2022) even though throughout its lifespan and/or the farming processes, this shrimp is exposed to a variety of conditions in its tidal environment (Wang et al. 2012). Environmental stresses have significant adverse effects on the health, welfare, and overall production of cultured species, and these stresses have been aggravated by recent climate changes (Reid et al. 2019). For marine ectotherms like shrimps, water temperature fluctuation is a typical abiotic stressor, and in cultured shrimp, temperature changes impact almost all biological, physiological, and genetic activities (Ren et al. 2021). Thermal stress can seriously harm many important organs of shrimp and is a leading cause of death. Thus, thermal stress has recently emerged as the greatest challenge to commercial *P. vannamei* culture.

Temperature fluctuations promote higher metabolic rates, which in turn raise oxygen consumption and ultimately produce reactive oxygen species (ROS) (An and Choi 2010; Lushchak and Bagnyukova 2006). The resulting ROS lead to

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the generation of a number of metabolites including, malondialdehyde (MDA), that can negatively impact cell lipids, proteins, and DNA (Halliwell and Gutteridge 1999), thereby enhancing cellular oxidative damage and resulting in oxidative-antioxidative system imbalance. In addition, thermal stress may change the expression levels of related heat shock proteins (HSPs) (Roberts et al. 2010), which are crucial protein chaperones involved in cellular activity in the organism. Aquatic organisms can preserve their normal development by enhancing the expression levels of HSPs in response to thermal stressors under natural circumstances (Cheng et al. 2015), thereby stimulating several protective and restoration mechanisms as well as antioxidant functions throughout the body that eliminate ROS. Glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and other enzymes (Ghaffarizadeh et al. 2022; Zhou et al. 2014) are all part of the crucial oxidative-antioxidative mechanism. Hence, increasing global temperature can result in irreversible changes that increase the urgency of fisheries and aquaculture to introduce adaptations to climatic changes by implementing immediate aquaculture-based actions to improve and expedite climate mitigation and adaptation strategies (Cheung et al. 2009; Pörtner and Knust 2007).

One appropriate tactic is to provide feed supplements which provide multifunctional health benefits. Selenium (Se) is a crucial micronutrient that contributes significantly to the profitability of the aquaculture industry and is frequently employed in many forms as a nutritional antioxidant. Organic Se (OSe) exhibits high biological activity and has high absorption and low toxicity rates (Wang et al. 2021; Yu et al. 2022), making it the ideal Se supplement and free radical scavenger; consequently, it has received a lot of attention from researchers studying feed supplements in shrimps (Kemal et al. 2023; Yu et al. 2021, 2022). One main source of OSe is Se-enriched yeast (Se-Y), which mostly contains selenomethionine (a naturally occurring chemical form of OSe) along with smaller amounts of other Se-containing items (Schrauzer 2006). Se-Y as a feed supplement may be safer and more feasible for *P. vannamei* compared to inorganic Se supplements. AlkoSel® and SelPlex®, both precursors of OSe, are commonly referred to as Se-Y products since they involve selenoproteins, with higher bioavailability and antioxidative properties. Those products are essentially dried baker's yeast that are produced by growing *Saccharomyces cerevisiae* strains on a Se-supplemented fermentation substrate, to obtain high selenomethionine contents (Bampidis et al. 2019; Burdock and Cousins 2010). The proteins produced by this process will include selenomethionine instead of methionine, which will be converted to selenocysteine during metabolism (Schrauzer 2006).

Se plays an important role in many physiological processes, including cellular membrane integrity, immune system improvement, and antioxidative defense functions. It is well-known that OSe can provide its antioxidant characteristics by participating in the bioproduction of the GPx enzyme, a major selenoprotein enzyme that facilitates the scavenging of harmful ROS (Yu et al. 2021, 2022). Additionally, OSe can protect against such oxidative stresses by stimulating the cell's enzymatic and non-enzymatic antioxidant systems, which directly scavenge the harmful ROS and other compounds associated with ROS generation (Yu et al. 2021, 2022). The majority of earlier investigations have clarified the role of OSe in the physiological and biochemical components of stress in crustaceans (Wang et al. 2021, 2019; Yu et al. 2023), but little is understood about the role of OSe in the thermal stress response of *P. vannamei*. In the study reported here, we tested the hypothesis that dietary supplementation with organic Se-Y can improve the antioxidant properties and enhance the expression levels of HSPs and some immune-related gene expression in *P. vannamei* tissues, including the gill, muscle, and hepatopancreas. These tissues were chosen for examination due to their physiological importance and their role in the balance of ROS and redox homeostasis (Logan and Buckley 2015; Trasviña-Arenas et al. 2013). To this end, under conditions of Se-Y supplementation and non-supplementation, we evaluated the ongoing shifts in the relative expression levels of the applicable genes in all of the evaluated organs, at various durations of heat stress following culture in the shrimp's thermal comfort zone.

Materials and methods

Ethical disclosure

Japanese legislation on the Use and Care of Experimental Animals was followed during this research utilizing *P. vannamei*. The Ethics Committee of Tokyo University of Marine Science and Technology (TUMSAT) gave consent to the study protocols.

Shrimp feeding trial and induced heat stress challenge

A total of 180 juvenile whiteleg shrimp (*P. vannamei*) were obtained from a private hatchery in Okinawa prefecture, each shrimp weighing 1.1 ± 0.08 g, and randomly allotted into six indoor rectangular fiberglass aquaria filled with 60 L of artificial saltwater, with 30 shrimp aquarium^{-1} (Briggs et al. 2004), in two replicates. These aquaria were equipped with a complete water recirculation system, with temperature control (28 °C), constant aeration (> 5.5 ppm), and pumped filtered artificial saltwater (salinity, 32 ± 1‰) during the entire experimental period. Prior to the feeding trial, shrimps were acclimatized at 28 °C for 7 days. A commercial *P. vannamei*

feed, GF03S (Krungthai Food Co., Ltd, Bangkok, Thailand) was provided ad libitum twice daily. In addition, supplementation of feed with 0.2 g kg^{-1} of AlkoSel 2000® (Lallemand Animal Nutrition Inc., Tokyo, Japan) and SelPlex 2000® (Alltech Inc. Tokyo, Japan) was shown to be the appropriate supplemental dose of Se-Y (Nugroho and Fotedar 2013). This amount was adequate to provide a ration of 0.4 mg Se kg^{-1} in the final feed. Following acclimatization, shrimp were fed either a control diet (Cont group) or control diet + 0.2 g kg^{-1} AlkoSel 2000® (Alko-fed group) or control diet + 0.2 g kg^{-1} SelPlex 2000® (Sel-fed group) for 30-days.

The room where the induced heat stress challenge (IHSC) experiments were performed contained twelve 15 L-fiberglass aquaria individually fitted with an underwater filter with aeration properties (Suisaku Eight S; Suisaku Co., Ltd., Tokyo, Japan) and submersed water-heaters (Tetra IC Thermo Heater 200W with cover; Spectrum Brands Japan, Tokyo, Japan). During the IHSC, the submersed digital aquarium heaters were used to maintain and stabilize the temperatures of the water in the aquaria at the proposed temperature, namely 33 °C. Following the feeding phase, the temperature was modified to assessed the tolerance of the juvenile *P. vannamei* shrimp to heat shock stress after the Se-Y treatment. Prior to the IHSC, shrimp juveniles were fasted for 12 h; then 40 shrimp per each dietary treatment (20 shrimp for each temperature point duration) were randomly netted from each of the feeding aquaria and transferred into the separate challenge aquaria system. Upon transfer of the shrimp, all aquaria were immediately subjected to a controlled IHSC by gradually increasing water temperature from 28 °C to 33 °C (1 °C every 1 h), following (with minor modifications) the protocol of Wu et al. (2008). The shrimp were kept under these conditions for either 1 h (T_1; a total of 6 h from the start of the temperature rise) or 6 h (T_2; a total of 11 h from the start of the temperature rise) during which time the shrimp were deprived of feed (Fig. 1a, and b). During this time, the shrimp were observed for signs of thermal stress, such as changes in normal coloration, disoriented swimming, wide and rapid ventilation, and flaring of gills.

Tissue sample collection

To assess the responses of the juvenile *P. vannamei* shrimp fed the two Se-Y diets to the IHSC, we randomly chose and dissected six shrimp from each dietary treatment before the stress challenge; after the IHSC, we also randomly chose and dissected six shrimp from each temperature point duration (T_1 and T_2). Tissue samples were obtained from the gill, muscle, and hepatopancreas; 50 mg of each tissue sample was rapidly suspended in 1000 µL RNAlater (Invitrogen, Thermo Fisher Scientific Japan, Tokyo, Japan) and left overnight at 4 °C for the qPCR gene expression analysis. The remaining tissues were utilized for biochemical analysis. Each sample was cooled instantly in dry ice and stored at − 80 °C until examination.

Measurement of MDA and glutathione concentrations

Concentrations of MDA in the shrimp tissues were measured colorimetrically in triplicate for each sample at 532 nm (Multiskan™ FC with Skan It™ software; Thermo Fisher Scientific Japan, Tokyo, Japan) using the commercial MDA Assay Kit (M496; Dojindo Molecular Technologies, Inc., Tokyo, Japan) in accordance with the manufacturer's instructions. Oxidized disulfide glutathione (GSSG) and reduced glutathione (GSH) in the tissue samples were also measured in triplicate with a Glutathione Assay Kit (G257 GSSG/GSH Quantification Kit; Dojindo Molecular Technologies, Inc.), following the manufacturer's recommendations.

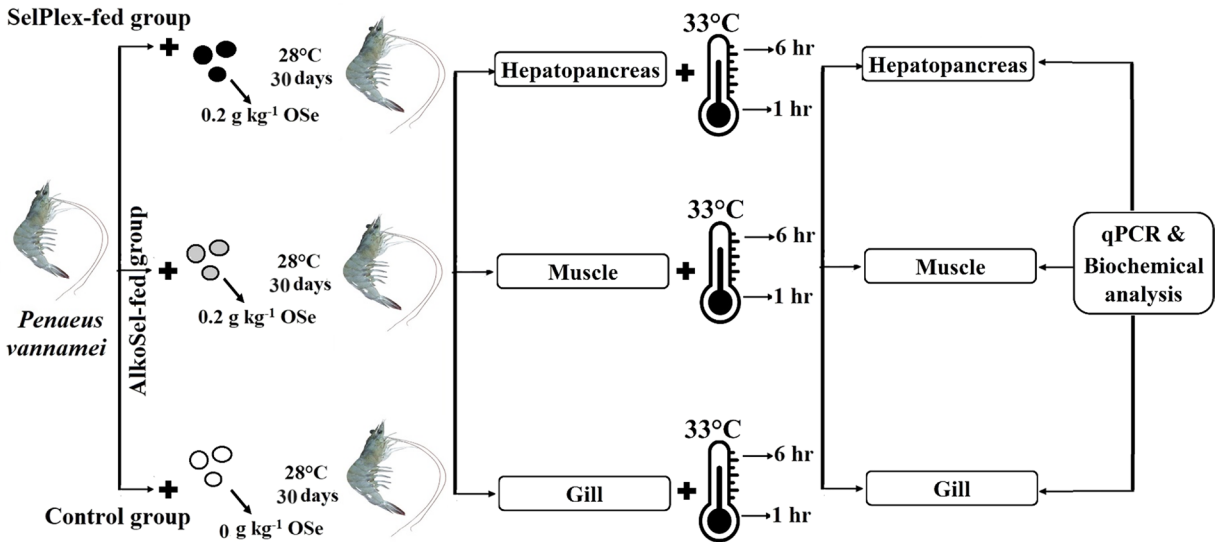
Isolation of total RNA

Following the manufacturer's instructions, total RNA was isolated from the tissue samples of the gill, muscle, and hepatopancreas using 500 µL RNAiso Plus (Takara Bio Inc., Kusatsu, Shiga, Japan). RNA integrity was ascertained by electrophoresis in a 1.5% RNAse-free agarose gel. The purity and quantity of the isolated total RNA was also assessed using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit® 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

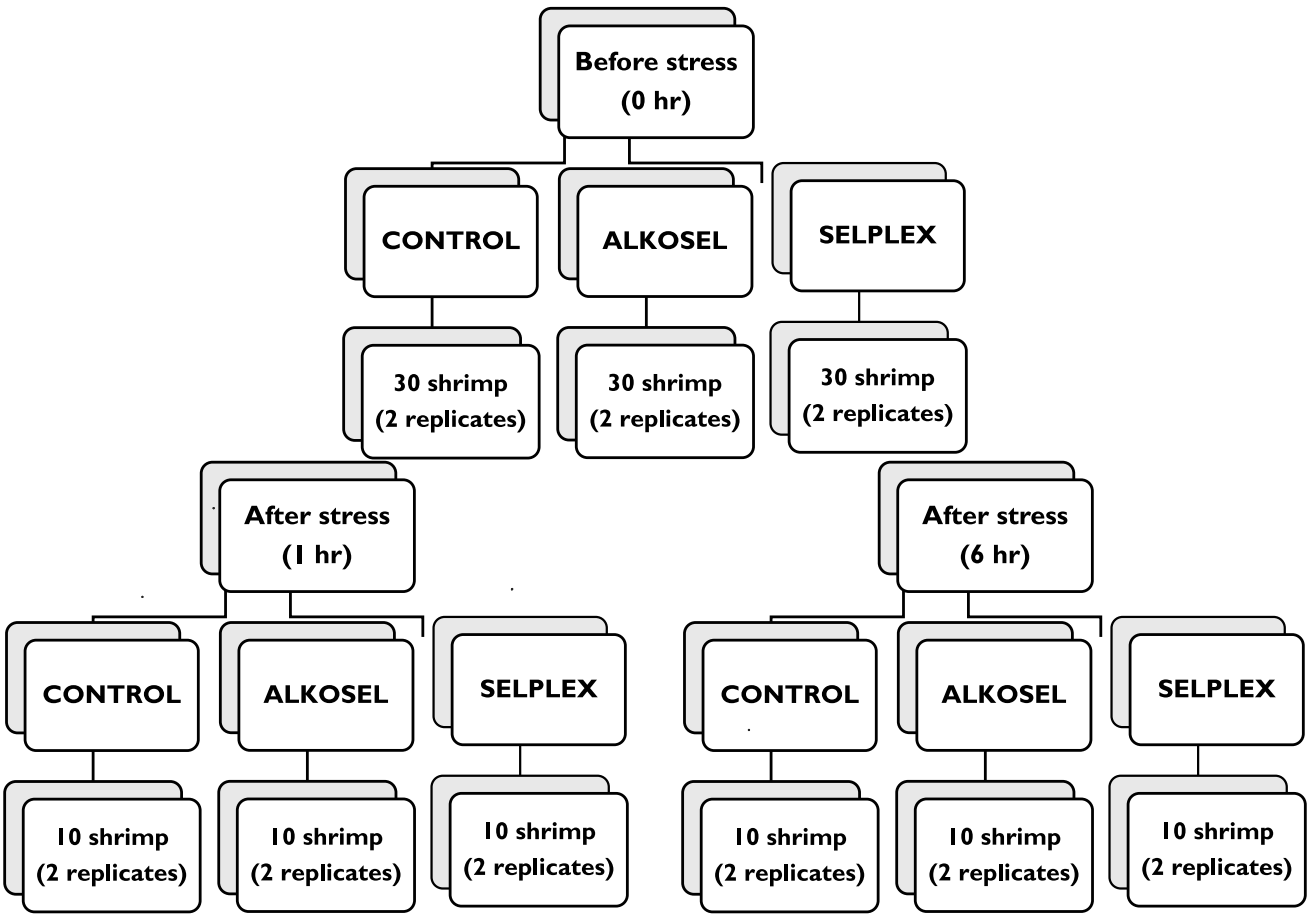
Quantitative real-time PCR gene expression analysis

High-quality isolated total RNA (1 µg) was reverse transcribed with a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Thermo Fisher Scientific Japan, Tokyo, Japan) following the manufacturer's recommendations. For complementary DNA (cDNA) transcription, the thermal cycling regimen was 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min, with holding at 4 °C. The resulting cDNAs were then diluted 1:4 with autoclaved distilled water and stored at − 30 °C until used for the quantitative real-time PCR (qPCR) steps. The expression patterns of certain HSPs, genes involved in the response to oxidative stress, and immune-related genes in all evaluated tissues were assessed using transcript-specific primers (Table 1). Since Se-Y supplements had no impact on the gene expression of *P. vannamei* elongation factor 1α (EF1α), this factor was employed as an internal control.

qPCR amplifications were conducted in a total volume of 20 µL cDNA solution, including 5 µL cDNA, with tissue



(a). General outline and an overview of our current work.



(b). Schematic design of the feeding trial and IHSC of *Penaeus vannamei* shrimp fed Se-Y-containing diets.

Fig. 1 **a** General outline and an overview of the current study. **b** Schematic design of the feeding trial and IHSC of *Penaeus vannamei* shrimp fed diets containing Se-Y. IHSC, Induced heat stress challenge; OSe, organic selenium; qPCR, quantitative real-time PCR; Se-Y, organic Se-enriched yeast

Table 1 Primer sequences for evaluated genes

No.	Primer name	Sequence (5′–3′)	References
1	Pv_EF1α	F: CCAGGGTGAAGCACAGCAAC R: CGACAAGCGAACCATCGAGA	NCBI
2	HSP70	F: CTCCTGCGTGGGTGTGTT R: GCGGCGTCACCAATCAGA	(Qian et al. 2012)
3	HSP90	F: TGGGCTTCTACTCCGCCTACC R: ACGGTGAAAGAGCCTCCAGCA	(Qian et al. 2012)
4	GPx	F: CGTGCAAAAAGGACCTTGGG R: ATACGCGATGCCCCTAACAC	(Abdel-Tawwab et al. 2022)
5	GST	F: GCAGGATGATGCCAGCAAGC R: TCAGGTTGAGTTCGACGCCC	NCBI
6	SOD	F: ATGGTACGGTTGGCGGTCTG R: GGTGGAAGTCCAAGGGGCTG	NCBI
7	GILT	F: AAAGCGCCGCTACACCTACT R: AACTCGTAGTCGCCCGTTCC	NCBI
8	AST	F: CCTTTAGCACTGCTGCTGCC R: AATGCGGAGTCCTCCAGTGC	NCBI

F Forward, *NCBI* National Center for Biotechnology Information, *R* reverse

samples (gill, muscle, and hepatopancreas) from six shrimp before and after the IHSC, respectively, using the THUNDERBIRD™ Next SYBR® qPCR Mix (Toyobo Co., Ltd., Osaka, Japan) in the Real-time QuantStudio 1 Real-Time PCR System (Applied Biosystems, Japan). The qPCR cycling regimen included a preliminary denaturation at 95 °C for 60 s, followed by 40 cycles of 95 °C for 15 s and 52 °C for 15 s, with a final extension at 72 °C for 30 s and a dissociation analysis step. The $2^{-\Delta\Delta Ct}$ comparative technique (Livak and Schmittgen 2001) was used to determine the relative expression patterns of messenger RNA (mRNA) for each selected gene.

Statistical analysis

Normality and homogeneity of variance tests were conducted before the statistical analysis. A two-way analysis of variance (ANOVA) was employed to assess the impacts of Se-Y supplementation, thermal stress exposure, and their interactions. The interaction between factors Se-Y treatments × thermal stress duration was tested by a 3 × 3 factorial design, with three Se-Y treatments (Cont-, Alko-, and Sel-fed groups) and three temperature point durations (0, 1, and 6 h). Then, to assess statistically significant variances between treatments, Tukey's multiple comparison test (TSHD) was applied with significance set at $P < 0.05$. For some trials, *t*-tests were used to find differences within the Se-Y treatment or thermal stress exposure groups when the ANOVA findings were not significant. The data were recorded as mean ± standard error of the mean (SEM) using R package scripts® Foundation for Statistical Computing, Vienna, Austria).

Results

Effects of OSe on MDA and glutathione contents of *P. vannamei* shrimp subjected to IHSC

The MDA content in all tested organs before and after 1 and 6 h of IHSC showed a similar pattern due to the effect of thermal exposure, Se-Y treatment, and their interaction, with the two Se-Y-supplemented groups showing significantly ($P < 0.01$) lower MDA levels compared to their respective Cont groups (see Table 2). Additionally, in terms of the extent of the IHSC on the examined tissues, the hepatopancreas was more affected than the gill and muscle. The MDA concentration was higher in the hepatopancreas than in the gill and muscle irrespective of Se-Y supplementation or not, or the impact of the IHSC.

Reduced and oxidized forms of glutathione were detected in all three organs (Table 3). With the addition of Se-Y to shrimp diets, the contents of GSH and GSSG varied between all tested organs under both normal and heat stress conditions. When shrimp were maintained in their thermal comfort zone, the GSH content was higher in the gill than in the muscle and hepatopancreas, while GSSG content was highest in the hepatopancreas. The GSH content in the gill and muscle significantly increased in the Alko- and Sel-fed groups compared to the Cont group; however, this increase was not statistically significant across groups for the hepatopancreas. As a result of the interaction between Se-Y supplementation and temperature treatments, the GSH/GSSG ratio was not significantly affected in the gill ($P > 0.05$), but it was significantly enhanced in the two other organs of the two Se-Y groups compared to the Cont group ($P < 0.01$). Remarkably, GSH and GSSG content varied dramatically in response to the interactions between thermal stress exposure

Table 2 Malondialdehyde content in the gill, muscle, and hepatopancreas of *Penaeus vannamei* shrimp fed Se-Y-containing diets under the induced heat stress challenge regimen

Organ tested	MDA concentration (μmol/L)								
	0 h of thermal stress			1 h of thermal stress			6 h of thermal stress		
	Control group ($n=3$ samples)	AlkoSel group ($n=3$ samples)	SelPlex group ($n=3$ samples)	Control ($n=3$ samples)	AlkoSel group ($n=3$ samples)	SelPlex group ($n=3$ samples)	Control group ($n=3$ samples)	AlkoSel group ($n=3$ samples)	SelPlex group ($n=3$ samples)
Gill	1.860 ± 0.29^{c}	0.732 ± 0.23^{d}	0.796 ± 0.21^{d}	$3.297 \pm 4.62e\text{-}03^{b}$	2.184 ± 0.19^{c}	2.269 ± 0.12^{c}	6.124 ± 0.03^{a}	3.537 ± 0.14^{b}	3.660 ± 0.05^{b}
Muscle	1.115 ± 0.28^{def}	0.500 ± 0.22^{f}	0.628 ± 0.04^{ef}	2.536 ± 0.01^{b}	1.552 ± 0.30^{cde}	1.632 ± 0.25^{bcd}	4.155 ± 0.15^{a}	2.346 ± 0.16^{bc}	2.412 ± 0.10^{bc}
Hepatopancreas	10.031 ± 0.23^{e}	6.492 ± 0.23^{f}	6.679 ± 0.21^{f}	21.682 ± 0.16^{b}	12.832 ± 0.04^{d}	13.271 ± 0.10^{d}	28.906 ± 0.03^{a}	16.858 ± 0.10^{c}	17.089 ± 0.27^{c}

Values are presented as the mean ± SEM. Values followed by different lowercase letters indicate significant differences ($P < 0.05$) among groups in response to the interactions between thermal stress exposure and Se-Y supplementation

MDA Malondialdehyde, *SEM* standard error of the mean, *Se-Y* organic Se-enriched yeast

and Se-Y supplementation. When compared to the Cont group, the relative GSH content in the Alko- and Sel-fed groups increased considerably at 1 and 6 h of heat stress. Furthermore, compared to their respective groups prior to stress, all tissues in the IHSC groups showed significantly higher GSSG levels at 1 and 6 h of heat stress, with significantly lower levels in the Se-Y-supplemented groups compared to the Cont group. In addition, while it was still significantly higher than in the non-stressed groups, the GSSG concentration in all groups and tissues at 1 h of thermal stress was marginally lower than that at 6 h of stress (Table 3).

OSe effects on the relative expression levels of HSP genes in *P. vannamei* shrimp subjected to IHSC

Figure 2 shows the effects of Se-Y on the relative expression levels of HSP70 in *P. vannamei* shrimp under thermal stress. Before the application of thermal stress (0 h), the gills of shrimp in the two treatment groups showed no significant effects of Se-Y supplementation on the relative expression levels of HSP70 compared with those of the Cont group (Fig. 2a), while the effects of supplementation were significant in the other organs (Fig. 2b, c). Compared with the expression levels before thermal stress, the relative expression levels of HSP70 were significantly upregulated in all groups at 1 and 6 h following the application of thermal stress in all evaluated organs. Furthermore, the expression levels of HSP70 mRNA in shrimp fed the Alko- and Sel-supplemented diets were significantly enhanced compared to those of the Cont group at 1 and 6 h of thermal stress in all the evaluated organs except the gill.

The expression levels of HSP90 mRNA in all groups also appeared to be upregulated after the application of thermal stress compared to before stress (Fig. 3). In the gill and muscle, HSP90 mRNA expression levels in shrimp fed diets supplemented with Alko and Sel, respectively, were higher than those of shrimp in the Cont group at 0 and 1 h, and the expression levels at 6 h were significantly higher than those of the other groups (Fig. 3a, b). However, for the hepatopancreas, there was no significant interaction in the relative expression levels of HSP90 mRNA between any of the Se-Y-fed groups or the Cont group either before or after stress (Fig. 3c).

Effects of OSe on the relative expression levels of antioxidant enzyme genes in *P. vannamei* shrimp subjected to IHSC

As shown in Fig. 4, the expression levels of GPx transcripts in all groups had a tendency to increase under the IHSC condition. Before stress, the expression levels of GPx transcripts in the gill and muscle of shrimp fed diets supplemented with Alko and Sel, respectively, showed a non-significant increase compared to those of the Cont group (Fig. 4a, b); in contrast, in the hepatopancreas, these expression levels were significantly higher than those in the Cont group (Fig. 4c). After 1 and 6 h of thermal stress, in all evaluated tissues (except muscle at 1 h), the expression of GPx transcripts in shrimp fed diets supplemented with Alko and Sel were significantly upregulated compared with those in the Cont group (Fig. 4).

The effects of Se-Y on the relative expression levels of GST and SOD in *P. vannamei* are shown in Figs. 5 and 6, respectively. The expression levels of GST and SOD mRNA in all groups followed the same pattern, with the tendency to be upregulated under the IHSC condition. Before stress, there were highly significant differences in the GST and SOD mRNA expression levels in all evaluated tissues among all groups. Following application of the IHSC, the GST and SOD mRNA expression levels (Figs. 5, 6, respectively) increased significantly in all tissues evaluated in shrimp fed the Alko- and Sel-supplemented diets, respectively, with significantly higher levels at 1 and 6 h in all evaluated tissues (except for gill GST at 1 h) in comparison to the Cont group;

Table 3 Glutathione content in the gill, muscle, and hepatopancreas of *Penaeus vannamei* shrimp fed the Se-Y-containing diets under the induced heat stress challenge regimen

Organ tested	0 h of thermal stress			1 h of thermal stress			6 h of thermal stress		
	Control ($n=3$)	AlkoSel ($n=3$)	SelPlex ($n=3$)	Control ($n=3$)	AlkoSel ($n=3$)	SelPlex ($n=3$)	Control ($n=3$)	AlkoSel ($n=3$)	SelPlex ($n=3$)
Gill									
GSH (μmol/L)	4.372 ± 1.08^{d}	7.114 ± 0.12^{c}	6.580 ± 0.09^{c}	7.302 ± 0.11^{c}	9.907 ± 0.10^{b}	9.622 ± 0.10^{b}	8.062 ± 0.11^{bc}	12.442 ± 0.14^{a}	12.485 ± 0.13^{a}
GSSG (μmol/L)	$0.866 \pm 8.30\text{e-}03^{f}$	0.552 ± 0.04^{g}	$0.622 \pm 7.33\text{e-}03^{g}$	2.692 ± 0.02^{b}	$1.197 \pm 7.33\text{e-}03^{e}$	1.285 ± 0.03^{e}	$6.105 \pm 5.69\text{e-}03^{a}$	$2.101 \pm 1.41\text{e-}03^{d}$	$2.208 \pm 8.02\text{e-}03^{c}$
GSH/GSSG	5.070 ± 1.28^{A}	$13.055 \pm 1.09^{*A}$	$10.581 \pm 0.09^{*A}$	2.713 ± 0.05^{B}	$8.276 \pm 0.05^{*B}$	$7.495 \pm 0.14^{*B}$	1.321 ± 0.02^{C}	$5.921 \pm 0.06^{*C}$	$5.655 \pm 0.06^{*C}$
Muscle									
GSH (μmol/L)	1.998 ± 0.17^{e}	$3.524 \pm 4.25\text{e-}03^{cd}$	3.543 ± 0.07^{c}	2.976 ± 0.07^{d}	6.531 ± 0.20^{b}	6.267 ± 0.10^{b}	3.400 ± 0.0^{cd}	9.172 ± 0.02^{a}	9.052 ± 0.12^{a}
GSSG (μmol/L)	1.020 ± 0.01^{e}	0.237 ± 0.05^{f}	0.300 ± 0.02^{f}	3.134 ± 0.02^{b}	1.662 ± 0.04^{d}	1.765 ± 0.02^{d}	4.862 ± 0.07^{a}	2.138 ± 0.04^{c}	2.228 ± 0.08^{c}
GSH/GSSG	1.957 ± 0.15^{c}	16.896 ± 4.65^{a}	11.910 ± 0.74^{ab}	0.949 ± 0.02^{c}	3.934 ± 0.16^{c}	3.550 ± 0.02^{c}	0.700 ± 0.02^{c}	4.294 ± 0.09^{bc}	4.072 ± 0.14^{c}
Hepatopancreas									
GSH (μmol/L)	1.891 ± 0.12^{c}	2.528 ± 0.12^{c}	2.471 ± 0.10^{c}	2.286 ± 0.09^{c}	5.291 ± 0.77^{b}	4.987 ± 0.02^{b}	2.374 ± 0.05^{c}	7.934 ± 0.16^{a}	7.790 ± 0.09^{a}
GSSG (μmol/L)	1.325 ± 0.02^{e}	0.538 ± 0.03^{f}	0.609 ± 0.05^{f}	4.566 ± 0.06^{b}	2.755 ± 0.08^{d}	2.821 ± 0.31^{d}	$5.786 \pm 9.55\text{e-}03^{a}$	$3.407 \pm 4.28\text{e-}03^{c}$	3.588 ± 0.06^{c}
GSH/GSSG	1.426 ± 0.08^{b}	4.726 ± 0.28^{a}	4.114 ± 0.37^{a}	0.501 ± 0.03^{c}	1.909 ± 0.23^{b}	1.807 ± 0.18^{b}	$0.410 \pm 9.67\text{e-}03^{c}$	2.329 ± 0.05^{b}	2.172 ± 0.04^{b}

Values are presented as the mean ± SEM. Values followed by different lowercase letters indicate significant differences ($P < 0.05$) among groups in response to the interactions between thermal stress exposure and Se-Y supplementation. Values followed by asterisks (*) are significantly different among the Se-Y treatment groups. Values followed by different uppercase letters are significantly different ($P < 0.05$) among thermal stress groups (*t*-test)

GSH Reduced glutathione, *GSSG* oxidized disulfide glutathione, *SEM* standard error of the mean, *Se-Y* organic Se-enriched yeast

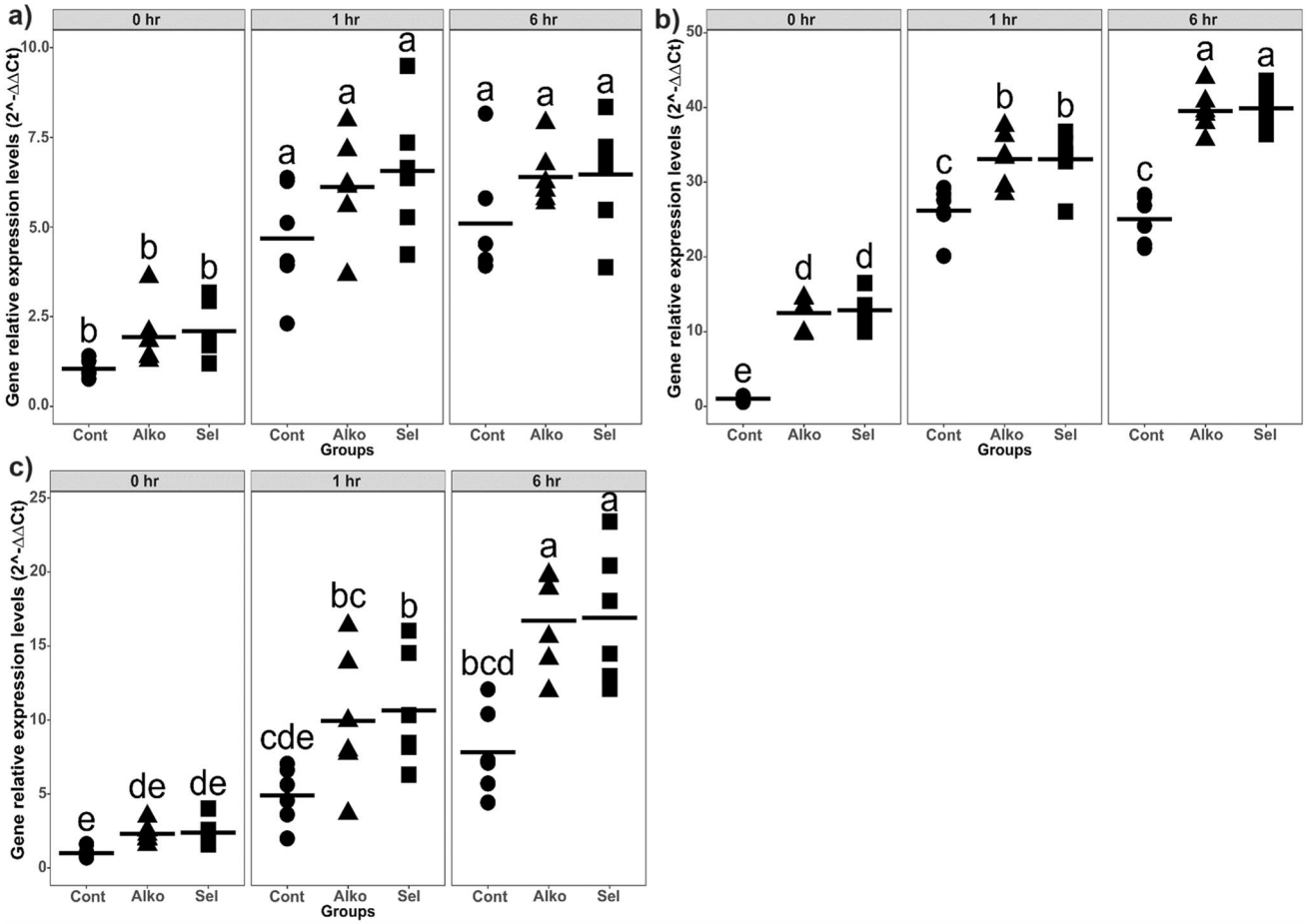


Fig. 2 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of HSP70 in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, mean ± SEM is shown ($n = 6$). Different lower-case letters indicate significant differences at $P < 0.05$ among groups. Cont, Control group; HSP, heat shock protein, SEM, standard error of the mean

in addition the expression levels of GST and SOD mRNA were significantly more upregulated at 6 h than at 1 h.

Effects of OSe on the relative expression levels of gamma-interferon inducible lysosomal thiol reductase-like in *P. vannamei* shrimp subjected to IHSC

Before application of the IHSC, the expression levels of gamma-interferon inducible lysosomal thiol reductase-like (GILT) mRNA expression showed a downregulation pattern in all tested tissues of shrimp in the two Se-Y-supplemented groups compared to those in the Cont group (Fig. 7), although the differences were non-significant. However, in all tested tissues, thermal stress exposure and Se-Y addition, as well as their interaction, had an effect on the GILT relative expression levels under the stress conditions, especially after 6 h (Fig. 7). Surprisingly, the relative expression levels of GILT were significantly upregulated in shrimp on the Se-Y-containing diets and under increasing stress time duration compared with Cont groups under the same conditions. Furthermore, heat stress increased GILT expression in the Se-Y group, whereas no significant changes were observed in the control group in all the evaluated tissues.

Effects of OSe on the relative expression levels of aspartate aminotransferase cytoplasmic-like in *P. vannamei* shrimp subjected to IHSC

The relative expression levels of aspartate aminotransferase cytoplasmic-like (AST) in the Alko- and Sel-fed groups showed a high downregulation pattern compared with the Cont group in all evaluated tissues before thermal stress (0 h), although the differences were non-significant (Fig. 8). Under the IHSC condition, with increasing duration of stress time (1 and 6 h), the AST relative expression levels were significantly increased in all groups compared with the Cont

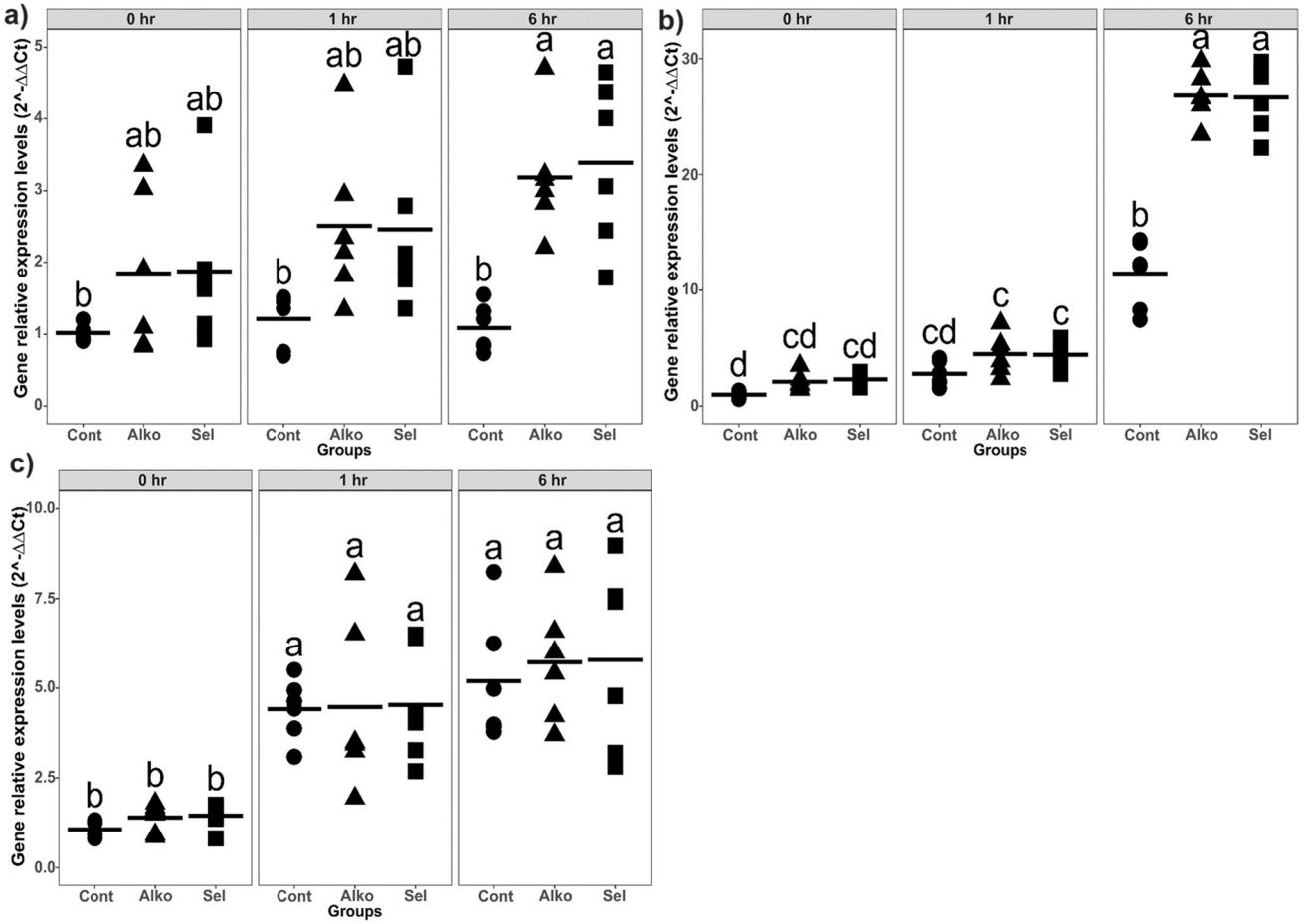


Fig. 3 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of HSP90 in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, the mean ± SEM is shown ($n = 6$). Different lowercase letters indicate significant differences at $P < 0.05$ among groups. Cont, Control group; HSP, heat shock protein, SEM, standard error of the mean

group before thermal stress (0 h). Moreover, AST mRNA expression levels of the Cont group after 1 and 6 h of IHSC were significantly upregulated compared to those of the *P. vannamei* shrimp fed the Se-Y-containing diets at the same time point of stress duration.

Discussion

The conundrum that *P. vannamei* shrimp are hampered by a variety of stressors in their environment, particularly temperature changes, makes it of outmost importance to find efficient strategies to lessen thermal stress in shrimp. Employing supplemental dietary feed additives to mitigate heatwave damage in aquatic organisms has become a growing area of interest for many researchers nowadays (Duan et al. 2017; Li et al. 2022; Naiel et al. 2021; Rezende et al. 2022; Rocha et al. 2021). It is commonly accepted that any external stress activates the antioxidant system, which then counteracts the generated free radicals. Thermal stresses, however, could provoke an increase in the generation of free radicals, which in turn leads to the production of higher levels of lipid peroxides, resulting in oxidative–antioxidative system imbalance and causing serious cell injury (Wang et al. 2012). In the present investigation, in all the evaluated tissues, GPx, GST, and SOD expression in *P. vannamei* shrimp of the Cont group had lower levels compared to those of the Alko- and Sel-fed groups, triggering an enhancement of MDA levels in the Cont group after 1 and 6 h of thermal stress (Figs. 4, 5, 6; Table 2). These outcomes prove that environmental thermal stress generates lipid peroxidation and oxidative–antioxidative system imbalance, resulting in oxidative damage in the *P. vannamei* shrimp in our study (Duan et al. 2017; Zhang et al. 2022; Zhou et al. 2010).

Since antioxidant defense mechanisms in aquatic animals are to some degree influenced by dietary elements (Zhou et al. 2014), the results of our trial showed that shrimp pretreatment with Se-Y has an antioxidative impact against

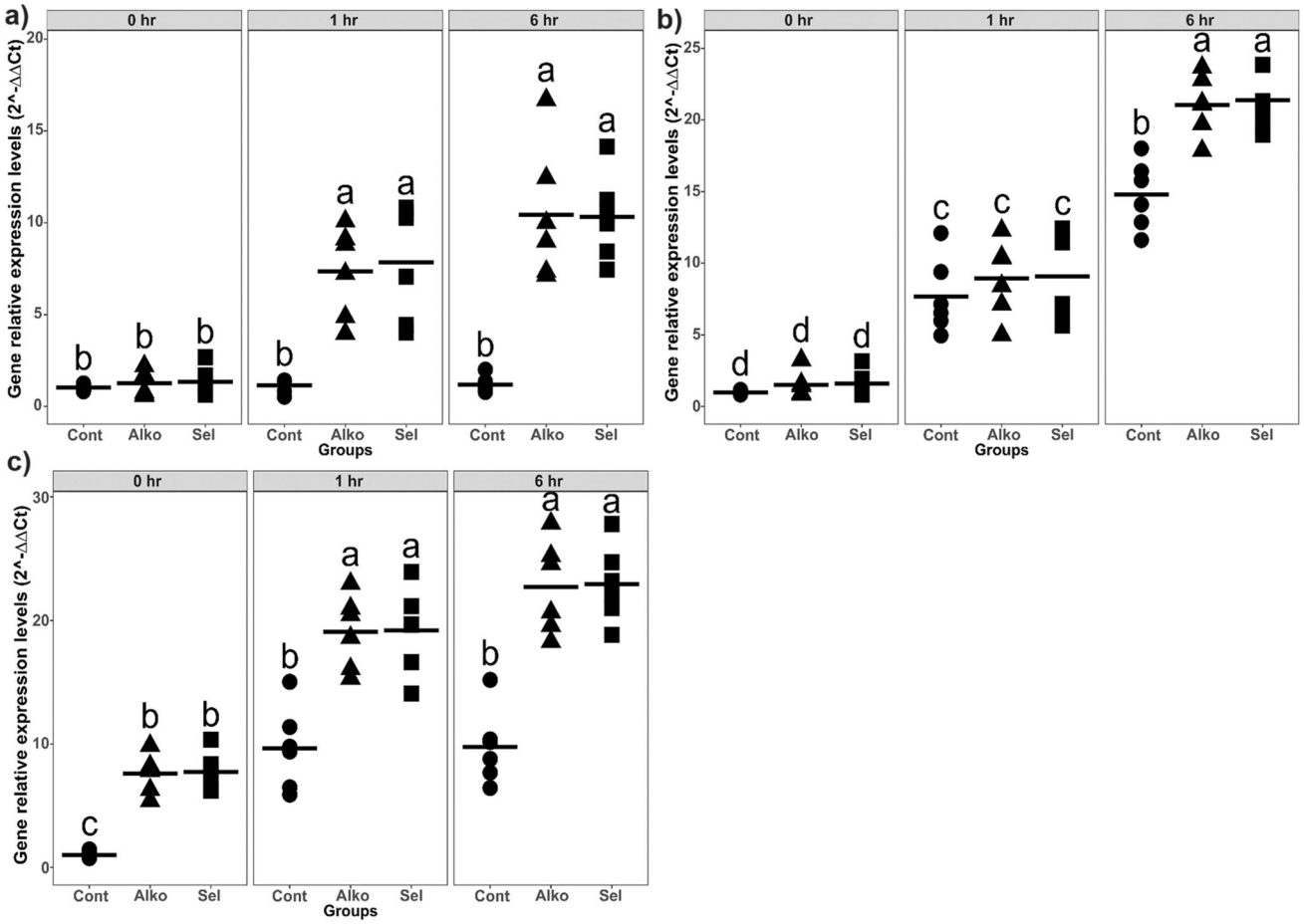


Fig. 4 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of GPx in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, the mean ± SEM is shown ($n = 6$). Different lowercase letters indicate significant differences at $P < 0.05$ among groups. Cont, Control group; GPx, glutathione peroxidase, SEM, standard error of the mean

thermal stress. We found that the supplementation of Se-Y to the feed could increase the expression levels of GPx, GST, and SOD in all tissues of *P. vannamei* shrimp after 1 and 6 h of stress compared to those of the Cont group, as well as promote the antioxidative mechanism ability, resulting in a lower MDA content compared to the Cont group. The findings in this study are in line with those reported by Long et al. (2017), who demonstrated how Se-Y supplementation to diets of Wuchang bream *Megalobrama amblycephala* under nitrite stress enhanced the transcriptional levels of antioxidant enzymes. Similarly, the authors of previous studies have reported that Se supplementation enhances the expression level of the antioxidant transcripts in the gill (Trevisan et al. 2011), muscle (Saleh and Ebeid 2019), and hepatopancreas (Yu et al. 2021).

Shrimp also have a coordinated antioxidant cascade that includes antioxidant enzymes (e.g. GPx, GST, and SOD) and non-enzymatic antioxidants (glutathione) to maintain adequate cellular redox homeostasis and particularly to fight off either provoked or spontaneous stressors (Parrilla-Taylor and Zenteno-Savín 2011). Cells primarily utilize glutathione as the most significant small molecular weight antioxidant; moreover, in *P. vannamei*, glutathione appears to have a significant antioxidant impact in response to thermal stress. First, our findings highlight that, within the thermal comfort zone and in the absence of any specific stressful circumstance, Se-Y supplementation appears to have a minimal impact on the glutathione content of the shrimp hepatopancreas, while it has a significant impact on its content in other tissues. Similar findings have been reported for the crayfish *Astacus astacus*, *Austropotamobius torrentium*, and *Orconectes limosus* (Kovačević et al. 2008). However, our outcomes are partly contrary to those observed by Kavitha and Devadasan (2005) who reported that supplementing the diet of *Penaeus monodon* with Se resulted in a significant improvement in the amount of GSH in the hepatopancreas and muscle tissues. Thus, we assume that Se-Y supplementation may enhance

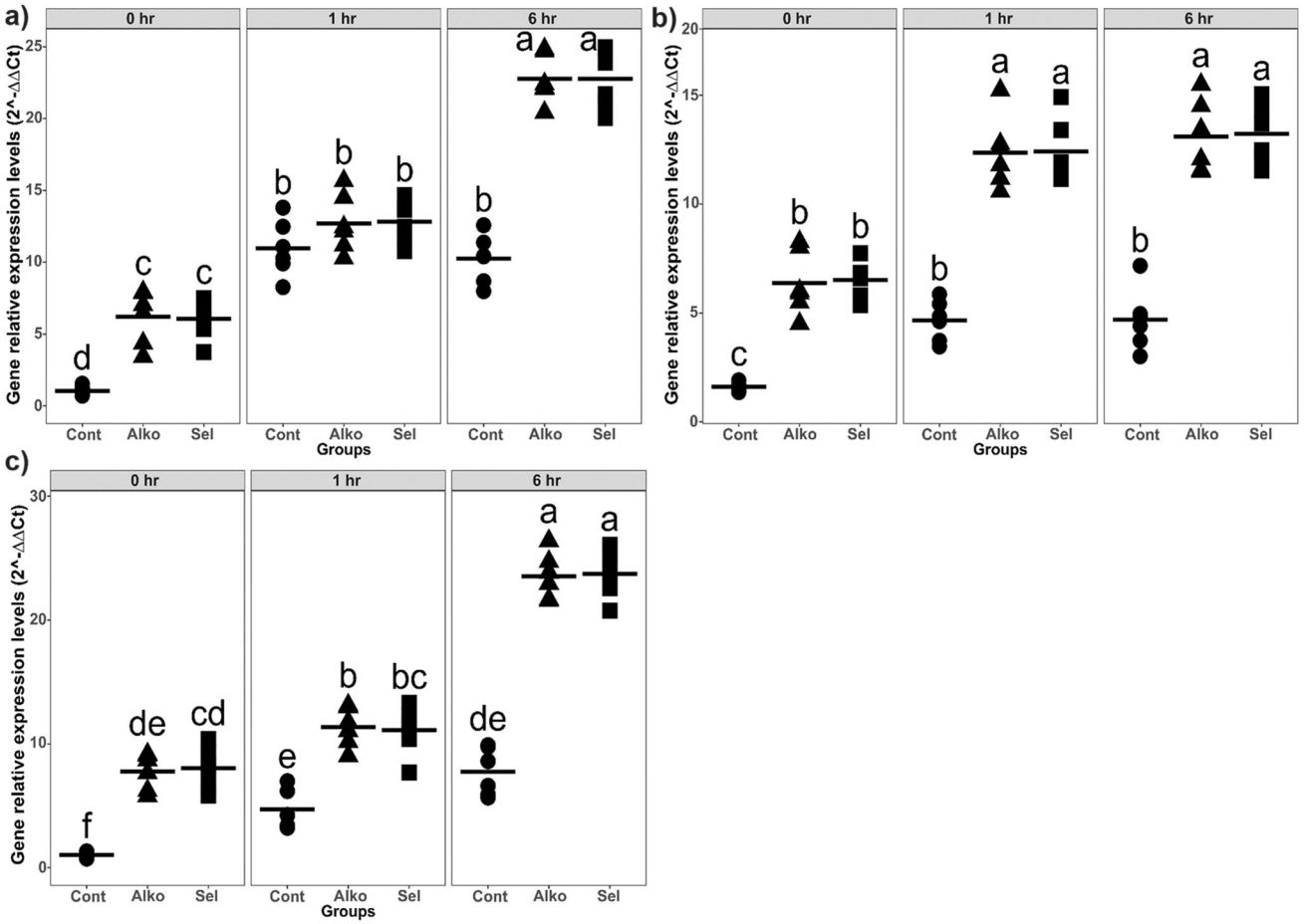


Fig. 5 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of GST in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, the mean ± SEM is shown ($n = 6$). Different lowercase letters indicate significant differences at $P < 0.05$ among groups. Cont, Control group; GST, glutathione-S-transferase; SEM, standard error of the mean

the antioxidant status of the shrimps by working in concert with the enzymatic and non-enzymatic components of antioxidants, which would account for the significant increase in the levels of GSH in the gill, which might have maintained the role of GPx or supported its function.

According to Masella et al. (2005), during thermal stress, the concentration of GSH will be reduced and the concentration of GSSG will be enhanced due to lipid peroxide reductions. However, in the present study, in all tested organs, Se-Y supplementation demonstrated a protective impact through the preservation of a constantly elevated level of GSH and a reduced GSSG level after 1 and 6 h of IHSC. Similarly, Qin et al. (2016) explained that Chinese mitten crabs *Eriocheir sinensis* fed nano-Se diets may have activated their antioxidant properties to reduce oxidative stress in their hypoxic environment through elevating their GSH content. Thus, this scenario demonstrates that shrimp groups fed a diet supplemented with Se-Y were able to effectively handle the IHSC and thereby limit related tissue damage through the enhancement of all antioxidant defense mechanisms.

The reported enhancement in the antioxidant status of *P. vannamei* may be the result of an increased Se content in shrimp tissues through the incorporation of Se into selenoproteins. Thioredoxin reductase is a crucially recognized selenoprotein that plays a significant role in the stimulation of signaling molecules, such as the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling cascade (Hoffmann 2007; Jingyuan et al. 2020). Nrf2 is a key component of the cellular antioxidant system and averts oxidative damage by regulating the antioxidant enzyme-related transcriptional factors, including GPx, SOD, and GST (Hoffmann 2007; Jingyuan et al. 2020). Another selenoprotein is selenomethionine, which is the predominant selenocompound in Se-Y and a component of the active core of the GPx enzyme that maintains the integrity of cellular membranes and organelles from superoxide radical-associated damage (Wang et al. 2019; Yu et al. 2022).

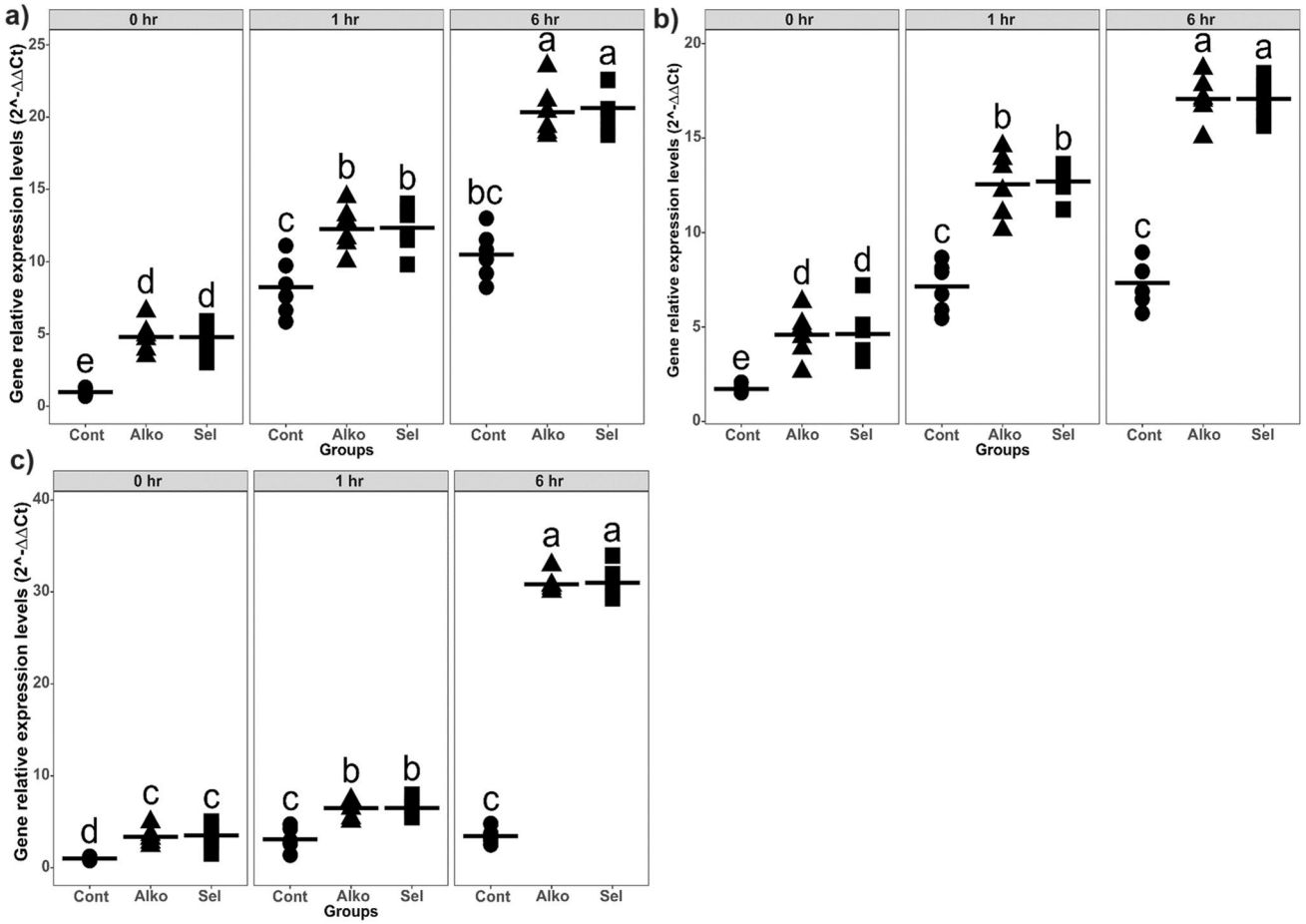


Fig. 6 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of SOD in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, the mean ± SEM is displayed ($n = 6$). Different lowercase letters indicate significant differences at $P < 0.05$ among groups. Cont, Control group; SEM, standard error of the mean; SOD, superoxide dismutase

It is worth noting that the commercial product used in this study was Se-Y. Previous studies have reported the role of yeast and yeast extracts against stresses in multiple aquatic organisms via modulating their antioxidant cascade (Jian et al. 2013; Reda et al. 2018; Tovar-Ramírez et al. 2010; Wang et al. 2019). In *P. vannamei*, studies have presented varying results on the effect of yeast on antioxidant cascade modulation. For example, in one study, even though *P. vannamei* shrimp fed yeast and yeast extract-containing diets exhibited no variations in their SOD activities compared to those fed the control diet, GPx activities significantly improved (Zheng et al. 2021). Red yeast supplementation also decreased the relative expression levels of SOD and catalase in the shrimp hemocytes, while it improved certain hepatopancreas antioxidant-related genes (Jian et al. 2013). Our experiment did not include yeast as a separate feed component, which is a limitation our evaluation of the effect of yeast on the modulation of antioxidant genes. In the context of this study, we conclude that the microelements in Se-Y may account for the enhanced expression of antioxidant transcriptional factors in shrimp fed the Se-Y-incorporated diets.

Prior research has demonstrated that HSPs may be induced as a cellular response to thermal stress in order to improve cellular adaptation to this stress (Cheng et al. 2015). Indeed, the expression levels of HSP70 and HSP90 in *P. vannamei* shrimp were strongly induced at 1 and 6 h of thermal stress. In addition, various studies have demonstrated that certain dietary supplements may promote the generation of HSPs in aquatic organisms (Zhou et al. 2014). Surprisingly, before and after stress, the expression levels of HSP70 and HSP90 mRNA in the Se-Y-treated groups were higher than those of the Cont group in all tested tissues, thereby boosting the protective effect on cells. Similarly, Li et al. (2022) discovered that under thermal stress conditions, HSPs were considerably upregulated in the Se-supplemented groups compared to the Cont group in rainbow trout *Oncorhynchus mykiss*. One possible explanation for how Se responds to stressors when used as a feed supplement is that it enhances

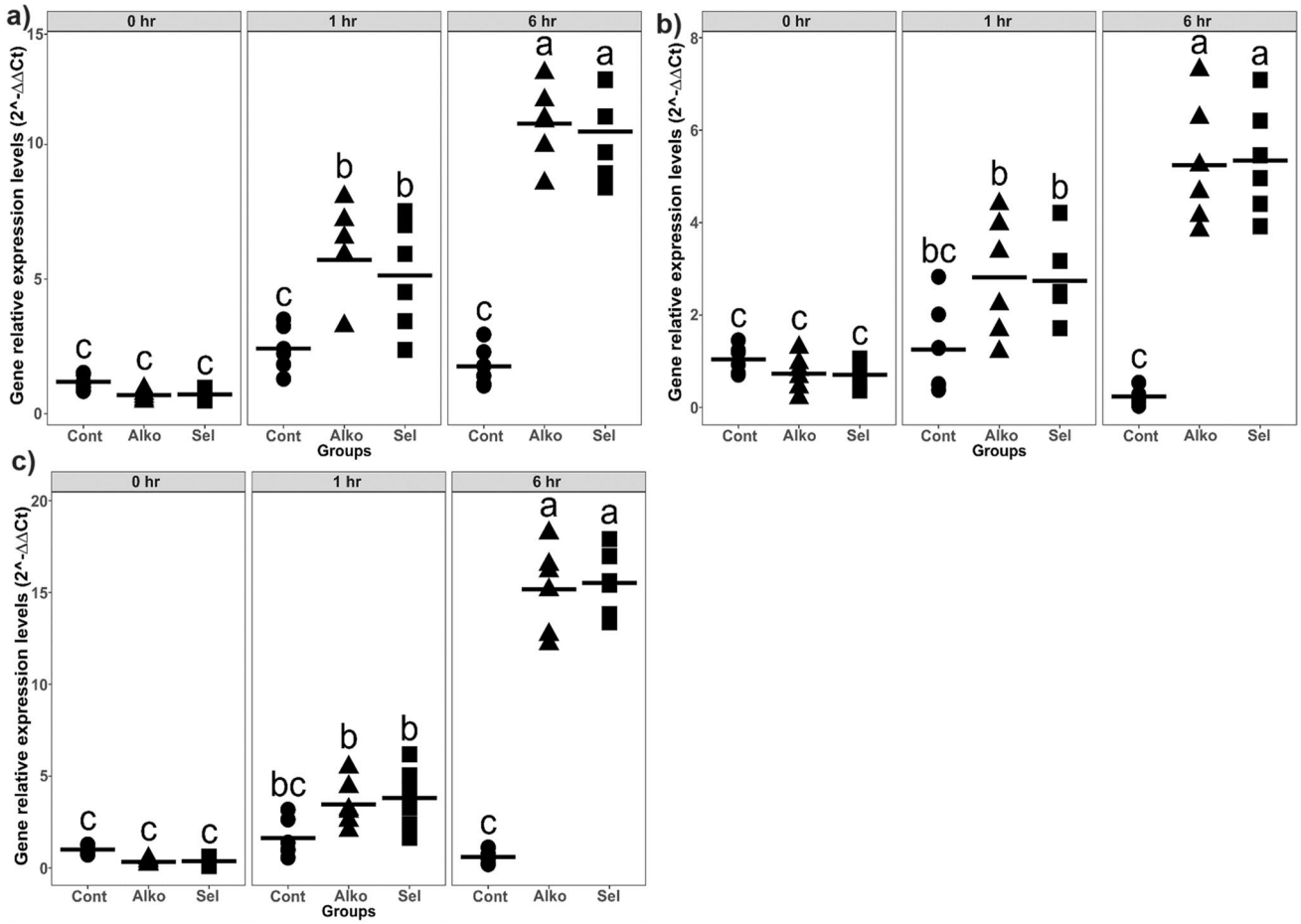


Fig. 7 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of GILT in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, the mean ± SEM is displayed ($n = 6$). Different lowercase letters indicate significant differences at $P < 0.05$ among groups. Cont, Control group; GILT, gamma-interferon-inducible lysosomal thiol reductase-like, SEM, standard error of the mean

glutamine amino acid content in reaction to thermal stress (Li et al. 2022). Glutamine can not only hinder the formation of ROS and suppress inflammation through switching on the glutamate-glutamine metabolic pathway, but it also promotes surface antigen synthesis and HSP production, as well as various other crucial activities (Matés et al. 2006). Overall, our findings indicate that when *P. vannamei* shrimp were subjected to thermal stress, the extended production of HSP70 and HSP90 by Se-Y supplementation might be more specifically associated with stress-triggered cytoprotection and long-lasting cellular adaptation.

Large-scale genomics studies offer an opportunity to assess universal patterns in Se applications in aquatic organisms. Such studies have recently identified a family of eukaryotic selenoproteins called GILT (Zhang and Gladyshev 2008). These proteins are now known to also control the cellular redox balance (Chiang and Maric 2011). However, sparse information in available in the literature on invertebrates in terms of GILT, and only a few studies have detailed the significance of GILT in the innate immune system response to bacterial, lipopolysaccharide, or viral challenges (Liu et al. 2019; Nualla-ong et al. 2017). In order to ascertain the functional role of GILT protein in the innate immune system of *P. vannamei* shrimp fed diets containing Se-Y with or without thermal stress, we performed a qPCR study. This qPCR study revealed that GILT transcripts show depressed expression patterns between shrimp fed the Se-Y-containing diets and those in the Cont group in the thermal comfort zone (0 h), although the differences were not significant. In Atlantic salmon fed soybean meal, GILT transcripts were downregulated in the early stage (Lilleeng et al. 2009). Thus, in the present study, it would seem that the depressed expression levels of GILT genes at 0 h (no thermal stress) in the groups receiving supplemental Se-Y indicate that the shrimp in our feeding trial were not subjected to any type of stress or microbial infection (normal rearing environmental state) before the thermal stress condition was applied. Moreover, it is probable that these transcripts may only be

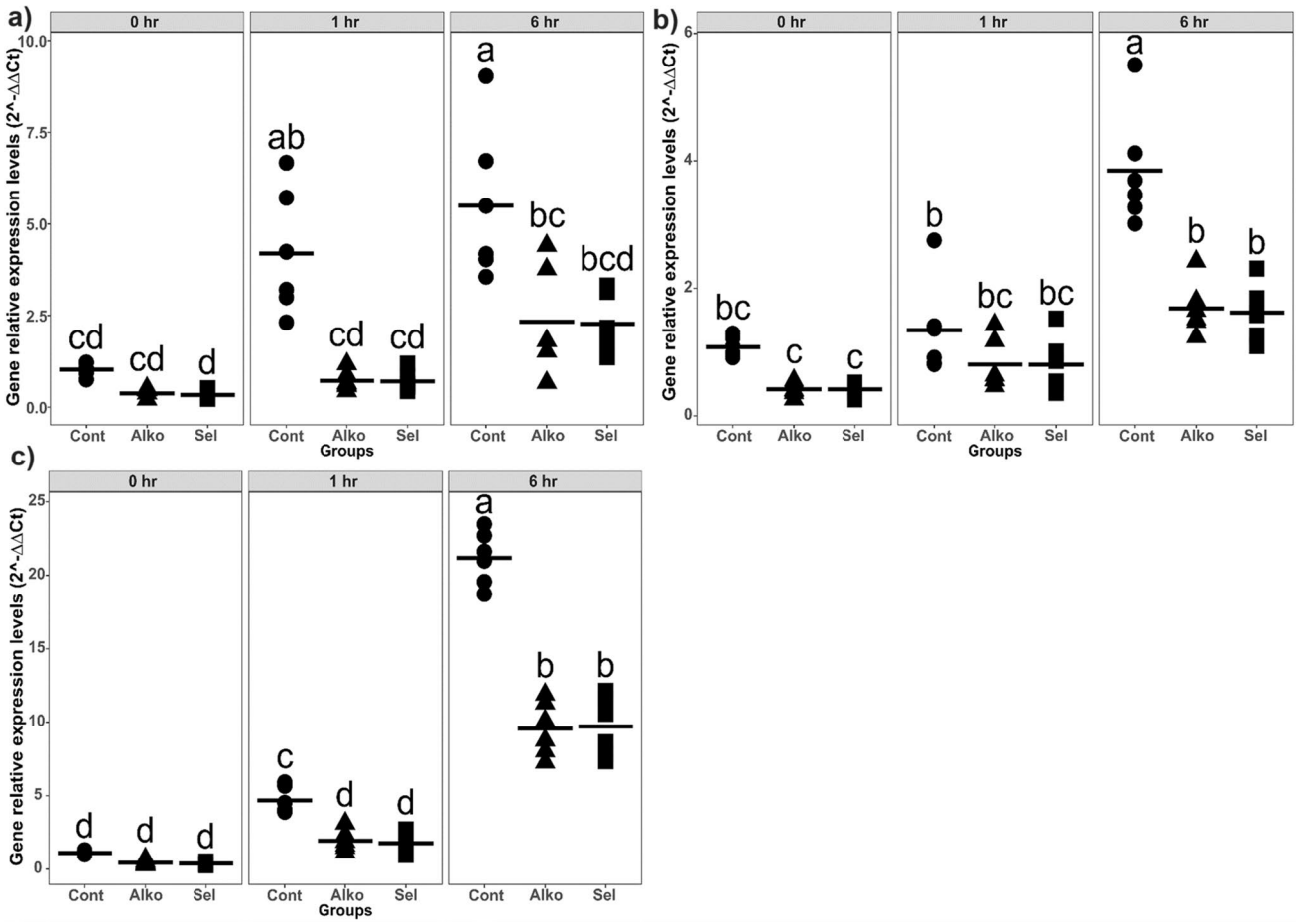


Fig. 8 Effects of AlkoSel (Alko) and SelPlex (Sel) supplementation on the relative expression levels of AST in the gill (**a**), muscle (**b**), and hepatopancreas (**c**) of *Penaeus vannamei* shrimp under thermal stress. For each group, the mean ± SEM is shown ($n = 6$). Different lowercase letters (a, b, c, and d) indicate significant differences at $P < 0.05$ among groups. AST, Aspartate aminotransferase cytoplasmic-like; Cont, control group; SEM, standard error of the mean

directly expressed by *P. vannamei* shrimp fed the two Se-Y-containing products in response to stressors, such as the thermal stress in our trial. Additionally, in all the examined tissues, it is worth noting that GILT expression levels were depressed in the Cont groups subjected to severe thermal stress (especially at 6 h). In this scenario, under conditions of thermal stress, salinity stress, or a combination thereof, the GILT transcripts of *Saccostrea glomerata* were slightly to significantly depressed (Ertl et al. 2019). Interestingly, the thermally induced ROS may affect the role of GILT proteins (Chiang and Maric 2011), possibly explaining why these gene expression levels were not regulated anymore in the thermally-stressed Cont groups and the ability of thermal stress to compromise the immune response. However, that the expression levels of GILT transcripts did increase in the Alko- and Sel-fed shrimp is good evidence of the enhanced OSe-related immunological responses in shrimp to mitigate thermal stresses. GILT proteins handle the cellular redox status through upregulating the expression levels and function of SOD enzymes, and these alterations are linked with the regulation of cellular homeostasis imbalance under stress (Chiang and Maric 2011). In their study, Gong et al. (2019) found significantly higher expression levels of GILT in largemouth bass *Micropterus salmoides* fed yeast hydrolysate in the diet after the post-challenge test.

Previous research found that variations in the activity or relative expression levels of AST protein, also known as glutamate oxaloacetate transaminase (GOT), frequently represents cellular damage in particular organs impacted by exposure to environmental pollution or stress (Krajnovic-Ozretic and Ozretic 1987; Liu et al. 2007). In our trial, the AST gene was expressed in all examined tissues before and after the IHSC, with the highest relative expression levels in the hepatopancreas, followed by the gill and muscle, respectively; these results are similar to those found in the Walking catfish *Clarias magur* (Patra et al. 2022). Our trial is the first study to describe the response of the AST gene in a crustacean to thermal stress after pretreatment with Se-Y supplementation. In our study,

the shrimp in the Cont groups under thermal stress were in a destructive phase, and long-term thermal stress exposure (6 h) exacerbated this degenerative stage, resulting in the production of much higher AST transcripts compared to other treatments. However, the lower estimated relative expression levels of the AST gene in the shrimp groups under thermal stress but fed the commercial Se-Y-containing products suggested that this feed additive makes all the evaluated organs of these groups perform better to help alleviate the negative impacts of thermal stress; similar results were found in the Tiger prawn *P. monodon* (Chien et al. 2003) and the Giant River prawn *Macrobrachium rosenbergii* (Liu et al. 2010). This can be explained by the concept that commercial Se-Y supplementation enhances glutamine amino acid levels in response to thermal stress, thus enhancing the non-specific immune responses of shrimp. Interestingly, these outcomes are supported by Hong et al. (1992), who reported that in acetaminophen-treated rats, glutamine supplementation is linked to lower levels of plasma transaminases and higher levels of hepatic GSH.

To summarize, by considering these issues, we focused on identifying substances that can alleviate or restore cellular damage related to thermal stress. The key function in explaining the mitigatory impact of Se-Y supplementation on thermal stress was the significant upregulation of the antioxidant indices and the high levels of GSH content, along with the enhancement of HSPs and GILT gene expression levels. The large decrease in MDA content and GSSG levels in tissues of *P. vannamei* shrimp fed the Se-Y-supplemented diets shows that thermal stress-induced ROS are nearly totally scavenged by the antioxidant cascades. Moreover, the conceivable therapeutic role of Se-Y supplementation against thermal stress-induced tissue damage and organ dysfunctions was also clarified by the lowered expression level of the AST gene.

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Declarations

Conflict of interest The authors have disclosed that there are no conflicts of interest.

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