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Dietary selenium sources differentially regulate selenium concentration, mRNA and protein expression of representative selenoproteins in various tissues of yellow catfish *Pelteobagrus fulvidraco*

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Abstract

The study was conducted to determine the effects of three dietary Se sources, such as sodium-selenite (S-S), seleno-yeast (S-Y) and seleno-methionine (S-M), on Se concentration, glutathione peroxidase (GPX) and TXNRD activities, and mRNA expression of fifteen representative selenoproteins, and protein expression of four endoplasmic reticulum-resided selenoproteins in a wide range of tissues of yellow catfish. Compared with S-S and S-M groups, dietary S-Y significantly decreased growth performance and feed utilisation of yellow catfish. Dietary Se sources significantly influenced Se contents in the spleen, dorsal muscle and the kidney, GPX activities in spleen, kidney, intestine, muscle and mesenteric fat, and TXNRD activities in the heart, intestine and mesenteric fat. Among ten tested tissues, dietary Se sources influenced mRNA expression of GPX4 and SELENOK in three tissues; GPX3, SELENOS and TXNRD2 in four tissues; SELENOF, SELENON and DIO2 in five tissues; SELENOM, GPX1/2 and TXNRD3 in six tissues; SELENOW in seven tissue and SELENOP and SELENOT in eight tissues. Based on these observations above, S-S and S-M seem to be suitable Se sources for improving growth performance and feed utilisation of yellow catfish. Dietary Se sources differentially influence the expression of selenoproteins in various tissues of yellow catfish. For the first time, we determined the expression of selenoproteins in fish in responses to dietary Se sources, which contributes to a better understanding of the functions and regulatory mechanisms of selenoporteins.

Key words: ***Pelteobagrus fulvidraco*: Selenium nutrition: Selenoprotein expression: Dietary Se sources**

Se is an essential trace element and plays many important roles in a wide range of biological functions, such as antioxidant defence, inflammatory modulation and production of thyroid hormones, in vertebrate, including fish[1,2]. Studies suggested that Se exerted its biological effects mainly through selenoproteins, which contained Se in the form of the 21st amino acid selenocysteine[3,4]. At present, selenoproteomes have been analysed in a variety of organisms and 24–25 selenoprotein genes were identified in humans and rodents[1]. Some of these proteins, such as glutathione peroxidase (GPX), iodothyronine deiodinases (DIO), methionine sulfoxide reductase and thioredoxin reductase (TXNRD), participate in maintaining cellular redox homoeostasis and protect cells from oxidative stress[1,5]. However, most members of the selenoproteins have not been functionally characterised[4,6]. Recently, based on the gene cloning and sequence analysis, we obtained more twenty-eight selenoproteins in yellow catfish *Pelteobagrus fulvidraco*, a widely cultured freshwater teleost, and the sequence information of some selenoproteins has been published[7]. Conservation of selenoproteins among species indicated the importance of this class of proteins.

Up to date, studies have determined collective responses of these selenoprotein genes to dietary Se concentrations in terrestrial vertebrates and found that the expression of some selenoproteins was differentially regulated by dietary Se availability[8–14]. These valuable data showed that the expression pattern of selenoproteins by dietary Se deficiency and excess varied with the selenoprotein genes, animal species and tissues[3,4,9–11,15]. Several studies have also explored the effects of dietary Se levels on the expression of selenoproteins in fish[16–18]. However, studies involved in their collective responses to different dietary Se sources were very scarce. An

Abbreviations: DIO, iodothyronine deiodinases; ER, endoplasmic reticulum; GPX, glutathione peroxidase; *rpl7*, ribosomal protein L7; S-M, seleno-methionine; S-S, sodium-selenite; S-Y, seleno-yeast; TXNRD, thioredoxin reductase; *ubce*, ubiquitin-conjugating enzyme.

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assessment of the regulation of the complete selenoproteome by dietary Se sources in fish will help to provide the basis for elucidating their biological functions of these selenoproteins.

Se occurs in both inorganic and organic forms in nature. Utilisation of organic and inorganic sources of Se is different. Compared with inorganic forms (such as selenite), organic Se sources, such as SeMet and Se-containing yeast, have excellent bioavailability and lower toxicity[19–25]. At present, effect of the different forms of Se (yeast, SeMet or selenite) on selenoprotein expression has not yet been well performed in fish. In the present study, we hypothesised that dietary Se sources differentially influence the expression of selenoproteins of yellow catfish. The objectives of the present study were to determine the effects of three dietary Se sources (sodium-selenite (S-S), seleno-yeast (S-Y) and seleno-methionine (S-M)) on Se accumulation, GPX and TXNRD activities, tissue expression profiles of fifteen selenoprotein genes (GPX1/2/3/4, TXNRD2/3, SELENOW, SELENOP, SELENOF, SELENOM, SELENOS, SELENON, SELENOK, SELENOT and DIO2) in yellow catfish. Based on available antibodies, effects of dietary Se sources on protein production of four selected selenoproteins (SELENOF, SELENOM, SELENOS and SELENON) were also determined in selected tissues. The SELENOF, SELENOM, SELENOS and SELENON resided in the endoplasmic reticulum (ER) lumen and played important roles in the function of ER because the ER was an important organelle responsible for protein and lipid synthesis and Ca storage within the cells[1,6]. The nomenclature of selenoprotein followed Gladyshev *et al.*[26], which was also approved by the HUGO Gene Nomenclature Committee. As fish are sensitive to dietary Se addition, revealing the regulation of dietary Se sources on their selenogenome will not only help understand the Se status but also provide useful clues to unveil the biological functions of these selenoproteins.

Materials and methods

Ethical standards

Our experimental procedures followed the institutional guide for the care and use of experimental animals of Huazhong Agricultural University and was approved by the Committee of Huazhong Agricultural University on the Ethics of Laboratory Animal.

Animals, diets, management and sample collection

Juvenile yellow catfish were purchased from Hubei Wuhan Fisheries Farm (Wuhan, Hubei Province, China). Three semi-purified diets were supplemented with S-S (214485, ≥99 % in purity; Millipore Sigma), S-Y (2000 ppm; Angel Yeast Co. Ltd) and S-M (3211-76-5, ≥98 % in purity; Millipore Sigma). Dietary Se contents were analysed, and the values were 0·25 mg/kg diet for three experimental diets (Table 1), which was considered to be optimal for meeting dietary Se requirement for yellow catfish[27]. In order to produce the diets, we finely ground all dry ingredients, weighed and mixed them for 10 min. Then, fish oil and maize oil were added, and they were thoroughly mixed for another 10 min. Finally, water was added and mixed to form a dough. The dough was passed through a pelletiser with a 2·0-mm-diameter die. The diets were dried in an oven until the moisture was reduced to <10 %.

Table 1. Feed formulation and proximate analysis of experimental diets*

	S-S	S-Y	S-M
Ingredients (g/kg)			
Casein	420	420	420
Gelatin	20	20	20
Fish oil	30	30	30
Maize oil	30	30	30
Wheat flour	250	250	250
Ascorbyl-2-polyphosphate	10	10	10
NaCl	10	10	10
$Ca(H_2PO_4)_2{\cdot}H_2O$	10	10	10
Vitamin premix	5	5	5
Mineral premix	5	5	5
Betaine	10	10	10
Cellulose	200	199·9998	199·994
Sodium-selenite	0·0005	0	0
Seleno-yeast	0	0·0002	0
Seleno-methionine	0	0	0·0006
Proximate analysis (g/kg DM basis)			
Moisture	94·5	92·2	97·9
Crude protein	425·7	430·5	428·3
Lipid	74·3	73·7	72·9
Ash	19·7	19·6	19·3
Se	0·0002513	0·0002506	0·0002514

* Vitamin premix mg provided/kg diet: retinyl acetate 3; cholecalciferol 0.025; dl-α-tocopheryl acetate 30; menadione nicotinamide bisulfite 7; thiamine hydrochloride 6; riboflavin 3; pyridoxine hydrochloride 12; D-calcium pantothenate 30; niacin 50; biotin 1; folic acid 6; cyanocobalamine 0.03. Trace mineral premix mg provided/kg diet: $Ca(H_2PO_3)_2{\cdot}H_2O$, 1000; $FeSO_4{\cdot}7H_2O$ 40; $ZnSO_4{\cdot}H_2O$ 100; $MnSO_4{\cdot}H_2O$ 40; $CuSO_4{\cdot}5H_2O$ 2; $CaIO_3{\cdot}6H_2O$ 3.

The experimental procedures were similar to those described in our previous study[28]. One hundred eighty juvenile fish (7·55 (SEM 0·03) g) were assigned to nine fibreglass tanks (20 fish/tank, 300 litre water volume) at ambient temperature (27 (SEM 1·5)°C) with a normal photoperiod (14 light–10 dark). Each diet was distributed randomly to triplicate tanks. The fish were fed 4 % of fish body weight per d twice daily at two equal meals (09.00 hours and 16.30 hours). This amount was close to the maximal daily ration of yellow catfish, based on feed consumption during the acclimation period of the study. Faecal matter was removed before feeding in the morning and afternoon. Fish were weighed once every 2 weeks, and the daily ration adjusted accordingly.

The experiment was carried out in a semi-static aquarium system and continuously aerated to maintain dissolved oxygen. In order to maintain good water quality, fresh water was renewed 80 % twice daily. Water quality parameters were monitored twice a week in the morning, and their values were followed below: pH 8·2 (SEM 0·2); dissolved oxygen 6·75 (SEM 0·23) mg/l; NH_4^+-N < 0·01 mg/l, NO_2-N < 0·01 mg/l. The experiment continued for 10 weeks. At the termination of the feeding experiment, yellow catfish were weighed and then fasted for 24 h. The survival, WG (weight again), FCR (feed conversion rate) and FI (feed intake) were determined. They were euthanised with MS-222 (100 mg/l) and dissected on ice to obtain the tissue samples. Three yellow catfish were collected randomly from each tank (nine fish for each treatment in total), and heart, brain, spleen, head kidney, intestine, dorsal muscle, mesenteric fat, gill, ovary

and testis were sampled to investigate the effects of dietary Se sources on Se deposition in nine tissues, GPX and TXNRD activity in ten tissues, fifteen selenoprotein gene transcription levels in ten tissues and four selenoprotein protein levels in five tissues of yellow catfish.

Sample analysis

Determination of selenium concentration and other nutritional parameters, and glutathione peroxidase and thioredoxin reductase activities. Diets and tissues' Se concentrations were determined using a hydride generation–atomic fluorescence spectrometer (AFS-8530; Haiguang Instruments). Briefly, total Se concentration in feed samples was determined by mineralisation of 1 g of sample in a mixture of 4 ml of 70 % HNO_3 (10014508; Sinopharm Chemical Reagent Co. Ltd) and 2 ml of 35 % H_2O_2 (10011208; Sinopharm Chemical Reagent Co. Ltd) at 85°C for 4 h within a closed vessel heating block system (Mars5, CEM). For tissue samples, the mass uptake was reduced to 250 mg digested by 2 ml of HNO_3 and 1 ml of H_2O_2. The solution was further used to determine the Se concentration according to the standard reference of Se (GSB04-1751-2004; National Research Center for Certified Reference Materials) by a hydride generation–atomic fluorescence spectrometer (AFS-8530; Haiguang Instruments)[29].

Other nutritional parameters consisted of moisture, ash, crude protein and lipid contents in the diets using standard methods. Crude protein was determined by the Kjeldahl method after concentrated H_2SO_4 digestion; crude lipid was determined by the ether-extraction method; moisture was determined by oven drying at 105°C for 24 h and ash content was determined using a muffle furnace at 550°C for 24 h.

The activities of GPX and thioredoxin reductases (TXNRD) were detected by using specific assay kits (A005-1-2 and A119-1-1) from the Nanjing Jiancheng Bioengineering Institute of China.

Real-time quantitative PCR and Western blot analysis. The quantitative PCR assays were conducted based on our published protocol[30]. Total RNA was extracted using a TRIzol reagent (Invitrogen) based on the acid guanidinium thiocyanate-phenol-chloroform extraction method. The integrity of total RNA was confirmed by agarose gel electrophoresis, and the purity of total RNA was measured by using a Nanodrop ND-2000 spectrophotometer. Total RNA was reverse-transcribed to cDNA with RT Primer Mix. The quantitative PCR assays were carried out in a quantitative thermal cycler (MyiQ 2 Two-Color Real-Time PCR Detection System; BIO-RAD) with a 20-μl reaction volume containing 10 μl SYBR Premix EX TaqTM II (TaKaRa), 1μl of diluted cDNA (10-fold), 10 mM each of forward and reverse primers 0·4 μl, and 8·2 μl H_2O. The gene specific primers are given in Table 2. The quantitative PCR programme included 10 min at 95°C and 40 cycles at 95°C for 15 s, 60°C for 1 min. We selected nine reference genes (*tbp*, *gapdh*, ribosomal protein L7 (*rpl7*), *18srRNA*, *elfa*, *hprt*, *b2m*, ubiquitin-conjugating enzyme (*ubce*) and *tuba*) to test their stability of mRNA expression. GeNorm was used to calculate the geometric mean of the best combination of two genes[31]. The $2^{-\Delta\Delta Ct}$ method was used for the quantification of quantitative PCR by using *rpl7* and *ubce* as two reference genes. The relative abundance was normalised to the S-S group.

Based on the protocols described in previous reports[30], cell lysates were prepared with RIPA buffer (Thermo Fisher

Table 2. Primer sets used for performing the quantitative real-time PCR analyses

Genes	Forward primer (5′-3′)	Reverse primer (5′-3′)	Accession no.
GPX1	CTCTCTGAGGCATGACGGTC	CCCAGGACGCACATACTTCA	MN062284
GPX2	GTCATTTGACACCCAAGC	CATAGTGATGCCACATTCTC	XM_027172340.1
GPX3	ATCTGGGTCTCTGTCCTGCT	TGACGGAAGGGAATGTGCAA	MN062285
GPX4	CTTGGGCAGAGCAATGTGTG	CTGCTCAGTGTACGTGGTGT	MN062286
SELENOW	GTACAGGCCCAAGTTCACCA	TCCCCGTTCTTCTTCGAGTG	MN062288
SELENOP	TGTGTGTGGAGACTGTGAGC	GACTATGCCTCGCTCCACTC	MN062289
TXNRD2	GGCACTACATGGGGTATCGG	ACTCTGTGGCCCCAATTCAG	MN062290
TXNRD3	AGACAAGGCTGGGGTGATTG	GACCGCAGCTACCATACTCC	MN062291
SELENOF	GCTGCGGAGGTGTATTTACTTTG	CCAGTTTTCATCCACAGACCTCA	XM_027150758.1
SELENOS	TCCGTGGTAATGCGTCAGG	TTTGTCCGTCTTGGGCTTC	XM_027163809.1
SELENOK	ACAGTAGGACACAGTCGCCA	GCTTGACGAGGGTCTGAAAGA	XM_027166810.1
SELENOM	TTTTATTGGCTGCGTTTC	TAGTAGTGGTTCAGGAGGAC	XM_027145607.1
SELENON	CCGCATCTGGGCTTTATTC	GCGACGCCTGTGAGTTTCT	XM_027143731.1
SELENOT	GCCTGCTCGCTTTCTCAC	CGATGCGGATGTCTGGGT	XM_027156829.1
DIO2	CCAGGCTTCTTCTCCAACTG	AGAGTTGGGAGCTGCTTCAC	XM_027157036.1
b2m	GCTGATCTGCCATGTGAGTG	TGTCTGACACTGCAGCTGTA	KP938520.1
gapdh	GCCTCCTGCACCAC AAACT	GGACCATCCACGGT CTTCT	KP893555
rpl7	GCGCCAGATCTTCAATGGAG	CTCATTCTGCCATGACCACG	KP893557
tbp	AGCAAAGAGTGAGGAGCAGT	ACTGCTGATGGGTGAGAACA	KP938525
18srRNA	TCATTCCGATAACGAACGAG	GGACATCTAAGGGCATCACA	KP893562
elfa	GTCTGGAGATGCTGCCATTG	AGCCTTCTTCTCAACGCTCT	KU886307.1
hprt	CCTCTCCGACTCACAGCTAG	GTCGCCATCTTCACC TCAAC	KP893556
ubce	GCCCGTGGAAGGATTCAAAA	AAGGCAGGTGGAGAGTATGG	KP893560
tuba	CACTTCCCTCTTGCCACCTA	ACGGTACAGGAGACAACAGG	KP893558

18srRNA, 18S ribosomal RNA; b2m, beta-2-microglobulin; DIO2, iodothyronine deiodinase 2; elfa, translation elongation factor; gapdh, glyceraldehyde-3-phosphate dehydrogenase; GPX, glutathione peroxidase; hprt, hypoxanthine-guanine phosphoribosyltransferase; rpl7, ribosomal protein L7; SELENO F, K, S, M, N, T, P, W, selenoprotein F, K, S, M, N, T, P, W; tbp, TATA-box-binding protein; tuba, tubulin alpha chain; TXNRD, thioredoxin reductase; ubce, ubiquitin-conjugating enzyme.

Scientific). Twenty-five-microgram protein was separated on 12 % SDS–polyacrylamide gels. After SDS–PAGE, the proteins were transferred to a PVDF membrane, blocked with 8 % (w/v) dry milk for 1h and then washed thrice with TBST buffer for 10 min each, followed by incubation with specific primary antibodies against SELENOF (ab124840; Abcam), SELENON (55333-1-AP; Proteintech Group), SELENOS (15591-1-AP; Proteintech Group), SELENOM (ab133681; Abcam) and GAPDH (10494-1-AP; Proteintech Group) overnight at 4°C and then processed with goat anti-rabbit IRDye 800CW secondary antibody (926-32211; Li-Cor Biosciences). The protein bands were visualised by a Vilber FUSION FX6 Spectra imaging system (Vilber Lourmat) and quantified by Image-Pro Plus 6.0 software (Media Cybernetics).

Statistical analysis

Before statistical analysis, all the data were evaluated for the normality with a Kolmogorov–Smirnov test. Bartlett's test was conducted to test the homogeneity of the variances among the treatments. One-factor ANOVA with Duncan's multiple range test was used to analyse the data. For all of the analyses, results are expressed as mean values with their standard errors and the significance level for differences was $P < 0{\cdot}05$. The analysis was carried out using the SPSS 19.0 for Windows (SPSS, Michigan Avenue).

Results

Growth performance

After feeding study, the survival was 100 % among three treatments (Table 3). Among the three dietary Se groups, FBW and WG were the lowest for fish fed the S-Y diet and showed no significant differences between the S-S and S-M groups; FCR was the highest for fish fed the S-Y diet and showed no significant differences between the S-S and S-M groups (Table 3).

Table 3. Three diets with different selenium sources influenced growth performance and feed utilisation of yellow catfish after 10 weeks (Mean values and standard deviations, *n* 3 replicate tank (IBW, FBW, WG, FCR and FI: replicates of 20 fish))

	Se supplementation					
	S-S		S-Y		S-M	
	Mean	SEM	Mean	SEM	Mean	SEM
IBW, g/fish	7·55	0·03	7·57	0·01	7·55	0·03
FBW, g/fish	38·4	0·5[a]	33·7[b]	0·7	37·4[a]	0·5
WG*, %	408·6[a]	8·7	345·1[b]	10·5	395·5[a]	6·8
FCR†	1·43[b]	0·02	1·55[a]	0·02	1·43[b]	0·01
FI‡, g/fish	44·1[a]	0·03	40·5[b]	0·74	42·8[a]	0·74
Survival, %	100·0	0·00	100·0	0·00	100·0	0·00

IBW, initial mean body weight; FBW, final mean body weight; WG, weight gain; FCR, feed conversion rate; FI, feed intake.
[a,b]Means in a row without a common superscript letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncans multiple range test).
* WG = (FBW–IBW)/IBW × 100 %.
† FCR = dry feed fed (g)/wet weight gain (g).
‡ FI = dry feed fed (g)/fish numbers.

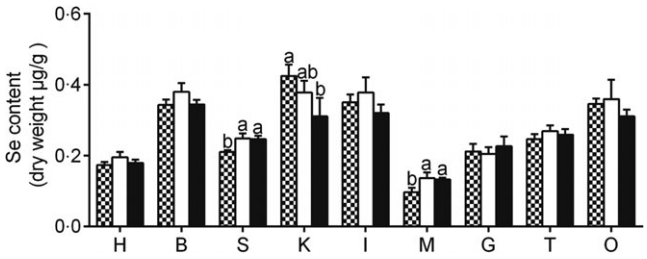

Fig. 1. Effects of three diets with different selenium sources on selenium concentration in the heart (H), brain (B), spleen (S), kidney (K), intestine (I), dorsal muscle (M), gill (G), testis (T) and ovary (O) tissues of *Pelteobagrus fulvidraco*. Values are mean with their standard errors, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). S-S, sodium-selenite; S-Y, seleno-yeast; S-M, Seleno-methionine. ▩, S-S; ▭, S-Y; ■, S-M

Selenium concentration

In the spleen and dorsal muscle, Se concentration was the lowest for fish fed the S-S diet and showed no significant difference between fish fed the S-Y and S-M diets. In the kidney, Se concentration was the highest for fish fed S-S diets and lowest for fish fed the S-M diet (Fig. 1). The three dietary Se sources did not significantly influence Se contents in the heart, brain, intestine, gill, testis and ovary tissues (Fig. 1).

Antioxidant enzyme activity

Three diets with different Se sources significantly influenced GPX activities in spleen, kidney, intestine, muscle and mesenteric fat, and TXNRD activities in the heart, intestine and mesenteric fat. GPX activities were lower in the spleen and kidney, and higher in the intestine, muscle and mesenteric fat for fish fed the S-S diet than those from other two groups ($P < 0{\cdot}05$) (Fig. 2(a)). In the heart, brain, kidney, gill, testis and ovary tissues, GPX activities presented no significant differences among the three treatments (Fig. 2(a)). TXNRD activities were the highest in the heart and mesenteric fat for fish fed the S-S diet and showed no significant differences between other two groups. TXNRD activity was the highest in the intestine for fish fed the S-S diet and lowest for fish fed S-M diet ($P < 0{\cdot}05$) (Fig. 2(b)).

mRNA expression of fifteen representative selenoprotein genes

Next, the expression of the fifteen representative selenoproteins (GPX1/2/3/4, TXNRD2/3, SELENOW, SELENOP, SELENOF, SELENOM, SELENOS, SELENON, SELENOK, SELENOT and DIO2) was determined in ten tissues (heart, brain, spleen, kidney, intestine, muscle, mesenteric fat, gills, testis and ovary). Different dietary Se sources significantly influenced mRNA expression of SELENOF in kidney, dorsal muscle, mesenteric fat, testis and ovary tissues but did not significantly influence mRNA expression of SELENOF in heart, brain, spleen, intestine and gills (Fig. 3(a)). mRNA expression of SELENOF was the highest in the kidney, muscle and testis for fish fed the S-S diet and showed no significant differences between other two groups. In the mesenteric fat, SELENOF mRNA expression was the lowest for fish fed the S-S diet and highest for fish fed the S-M diet. In

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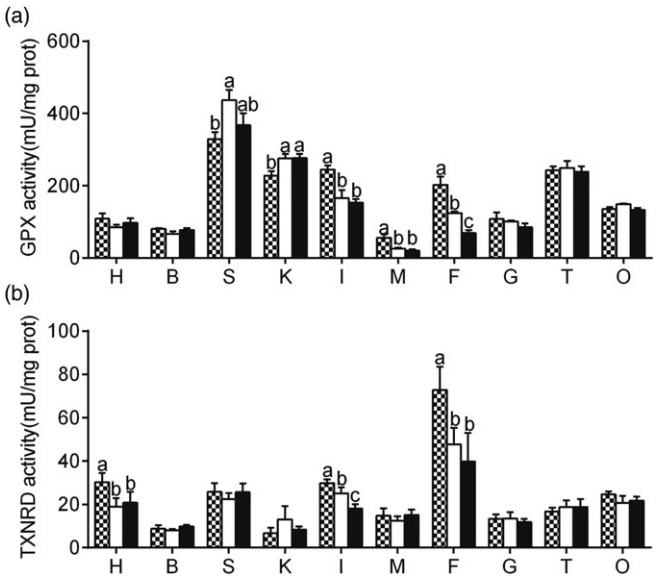

Fig. 2. Effects of three diets with different selenium sources on glutathione peroxidase (GPX) (a) and thioredoxin reductases (TXNRD) (b) activities in the heart (H), brain (B), spleen (S), kidney (K), intestine (I), dorsal muscle (M), mesenteric fat (F), gill (G), testis (T) and ovary (O) of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). S-S, sodium-selenite; S-Y, seleno-yeast; S-M, seleno-methionine. ▨, S-S; □, S-Y; ■, S-M.

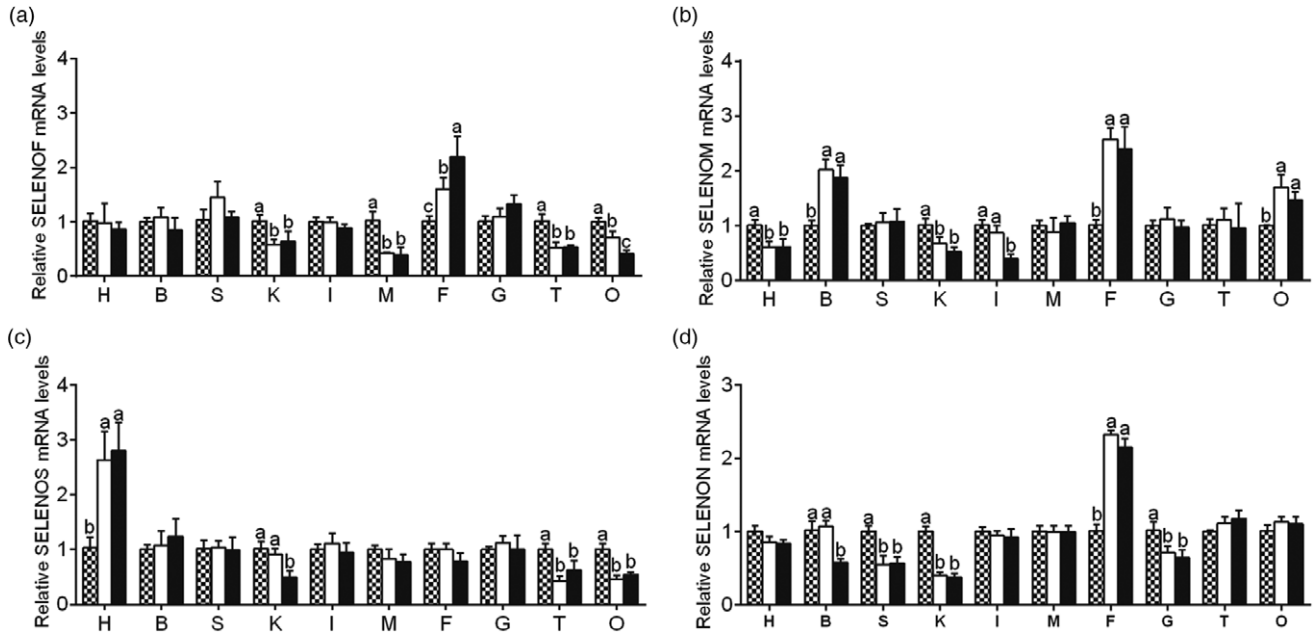

Fig. 3. Effects of three diets with different selenium sources on mRNA expression of SELENOF (A), SELENOM (b), SELENOS (C), SELENON (D) across heart (H), brain (B), spleen (S), head kidney (K), intestine (I), dorsal muscle (M), mesenteric fat (F), gill (G), testis (T) and ovary (O) of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). S-S, sodium-selenite; S-Y, seleno-yeast; S-M, seleno-methionine. ▨, S-S; □, S-Y; ■, S-M.

contrast, in the ovary, SELENOF mRNA expression was the highest for fish fed the S-S diet and lowest for fish fed the S-M diet (Fig. 3(a)).

For SELENOM, in the heart and kidney, its mRNA expression was the highest for fish fed S-S diets and showed no significant difference in fish fed the S-Y and S-M diets (Fig. 3(b)). In the brain, mesenteric fat and ovary tissue, the SELENOM mRNA expression was the lowest for fish fed S-S diets and showed no significant difference in fish fed the S-Y and S-M diets (Fig. 3(b)). In the intestine, the SELENOM mRNA expression was lowest for fish fed the S-M diet and showed no significant differences between other two groups.

Three diets with different Se sources significantly influenced SELENOS mRNA expression in the heart, kidney, testis and ovary

tissues, but not the brain, spleen, intestine, muscle, mesenteric fat and gills. SELENOS mRNA expression is the lowest in the heart, and highest in the testis and ovary tissues, for fish fed the S-S diet and showed no significant differences between other two groups (Fig. 3(c)). Kidney SELENOS mRNA expression is the lowest for fish fed the S-M diet and showed no significant differences between the S-S and S-Y group.

For SELENON, three diets with different Se sources significantly influenced its mRNA expression in the brain, spleen, kidney, mesenteric fat and gills, but not the heart, intestine, muscle, testis and ovary tissue (Fig. 3(d)). In the spleen, kidney and gill, SELENON mRNA expression was the highest for fish fed the S-S diet and showed no significant differences between other two groups. In the brain, SELENON mRNA expression was the lowest for fish fed the S-M diet and showed no significant differences between other two groups. In the mesenteric fat, the SELENON mRNA expression was the lowest for fish fed S-S diets and showed no significant difference in fish fed the S-Y and S-M diets (Fig. 3(d)).

GPX1 mRNA expression was the highest in the brain and spleen tissues, and lowest in the mesenteric fat for fish fed the S-M diet and showed no significant difference between other two groups (Fig. 4(a)). GPX1 mRNA expression was the lowest in kidney and muscle for fish fed S-S diet and showed no significant differences between other two groups (Fig. 4(a)). For GPX2, its mRNA expression is the lowest in the spleen and highest in mesenteric fat for fish fed S-M diet and showed no significant differences between other two groups (Fig. 4(b)). GPX2 mRNA expression was the highest in the kidney, and the lowest in the muscle and gills for fish fed the S-S diet, and showed no significant differences between other two groups. In the testis, GPX2 mRNA expression is the highest for fish fed S-Y diet and showed no significant differences between other two groups (Fig. 4(b)).

GPX3 mRNA expression showed significant differences in the spleen, intestine, mesenteric fat and ovary tissues for fish fed three dietary Se sources (Fig. 4(c)). Spleen GPX3 mRNA expression was the highest for fish fed the S-S diet and showed no significant differences between other two groups. GPX3 mRNA expression in the intestine and mesenteric fat was the lowest for fish fed the S-M diet and showed no significant differences between other two groups. Ovarian GPX3 mRNA expression was the lowest for fish fed the S-S diet and highest for fish fed the S-M diet (Fig. 4(c)). GPX4 mRNA expression in the mesenteric fat was the highest for fish fed the S-Y diet and showed no significant differences between other two groups (Fig. 4(d)). GPX4 mRNA expression in the testis was the highest for fish fed the S-M diet and showed no significant differences between other two groups. Ovarian GPX4 mRNA expression is the lowest for fish fed the S-M diet and showed no significant differences between other two groups (Fig. 4(d)).

SELENOW mRNA expression was the lowest in the brain, intestine, muscle, mesenteric fat and ovary for fish fed the S-M diet and showed no significant differences between other two groups (Fig. 4(e)). In the kidney, SELENOW mRNA expression is the highest for fish fed the S-S diet and showed no significant differences between other two groups. In the gills, SELENOW mRNA expression is the highest for fish fed the S-Y diet and lowest for fish fed the S-M diet (Fig. 4(e)).

Three diets with different Se sources significantly influenced SELENOP mRNA expression in the heart, brain, spleen, kidney, intestine, mesenteric fat and gills, but not testis and ovary tissues (Fig. 4(f)). SELENOP mRNA expression in the heart, kidney and gills was the lowest, in the spleen was the highest for fish fed the S-S diet and showed no significant differences between other two groups. In the brain, intestine and muscle, SELENOP mRNA expression was the highest for fish fed the S-M diet and showed no significant differences between other two groups. SELENOP mRNA expression in the mesenteric fat was the lowest for fish fed the S-M diet and showed no significant differences between other two groups (Fig. 4(f)).

TXNRD2 mRNA expression was the lowest in the heart and mesenteric fat for fish fed the S-S diet and highest for fish fed S-M diet (Fig. 4(g)). Spleenic TXNRD2 mRNA expression was the highest for fish fed the S-S diet and showed no significant differences between other two groups. Kidney TXNRD2 mRNA expression was the highest for fish fed the S-M diet and showed no significant differences between other two groups (Fig. 4(g)). TXNRD3 mRNA expression was the lowest in the spleen and muscle for fish fed the S-M diet and showed no significant differences between other two groups. TXNRD3 mRNA expression was the highest in the intestine, testis and ovary, the lowest in mesenteric fat for fish fed the S-S diet and showed no significant differences between other two groups (Fig. 4(h)).

Three diets with different Se sources significantly influenced SELENOK mRNA expression in the brain, mesenteric fat and gills, but not in other tissues (Fig. 4(i)). SELENOK mRNA expression in the brain was the lowest for fish fed the S-M diet and showed no significant differences between other two groups. In the mesenteric fat and gills, SELENOK mRNA expression was higher for fish fed the S-Y diet than those fed the S-S and S-M diets (Fig. 4(i)).

For SELENOT, in the heart, brain, intestine and gills, its mRNA expression was the lowest for fish fed the S-S diet and showed no significant differences between other two groups (Fig. 4(j)). In the kidney and muscle, SELENOT mRNA expression was the highest for fish fed the S-S diet and showed no significant differences between other two groups. Spleenic SELENOT mRNA expression was higher for fish fed the S-Y diet than those fed other two diets. In the mesenteric fat, SELENOT mRNA expression was the highest for fish fed the S-M diet and showed no significant differences between other two groups (Fig. 4(j)).

DIO2 mRNA expression showed significant differences in the brain, intestine, mesenteric fat, testis and ovary tissues for fish fed three Se sources (Fig. 4(k)). In the brain, mesenteric fat and testis, DIO2 mRNA expression was the highest for fish fed the S-M diet and showed no significant differences between other two groups. In the intestine, DIO2 mRNA expression was the lowest for fish fed the S-S diet and showed no significant differences between other two groups. In the ovary, DIO2 mRNA expression was the highest for fish fed the S-S diet and showed no significant differences between other two groups (Fig. 4(k)).

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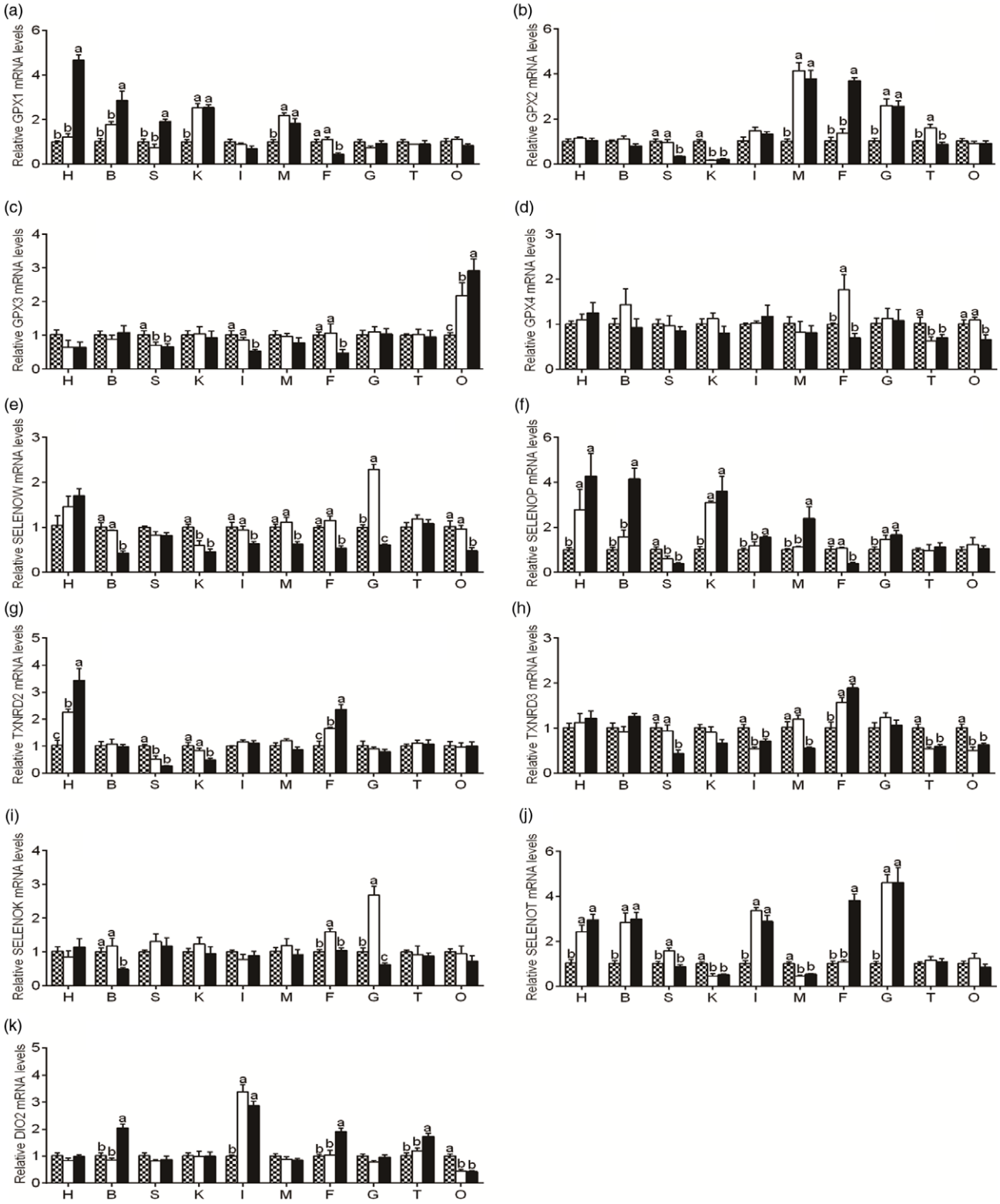

Fig. 4. Effects of three diets with different selenium sources on mRNA expression of GPX1 (A), GPX2 (B), GPX3 (C), GPX4 (D), SELENOW (E), SELENOP (F), TXNRD2 (G), TXNRD3 (H), SELENOK (I), SELENOT (J) and DIO2 (K) across heart (H), , brain (B), spleen (S), head kidney (K), dorsal muscle (M), mesenteric fat (F), anterior intestine (I), gill (G), testis (T) and ovary (O) of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). GPX, glutathione peroxidase; TXNRD, thioredoxin reductases; SELENOW, selenoprotein W; SELENOP, selenporotein P; SELENOK, selenoprotein K; SELENOT, selenoprotein T, DIO2, iodothyronine deiodinase 2; S-S, sodium-selenite; S-Y, seleno-yeast; S-M, Seleno-methionine. ▩, S-S; □, S-Y; ■, S-M.

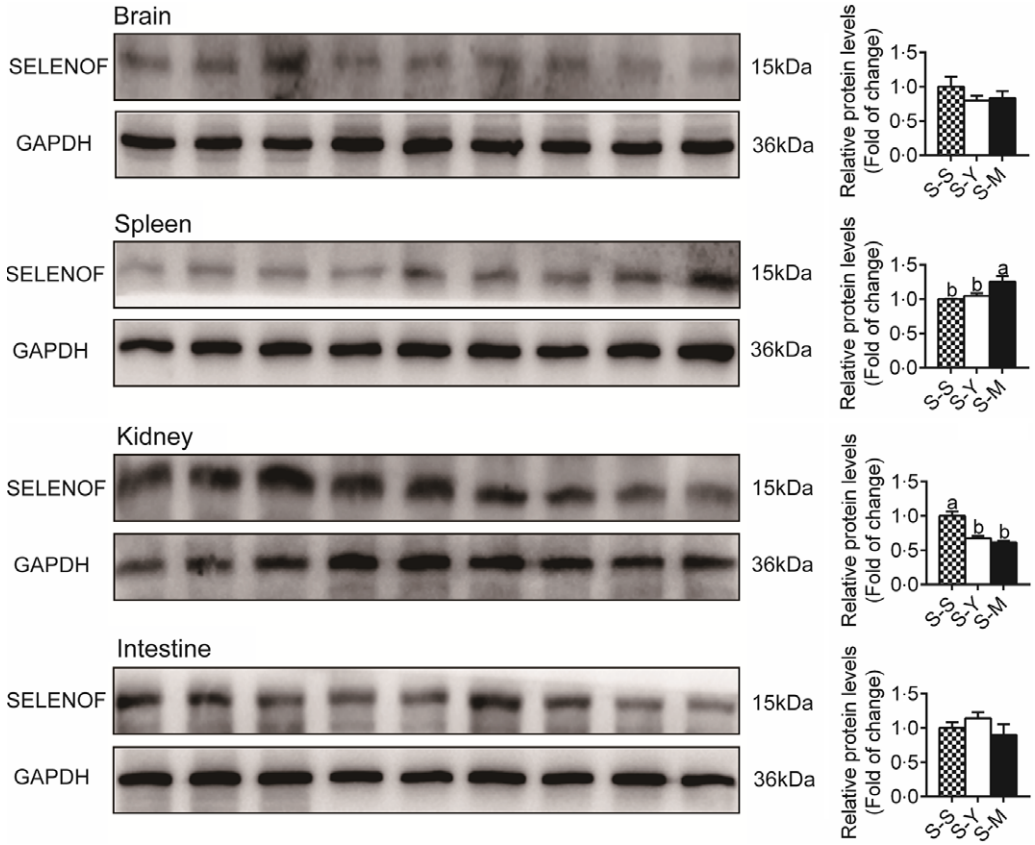

Fig. 5. Effects of three diets with different selenium sources on SELENOF expression levels in heart, brain, spleen, kidney, intestine tissues of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). ▩, S-S, sodium-selenite; □, S-Y, seleno-yeast; ■, S-M, Seleno-methionine.

Protein expression of four endoplasmic reticulum-related genes

Next, western blotting was used to detect the expression levels of the four representative ER selenoproteins (SELENOF, SELENOM, SELENOS, SELENON) in five tissues (heart, brain, spleen, kidney and intestine) of fish fed three Se sources (Figs. 5-8). Dietary Se sourced did not significantly influence SELENOF protein expression in the brain and intestine (Fig. 5). In the heart, SELENOF protein expression was the lowest for fish fed the S-Y diet and showed no significant differences between the S-S and S-M groups. In the spleen, SELENOF protein expression was the highest for fish fed the S-M diet and showed no significant differences between the S-S and S-Y groups. In the kidney, SELENOF protein expression was the highest for fish fed the S-S diet and showed no significant differences between the S-Y and S-M groups (Fig. 5).

SELENOM protein expression showed no significant differences in the brain and kidney for fish fed three diets (Fig. 6). In the heart, SELENOM protein expression was the highest for fish fed the S-S diet and showed no significant differences between the S-Y and S-M groups. In the spleen, SELENOM protein expression was the lowest for fish fed the S-M diet and showed no significant differences between the S-S and S-Y groups. In the intestine, SELENOM protein expression was the highest for fish fed the S-S diet and showed no significant differences between the S-Y and S-M groups (Fig. 6).

SELENOS protein expression showed no significant differences in the heart and intestine for fish fed three diets (Fig. 7). In the brain, SELENOS protein expression was the lowest for fish fed the S-S diet and showed no significant differences between the S-Y and S-M groups. In the spleen, SELENOS protein expression was the highest for fish fed the S-S diet and showed no significant differences between the S-Y and S-M groups. In the kidney, SELENOS protein expression was the lowest for fish fed the S-M diet and showed no significant differences between the S-S and S-Y groups (Fig. 7).

Three diets with different Se sources did not significantly influence SELENON protein expression in the spleen (Fig. 8). In the heart and brain, SELENON protein expression was the highest for fish fed the S-S diet and showed no significant differences between the S-Y and S-M groups. In the kidney and intestine, SELENON protein expression was the lowest for fish fed the S-M diet and showed no significant differences between the S-S and S-Y groups (Fig. 8).

Discussion

In the present study, compared with S-S and S-M groups, dietary S-Y significantly decreased growth performance and feed utilisation of yellow catfish. Thus, S-S and S-M seem to be optimal Se sources for yellow catfish. Kucukbay *et al.*[32] reported that fish

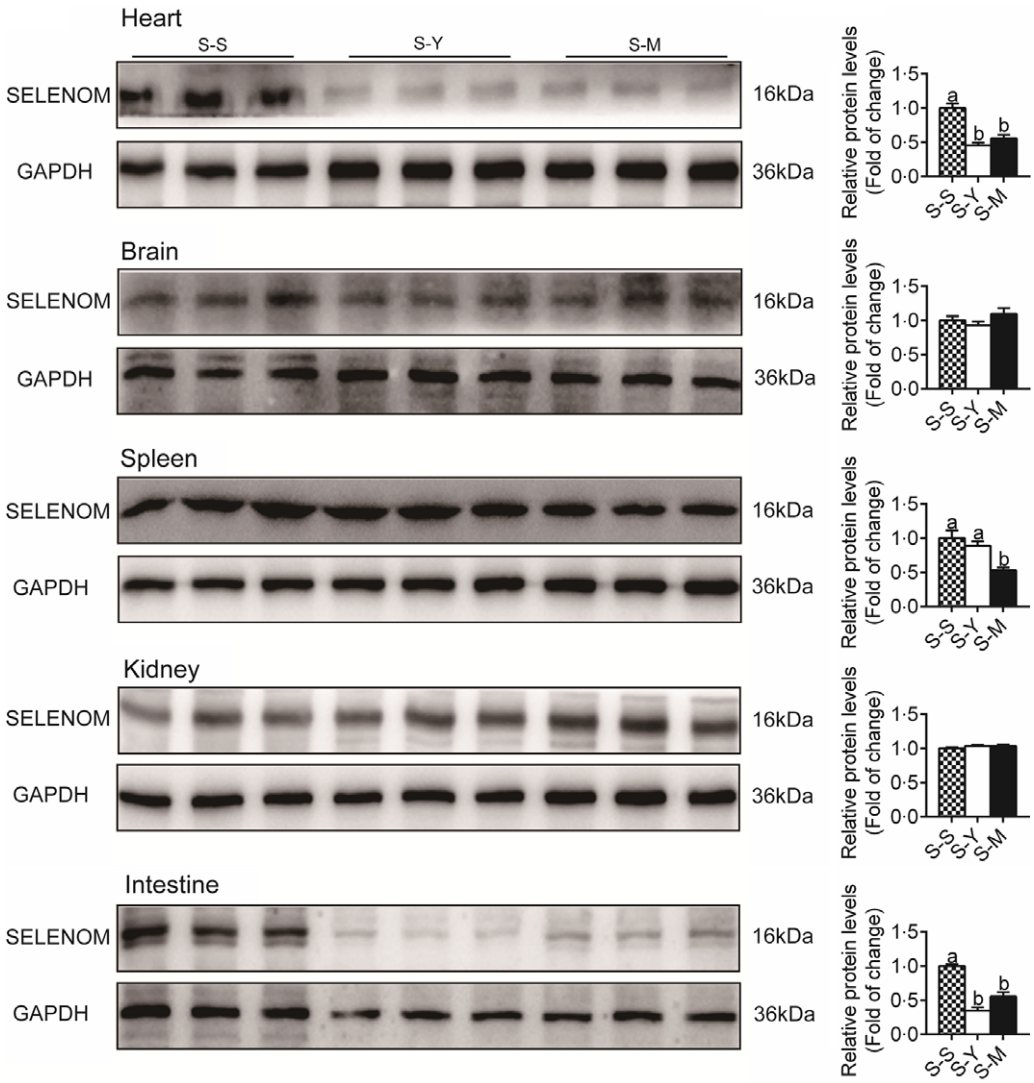

Fig. 6. Effects of three diets with different selenium sources on SELENOM expression levels in heart, brain, spleen, kidney, intestine tissues of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). ▩, S-S, sodium-selenite; □, S-Y, seleno-yeast; ■, S-M, Seleno-methionine.

given diets containing organic Se had a better growth because the organic Se was better absorbed than the inorganic source. This study also indicated that Se concentration was lower in the spleen and dorsal muscle for fish fed the S-S diet than those for fish fed the S-Y and S-M diets and that Se concentration was the highest for fish fed S-S diets in the kidney and lowest for fish fed the S-M diet. This is one of the few studies involved in the wide tissue distribution of Se content in animals in responses to dietary Se sources. Wide differences between tissues in Se concentration and sensitivity to dietary Se levels are well known, as observed by Barnes *et al.*[10]. Studies mentioned that organic Se are better absorbed and retained than inorganic forms[5]. Lorentzen *et al.*[33] reported that muscle Se concentrations were highest in the groups given S-M compared with that given selenite in Atlantic salmon. This study indicated that the three dietary Se sources did not significantly influence Se contents in the heart, brain, intestine, gill, testis and ovary tissues, suggesting the homoeostatic regulation in these tissues.

In the present study, GPX activities were lower in the spleen and kidney, and higher in the intestine, muscle and mesenteric fat for fish fed the S-S diet than those from other two groups; in the heart, brain, kidney, gill, testis and ovary tissues, GPX activities presented no significant differences among the three treatments. GPx is a biochemical marker to evaluate dietary Se requirement[34]. GPx regulate hydrogen peroxide and other hydroperoxides, affecting signalling and protecting against oxidative injury[2]. Several studies indicated that organic Se, such as selenomethione, increased GPx activities compared with S-S[2,4]. TXNRD is TXNRD which regulated thiol redox status by reducing thioredoxin and protects cells from oxidation[35,36]. This study indicated that TXNRD activities were the highest in the heart and mesenteric fat for fish fed the S-S diet and showed no significant differences between other two groups. Moreover, TXNRD activity is the highest in the intestine for fish fed the S-S diet and lowest for fish fed S-M diet. As TXNRD code for antioxidant enzyme or protein that may control the quality of cellular

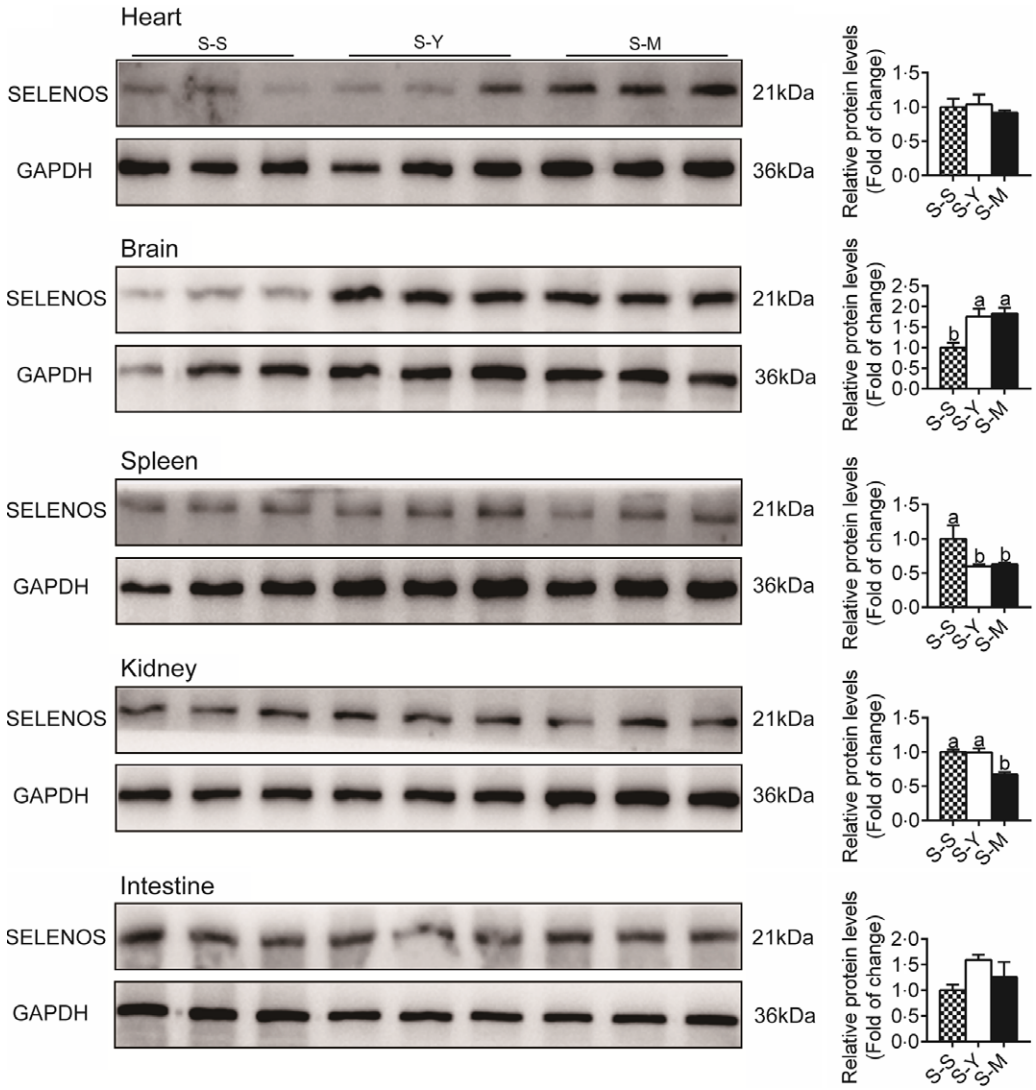

Fig. 7. Effects of three diets with different selenium sources on SELENOS expression in heart, brain, spleen, kidney, intestine tissues of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). ▩, S-S, sodium-selenite; ▭, S-Y, seleno-yeast; ■, S-M, Seleno-methionine.

proteins[1,37], its intriguing elevation might be induced by the elevated oxidative stress.

In the present study, three diets with different Se sources influenced mRNA expression of GPX4 and SELENOK in three tissues; GPX3, SELENOS and TXNRD2 in four tissues; SELENOF, SELENON and DIO2 in five tissues; SELENOM, GPX1/2 and TXNRD3 in six tissues; SELENOW in seven tissue and SELENOP and SELENOT in eight tissues. For the first time, we determined the effects of dietary Se sources on mRNA expression of representative selenoprotein among many tissues, which makes the comparison rather difficult. Several other studies explored the effects of dietary Se levels on mRNA expression of selenoproteins and found that the changes of selenoprotein expression in responses to dietary Se addition were selenoprotein- and tissue-dependent[4,9,12,38,39]. Moreover, Liu *et al.*[12] pointed out that no single selenoprotein gene exhibited any common response to dietary Se across various tissues and that no single tissue showed any common response to dietary Se across various selenoprotein genes. Among these selenoproteins, GPx1 is the most abundant one and considered as a biomarker of Se status because its expression shows a dramatic decrease in response to Se deficiency and increase in responses to Se repletion[2,11]. Meantime, this study did not find a parallel increase in the expression levels of most selenoproteins in responses to tissue Se contents. Similar results have widely reported in other studies[9,10,38]. For example, Akahoshi *et al.*[4] reported that Se-excess feeding induced massive Se accumulation but did not result in a parallel increase in the expression levels of most selenoproteins in tested organs. Moreover, animal species-dependent expression of selenoproteins in responses to dietary Se addition was also reported. For example, a number of selenoprotein genes, including GPx4, SELENOF, SELENON, SELENOO, SELENOS, SELENOT, or SELENOX, were regulated by dietary Se in chicks[9], but not in rodents[10,38]. Thus, apart

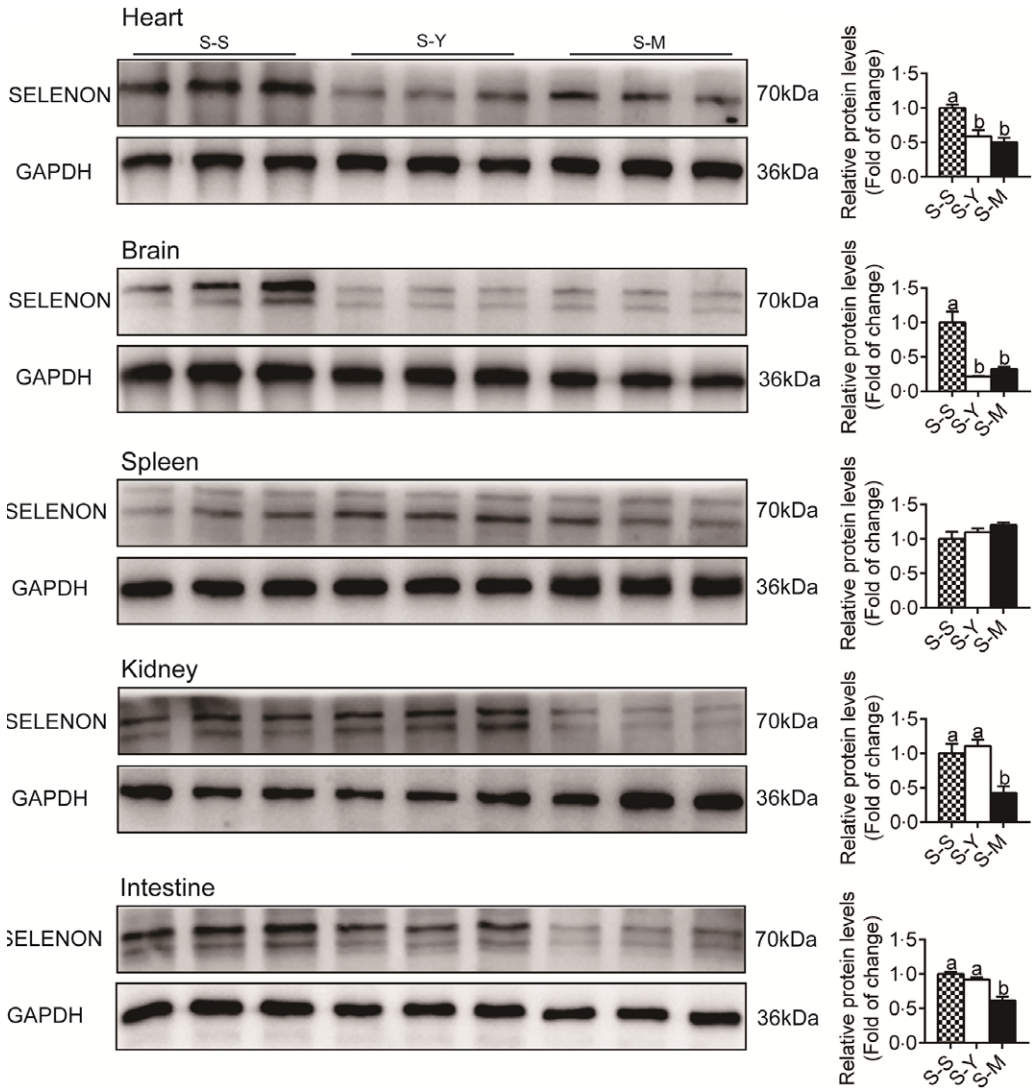

Fig. 8. Effects of three diets with different selenium sources on SELENON expression levels in heart, brain, spleen, kidney, intestine tissues of *Pelteobagrus fulvidraco*. Values are mean with their standard error, $n = 3$ replicate tanks. Labelled means without a common letter differ, $P < 0{\cdot}05$ (one-factor ANOVA, Duncan's *post hoc* test). ▭, S-S, sodium-selenite; ▭, S-Y, seleno-yeast; ■, S-M, Seleno-methionine.

from possible experimental differences, some of these features may imply a unique fish selenogenome derived by an evolution divergence from the mammalian species. This study indicated that dietary Se sources influenced mRNA expression of SELENOP and SELENOT in eight tissues, indicating that SELENOP and SELENOT seem to be more sensitive to dietary Se sources. SELENOP is primarily an Se transporter glycoprotein and helps sustain Se export to other tissues for proteins critical for normal metabolism[2]. SELENOT exerts a key redox function that controls protein processing in the ER, allowing cells to cope with oxidative stress and to ensure ER homoeostasis[6,40]. On the other hand, this study also indicated the lack of correlation of mRNA levels with GPx/TXNRD enzymes activities under different Se sources, suggesting that GPx/TXNRD regulation was not under transcription control, as suggested by other studies[41,42].

The protein levels are more relevant to physiological functions or metabolic phenotypes. In the present study, we identified appropriate antibodies from multiple sources to assay four selenoproteins (SELENOF, SELENOM, SELENOO and SELENON). These selenoproteins are localised to the ER and predominantly contribute to the Ca ion signalling, the protein folding and ER-associated degradation[6,37]. The present study indicated that three diets with different Se sources significantly influenced the protein expression of SELENOF in the heart, the spleen and the kidney; SELENOM in the heart, the spleen and the intestine; SELENOS in the brain, the spleen and the kidney and SELENON in the heart and brain, the kidney and intestine. Thus, three diets with different Se sources probably influenced the regulation of ER homoeostasis, which resulted in the changes of physiological function. This study indicated that changes at the protein expression were not generally parallel with their mRNA changes, in agreement with other studies[15]. The inconsistent abundances of their mRNA and protein across the tissues may result from complicated mechanisms regulating

the following processes: transcription, mRNA decay, translation, amino acid properties and protein degradation[43,44].

In summary, S-S and S-M seem to be optimal Se sources for improving growth performance and feed utilisation of yellow catfish. Three diets with different Se sources differentially influence the expression of selenoproteins in various tissues of yellow catfish. For the first time, we determined the expression of selenoproteins in fish in responses to dietary Se sources, which contributes to a better understanding of the functions and regulatory mechanisms of selenoporteins. Further investigation remained to elucidate the mechanism for the differences in their expression, which could provide important new insights into the molecular mechanisms of metabolic activation and selenoprotein function.

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The authors' responsibilities were as follows: Z. L. and X.-J. X. designed the experiments; X.-J. X. carried out animal and sample analysis with the help of D.-G. Z., T. Z. and Y-H X.; X.-J. X. and Z. L. analysed data; X.-J. X.: wrote the manuscript and Z. L. revised the manuscript; X.-J. X. and Z. L. had primary responsibility for the final content; and all the authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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