Effects of dietary glutathione on the growth performance, skin mucus antioxidant capacity, and immune responses of juvenile taimen *Hucho taimen*

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Abstract
A 49-day feeding trial was conducted to examine the effects of dietary glutathione on growth performance, skin antioxidant capacity, immune responses, and gene expression in juvenile taimen (*Hucho taimen*). The experimental fish had a mean initial weight of 5.36±0.13 g and were fed diets (approximately crude protein 460 g/kg, gross energy 22 MJ/kg) containing graded amounts of glutathione (0–control, 100, 200, 400, and 800 mg/kg). Each group had 3 replicates with 500 fish per tank (500 L). Fish were fed four times daily until evident satiation. After the feeding trial, 60 fish from each tank were challenged with lipopolysaccharide for 7 days, and the skin mucus antibacterial activity, antioxidant ability, and skin gene expression were also determined. The weight gain (WG, 3642.33–3843.67 g) and survival rate (SR, 93.07–94.19%) were all significantly improved in fish fed dietary glutathione (100–800 mg/kg) compared with the control group (WG, 3414.30 g; SR, 90.61%) (*P* < 0.05). After a 7-day lipopolysaccharide (3.00 mg/kg body weight) challenge, compared with the control group, dietary glutathione (100–800 mg/kg) significantly increased skin mucus protein, lysozyme activity (LZM, 2.67–2.74 U/mg; control, 2.11 U/mg), alkaline phosphate activity (ALP, 5.09–5.34 U/L; control, 4.50 U/L), minimal inhibitory concentration (MIC, 100–200 μL/mL; control, > 200 μL/mL), and antimicrobial activity (7.72–9.11 mm; control, 7.19 mm) (*P* < 0.05). Furthermore, dietary glutathione (100–800 mg/kg) also improved skin mucus antioxidant capacity and superoxide dismutase (SOD, 9.45–9.52 U/L; control, 7.51 U/L), and glutathione peroxidase (GSH-px, 9.68–10.11 U/L; control, 8.68 U/L) was significantly higher (*P* < 0.05), whereas the malondialdehyde (MDA, 0.18–0.20 nmol/L; control, 0.23 nmol/L) concentration was lower (*P* < 0.05). Additionally, dietary glutathione (100–800 mg/kg) significantly decreased the expression levels of skin tumor necrosis factor (*TNF-α*), interleukin 1β (*IL-1β*), interleukin 6 (*IL-6*), and interleukin 8 (*IL-8*), but increased the expression levels of interleukin 10 (*IL-10*) and nuclear factor-kappa-B inhibitor alpha-like (*IκBα*). In conclusion, dietary glutathione (100–800 mg/kg) improved the growth performance and decreased the lipopolysaccharide-induced skin inflammatory response, indicating that glutathione has the potential for anti-inflammatory effects on juvenile *H. taimen*.

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**Keywords**  Glutathione · Feed additives · Skin inflammation · Gene expression · *Hucho taimen*

**Introduction**

In intensive aquaculture, fish may be subjected to a variety of environmental factors, such as high temperature, overcrowding, poor water quality, and pathogens, that act as stressors (Barton 2002). This can result in immunosuppression, increased susceptibility to different diseases, and significant economic losses. The extensive use of disinfectants and antibiotics to counteract this can give undesirable effects such as the development of bacterial resistance and the presence of residues in aquatic products (Cabello 2006). As a result, nutritional approaches have been suggested as ways to enhance the stress resistance and immune responses of farmed fish.

Glutathione, a tripeptide consisting of glutamate, cysteine, and glycine residues (Rahman and MacNee 2000), can protect organisms from toxicity and disease as well as oxidative damage (Doyotte et al. 1997; Ponsoda et al. 1999; Will et al. 1999; Raghunathan et al. 2007). Studies on fish have revealed that glutathione can improve growth, antioxidant capacity, and immunity in species such as European seabass (*Dicentrarchus labrax*) (Zambonino-Infante et al. 1997), Nile tilapia (*Oreochromis niloticus*) (Zhou et al. 2013), grass carp (*Ctenopharyngodon idella*) (Ming et al. 2019), and Atlantic salmon (*Salmo salar*) (Ma et al. 2019). However, reports regarding the effects of glutathione on fish immune responses are still scarce.

Skin mucus, a key component of fish immunity, contains both adaptive and innate immune components such as lysozymes (LZM), proteases, immunoglobulins, complement and C-reactive proteins, lectins, and proteolytic enzymes (Subramanian et al. 2007). Due to the protective role of fish skin mucus as the first line of defense against environmental stressors and pathogens, any improvement in the mucosal immunity obtained through dietary supplementation can be considered of interest. For instance, previous research has shown that skin mucus immune reactions were improved in rainbow trout (*Oncorhynchus mykiss*) (Taee et al. 2017), barb (*Puntius sophore*) (Patel et al. 2020), and Nile tilapia (*Oreochromis niloticus*) (Zhou et al. 2013), grass carp (*Ctenopharyngodon idella*) (Ming et al. 2019), and Atlantic salmon (*Salmo salar*) (Ma et al. 2019). However, reports regarding the effects of glutathione on fish immune responses are still scarce.

Taimen (*Hucho taimen*) is a promising salmonid species for aquaculture in China. However, *H. taimen* is vulnerable to adverse environmental stress such as excess ultraviolet radiation, resulting in skin ulcers in summer, which can be a significant hindrance to farming efficiency. Therefore, this study was conducted to investigate the possible role of glutathione in modulating the growth performance, skin mucus antioxidant capacity, immune responses, and skin gene expression of juvenile *H. taimen*.

**Materials and methods**

**Diet preparation**

Experimental diets with five glutathione supplementation levels of 0 (G1, control), 100 (G2), 200 (G3), 400 (G4), and 800 (G5) mg/kg were prepared. According to spectrophotometric analysis, dietary glutathione concentrations were 0.25 (G1), 98.35 (G2), 201.36
(G3), 398.66 (G4), and 796.98 (G5) mg/kg diets, respectively. The key protein sources in the experimental diets were casein and gelatin, which provided 459.6–460.2 g/kg of dietary protein. Also, 93.3–94.8 g/kg of dietary lipid was supplied by fish oil. The ingredient composition and proximate analysis of the diets are shown in Table 1.

Diets were produced as follows: the ingredients were finely ground (<250 µm) before being blended with vitamins and minerals. All the ingredients were thoroughly mixed for 15 min and mixed again for 10 min after the addition of the lipid source. The resultant dough was made into feed pellets with a diameter of 1.2 mm using a small-scale extruder (G250, Machine Factory of Muyang, China) and dried in a ventilated oven at 45 °C for approximately 12 h. The pellets were then frozen at −20 °C until required.

The proximate composition of the diets was determined using the methods described by AOAC (2012). Crude protein (N × 6.25) was measured using the Kjeldahl method (KDN-102C Autoanalyzer, Xianjian, China). The ether-extraction process was used to measure crude lipid with the Soxtec System (SXT-06-analyzer, Hongji, China). Moisture was measured by oven drying for 6 h at 105 °C. Ash was placed in a muffle furnace.

### Table 1  Formulation and chemical proximate composition of the experimental diets

| Ingredients (g/kg)                        | Dietary glutathione level |
|------------------------------------------|---------------------------|
|                                          | Control  | 100    | 200    | 400    | 800    |
| Gelatin 1                                | 50       | 50     | 50     | 50     | 50     |
| Casein 2                                 | 450      | 450    | 450    | 450    | 450    |
| Dextrin                                  | 250      | 250    | 250    | 250    | 250    |
| Fish oil 3                               | 90       | 90     | 90     | 90     | 90     |
| Starch                                   | 120.5    | 120.5  | 120.5  | 120.5  | 120.5  |
| Calcium dihydrogen phosphate (Ca(H2PO4)2·H2O) | 20       | 20     | 20     | 20     | 20     |
| Butylated hydroxytoluene (BHT)           | 0.5      | 0.5    | 0.5    | 0.5    | 0.5    |
| Vitamin premix 4                         | 3        | 3      | 3      | 3      | 3      |
| Mineral premix 5                         | 6        | 6      | 6      | 6      | 6      |
| Glycine                                  | 10       | 9.9    | 9.8    | 9.6    | 9.2    |
| Glutathione (mg/kg)                      | 0        | 100    | 200    | 400    | 800    |
| Nutrient levels (measured value)         |          |        |        |        |        |
| Glutathione (mg/kg)                      | 0.25     | 98.35  | 201.36 | 398.66 | 796.98 |
| Moisture                                 | 81.6     | 82.3   | 80.9   | 82.1   | 81.8   |
| Crude protein                            | 460.2    | 459.6  | 461.1  | 460.2  | 460.5  |
| Crude lipid                              | 94.5     | 94.8   | 93.3   | 94.2   | 93.6   |
| Crude ash                                | 24.65    | 24.66  | 24.32  | 24.61  | 24.22  |
| Gross energy (MJ/kg)                     | 22.23    | 22.16  | 22.09  | 22.14  | 22.06  |

1Zhiyuan Chemical Agent Company, Tianjin, China
2Vitamin-free casein, Sigma, America
3Huludao Chia Tai Feed Corporation, Huludao, China
4Vitamin premix (mg/kg): alpha-tocopherol 100, ascorbic acid 200, retinol acetate 5.2, menadione sodium bisulfate 5, thiamin 25, cholecalciferol 0.07, pyridoxine 25, riboflavin 40, nicotinic acid 275, cyanocobalamin 0.8, biotin 5, folic acid 8, pantothenic acid 100
5Mineral premix (mg/kg): KCl 1500, MgSO4·7H2O 2000, CuSO4·5H2O 20, FeSO4·7H2O 1000, ZnSO4·7H2O 150, MnSO4·4H2O 100, NaCl 500, KI 3, Na2SeO3 3, CoCl2 5
at 550 °C for 12 h. The energy content of the diet was determined using bomb calorimetry (XRY-1A, Jingmi, China). Spectrophotometric kits (cat. no. A061-1) were purchased from the Chinese Nanjing Jiancheng Institute of Bioengineering and used to analyze dietary glutathione concentration according to the manufacturer’s instructions.

**Experimental procedures**

*H. taimen* juveniles were provided by the Bohai Coldwater Fish Research Station of Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, China. The fish were cultured in flow-through aquaria supplied by spring water (flow rate, 1.0 L/s). After disinfection with 2 g/kg salt for 10 min, the fish were stocked in 15 cylindrical tanks (water volume, 500 L) for 14 days at a stocking density of 600 fish per tank. During this period, fish were fed the G1 diet four times per day (08:00, 11:00, 14:00, and 17:00).

Before the feeding trial, the fish were starved for 24 h. The fish from each tank were weighed as a batch. Fish (initial weight 5.36 ± 0.13 g) were randomly distributed into another 15 tanks (water volume, 500 L) with 500 juveniles per tank. Each experimental diet was randomly assigned to 3 tanks, and the fish were hand-fed to apparent satiation four times daily (08:00, 11:00, 14:00, and 17:00). After feeding for 1 h, all the feed residues were collected, dried, weighed, and recorded. Water temperature (11.3–15.8 °C), pH (7.2–7.5), dissolved oxygen (7.6–9.6 mg/L), and ammoniacal nitrogen (<0.2 mg/L) in the tanks were monitored daily using a YSI 556MPS multi-probe field meter (Geo-tech, USA). The dead fish were also recorded daily to calculate the feed conversion rate (FCR). This feeding trial lasted for 49 days.

**Growth and survival**

After a 24-h starvation period at the end of the feeding trial, fish in each tank were counted to obtain the survival (SR) and weighed as a batch to estimate the FCR. The fork length and body weight of a random sample of 30 fish from each tank were determined to calculate the condition factor (CF).

The variables were calculated as follows:

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\text{Weight gain (WG, g)} = (\text{final tank biomass, g}) - (\text{initial tank biomass, g}); \quad \text{FCR} = (\text{dry dietary intake, g}) / (\text{weight gain, g}); \quad \text{SR (％)} = 100 \times (\text{final amount of fish}) / (\text{initial amount of fish}); \quad \text{CF} = 100 \times [(\text{body weight, g}) / (\text{total length, cm})^3].
\]

**Challenge test**

After the 49-day feeding trial, 60 fish from each tank were challenged for 7 days by intraperitoneally injecting 100 μL of lipopolysaccharide (3.00 mg/kg of body weight, Sigma, USA) diluted in sterile phosphate buffer (Haukenes and Barton 2004). The challenged fish were placed in their respective tanks to obtain skin or skin mucus samples for immunity determination.
Skin gene expression

After starvation for 24 h, the 3 skin specimens were randomly selected from each tank. The skin was removed from the fish’s left side and spanned 0.2 cm ventrally from the mid-dorsal line. RNAiso Plus Reagent (TaKaRa, Dalian, China) was used to extract total RNA from the skin (approximately 80 mg) according to the manufacturer’s instructions. A spectrophotometer was used to examine the absorbance at 260 nm to determine the RNA concentration. Using agarose gel electrophoresis, the RNA integrity was determined, and the absorbance ratio at A260 nm/A280 nm ranged from 1.8 to 2.0. Tumor necrosis factor (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), nuclear factor-kappa-B inhibitor alpha-like (IκBα), mucins 2 (MUC-2), Claudin-1, zonula occludins 1 (ZO-1), and Occludin expression levels were determined using quantitative real-time PCR (ABI 7500, USA) with a reaction volume of 20 µL, including 10 µL of SYBR® Premix Ex Taq (TaKaRa, Dalian, China), and 0.8 µL for quantitative real-time PCR. Specific primers were constructed based on sequences cloned and published in the O. mykiss gene bank (Table 2). The cycling conditions were 95 °C for 30 s followed by 35 cycles of 95 °C for 5 s, 59 °C for 10 s, and 72 °C for 30 s. The housekeeping gene (β-actin) was chosen as a reference gene to normalize the results. 2^{-ΔΔCt} was used to measure the expression values.

Table 2  Primers used for quantitative real-time PCR

| Genes   | Forward primer sequences (5′–3′) | Reverse primer sequences (5′–3′) | PCR fragment length (bp) | Accession number |
|---------|---------------------------------|---------------------------------|--------------------------|-----------------|
| TNF-α   | GTACCAGACGCTGCTCAACT           | CAGGCTAAACACTGCTTCCCA        | 115                      | XM_020468340.2  |
| IL-1β   | CCGTGGACATGTGTCAGAAA          | GTGTCACAGTGTTTGCAGACC       | 116                      | XM_020475860.2  |
| IL-6    | CTCCATCCACACACACCTCTC        | ACACCTCGACACATGACTG         | 96                       | XM_020507339.2  |
| IL-8    | TGAGACGGAGACGCAGACGTAA       | AGGGCTGACATCCACACAA         | 136                      | XM_020475139.2  |
| IL-10   | GTCACTGAGAGGACGTCCG          | ATAGCGTACACACCCGATGG        | 134                      | XM_031825856.1  |
| IκBα    | AAGTTGGCTGCATCACATGGGT       | CCAGACTTCCACATGCGG          | 184                      | XM_020454854.2  |
| MUC2    | CTCAGGTTGTTAGGTGGAGGA        | CCAGCCTCTCTCCTCTCTGTC       | 87                       | XM_029752056.1  |
| Claudin-1 | GGACAACATCATCACAGGCGCC    | CTGACACTCCCATCCCGGTATGGTT  | 124                      | XM_029726273.1  |
| ZO-1    | CCGCTTACGCCTACTGTAGG         | GGTGTCGTAGTCGAACTGGA        | 97                       | XM_029757389.1  |
| Occludin | GCAGGAACTGTTGGAGACGCACTACA | TCCCTCCTGCAGATCGATC          | 133                      | XM_029709697.1  |
| β-actin | CTCTGCTGATGTTGTTGCCACC      | CGATGTCACGACGATTTCCCC       | 176                      | XM_044191757.1  |

*β-actin gene as a housekeeping gene was used as an internal reference
Skin mucus sampling

Following the collection of skin samples, the other fish in each tank were anesthetized with a 5 mg/L clove oil solution before the collection of skin mucus, as described by Ross et al. (2000). Afterward, individuals were transferred into polyethylene bags containing 10 mL of 50 mmol sodium chloride (NaCl). After 2 min, the skin mucus was collected, placed in a sterile tube (15 mL), and centrifuged for 10 min at 1500 $\times g$ and 4 °C. After that, the surfactant was divided into two portions: half was measured for skin mucus protein and enzyme activity, and the other half for antibacterial activity. All surfactants were stored at 80 °C for further analysis.

Skin mucus protein and enzyme activity

For skin mucus from each treatment ($n=3$), the total mucus protein was determined using the method of Lowry et al. (1951). The standard was bovine serum albumin, and the spectrophotometer absorbance was determined at 750 nm. Spectrophotometric kits were bought from the Chinese Nanjing Jiancheng Institute of Bioengineering and were used to analyze the malonaldehyde content (MDA, cat. no.A003-1), and the activity of the enzymes: LZM (cat. no.A059-2), alkaline phosphatase (ALP, cat. no.A059-2), glutathione peroxidase (GSH-px, cat. no.A006-2), superoxide dismutase (SOD, cat. no.A001-1), and catalase (CAT, cat. no.A007-1).

Skin mucus antibacterial activity

Two bacterial strains (Yersinia ruckeri and Aeromonas hydrophila) were used to test the antimicrobial properties of skin mucus from each treatment ($n=3$). A traditional disc diffusion approach was used for assessing antimicrobial properties (Hellio et al. 2002). In brief, the bacteria were cultivated for 24 h at 37 °C, and then 0.1 mL of each broth culture medium ($1.5 \times 10^8$ CFU/mL) was cultured on nutrient agar. Paper discs (6 mm in diameter) were impregnated with 100 μL of the skin mucus samples, mounted on the medium, and incubated for 24 h at 37 °C. The diameter of the growth inhibition region was measured using a ruler. Antimicrobial activity was indicated by a clear zone around the disks. The visual observation method was used to determine the minimum inhibitory concentration (MIC) of the mucus samples (Hellio et al. 2002).

Data statistical and analysis

After normality and homogeneity checking, one-way variance analysis (ANOVA) and Duncan’s multiple range test were used to examine the data. $P$ values $<0.05$ were considered significantly different. The SPSS statistical package 23.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The GraphPad Prism software version 9.0 is used to draw column graphs.
Results

Growth performance

The WG (3642.33–3843.67 g) and SR (93.07%–94.19%) were all significantly improved in fish fed glutathione (100–800 mg/kg) compared with the control group ($P < 0.05$) (Table 3). However, there were no differences in the FCR and CF among the groups ($P > 0.05$). After a 7-day challenge with lipopolysaccharide, there was no death, indicating that the doses used were safe for determining immune responses.

Skin immunity

The skin mucus antimicrobial activities are presented in Tables 4–5. Compared with the control group, the skin mucus MIC was significantly lower in the dietary glutathione (100–800 mg/kg) supplementation groups ($P < 0.05$), indicating significant

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**Table 3** Growth performances of *H. taimen* fed the experimental diets

| Indices       | Dietary glutathione level |
|---------------|---------------------------|
|               | Control 100 200 400 800   |
| IBW (g)       | 5.33±0.12 5.40±0.10 5.38±0.23 5.29±0.11 5.38±0.16 |
| ITB (g)       | 2666.67±62.12 2698.33±49.07 2688.33±115.4 2645.00±52.68 2691.67±77.51 |
| FBW (g)       | 12.76±0.35 13.17±0.95 12.87±0.51 13.34±0.17 13.48±0.08 |
| FTB (g)       | 6080.97±170.54 6340.67±59.28 6325.67±75.12 6454.67±96.54 6535.33±71.50 |
| WG (g)        | 3414.30±108.59 3642.33±92.35 3637.33±40.53 3809.67±46.80 3843.67±50.12 |
| CF            | 0.66±0.02 0.68±0.03 0.70±0.04 0.69±0.01 0.68±0.05 |
| FCR           | 1.12±0.01 1.15±0.06 1.11±0.02 1.16±0.05 1.12±0.01 |
| SR (%)        | 90.61±0.61 93.07±1.25 93.53±0.38 93.82±0.27 94.19±0.87 |

Values are means ± S.D. of three replicates ($n = 3$). Data in a row assigned different letters to denote significant differences ($P < 0.05$).

$^{1}$IBW, initial body weight

$^{2}$ITB, initial tank biomass

$^{3}$FBW, final body weight

$^{4}$FTB, final tank biomass

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**Table 4** The antibacterial activity of the skin mucus (growth inhibition zone diameter [mm]) of *H. taimen*

| Dietary glutathione level | Control 100 200 400 800 |
|---------------------------|---------------------------|
| *A. hydrophila*           |                           |
|                           | 7.19±0.11                 |
|                           | 7.82±0.65                 |
|                           | 9.11±0.15                 |
|                           | 9.04±0.22                 |
|                           | 9.01±0.20                 |
| *Y. ruckeri*              |                           |
|                           | 6.32±0.35                 |
|                           | 7.93±0.08                 |
|                           | 7.86±0.09                 |
|                           | 7.84±0.22                 |
|                           | 7.72±0.11                 |

Values are means ± S.D. of three replicates ($n = 3$). Data in a row assigned different letters to denote significant differences ($P < 0.05$).
improvements in antibacterial activity against *Y. ruckeri* and *A. hydrophila* through dietary glutathione supplementation.

Regarding the mucus protein content and enzyme activity, the amount of skin mucus protein and the ALP and LZM activity in the treatment groups (100–800 mg/kg glutathione supplementation) were higher than in the control group. These findings suggest a positive effect of dietary glutathione supplementation on skin mucus protein content, ALP, and LZM activity (*P* < 0.05) (Table 6; Fig. 1).

### Table 6  Skin mucus parameters of *H. taimen* fed the experimental diets

| Indices   | Dietary glutathione level | Control  | 100   | 200   | 400   | 800   |
|-----------|---------------------------|----------|-------|-------|-------|-------|
| SOD (U/L) |                           |          |       |       |       |       |
|           |                           | 7.51 ± 1.10a | 9.45 ± 0.07b | 9.51 ± 0.27b | 9.52 ± 0.11b | 9.49 ± 0.02b |
| CAT (U/L) |                           | 0.05 ± 0.01a | 0.08 ± 0.02ab | 0.10 ± 0.02b | 0.10 ± 0.01b | 0.10 ± 0.02b |
| GSH-px (U/L) |                        | 8.68 ± 0.50a | 9.68 ± 0.49b | 9.96 ± 0.02b | 9.91 ± 0.12b | 10.11 ± 0.08b |
| MDA (nmol/L) |                        | 0.23 ± 0.01b | 0.18 ± 0.01a | 0.19 ± 0.01a | 0.20 ± 0.01a | 0.20 ± 0.02a |
| ALP (U/L)  |                           | 4.50 ± 0.43a | 5.09 ± 0.14b | 5.34 ± 0.19b | 5.29 ± 0.09b | 5.27 ± 0.07b |
| LZM (U/mg) |                           | 2.11 ± 0.06a | 2.74 ± 0.11c | 2.47 ± 0.16b | 2.65 ± 0.18bc | 2.67 ± 0.08bc |

Values are means ± S.D. of three replicates (*n* = 3). Data in a row assigned different letters to denote significant differences (*P* < 0.05).

**Fig. 1**  The skin mucus total protein levels (mg/mL) of juvenile *H. taimen* fed a diet supplemented with different levels of glutathione. Values are means ± S.D. of three replicates (*n* = 3). Data in a row assigned different letters to denote significant differences (*P* < 0.05).
Antioxidant capacity

The skin mucus in the treatment groups (100–800 mg/kg glutathione supplementation) exhibited significant antioxidant activity (Table 6). In contrast to that in the control group, the skin mucus of the treatment groups showed higher SOD and GSH-px activity; the CAT activity from the 200 and 800 mg/kg glutathione supplementation treatments was also significantly higher ($P<0.05$). The MDA concentration was reduced by dietary glutathione supplementation (100–800 mg/kg) ($P<0.05$).

Skin gene expression

Skin TNF-$\alpha$, IL-$1\beta$, IL-$6$, and IL-$8$ gene expression were significantly lower in fish fed 400–800 mg/kg glutathione compared with the control group, whereas IL-$10$ expression increased ($P<0.05$) (Fig. 2). When dietary glutathione levels were increased to 200 mg/kg, a significant increase in IκBα was observed ($P<0.05$) (Fig. 2). There were no significant differences in the MUC-2, Claudin-1, Occludin, and ZO-1 gene expression ($P>0.05$) (Fig. 3).
Discussion

Growth performance

In this study, the fish that were fed a semi-purified glutathione diet showed an increase in WG and SR. Similar results were reported for *C. idella* (Ming et al. 2019) and *S. salar* (Ma et al. 2019). This may be related to glutathione being involved in the transfer of amino acids in vivo and in raising the gene mRNA expression levels (IGF-1, etc.) in fish cells to promote development (Ming et al. 2015). Moreover, dietary glutathione functions as an antioxidant, enhancing antioxidant capacity, immunity, and antistress processes, keeping the body healthy and finally leading to a rise in growth performance (Zhou et al. 2013). In addition, previous studies on *S. salar* (Ma et al. 2019), *C. idella* (Ming et al. 2015), and *O. niloticus* (Zhou et al. 2013) showed that excess glutathione in the diet has a negative influence on growth performance. Excess glutathione in the body can react with halogenated olefins and anthraquinones, causing cytotoxicity (Monks et al. 1990). Meanwhile, excess glutathione may induce DNA damage and oxidative stress (Thomas et al. 1998). In the present study, there were no significant changes in the fish fed 400–800 mg/kg dietary glutathione. Inconsistent findings on the impact of glutathione in this study may be attributed to differences in species, size, dosage, and types of studies.

Skin immunity

Previous studies have shown that skin mucus components in species such as *O. mykiss* (Sheikhzadeh et al. 2011), common carp (*Cyprinus carpio*) (Hoseinifar et al. 2019), and Caspian white fish (*Rutilus frisii kutum*) (Hoseinifar et al. 2014) can be improved by including immunostimulants in the diet. The antibacterial skin mucus activity of probiotic-fed black swordtail (*Xiphophorus helleri*) was also improved (*P* < 0.05) (Hoseinifar et al. 2015). In this study, an improvement in skin mucus antibacterial activity against *Y. ruckeri* and *A. hydrophila* was detected in association with dietary glutathione supplementation. The possible underlying antimicrobial mechanism is that glutathione has a structure similar to the *Penicillium* antibiotic precursors, which act as inhibitors of the D-alanyl-D-alanine-carboxypeptidase enzyme (DacC) that catalyzes the conversion of glycopeptides (D-alanyl-alanine) to peptidoglycan in cell walls (Mustikaningtyas et al. 2021).

In salmonids, skin mucus protein is known to increase the level of a protein involved in oxidative defense, motility, and general stress responses (Ræder et al. 2007). In the present study, the high level of skin mucus protein was associated with dietary glutathione supplementation. Meanwhile, the activity of skin mucus LZM and ALP in treatment groups was also affected (*P* < 0.05). Similar to *O. mykiss* (Sheikhzadeh et al. 2011) and *R. frisii kutum* (Hoseinifar et al. 2014), the increased levels of skin mucus protein, LZM, and ALP in *H. taimen* suggest that dietary glutathione may trigger such an innate immune response (Biller and Takahashi 2018).

Skin antioxidant capacity

The antioxidant enzymes such as SOD, CAT, and GSH-px are stimulated as a cellular defense mechanism and are a significant safety mechanism for cellular oxidative damage reduction (Tabrez and Ahmad 2009). In the present study, the skin mucus of juvenile *H. taimen* fed dietary glutathione demonstrated an improved antioxidant ability, and the
skin mucus antioxidant reaction was obvious in the treatment groups that received dietary glutathione levels of 100–800 mg/kg \((P < 0.05)\). MDA is an oxidation metabolite in the body that reflects tissue and cell damage caused by free radicals (Seifried et al. 2007). A lower MDA content and increased SOD activity in skin mucus from the treatment groups in the present study suggest that dietary glutathione has a positive effect on \(H. \text{ taimen}'s\) antioxidant capacity under the combined activity of an enzymatic and nonenzymatic antioxidant system. Furthermore, a dietary glutathione level of 800 mg/kg in this study did not affect the body’s antioxidant metabolism balance, and the mechanism by which excess glutathione reduces \(H. \text{ taimen}'s\) antioxidant ability needs further investigation.

**Skin gene expression**

Pro-inflammatory cytokines including \(\text{TNF-}\alpha, \text{IL-}\ 1\beta, \text{IL-}\ 6, \text{IL-}\ 8,\) and \(\text{IL-10}\) are involved in the initiation and regulation of inflammatory responses (Neurath 2014). In our study, after the 7-day lipopolysaccharide challenge, skin \(\text{TNF-}\alpha, \text{IL-}\ 1\beta, \text{IL-}\ 6,\) and \(\text{IL-8}\) gene expression showed a significant decrease by dietary glutathione supplementation (100–800 mg/kg), whereas \(\text{IL-10}\) expression showed the opposite trend. These suggested that dietary glutathione had the potential to reduce inflammation. Similar results were found in the study of \(C. \text{ carpio}\) (Hoseinifar et al. 2019). Moreover, dietary glutathione inhibited the breakdown of \(\text{IkBa}\). The nuclear factor kappa B (\(\text{NF-}\alpha\text{B}\)) signalling pathway is important in the regulation of non-specific immunity. \(\text{IkB}\) is a critical component of the \(\text{NF-}\alpha\text{B}\) complex, and its breakdown can cause the production of pro-inflammatory cytokines (DiDonato et al. 2012). Therefore, these findings suggest that the anti-inflammatory effects of dietary glutathione in the skin may be due to its suppression of the \(\text{TNF-}\alpha/\text{NF-}\alpha\text{B}\) signalling system.

Tight junctions, which are made up of the membrane proteins claudins, occludin, and cyclin ZOs, are the primary connectors between mucosal epithelial cells and perform critical functions in maintaining the mechanical integrity and the mucosal barrier function (Pummi et al. 2004). Pro-inflammatory factors such as \(\text{TNF-}\alpha, \text{IL-}\ 1\beta, \text{IL-}\ 6,\) and \(\text{IL-8}\) play a key role in triggering the chain reaction of tight junction damage (Al-Sadi et al. 2013). In this study, the \(\text{Claudin-1, Occludin, and ZO-1}\) gene expression levels had no significant differences among the treatment groups. This implies that dietary glutathione supplementation may not impact the expression activity of skin mucosal cell tight junction structural protein genes. Therefore, dietary glutathione supplementation may affect the skin by mainly decreasing the production of inflammatory cytokines rather than tight junctions.

**Conclusion**

In conclusion, dietary glutathione (100–800 mg/kg) improved the growth performance and protected the skin of juvenile \(H. \text{ taimen}\) from oxidative damage by enhancing SOD, GSH-Px, and CAT activities. Skin non-specific immunity was also enhanced by mucus protein, LZM, ALP, and antimicrobial activity. Moreover, dietary glutathione may act against skin damage mainly through the \(\text{TNF-}\alpha/\text{NF-}\alpha\text{B}\) signaling pathway due to significantly upregulated \(\text{IkBa}\) and \(\text{IL-10}\) expression levels while downregulating \(\text{TNF-}\alpha, \text{IL1}\beta, \text{IL-}\ 6,\) and \(\text{IL-8}\) expression levels. These results showed that glutathione may be utilized as a functional component in fish feed to protect against skin damage in juvenile \(H. \text{ taimen}\).
Author contribution Chang’an Wang designed the study and wrote the manuscript. Xiaoxia Lu assisted with sampling. Ze Fan tested the samples and analyzed the results. Haibo Jiang analyzed the results. Shicheng Han carried out the rearing work. Yang Liu assisted with sampling. Hongbai Liu provided guidance. All the authors read and approved the final manuscript.

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Data availability The data will be provided upon direct request to the authors.

Code availability Not applicable for that section.

Declarations

Ethics approval The study was approved by the ethics committee on animal use of the Protection of Animal Health and the Instructions for the Granting of Permits for Scientific Experimental Animals (Ethics approval number: SCXK (YU) 2005–0001).

Consent to participate All authors agreed to participate in this research.

Consent for publication All authors agreed to publish this paper.

Conflict of interest The authors declare no competing interests.

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