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Original Research Article

Dietary geniposide supplementation could enhance hepatic lipid metabolism, immunity, antioxidant capacity, and ammonia stress resistance in turbot (*Scophthalmus maximus*)

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ABSTRACT

This study aimed to evaluate the effects of dietary geniposide supplementation on growth performance, lipid metabolism, health status, and ammonia stress resistance in turbot (*Scophthalmus maximus*). Four hundred fifty fish were randomly allocated into 5 treatments with triplicate tanks (30 fish per tank). They were hand-fed to apparent satiety for 56 d with a basal diet (GP0) or diets containing 100, 200, 400, and 800 mg/kg geniposide (termed as GP100, GP200, GP400, GP800, respectively). After the conclusion of the feeding trial, the fish were exposed to ammonia stress for 96 h. The results showed that the growth performance were not affected by geniposide ($P > 0.05$). Dietary supplementation with geniposide decreased crude lipid in viscera without liver, subcutaneous adipose tissue (SAT), and the liver, as well as triglyceride concentrations in plasma, the liver and SAT ($P < 0.05$). Dietary supplementation with 400 and 800 mg/kg geniposide significantly down-regulated lipogenesis-related gene expression, as well as fatty acid uptake-related gene expression, while significantly up-regulated triglyceride secretion-related gene expression in the liver compared with the control group ($P < 0.05$). The GP800 group exhibited a significant reduction in plasma malondialdehyde contents compared with the control group, while both the GP200 and GP800 groups showed a significant increase in plasma complement C3 activities ($P < 0.05$). Furthermore, there was a notable enhancement in plasma lysozyme and total superoxide dismutase levels in the geniposide supplemented groups compared to the control group ($P < 0.05$). Additionally, a significant decrease in the mRNA level of pro-inflammatory cytokine and a remarkable increase in the mRNA expression of anti-inflammatory cytokines were discovered in geniposide supplemented groups relative to the control group ($P < 0.05$). Cumulative survival rates after ammonia stress in the GP400 and GP800 groups were statistically higher than that in the control group ($P < 0.05$). In conclusion, dietary geniposide supplementation could reduce lipid deposition in turbot by regulating lipid metabolism and transportation, and remarkably enhance immunity, antioxidant ability, and resistance to ammonia stress in turbot. Based on the quadratic regression analyses, the optimal concentrations of geniposide were estimated to be 545.21 to 668.41 mg/kg feed.

1. Introduction

Turbot (*Scophthalmus maximus*) is a highly welcomed marine fish for human consumption due to its high-quality muscular protein and skin collagen, as well as flavorful taste (Zou et al., 2019). In recent years, the aquaculture production of this marine flatfish has mainly occurred in Europe and China, with a total output reaching 59,615 tonnes (FAO, 2020). However, turbot has been

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increasingly exposed to various stressors during aquaculture practices, such as deteriorating water quality and extreme temperature fluctuations (Sun et al., 2022). Amongst these stressors, ammonia nitrogen concentration is often high in the intensive culture system of turbot farms due to the use of high-protein diets, high stocking density, and overfeeding (Foss et al., 2007). These factors may lead to a range of abnormal physiological responses and ultimately compromise the health status of the fish. Consequently, these stressors make fish highly vulnerable to additional stressors or pathogenic microorganisms (Naylor et al., 2021). Apart from adverse environmental conditions, the utilization of high-energy feed and overfeeding management are commonly practiced in some turbot farms, leading to potential lipid over-accumulation in fish bodies and compromised immune and antioxidant capacities (Jia et al., 2020; Jin et al., 2019), thereby increasing the risk of diseases (Naiel et al., 2023). In the context of the global ban on antibiotic use in aquaculture (Ng and Koh, 2017), functional feed additives are increasingly being utilized to optimize the growth and health status of farmed fish (Hoseinifar et al., 2017).

Amongst diverse functional feed additives, phytogenic extracts have attracted growing attention ascribing to their bioactive compounds, safety, and eco-friendliness, as well as multifunctional benefits (Dawood et al., 2022). Geniposide, an emerging feed supplement in animal feed, is an iridoid glycoside chiefly extracted from the fruit of cape jasmine (*Gardenia jasminoides*) (Liu et al., 2022). Geniposide has practicality and feasibility in the feed industry due to its relatively high melting point (161.53 °C) (Chen et al., 2020), which exceeds the upper limit (around 120 °C) of the conditioning temperature in the manufacturing of extruded feed. From the perspective of geniposide's functionality, it plays a versatile role in not only ameliorating lipid metabolic disorders but also exerting anti-inflammatory and antioxidant effects in mammals (Shan et al., 2017). Numerous studies have corroborated that geniposide exerts suppressive effects on lipid accumulation in mammals by inhibiting lipogenesis, promoting lipolysis, and regulating lipid transportation (Gao and Feng, 2022; Ma et al., 2011). The modulatory effects of geniposide in lipid metabolism have been reported to be associated with several key transcriptional regulators, such as peroxisome proliferators-activated receptors (PPARs) and sterol regulatory element binding transcription factor-1 (SREBP-1) (Kojima et al., 2011; Nam et al., 2014). In addition, geniposide could also suppress excessive inflammatory responses in tissues by inhibiting canonical inflammatory-related pathways such as nuclear factor kappa B (NF-κB) p65, and p38 mitogen-activated protein kinase (MAPK) signaling pathways. Moreover, geniposide could protect tissues against oxidative stress by activating nuclear factor erythroid 2-related factor-2 (Nrf2) and its downstream antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Shen et al., 2020). Therefore, geniposide probably serves as a promising feed additive in aquafeeds to enhance health status due to its practicability and multifunctionality.

Until now, there are very few studies reporting the efficacy of dietary supplementation of geniposide in fish. In grass carp (*Ctenopharyngodon idella*), dietary administration of geniposide at 400 to 600 mg/kg reduced circulating triglycerides level and increased collagen content in muscle and skin (Sun et al., 2017). The application of geniposide at 100 mg/kg was proved to enhance innate immune response and disease resistance in crucian carp (*Carassius carassius*) (He et al., 2020). Previous research on fish has confirmed that geniposide has similar influences on lipid metabolism and the overall health of fish. Given the emerging challenges in turbot aquaculture and the pleiotropic effects of geniposide, this study aimed to investigate the effects of dietary supplementation of geniposide within the dosage range of 100 to 800 mg/kg on lipid metabolism, immune response, antioxidant capacity, and ammonia stress resistance in turbot. These findings would deepen our comprehension of the multi-effectiveness of dietary geniposide in fish and establish a theoretical foundation for incorporating this functional feed supplement into aquafeeds.

2. Materials and methods

2.1. Animal ethics statement

The experimental procedures implemented in the current study strictly adhered to the guidelines of the Biomedical Ethics Committee of Hebei Normal University (Shijiazhuang, Hebei), with the approved protocol code of 218,025.

2.2. Experimental diet preparation

The geniposide (purity: 99.82%) derived from the *G. jasminoides* fruit was procured from Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, China). Five doses of geniposide at 0, 100, 200, 400, and 800 mg/kg were incorporated into a normal basal diet to formulate five isonitrogenous (47.84% crude protein), isolipidic (11.25% crude lipid), and iso-energetic (20.40 MJ/kg gross energy) experimental diets (termed as GP0, GP100, GP200, GP400, and GP800), respectively, meeting the dietary macronutrients requirement in turbot according to previous review (Cui et al., 2023). The diet formula and proximate chemical composition of the five experimental diets are detailed in Table 1. All the macro-ingredients were pulverized and sieved through an 80-mesh sieve. Subsequently, all feed ingredients except fish oil were accurately weighed and thoroughly mixed in ascending order of usage amount, and then fish oil was further kneaded and blended sufficiently with the feedstuff mixture. Thereafter, a certain amount of distilled water (27% of the mixture's weight) was supplemented into the commixture in a blender to form a soft dough. The dough was pelletized using a pelletizer to produce diets with a 2-mm diameter (Youyi Machinery Factory, Shandong, China). Finally, all diets were air-dried with an electric fan at room temperature and placed in sealed plastic bags at −20 °C until use.

2.3. Fish and feeding management

A total of 500 turbot juveniles were procured from a local fish farm and transported to an aquaculture system of Ruihui Aquaculture Company in Tangshan, Hebei province. Upon their arrival, the juveniles were temporarily cultured in a seawater system to acclimatize to the rearing environment. During the acclimatization period, the fish were hand-fed to apparent satiation with the control diet twice daily (08:30, 14:30) for 2 weeks. Afterward, fish were subjected to 24-h deprivation, and 450 healthy fish with homogenous size (average body weight: 5.19 ± 0.14 g) and without deformity and symptoms were randomly selected and distributed into 15 tanks (length × height × width: 100 cm × 80 cm × 70 cm, water capacity: 400 L) with three replicas per treatment and 30 fish per tank. Each tank was equipped with an air stone for continuous aeration. Each diet was offered at random to three tanks twice a day until satiation. Any unconsumed diets were siphoned out and collected after 1 h of a meal, then dried at 105 °C in a ventilated oven to correct for the actual feed consumption. The water utilized in the aquaculture system was natural seawater filtrated through sand filters. Two-thirds of the seawater volume in each tank was renewed twice daily. Throughout the 8-week feeding trial, water temperature was monitored daily, while other water quality parameters were assessed weekly. The dissolved oxygen was

Table 1
Diets formulation and analyzed chemical compositions of experimental diets (dry matter basis, %).

Ingredients	GP0	GP100	GP200	GP400	GP800
Russian white fishmeal[1]	30.00	30.00	30.00	30.00	30.00
Chicken meal[2]	9.00	9.00	9.00	9.00	9.00
Squid liver powder[3]	5.00	5.00	5.00	5.00	5.00
Cottonseed protein concentrate[4]	8.00	8.00	8.00	8.00	8.00
Wheat gluten meal[5]	6.00	6.00	6.00	6.00	6.00
Peanut meal[6]	5.00	5.00	5.00	5.00	5.00
Extruded soybean meal[7]	3.70	3.70	3.70	3.70	3.70
Yeast powder[8]	5.00	5.00	5.00	5.00	5.00
Pregelatinized corn starch[9]	15.00	15.00	15.00	15.00	15.00
Fish oil[10]	5.50	5.50	5.50	5.50	5.50
Soybean phospholipid[11]	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate	2.50	2.50	2.50	2.50	2.50
Limestone	0.50	0.50	0.50	0.50	0.50
Potassium chloride	0.50	0.50	0.50	0.50	0.50
Mineral and vitamin premix[12]	2.00	2.00	2.00	2.00	2.00
Choline chloride	0.20	0.20	0.20	0.20	0.20
Betaine	0.10	0.10	0.10	0.10	0.10
Microcrystalline cellulose	1.00	0.99	0.98	0.96	0.92
Geniposide	0.00	0.01	0.02	0.04	0.08
Sum	100.00	100.00	100.00	100.00	100.00
Analyzed chemical composition					
Crude protein	47.71	47.70	47.94	47.81	48.07
Crude lipid	11.68	11.29	11.21	11.46	10.64
Ash	12.02	12.12	12.20	12.15	12.19
Gross energy, MJ/kg	20.33	20.39	20.45	20.49	20.40

GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively.

[1] Russian white fishmeal (66.30% CP, 7.01% crude lipid, 18.61% ash, 19.99 MJ/kg gross energy on dry matter basis): JSC, Okeanrybflot, Petropavlovsk Kamchatsky, Russia.

[2] Chicken meal (66.27% CP, 10.99% crude lipid, 12.14% ash, 22.58 MJ/kg gross energy): Fieldale Farms Corporation, USA.

[3] Squid liver powder (45.86% CP, 7.08% crude lipid, 26.04% ash, 15.71 MJ/kg gross energy): Cangzhou Haitong Bio-Feed Co. Ltd., Hebei, China.

[4] Cottonseed protein concentrate (63.34% CP, 1.20% crude lipid, 7.99% ash, 19.91 MJ/kg gross energy): Handan Chenguang Biotechnology Co. Ltd., Hebei, China.

[5] Wheat gluten meal (80.01% CP, 0.56% crude lipid, 0.87% ash, 23.95 MJ/kg gross energy): Kaifeng Shangdu Co. Ltd., Henan, China.

[6] Peanut meal (48.12% CP, 0.40% crude lipid, 6.18% ash, 17.90 MJ/kg gross energy): Shandong Luhua Group Co. Ltd., Shandong, China.

[7] Extruded soybean meal (40.95% CP, 21.36% crude lipid, 2.57% ash, 23.74 MJ/kg gross energy): Hebei Haitai Technology Co. Ltd., Hebei, China.

[8] Yeast powder (49.63% CP, 1.55% crude lipid, 6.43% ash, 19.39 MJ/kg gross energy): Hebei Ruiqi Biotechnology Co. Ltd., Hebei, China.

[9] Pregelatinized corn starch (0.90% CP, 0.31% crude lipid, 0.21% ash, 17.20 MJ/kg gross energy): Ningjin Jiahe Energy Saving Material Co. Ltd., Shandong, China.

[10] Fish oil: Rongcheng Haiyuan Fish Oil & Aquatic Products Co. Ltd., Shandong, China.

[11] Soybean phospholipid: Shandong Huierjia Biochemical Co. Ltd., Shandong, China.

[12] One kilogram of premix supplies the following vitamins and minerals: retinyl acetate, 75 mg; vitamin D_3, 19.85 mg; α-tocopheryl acetate, 24,000 mg; menadione nicotinamide bisulfite (MSN), 75 mg; thiamin hydrochloride, 100 mg; riboflavin, 250 mg; pyridoxine hydrochloride, 125 mg; cyanocobalamin, 2.5 mg; myo-inositol, 40,000 mg; calcium-D-pantothenate, 750 mg; niacin, 1000 mg; folic acid, 100 mg; Biotin, 75 mg; ascorhyl acetate, 10,000 mg; $CuSO_4 \cdot 5H_2O$, 982.22 mg; $FeSO_4 \cdot 7H_2O$, 24888.99 mg; $MnSO_4 \cdot H_2O$, 2307.34 mg; $ZnSO_4 \cdot 7H_2O$, 13316.24 mg; $CoCl_2 \cdot 6H_2O$, 305.30 mg; KI, 294.09 mg; Na_2SeO_3, 109.52 mg. All compounds were diluted with zeolite to one kilogram.

maintained above 5 mg/L, water temperature, pH, and salinity ranged from 19 to 21 °C, 7.7 to 8.0, and 15‰ to 16‰, respectively, and ammonia concentration was kept below 0.02 mg/L. The photoperiod in all tanks was set at 12-h light and 12-h dark (12L:12D), with the light switched on at 07:00 and off at 19:00.

2.4. Sample collection

At the end of the feeding trial, the turbot in each tank were enumerated and bulk-weighed post 24-h fasting period to calculate growth metrics, such as final body weight (FBW), specific growth ratio (SGR), weight gain rate (WGR), feed intake (FI), and feed conversion ratio (FCR). Subsequently, seven fish from each tank were chosen at random and anaesthetized using 100 mg/kg MS-222 (Green Heng Xing Biotech Co., Ltd., Beijing, China). Two fish per tank were selected at random and stored at −20 °C for subsequent analysis of whole-fish body chemical composition, including crude protein, crude lipid, ash and moisture. The body length and weight of other five fish were individually measured to compute the condition factor (CF). Afterward, blood was withdrawn from the caudal vein using a 1.5-mL sterile syringe and collected in a disinfected sharp-bottomed centrifuge tube containing heparin sodium. Plasma was isolated by centrifugation at 4000 × *g* at 4 °C for 10 min and preserved in a freezer at −80 °C for further analyses of hematological parameters, such as triglycerides (TG) and malonaldehyde (MDA) levels, as well as lysozyme (LZM), complement3 (C3), and total superoxide dismutase (T-SOD) activities. Afterward, the viscera and livers of the five phlebotomized fish were separated and weighed to calculate the viscerosomatic index (VSI) and hepatosomatic index (HSI). The weighed liver was then divided into two portions, with the larger portion being wrapped in tin foil, snap-frozen in liquid nitrogen, and then preserved at −80 °C for the measurement of hepatic TG content and expression levels of targeted genes. The smaller portion was fixed in a 4% paraformaldehyde solution for histomorphological examination using Oil red O staining. Additionally, the subcutaneous adipose tissue (SAT) of the aforementioned fish was also dissected and stored at −20 °C for the analysis of TG content. Meanwhile, the livers, SAT, and viscera without liver (VWL) of another three fish per tank were separated to determine crude lipid content.

2.5. Ammonia stress experiment

Following the conclusion of the feeding experiment, the remaining fish (18 fish per tank) after sampling were subjected to an ammonia stress test lasting 96 h. Ammonium chloride (purity: 99.5%) was utilized to prepare the solution containing 17 mg/L of ammonia, a concentration determined to be 96-h median lethal concentration in turbot following ammonia stress as established in a pre-test. During the ammonia stress test, the fish were not provided with any feed and only the air pump was employed to oxygenate the water. The concentrations of ammonia in each tank were monitored and adjusted every 6 h. The number of dead fish in each tank was recorded every 12 h over the course of 96 h.

2.6. Biochemical analyses

The proximate chemical composition of test diets and fish samples was analyzed in accordance with the standard protocols of the AOAC (AOAC, 2023). Briefly, moisture level was examined by drying at 105 °C in a ventilated oven for 3 h until constant weight (method 934.01). Crude protein concentration (N × 6.25) was determined using the Kjeldahl method with a Kjeltec 8420 Analyzer (FOSS Tecator, Hillerød, Denmark) (method 954.01). Crude lipid concentration was determined by Soxhlet extraction method with petroleum ether (method 920.39). Ash content was analyzed by burning in a muffle furnace at 550 °C for 3 h (method 942.05). Gross energy was evaluated by a Parr6200 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL, USA) with benzoic acid as a standard. The frozen samples of liver, SAT, and VWL were freeze-dried, and crude lipid contents in these samples were determined using chloroform/methanol-based classical lipid extraction methodology (Folch et al., 1957). The plasma LZM, T-SOD, C3 activities, and MDA content, as well as TG in plasma, liver and SAT were determined spectrophotometrically using

commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7. Histomorphological observation in the liver using Oil red O staining

Three fresh turbot liver samples per replicate were fixed in 4% paraformaldehyde for more than 24 h for liver tissue sectioning and staining. In brief, the fixed samples were dehydrated in an alcohol series (75%, 85%, 90%, 95%, 100%), cleared twice in xylene (10 min/time), and embedded in paraffin wax. The paraffin-embedded tissue blocks were chilled at −20 °C, trimmed with a surgical knife, and sliced on a Leica RM2016 automatic rotary microtome (Shanghai, China) at a thickness of 4 μm. The sections were mounted onto slides, dried at 60 °C in an oven, and stored at −20 °C. The frozen hepatic sections were restored to room temperature and fixed in 4% paraformaldehyde for 15 min. The sections were stained without light for 10 min using Oil red O, differentiated in 60% isopropanol, and re-stained with hematoxylin. Images of Oil red O-stained sections were captured with a Zeiss microscope (Oberkochen, Germany).

2.8. cDNA preparation and quantitative real-time PCR analysis

Around 50 mg of liver tissue from the turbot was thoroughly ground on ice, and total RNA was extracted using the Trizol method. The concentration and purity of the total RNA were evaluated using absorbance at 260 and 280 nm with a NanoDrop 2000 ultramicrospectrophotometer (Thermo Scientific, Wilmington, DE, USA) and gel electrophoresis. The mRNA in the sample was transcribed to cDNA using a First Strand Synthesis Kit (Thermo Scientific, Wilmington, DE, USA) following the manufacturer's instructions. Gene sequences of turbot were obtained from the NCBI database, and primer design was conducted using Primer 5 software. The name, primer sequence, amplicon size, optimal annealing temperature, and accession number of target genes are shown in Table S1. The β-actin gene was used as a housekeeping gene. A SYBR Green quantitative PCR kit and a quantitative thermal cycler (CFX96TM Real-Time System, BIORAD, USA) were used for the real-time quantitative PCR assay. The reaction system contained 2 μL of cDNA template, 10 μL of 2 × TransStart Top Green qPCR SuperMix, 0.4 μL each of the forward and reverse primers, and ultra-pure water to reach a final volume of 20 μL. The β-actin was applied as the internal reference gene to normalize expressions of target genes. The relative transcriptional level of the target genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Vandesompele et al., 2002).

2.9. Statistical analysis

The experimental data were analyzed using SPSS software (IBM SPSS Statistics 19.0, IBM, USA). Shapiro–Wilk and Levene's tests were conducted to evaluate the normality and homogeneity of variances of the data. All results were analyzed using one-way analysis of variance (one-way ANOVA) and the statistical model used was as follows:

$Y_{ij} = \mu + T_i + e_{ij}$,

where Y_{ij} is the observation of dependent variables, μ is the overall mean, T_i is the fixed effect of treatment, e_{ij} denotes the residual error for the observation. Tukey's method was used for multiple comparisons of the mean values among treatments. The significance level was set at $P \leq 0.05$. Additionally, regression analyses were performed to evaluate the linear and quadratic relationships between responsive variables and varying dietary geniposide levels. Two fish from each replicate of the dietary treatment were randomly selected and pooled as a sub-sample, and three sub-samples were analyzed for final body composition. The crude lipid contents of the liver, SAT, and VWL, as well as hepatic Oil red O staining of three fish from each tank were measured, and their mean values were used for statistical analysis. Regarding body morphology, triglycerides in different tissues, plasma parameters, and gene relative expression, five fish from each tank were examined, and their mean values were used for statistical analysis.

3. Results

3.1. Growth performance and morphological parameters

The growth performance and morphology of turbot fed five experimental diets are listed in Table 2. Geniposide supplementation did not affect FBW, WGR, SGR, and SR ($P > 0.05$). The FI and FCR

Table 2
Effects of dietary supplementation of geniposide at five doses on growth performance and morphological parameters in juvenile turbot.

Item	GP0	GP100	GP200	GP400	GP800	SEM	*P*-value (A)[9]	*P*-value (L)[10]	*P*-value (Q)[10]
IBW, g/fish	5.22	5.17	5.22	5.17	5.17	0.035	0.977	0.678	0.921
FBW, g/fish	28.80	30.39	29.89	30.84	30.77	0.427	0.607	0.153	0.326
WGR[1], %	450.74	488.83	473.35	497.95	495.48	9.784	0.585	0.161	0.344
SGR[2], %/d	3.05	3.16	3.12	3.19	3.18	0.030	0.576	0.159	0.339
FI[3], g/(fish·d)	2.16	2.13	2.14	2.06	2.03	0.019	0.137	0.011	0.035
FCR[4]	0.87	0.84	0.86	0.81	0.80	0.011	0.153	0.013	0.050
CF[5], g/cm^3	3.52^a	3.31^{ab}	3.33^{ab}	3.35^{ab}	3.19^b	0.035	0.021	0.007	0.027
VSI[6], %	6.57^a	6.42^a	6.53^a	5.90^b	5.91^b	0.083	<0.001	<0.001	<0.001
HSI[7], %	1.95^b	2.24^a	1.96^b	2.09^{ab}	1.64^c	0.056	<0.001	0.046	0.002
SR[8], %	97.78	96.67	97.78	97.78	98.89	0.703	0.937	0.523	0.712

IBW = initial body weight; FBW = final body weight; WGR = weight gain rate; SGR = specific growth rate; FI, = feed intake; FCR = feed conversion ratio; CF = condition factor; VSI = viscerosomatic index; HSI = hepatosomatic index; SR = survival rate; SEM = standard error of the mean.
GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively.
Different superscript letters in a row based on Tukey's multiple comparison test indicate significant variations among treatments ($P < 0.05$).
[1] WGR (%) = 100 × (FBW − IBW)/IBW.
[2] SGR (%) = 100 × [ln (FBW) - ln (IBW)]/days.
[3] FI (g/fish per day) = 100 × total feed intake/days/[(IBW + FBW)/2].
[4] FCR = dry feed intake/(FBW − IBW).
[5] CF (g/cm^3) = 100 × (body weight/body length^3).
[6] VSI (%) = 100 × viscera weight/FBW.
[7] HSI (%) = 100 × liver weight/FBW.
[8] SR (%) = 100 × (final fish number/initial fish number).
[9] This *P*-value calculated by one-way ANOVA (A) indicates if there existed significant differences among groups.
[10] These two *P*-values denote statistical significance of linear (L) and quadratic (Q) relationships between responsive variable and independent variable, respectively.

Table 3
Effects of dietary supplementation of geniposide at five doses on whole-body chemical composition in juvenile turbot (wet weight basis, %).

Item	GP0	GP100	GP200	GP400	GP800	SEM	*P*-value (A)	*P*-value (L)	*P*-value (Q)
Crude protein	14.76	14.82	14.84	14.65	14.73	0.027	0.173	0.237	0.385
Crude lipid	4.69	4.62	4.65	4.11	4.14	0.094	0.076	0.01	0.035
Ash	3.34	3.35	3.36	3.36	3.29	0.027	0.958	0.711	0.733
Moisture	76.79	76.91	76.88	77.39	77.27	0.107	0.321	0.053	0.166

SEM = standard error of the mean; A = one-way ANOVA, analysis; L = linear regression analysis; Q = quadratic regression analysis.
GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively.

decreased with increasing dietary geniposide levels (linear, quadratic; $P \leq 0.05$). Additionally, CF and HSI in the GP800 group, and VSI in the GP400 and GP800 groups, were significantly lower than those in the control group ($P < 0.05$).

3.2. Whole-fish body chemical composition

The effects of dietary geniposide supplementation on the whole-fish body chemical composition of turbot are exhibited in Table 3. The crude protein, ash, and moisture levels were not influenced by dietary geniposide ($P > 0.05$). The content of crude lipid tended to decrease with increasing dietary geniposide levels ($P = 0.076$).

3.3. Contents of crude lipid and triglycerides in various organs and plasma, as well as oil red O staining of liver

Crude lipids and triglycerides in various organs and plasma of turbot are presented in Table 4. The crude lipid in VWL of the GP800 group, as well as in the liver and SAT of all geniposide-supplemented groups, were significantly decreased than that in the control group ($P < 0.05$). Dietary administration of geniposide at 400 and 800 mg/kg reduced triglycerides in plasma, liver, and SAT relative to those in the control group ($P < 0.05$).

Photomicrographs of liver sections stained with Oil red O are illustrated in Fig. 1. The hepatic lipid droplets were stained red with Oil red O, and the area percentages in the sections of GP400 and GP800 groups were significantly lower than that in the control group ($P < 0.05$).

3.4. Lipid metabolism-related gene expression in the liver

Considering that the hepatic concentrations of crude lipid and triglycerides were significantly influenced by dietary geniposide, we further examined the hepatic mRNA levels of genes involved in lipogenesis, fatty acid oxidation, lipid transportation, and esterification (Fig. 2). There was significant decrease in mRNA levels of transcription factors (*pparγ* and *srebp1*), lipogenic gene (*acly*), and the fatty acid desaturation gene (*scd*) in fish fed diets containing 400 and 800 mg/kg geniposide compared to those fish fed the control diet ($P < 0.05$). In terms of FA oxidation, mRNA expression of *pparα*, a main regulator of fatty acids β-oxidation, was improved with the dietary addition of geniposide ($P < 0.05$). Additionally, the esterification of diglycerides to triglycerides (*dgat*) in the liver was remarkably suppressed in geniposide-supplemented groups compared to that in the control group ($P < 0.05$). Regarding lipid transport, mRNA expression of fatty acid uptake genes (*cd36*, *fabp-1*) and apolipoprotein gene (*apoa1*) were significantly down-regulated in fish fed diets containing geniposide compared to those fed the control diet ($P < 0.05$). However, another apolipoprotein gene (*apoa4*) was significantly up-regulated in GP400 and GP800 groups compared to that in the control group ($P < 0.05$). The regression analysis showed a decreasing trend in *cd36*, *fabp-1* and *apoa1* as geniposide concentration increased in the diet ($P < 0.05$).

3.5. Antioxidant parameters in plasma and related gene expression in liver

Table 5 and Fig. 3 illustrate the influences of dietary geniposide supplementation on antioxidant parameters in plasma and related gene expression in the liver of turbot. A significant decrease in plasma MDA content in the GP800 group, along with a remarkable increment in plasma T-SOD activity in geniposide-supplemented groups were discovered compared to the control group ($P < 0.05$). The mRNA expressions of hepatic *nrf2* and *gpx* were significantly up-regulated in turbot fed geniposide diets compared with the control group ($P < 0.05$). Significantly higher mRNA expressions of *cat* and *sod* were found in GP200 and GP800 groups compared with counterparts in the control group ($P < 0.05$).

3.6. Immune indicators in plasma and related gene expression in the liver

Table 5 and Fig. 4 exhibit the impacts of dietary geniposide supplementation on plasma immune indicators and related gene expression in the liver of turbot. A significant increment in plasma LZM activity in the geniposide-supplemented groups and a remarkable improvement in plasma complement C3 activity in the

Table 4
The contents of crude lipid and triglycerides in different organs and plasma of juvenile turbot fed experimental diets.

Item	GP0	GP100	GP200	GP400	GP800	SEM	*P*-value (A)	*P*-value (L)	*P*-value (Q)
Crude lipid, % dry matter basis									
VWL	20.44^{a}	20.21^{ab}	18.38^{ab}	19.33^{ab}	18.22^{b}	0.297	0.016	0.005	0.022
Liver	64.98^{a}	55.30^{b}	55.89^{b}	52.52^{bc}	49.02^{c}	1.474	<0.001	<0.001	<0.001
SAT	63.31^{a}	52.81^{cd}	50.46^{d}	56.30^{bc}	57.13^{b}	1.209	<0.001	0.317	<0.001
Triglycerides									
Plasma, mmol/L	4.92^{a}	4.00^{ab}	2.16^{c}	2.78^{bc}	2.47^{bc}	0.307	0.001	0.001	0.001
Liver, mmol/g prot	0.90^{a}	0.86^{a}	0.60^{b}	0.61^{b}	0.57^{b}	0.040	<0.001	<0.001	<0.001
SAT, mmol/g prot	1.34^{a}	1.10^{b}	1.16^{ab}	1.08^{bc}	0.86^{c}	0.044	0.001	<0.001	0.001

VWL = viscera without liver; SAT = subcutaneous adipose tissue; SEM = standard error of the mean. A = one-way ANOVA, analysis; L = linear regression analysis; Q = quadratic regression analysis.
GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively.
Different superscript letters in a row based on Tukey's multiple comparison test indicate significant variations among treatments ($P < 0.05$).

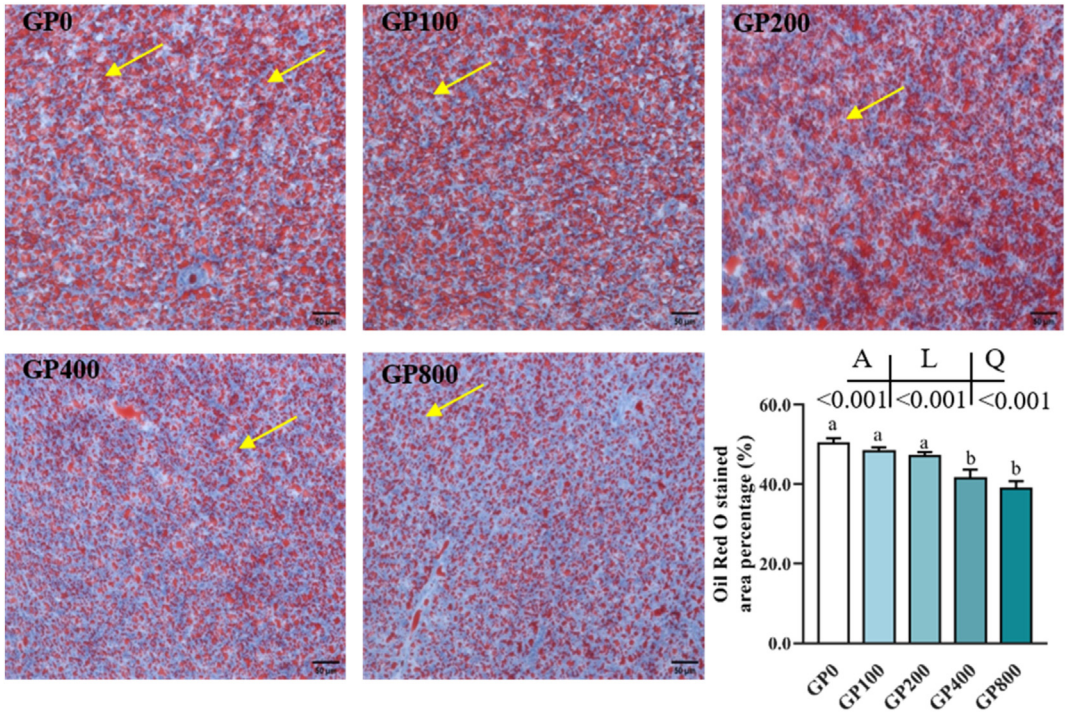

Fig. 1. Representative photomicrographs of Oil red O-stained liver section of juvenile turbot fed the experimental diets containing different doses of geniposide over an 8-week period. The scale bar was 50 μm. The red drops pointed by the yellow arrowhead indicate the presence of lipid droplets, and quantification of area percentage of Oil red O staining was measured by Image J software. GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively. A = one-way ANOVA analysis; L = linear regression analysis; Q = quadratic regression analysis. The corresponding *P*-values are just below the capitals. The lower case letters based on Tukey's multiple comparison test indicate significant differences among treatments ($P < 0.05$). All data are shown as mean ($n = 3$).

GP200 and GP800 groups were discovered compared to those in the control group ($P < 0.05$). A significant decrease in the mRNA level of the pro-inflammatory cytokine gene (*tnf-α*) and a notable increase in the transcriptional level of the anti-inflammatory cytokine gene (*tgf-β* and *il-10*) were induced by dietary supplementation of geniposide compared to control group ($P < 0.05$).

3.7. Survival rate under ammonia stress

The results of a 96-h ammonia stress experiment conducted post formal feeding trial are exhibited in Fig. 5. We discovered that fish death began to occur at 12 h following ammonia stress. In addition, after 84-h ammonia stress, the cumulative survival rate of turbot in each group tended to stabilize. At the termination of the stress trial, the cumulative survival rates of turbot in the GP400 and GP800 groups were significantly higher than that in the GP0 group ($P < 0.05$). The mean cumulative survival rates in GP0, GP100, GP200, GP400 and GP800 groups were 50.02%, 66.05%, 51.83%, 80.44% and 94.19%, respectively.

3.8. Determination of optimal supplemental level in turbot diet

Based on crude lipid in the liver, plasma triglycerides, and plasma LZM activity against dietary geniposide levels, the break point based on quadratic regression analysis was estimated to be 668.41, 545.21, and 576.56 mg/kg (Fig. 6).

4. Discussion

4.1. Growth performance and feed utilization

In recent decades, the role of phytogenic extracts in potentiating animal performance and health has been extensively studied due to their natural origin, minimal side effects, and multifaceted benefits (Dawood et al., 2022). Therefore, phytogenic extracts may constitute a substantial part of nutritional strategies to guarantee the quality and safety of animal products (Piao et al., 2023). Geniposide, a plant extract, has been extensively studied in terrestrial animals (Liu et al., 2022), but its efficacy in aquaculture animals was rarely researched (He et al., 2020; Sun et al., 2017). This study investigated the potency of geniposide on the growth performance and feed utilization in turbot. However, the use of geniposide did not improve the growth performance of juvenile turbot, which is consistent with previous study in grass carp (Sun et al., 2017). Similarly, the supplementation of gardenia pomace containing geniposide in the pig diet also did not affect average daily gain (Zou et al., 2022). FCR showed a slight decreasing trend as dietary geniposide levels increased in turbot diets. The slight improvement in feed utilization is probably correlated with a healthier fish body, and more dietary energy was saved for nutrient deposition. This was also corroborated by marginally decreased feed intake, and unchanged growth performance, as well as enhanced antioxidant ability, immunity, and ammonia stress resistance in the current study. These findings suggest that dietary geniposide administration in diets did not possess a growth-promoting effect but had a

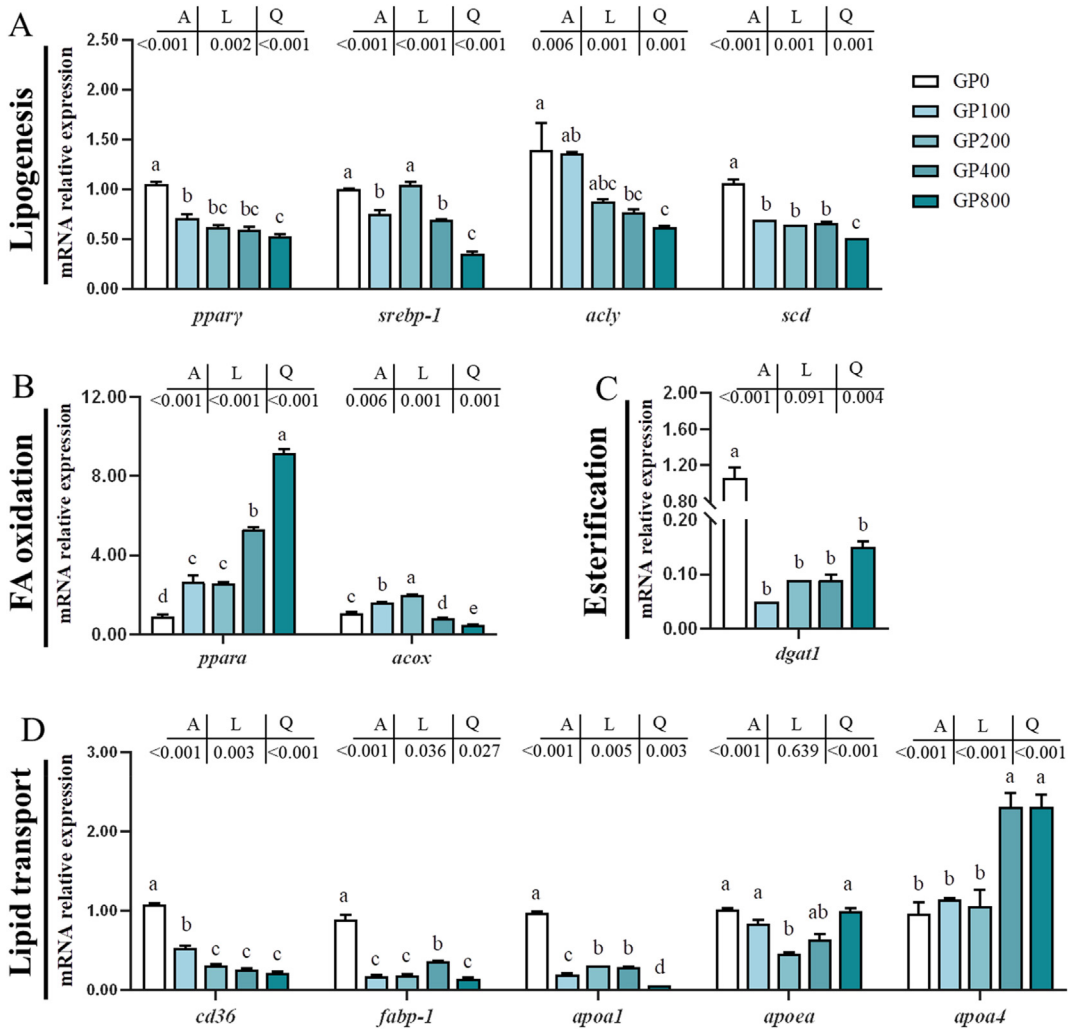

Fig. 2. Results of lipid metabolism-related parameters including lipogenesis, fatty acid oxidation, esterification, and lipid transportation in liver of turbot fed diets containing different doses of geniposide. The mRNA expressions of lipogenesis-related genes (A), fatty acids oxidation-related genes (B), esterification-related gene (C) and lipid transport-related genes (D). *pparγ* = peroxisome proliferator-activated receptor γ; *srebp1* = sterol regulatory element binding transcription factor 1; *acly* = ATP citrate lyase; *scd* = stearoyl-CoA desaturase; *pparα* = peroxisome proliferators-activated receptors α; *acox* = acyl-CoA oxidase; *dgat1* = diacylglycerol O-acyltransferase 1; *cd36* = cluster of differentiation 36; *fabp1* = fatty acid-binding protein 1; *apoa1* = apolipoprotein A-I; *apoa4* = apolipoprotein A-IV; *apoe* = apolipoprotein E. GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively. A = one-way ANOVA analysis; L = linear regression analysis; Q = quadratic regression analysis. The corresponding *P*-values are just below the capitals. Differnt lower case letters based on Tukey's multiple comparison test indicate significant differences among treatments ($P < 0.05$). All data are shown as mean ($n = 3$).

Table 5
Effects of dietary supplementation of geniposide at five doses on plasma immunity and antioxidant-related parameters of juvenile turbot.

Item	GP0	GP100	GP200	GP400	GP800	SEM	*P*-value (A)	*P*-value (L)	*P*-value (Q)
MDA, nmol/mL	14.75^{a}	12.40^{ab}	12.56^{ab}	10.73^{ab}	7.78^{b}	0.810	0.049	0.002	0.009
T-SOD, U/mL	56.41^{c}	64.52^{b}	65.38^{b}	67.44^{ab}	71.23^{a}	1.371	<0.001	<0.001	<0.001
LZM, μg/mL	7.46^{c}	9.10^{b}	10.32^{a}	8.92^{b}	9.96^{ab}	0.282	<0.001	0.01	0.004
C3, mg/L	12.91^{c}	14.81^{bc}	20.09^{a}	14.31^{bc}	18.65^{ab}	0.840	0.004	0.061	0.118

MDA = malonaldehyde; T-SOD = total superoxide dismutase; LZM = lysozyme; C3 = complement 3; SEM = standard error of the mean. A = one-way ANOVA, analysis; L = linear regression analysis; Q = quadratic regression analysis.
GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively.
Different superscript letters in a row based on Tukey's multiple comparison test indicate significant variations among treatments ($P < 0.05$).

slightly feed-saving effect on animals. Additionally, the CF, VSI, and HSI of fish fed a diet containing 800 mg/kg geniposide were significantly lower than those fed the control diet. The values of histological parameters often correlate with lipid accumulation in important lipid storage tissues in fish (Arfsten et al., 2010). Therefore, the impact of dietary geniposide on lipid metabolism in turbot was further investigated.

4.2. Lipid metabolism

The crude lipid of fish body tended to decrease in a linear pattern as the dietary geniposide increased. Meanwhile, geniposide supplementation also resulted in a decrease in crude lipid contents of VWL, liver, and SAT. Consistently, dietary addition of geniposide at 0.1% and 0.3% showed a preventive effect of lipid accumulation in

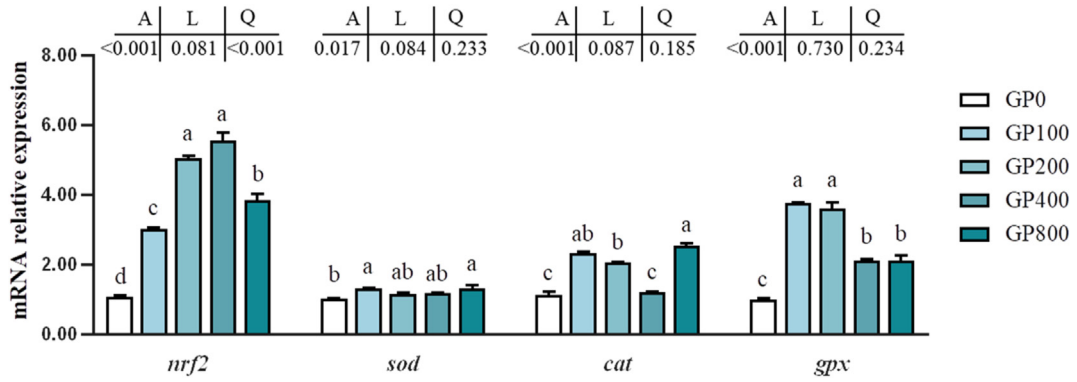

Fig. 3. The effects of dietary supplementation of geniposide at five doses on relative expression levels of genes associated with antioxidant ability in liver of turbot. *nrf2* = nuclear factor erythroid 2-related factor 2; *sod* = superoxide dismutase; *cat* = catalase; *gpx* = glutathione peroxidase. GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively. A = one-way ANOVA analysis; L = linear regression analysis; Q = quadratic regression analysis. The corresponding *P*-values are just below the capitals. Differnt lower case letters based on Tukey's multiple comparison test indicate significant differences among treatments ($P < 0.05$). All data are shown as mean ($n = 3$).

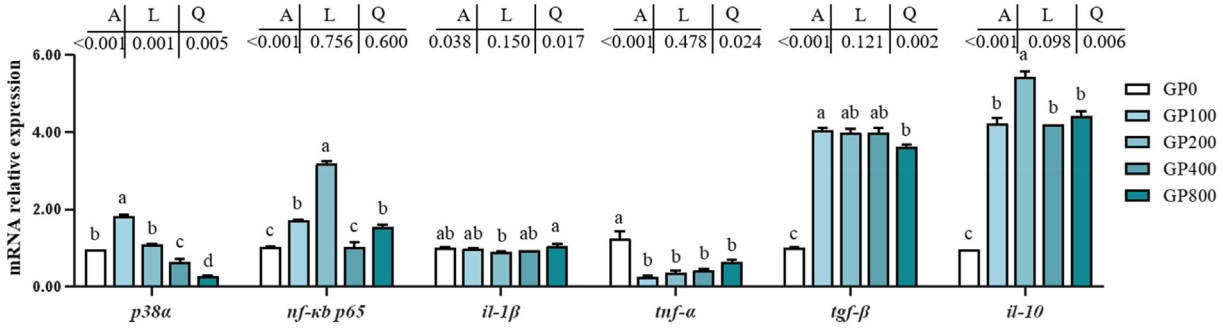

Fig. 4. The results of hepatic inflammatory response including inflammatory regulators, pro-inflammatory cytokines, anti-inflammatory cytokines in turbot. The mRNA expression of inflammatory cytokines-related gene. *p38α* = p38 mitogen-activated protein kinase α; *nf-κb p65* = nuclear factor kappa B p65; *il-1β* = interleukin-1β; *tnf-α* = tumor necrosis factor-α; *tgf-β* = transforming growth factor-β; *il-10* = interleukin-10. GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively. A = one-way ANOVA analysis; L = linear regression analysis; Q = quadratic regression analysis. The corresponding *P*-values are just below the capitals. Differnt lower case letters based on Tukey's multiple comparison test indicate significant differences among treatments ($P < 0.05$). All data are shown as mean ($n = 3$).

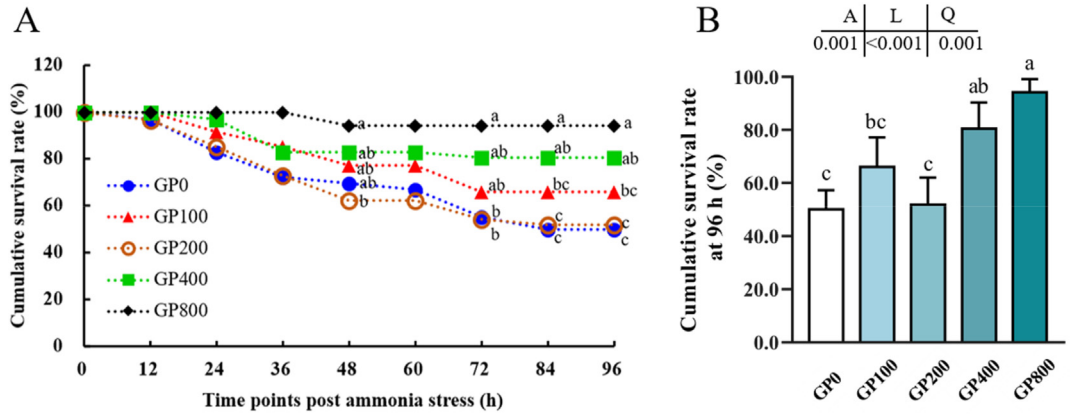

Fig. 5. Effects of dietary administration of geniposide on cumulative survival rate of turbot after exposure to ammonia stress. Cumulative survival curve over 96 h (A) and final cumulative survival rate at 96 h (B). GP0, GP100, GP200, GP400, and GP800 are designated as experimental diets containing 0, 100, 200, 400, and 800 mg/kg geniposide, respectively. A = one-way ANOVA analysis; L = linear regression analysis; Q = quadratic regression analysis. The corresponding *P*-values are just below the capitals. Differnt lower case letters based on Tukey's multiple comparison test indicate significant differences among treatments ($P < 0.05$). All data are shown as mean ($n = 3$).

the viscera and liver of obese Type 2 diabetic mice (Kojima et al., 2011). Likewise, dietary administration of geniposide at 600 and 800 mg/kg remarkably decreased muscular lipid content in grass carp (Sun et al., 2017). This lipid-lowering effect of geniposide was probably correlated with its regulatory role in lipid metabolism. Triglycerides (TG), the major form of lipids to store and provide energy, constitutes approximately or more than 50% of total lipids in the liver and adipose tissues of fish (Wang et al., 2022a). The TG contents in plasma, liver, and SAT of turbot tended to decrease as dietary geniposide increased. Similarly, the study in rats fed a high-

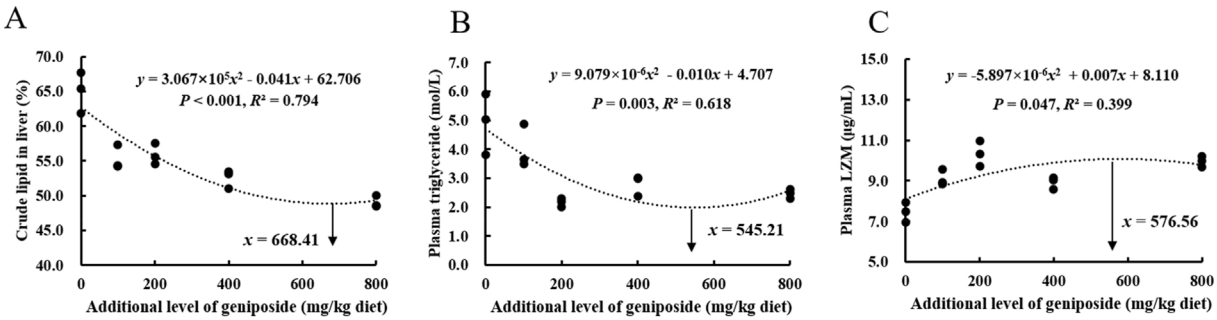

Fig. 6. The quadratic regression analysis to determine the appropriate dose of dietary supplementation of geniposide based on the following representative parameters in turbot juveniles, crude lipid in the liver (A), plasma triglycerides (B), plasma LZM (C). The optimal dose of geniposide was taken as the abscissa of the breakpoint in the curve equation. LZM = lysozyme.

fat diet found that the dietary addition of geniposide exerted a triglycerides-lowering effect in the liver (Ma et al., 2011). The result of Oil red O staining also revealed that the area percentage of lipid droplets in the liver section of fish fed geniposide-supplemented diets was visibly smaller than that in fish fed the control diet. These results collaboratively indicated an interesting metabolic phenotype where geniposide addition in turbot diet could decrease TG contents in some tissues (plasma, liver, and SAT) of the fish body. Therefore, the underlying mechanism of the TG-lowering effect of geniposide in turbot was further investigated.

In the present study, the addition of geniposide in turbot led to a significant decreasing tendency in hepatic FA de novo synthesis-related gene expression, including transcriptional factors (peroxisome proliferator-activated receptor γ (*pparγ*) and sterol regulatory element binding transcription factor-1 (*srebp-1*), and key enzymes (ATP citrate lyase [ACLY] and stearoyl-CoA desaturase [SCD]). As reported previously, the combination of geniposide and chlorogenic acid significantly reduced TG and free FA in the liver of mice fed a high-fat diet by suppressing gene and protein expressions of SCD-1 (Chen et al., 2021). An in vitro study in the HepG2 cell line also demonstrated that genipin, the aglycone of geniposide, could decrease FA synthesis via inhibiting expressions of *srebp-1c* and its downstream genes (*acc1* and *fas*) (Wang et al., 2022b). Diacylglycerol O-acyltransferase (DGAT) catalyzes the terminal step of TG biosynthesis (Eichmann and Lass, 2015), and there are two isoforms (DGAT1 and DGAT2) in animals (Yen et al., 2008). More interestingly, TG synthesized by DGAT1 is preferentially channeled to oxidation, whereas TG synthesized by DGAT2 is destined for very low-density lipoprotein assembly in primary hepatocytes (Li et al., 2015). In the current study, hepatic mRNA expression of *dgat1* was notably down-regulated by dietary geniposide addition. These results were suggestive of a significantly weakened lipid synthesis in the liver of turbot fed diets containing geniposide. In terms of lipid catabolism, PPARα is a key nuclear receptor transcription factor that regulates the expression of FA β-oxidation genes in tissues (especially in the liver and heart) (Kersten and Stienstra, 2017). In the current study, the hepatic transcript of *pparα* was significantly up-regulated in the geniposide-supplemented groups compared with the control group. A previous study showed that geniposide pretreatment at different concentrations (65, 130, 260 μmol/L) facilitated the protein expression of PPARα in HepG2 cells (Shen et al., 2020).

In our work, geniposide supplementation in turbot diet remarkably down-regulated hepatic mRNA levels of *cd36* and *fabp-1* compared with the placebo group. Cluster of differentiation 36 (CD36) and fatty acid-binding protein (FABP) are the main transporters facilitating the entry of long-chain fatty acids from plasma into hepatocytes (Zeng et al., 2022). It is also noteworthy that the main lipid source used in the current study was fish oil, which was rich in long-chain polyunsaturated fatty acids (docosahexaenoic acid and eicosapentaenoic acid). Similarly, research in mice showed that geniposide can reduce hepatic TG content by decreasing the protein expression of CD36 (Qiu et al., 2021). Therefore, geniposide supplementation in diets could reduce lipid content by down-regulating lipogenesis and up-regulating lipolysis, as well as inhibiting hepatocyte FAs uptake and trafficking. Additionally, mounting evidence points to the modulatory effects of apolipoproteins on TG deposition in the liver in mammals (Karavia et al., 2012; Liu et al., 2024). Apolipoprotein A-I (apoA-I) is considered the primary protein constituent of high-density lipoprotein (HDL), and the HDL metabolic pathway is a central contributor to the accumulation of dietary TG in the liver (Karavia et al., 2012). The expression of apolipoprotein A-IV (apoA-IV) in mouse liver promotes TG secretion and reduces hepatic lipid content by enhancing very low-density lipoprotein particle expansion (VerHague et al., 2013). In the current study, a high dose of geniposide in turbot diet significantly down-regulated the transcriptional level of *apoa1* and up-regulated the mRNA expression of *apoa4* in the liver. Therefore, geniposide administration in a turbot diet could also modulate apolipoprotein expression to reduce hepatic TG content. Taken together, these results suggest that the use of geniposide at 200 to 800 mg/kg significantly decreased hepatic TG content mainly by inhibiting lipogenesis, reducing FA uptake, and modulating lipoprotein expression.

4.3. Immune response, antioxidant ability and ammonia stress resistance

In the present study, a significant decreasing trend in plasma MDA content and a significant increasing tendency in plasma T-SOD activity were observed as dietary geniposide increased. This finding is consistent with previous research in crucian carp (He et al., 2020) and some mammals (Shen et al., 2022; Yang et al., 2021). The Nrf2-Keap1 signaling pathway is a canonical pathway involved in modulating the antioxidant network by activating the expression of an array of antioxidant genes, such as *sod*, *cat*, *gpx* (Nguyen et al., 2009). In the present study, dietary geniposide supplementation significantly increased the mRNA expressions of *nrf2* and *gpx*, and transcriptional levels in *sod* and *cat* were significantly up-regulated in the GP800 group compared with the control group. Likewise, geniposide has been shown to possess an antagonistic effect on $CdCl_2$-induced oxidative stress by enhancing Nrf2 protein expression and downstream antioxidant enzyme activities (He et al., 2019). Our study indicates that geniposide enhances antioxidant ability partly by activating the Nrf2 signaling pathway in turbot.

In the current study, a significantly improved plasma LZM activity in geniposide-supplemented groups, and a significantly increased plasma complement C3 in GP200 and GP800 groups were discovered compared with those in the control group. Lysozyme and C3 are important defense components of the fish innate immune system. It is generally acknowledged that NF-κB and p38α MAPK are central players in the generation of immune and inflammatory responses in vertebrates (Martín-Blanco, 2000; Tripathi and Aggarwal, 2006). The liver is an organ with predominant innate immunity through the biosynthesis of numerous pathogen-recognition receptors and complement components (Gao et al., 2008). In our work, gene expressions of *p38α* and *NF-κB p65* in the liver were significantly up-regulated in the GP100 group compared with the control group. In the meantime, the dietary addition of geniposide remarkably up-regulated mRNA expressions of anti-inflammatory cytokines (*il-10* and *tgf-β*) and down-regulated the transcriptional level of pro-inflammatory cytokine (*tnf-α*) in the liver of turbot compared with the control group. Similarly, the research conducted on rats also confirmed that geniposide exerts neuroprotective functions against traumatic brain injury by inhibiting activities of NF-κB p65 and p38 MAPK and modulating inflammatory cytokine levels (interleukin [IL]-10, IL-1β, IL-6, and IL-8) (Yuan et al., 2020). The modulatory effects of geniposide on inflammatory responses have also been verified in other studies (Chen et al., 2015, 2022). These results suggest that there are rather mild inflammatory responses in the liver of turbot fed geniposide-containing diets under normal conditions.

High concentration of ammonia is one of the main stressors for fish especially those cultured in recirculating aquaculture systems. This not only affects the health status of the fish but also potentially leads to the occurrence of mortality events due to weakened immune function and disrupted oxidative balance in the fish body (Ip and Chew, 2010). In the current research, the dietary addition of geniposide at 400 and 800 mg/kg significantly improved the cumulative survival rate of turbot after exposure to ammonia stress compared to the control group. It is commonly believed that anti-stress ability is highly correlated with obesity in humans (Foss and Dyrstad, 2011). The enhanced stress resistance against ammonia in turbot fed high doses of geniposide, especially at 400 and 800 mg/kg, was partly attributable to relatively lower lipid content in some fish tissues. Additionally, accumulating evidence have demonstrated that ammonia stress could result in excessive production of reactive oxygen species (ROS) in aquatic animals (Cheng et al., 2015; Zhang et al., 2018), thereby impairing immune function and over-consuming antioxidants (including enzymes). Therefore, the enhanced anti-stress ability in the geniposide-supplemented groups may also be partly attributed to the improved antioxidant capability and immunity in the current study.

5. Conclusion

In summary, dietary supplementation of geniposide has notably reduced lipid deposition in turbot by regulating lipid metabolism and transportation, while also remarkably enhancing immunity, antioxidant ability, and resistance to ammonia stress in turbot. The optimal supplementation levels of dietary geniposide for turbot were estimated to range from 545.21 to 668.41 mg/kg, based on hepatic crude lipid content, plasma triglycerides, and LZM.

CRediT authorship contribution statement

Haoran Sun: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Tongtong Yue:** Methodology, Investigation. **Yuqing Hou:** Investigation, Formal analysis. **Tao Li:** Investigation. **Zhi Li:** Data curation. **Haiyan Liu:** Supervision, Funding acquisition. **Peiyu Zhang:** Writing – review & editing, Project administration, Data curation, Conceptualization.

Data availability statement

All data are available from the corresponding author by request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.11.010.

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