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Research Article

Plant-Based Additivities Improved the Growth Performance and Immune Response, and Mitigated the Inflammatory Signalling in Channel Catfish Fed a High-Fat Diet

Hesham Eed Desouky,[1,2] **Guang-zhen Jiang**,[1] **Kenneth Prudence Abasubong**,[1] **Yong-Jun Dai**,[1] **Xiangyang Yuan**,[1] **Jean-Jacques Y. Adjoumani**,[1] **and Wen-bin Liu**[1]

[1]*Key Laboratory of Aquatic Nutrition and Feed Science of Jiangsu Province, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China*
[2]*Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Beheira, Damanhour 22713, Egypt*

Correspondence should be addressed to Guang-zhen Jiang; jianggz@njau.edu.cn

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This study was conducted to understand the effects of glycyrrhetinic acid, berberine, and resveratrol on growth and inflammatory signalling in channel catfish fed a high-fat diet (HFD). Fish (average weight, 20 ± 0.15 g) were distributed randomly in 15 tanks (11 fish per tank). Fish were allocated randomly to five experimental diets: (control diet (ND); high-fat diet (HFD); high-fat diet + 0.3 mg/kg glycyrrhetinic acid (HFD + GA); high-fat diet + 50 mg/kg berberine (HFD + B); and high-fat diet + 400 mg/kg resveratrol (HFD + R). Each diet was tested in triplicates for eight weeks. The final body weight (FBW), body weight gain (BWG), specific growth rate (SGR), and condition factor (CF) were significantly lower in fish fed HFD. In contrast, opposite trends were observed in the feed conversion ratio (FCR), hepatosomatic index (HSI), visceralsomatic index (VSI), and mesenteric fat index (MFI). Fish fed HFD showed a higher trend ($P < 0.05$) in plasma glucose, cortisol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), liver total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), while opposite trends were found in total protein (TP) and high-density lipoprotein (HDL). These parameters were all enhanced by feeding the additive-supplemented diets. Liver superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), plasma lysozyme (LYM), myeloperoxidase (MPO), alkaline phosphatase (AKP), acid phosphatase (ACP) activities, and immunoglobulin M (IgM) levels were all lower in fish fed HFD as compared to the control group. However, supplementing GA, berberine, and resveratrol restored these parameters to similar levels to the control group. Upregulated gene expression of interleukin 1β (IL-1β), nitric oxide (NOS), nuclear factor-kappa B (NF-κB), X box-binding protein 1 (XBP1s), coupled with tumour necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), was observed in fish fed HFD, whereas reverse trends were observed in the additive-supplemented groups as compared to the control group. Overall, glycyrrhetinic acid, berberine, and resveratrol could reduce endoplasmic reticulum stress and inflammation and enhance the immune response in channel catfish fed HFD.

1. Introduction

The sustainable development of animal feeding in China is being hampered by a lack of resources and rising protein feed prices [1]. The focus of global research has been shifted to finding other protein sources to replace fishmeal (FM) in the diet [2]. Lipids, as one of the primary nutritional components in fish diets, plays a critical role in fish growth as they contain the essential fatty acids needed by them [3, 4]. The impact of dietary lipid levels on the growth of different fish species has been previously studied. Various fish species, such as largemouth bass (*Micropterus salmoides*), juvenile giant croaker (*Nibea japonica*), juvenile northern whiting (*Sillago sihama*), silver barb fingerlings (*Puntius gonionotus*),

etc., have been discovered to require an optimum lipid level for growth [5–8]. However, inadequate dietary lipid levels may lead to a lack of essential fatty acids, ultimately impacting the fish growth [6, 8].

Aquaculture is currently using feeds with increased lipid contents. It is well-established that an optimum dietary lipid intake stimulates fish development [5, 6, 9–12]. However, excess dietary fat adversely impacts fish growth and immunity [5, 13, 14]. High dietary lipid levels hinder the body's ability to absorb nutrients and frequently cause abnormal lipid accumulation in fish [4, 14]. Excessive dietary lipids have been observed to reduce feed intake, impede fish growth, and suppress fish immunological responses [15, 16]. Furthermore, an accumulation of lipids may cause fatty livers and poor carcass quality [9]. Additionally, dietary lipid levels and fish immunity are highly connected [17–19]. For example, research has demonstrated that high-fat (HF) feeding inhibited immunological markers, which are linked to increased lipid peroxidation and subpar growth in blunt snout bream (*Megalobrama amblycephala*) [20]. Grass carp (*Ctenopharyngodon idella*) fed HFD showed decreased nonspecific immunological markers linked to a higher oxidative condition [17]. A similar result was also found in turbot (*Scophthalmus maximus*) [21] and marbled rockfish (*Sebastiscus marmoratus*) [22]. Therefore, finding an efficient solution to enhance fish health with HF feeding is very important.

Due to their direct connection to fish immunology, fish livers are particularly vulnerable to oxidative stress [23]. Reactive oxygen species (ROS) production is continuously elevated under oxidative stress, further compromising the fish immunity. Earlier studies have shown that Atlantic salmon, walleye pollock, and giant yellow croaker have antioxidant characteristics when their diets contain optimum amounts of dietary lipids [12, 24, 25]. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and related enzymes are only a few efficient mechanisms that organisms have evolved to maintain a balance between lipid peroxides and the antioxidant system. These enzymes are also involved in the immune system [26]. Interleukins (ILs), tumour necrosis factors (TNFs), chemokines, and interferons (IFNs), which work through cytokine receptors, are cytokines that contribute to certain characteristics, including anti-inflammatory, proinflammatory, and pathogen-killing activities [27]. The immunostimulant regulates the production of TNF-α in fish [26]. Moreover, an anti-inflammatory cytokine called interleukin 1 beta (IL-1) promotes inflammation [28].

It has been shown that several additives are useful for preventing fat accumulation, enhancing antioxidant capacity, enhancing immune response, and regulating lipid metabolism without impairing the growth performance of fish fed on HFD. These additives include betaine [29], berberine [20], prebiotics [30], resveratrol [31], and glycyrrhetinic acid [31]. As a result of their safety for both humans and animals, resveratrol, glycyrrhetinic acid, and berberine have received much attention. Glycyrrhetinic acid is the aglycon of glycyrrhizin (GL), a naturally occurring triterpene saponin made up of the active ingredient in licorice (*Glycyrrhiza* spp.) [32]. In both humans and rats, studies have revealed that glycyrrhetinic acid (GA) can lower internal fat accumulation and enhance lipolysis [33]. GA dramatically increased hepatic fatty acid transport and oxidation, which resulted in hepatic lipid accumulation and metabolic dysfunction in *Megalobrama amblycephala* farming [32]. Desouky et al. observed an improvement in the lipid metabolism and growth performance of channel catfish fed HFD-supplemented with GA [34].

Resveratrol belongs to a class of substances known as polyphenols. They are believed to function similarly to antioxidants. With several pharmacological effects, including anti-inflammatory, antihypertensive, and antiproliferative ones [35, 36], berberine has been used in clinical settings as a nonprescription medication to treat infections [20]. Additionally, it has been shown by Choi et al. to improve anti-inflammatory characteristics, lower cholesterol, and increase insulin resistance [37]. It is extremely important to investigate the role of GA as a useful strategy for mitigating lipid disorders in fish without impairing growth performance.

Channel catfish is a species of *Ictaluridae* fish that originated from North America and is now found in many waters throughout Europe (including the Czech Republic and Romania) and certain regions of Asia (China, Malaysia, and Indonesia) [38]. Nowadays, most feeds designed for channel catfish contain a considerable amount of lipids to spare protein. However, these feeds are linked to poor growth, typically accompanied by increased oxidative stress, endoplasmic reticulum stress, and immunological suppression, which may ultimately results in disease and high mortality. As a result, it is important to investigate practical strategies for this species to promote growth, decrease oxidative stress, and raise disease resistance when they are fed HFDs. Therefore, in this study, we assessed the effects of several functional additives on antioxidants, immune response, and endoplasmic reticulum function in channel catfish fed HFD.

2. Materials and Methods

2.1. Experimental Diets. Five experimental diets were designed: control diet, HFD, and HFD supplemented with 0.3 mg/kg GA, 50 mg/kg berberine, and 400 mg/kg resveratrol, respectively. According to earlier research, juvenile channel catfish had a lipid requirement of between 4% and 7%. Therefore, a lipid content of over 7% is considered high in fat content [29, 39]. The levels of GA, berberine, and resveratrol used in this study were adopted by Jiang et al. [32], Chen et al. [20], and Zhang et al. [31] with slight modifications. The supplier of GA powder was Nanjing Zelang Medical Technology (Nanjing, Jiangsu, China), whereas berberine powder and resveratrol powder were purchased at Sigma-Aldrich China, and they were 95% pure. Fish and soybean oil were used equally as lipid sources, with fish, soybean, cottonseed, and rapeseed serving as the main protein sources. For around 40 minutes, the materials, including GA, berberine, and resveratrol powders, were well blended with soybean and fish oil. The powdered diets were

then mixed with water to create stiff dough. The pelleted laboratory machine was used to pellet the dough before being air-dried. Dietary samples were kept at −20°C for proximate analysis. Table 1 displays the diet formulation and approximate composition.

2.2. Fish and Feeding Trial. Channel catfish fingerlings were collected from the Institute of Freshwater Fisheries Research, Fishery Sciences Academy of China (Jiangsu, China). The feeding experiment was conducted in our lab's indoor recirculating aquaculture system. Before the experiment, fingerlings were raised in tanks and fed on a commercial diet for two weeks to acclimate them to the test conditions. After the acclimation, fish of varying weights (average weight, 20 ± 0.15 g) were distributed randomly in 15 tanks ($3 \times 0.8 \times 0.8$ m) at stocking 11 fish per tank density. The five experimental diets each received a random number of fish. For eight weeks, each diet was examined in triplicate. Fish were fed thrice daily, at 8:00, 12:00, and 17:00 h, until they appeared satisfied. A 1 mm gauze disc with an 80 cm diameter was installed at the bottom of each tank, where the unconsumed pellet from each replicate was collected after feeding for 30 minutes to measure feed intake. The water quality was maintained by replacing a third of the tank's water with dechlorinated freshwater every two weeks. The following parameters were maintained to ensure optimal conditions: water temperature 28 ± 0.34°C, pH 7.5 ± 0.22, ammonia-nitrogen level 0.27 mg/L, dissolved oxygen 6.9 ± 0.31 mg/L, photoperiod 12 h light/dark cycle, constant aeration, and recirculation of water. In this study, there were no deaths noted.

2.3. Sample Collection. Fishes in each tank were denied food for 24 hours after 56 days of feeding before being sampled. The total number of fishes was then determined, and each fish's weight and length were recorded for growth performance. After that, four individuals from each replicate were randomly selected, weighed, and their blood was technically removed using heparinized syringes injected into the caudal vein. After that, the speed of 3000 g at 4°C for 10 min was used to centrifuge the sample, and the supernatant was quickly siphoned using a pipette and stored at −20°C for biochemical examination. Similarly, the individual liver and viscera were rapidly removed and stored at −20°C for enzymatic study. Liver samples from another three fishes from each replicate were sampled in liquid nitrogen for 3 hours and later transferred to −80°C for gene expression analysis. Another two fishes from each replicate were sampled for transmission electron microscopy (TEM) analysis. Two fishes per tank were randomly selected and frozen at −20°C to assess the body composition.

All the fishes were technically anesthetized with a concentration of 100 mg/L tricaine methanesulfonate solution to eliminate any occurrence of stress during experimental sampling.

2.4. Proximate Analysis of the Diets and the Whole Fish Body. According to AOAC (1990) [40], diets and fish were analyzed to determine their proximate composition. The moisture content was measured after drying at 65°C and then 105°C to prevent dry matter loss. The Kjeldahl technique (nitrogen × 6.25) was used to determine the crude protein using (FOSS KT260, Switzerland). Crude lipid was determined by the ether extraction method using a Soxtec Auto Extraction Unit. A combustion method was used to measure the amount of ash for four hours at 550°C.

2.5. Biochemical Analysis. Biochemical parameters were analyzed using commercial assay kits (ref. no. A012) developed by the Jiancheng Institute of Bioengineering (Nanjing, China) and performed within 24 hours of sample collection. Plasma glucose level was determined by the glucose oxidase method, as described by Asadi et al. [41]. Plasma cortisol concentration was estimated by a validated radioimmunoassay (RIA) method for fish, as defined by Winberg and Lepage [42], using a commercial Iodine cortisol RIA kit (ref. no. KD005-0049) produced by Beijing North Institute of Biological Technology (Beijing, China) [43]. Based on the Rietman and Frankel method [44], the activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined. Plasma total protein (TP) and albumin (ALB) contents were assessed based on the Biuret and BCG dye-binding techniques and the bromocresol green binding technique [45], respectively.

The liver was precisely measured and homogenized using a tissue homogenizer in an ice bath of nine volumes (v/w) of cold saline. The extract was then centrifuged for 10 min at 4°C at 3000 rpm/min and the supernatant was stored for subsequent analysis at 20°C. According to the manufacturer's instructions, Jiancheng Institute of Bioengineering (Nanjing, China) provided commercial assay kits for measuring the liver's TG, TC, LDL, and HDL levels.

2.6. Determination of Antioxidant Capacity. The liver sample was precisely weighed, homogenized in 10 volumes using a speed of 3000 g at 4°C for 10 min., and then centrifuged. The supernatant was rapidly removed and kept at −80°C in a pipette for enzymatic analysis.

Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and malondialdehyde (MDA) content were all measured using a commercial test kit according to the instruction manuals. This kit was acquired from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.7. Measurement of Plasma Immunity Parameters. An ELISA (enzyme-linked immunosorbent assay) test kit for fish detection was used to measure the total plasma immunoglobulins (IgM) concentration, as described by

Table 1: Formulation and proximate composition (%) of the experimental diets.

Ingredients	ND	HF	HF + R	HF + GA	HF + B
Fishmeal	8	8	8	8	8
Soybean meal	30	30	30	30	30
Rapeseed meal	11.4	11.4	11.4	11.4	11.4
Cottonseed meal	15	15	15	15	15
Mixed oil[a]	4.92	10.02	10.02	10.02	10.02
Corn starch	27.17	22.07	22.07	22.07	22.07
Dicalcium bisphosphate	1.8	1.8	1.8	1.8	1.8
Salt	0.4	0.4	0.4	0.4	0.4
Premix[b]	1	1	1	1	1
Ethoxyquin	0.3	0.3	0.3	0.3	0.3
Resveratrol (mg/kg)	0	0	400	0	0
Glycyrrhetinic acid (mg/kg)	0	0	0	0.3	0
Berberine (mg/kg)	0	0	0	0	50
Proximate composition					
Crude protein	33.02	33.05	33.01	32.98	33
Ether extract	5.97	11.01	10.98	11.03	11.01
Gross energy (MJ/kg)	15.60	18.50	18.40	18.40	18.50

[a]Fish oil and corn oil (1 : 1). [b]Premix supplied the following minerals (g/kg) and vitamins (IU or mg/kg): $CuSO_4 \cdot 5H_2O$, 2.0 g; $FeSO_4 \cdot 7H_2O$, 25 g; $ZnSO_4 \cdot 7H_2O$, 22 g; $MnSO_4 \cdot 4H2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; Vitamin A, 900,000 IU; Vitamin D, 200,000 IU; Vitamin E, 4,500 mg; Vitamin K3, 220 mg; Vitamin B1, 320 mg; Vitamin B2, 1,090 mg; Niacin, 2,800 mg; Vitamin B5, 2,000 mg; Vitamin B6, 500 mg; Vitamin C, 5,000 mg; Pantothenate, 1,000 mg; Folic acid, 165 mg; Choline, 60,000 mg.

Abasubong et al. [30]. According to the Yano method (1992) [46], the disodium phenyl phosphate technique was used to measure the activity of AKP and plasma acid phosphatase (ACP). Stolen's turbidimetric method was slightly modified to detect the activity of the lysozyme (LYM) [47]. Myeloperoxidase (MPO) activity was assessed by Bradley et al. using the dianisidine-H_2O_2 method [48].

2.8. TEM Analysis. Three fishes' livers were removed from each tank and examined. The samples were fixed for 4 hours at 4°C in phosphate buffer saline (PBS), pH 7.5, with 25 g·L^{-1} of glutaraldehyde and 40 g·L^{-1} of formaldehyde. They were then cleaned three times for 20 minutes using PBS. Tissues were dehydrated by successive immersions (20 min each) in gradient ethanol solutions ranging from 50% to 100% (v/v) after a postfixation with 20 g·L^{-1} osmium tetroxide for 2 hours. Samples were immersed for two hours and stirred in a 1 : 1 ethanol to EPON resin mixture. They were then put into pure EPON resin for 24 hours, and at 60°C, they were polymerized. On 700 copper meshes, the ultrafine incisions were laid out and dyed with lead citrate and uranyl acetate. The mesh was observed using a Zeiss 10°C transmission electron microscope operating at 100 kV (Carl Zeiss, Barcelona, Spain). Fields were captured at a magnification of approximately 16000 visualization.

2.9. Real-Time Quantitative PCR Analysis. According to the manufacturer's instructions, total RNA from the hepatopancreas was extracted using RNAiso Plus (Takara Co. Ltd., Dalian). After that, the final RNA concentration was calculated using a NanoDrop 1000 (Thermo Fisher Scientific, USA) device. The First Strand cDNA Synthesis Kit from Takara Co. Ltd., Dalian, was used to reverse-transcribe pure RNA into cDNA following the manufacturer's instructions. As an internal control, the housekeeping gene β-actin was used to modify the quantity of input cDNA. Utilizing Takara Co. Ltd.'s SYBR Premix EX Taq, the mRNA level was determined utilizing real-time quantitative PCR. Using the Primer 6.0 program, primers for the genes XBP1, NF-B, iNOS, Il, TNF-, and INF- in the liver of channel catfish were created. These primers are shown in Table 2. In a Mini Option real-time detector, qPCR was conducted (Bio-RAD, USA). The final volume of the PCR reaction was 25 L, which included 2 L of cDNA sample, 12.50 L of 2 SYBR Green I Master Mix (Takara, Dalian, China), 0.5 L of each primer, and 9.5 L of dH_2O. The 45 cycles of 10 s at 95°C and 20 s at 60°C in the qPCR process were separated by 3 min at 95°C. A melting curve analysis of the amplification products was carried out to confirm that the desired products had been acquired after each PCR experiment. It was done in duplicate for each sample. Without the addition of the template, PCR reactions were utilized as negative controls. After the reaction, the fluorescence data were transformed into Ct values. The $2^{-\Delta\Delta CT}$ techniques were used to normalize each gene expression content to B-actin [49].

2.10. Statistical Analyses. One-way analysis of variance (ANOVA) was applied to all the data using SPSS software (Ver 23.0; SPSS, Inc., Chicago, IL, USA) for Windows after testing the homogeneity of variances with the Levene test. The Tukey's HSD (honestly significant difference) test was used to rank the means. Percentage data were arcsine transformed before the ANOVA and reversed afterward [50]. All data are presented as the means ± SEM (standard errors of the mean) of three replicates. Significance was declared at a P value equal to or less than 0.05.

Table 2: Real-time PCR primer sequences.

Genes	Forward	Reversed	Accession number
IFN-γ	CAGCAGTGACTTCAGCCAAA	GCCTCAGAGTACGCCATCAT	BM494266
IL-1β	AAAAATGGCCAGCCTGTATG	CAGCCCGGGTATTTAACTGA	EE993591
iNOS	CTCTGACCCCTGTGTTCCAC	GCCTTTGAGAGAAGACCGCT	KF704364
NF-κB	GAGTTCCCGAGCTTTTCCGA	AAGTAGCCAAAGTGCCGTCT	KF57202
XBP1	GGATCTGTATGCCAACACTGT	CAGGTGGGGCAATGATCTTAA	XM_017454668.1

IFN-γ, interferon-gamma; IL-1β, interleukin 1; TNF-α, tumour necrosis factor-alpha; iNOS, nitric oxide (NO): NF-κB, nuclear factor-kappa B; XBP1s, spliced X box-binding protein 1.

3. Results

3.1. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Growth Performance and Feed Utilization. As can be seen in Table 3, SR and TFI were not affected by dietary treatment. However, FBW, BWG, SGR, and CF were significantly lower ($P < 0.05$) in fish fed on HFD, whereas the opposite trend was seen in FCR. In contrast, these parameters were enhanced in the supplemented groups with the highest level observed in fish provided with HFD + GA.

3.2. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Biometric Parameters. As shown in Table 4, fish fed on HFD obtained a significantly higher ($P < 0.05$) HSI, VSI, and MFI, whereas the opposite was true for the condition factor. The supplement maintained these parameters at the control level.

3.3. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Proximate Composition. As seen in Table 5, moisture and ash content were not affected by dietary treatment. However, crude protein was higher ($P < 0.05$) in fish fed on GA than those fed on HFD, but no differences ($P > 0.05$) were attributed to other groups. Meanwhile, fish fed on HFD obtained higher EE than the control, but fish fed supplements showed no significant difference compared to the control, with the lowest seen in fish provided on GA and berberine diets ($P < 0.05$).

3.4. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Liver Analysis. In Table 6, fish fed on HFD was significantly higher ($P < 0.05$) in the liver, TC, TG, and LDL, whereas the opposite pattern was observed in HDL activities. These parameters were all enhanced by feeding the supplemented diets.

3.5. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Liver TEM. In Figure 1, fish fed the control diet showed normal ultrastructure in their livers. A big, spherical nucleus was centrally placed within a modest amount of cytoplasm in each hepatocyte. Fish fed the control diet showed normal ultrastructure in their livers. A big, spherical nucleus was centrally placed within a modest cytoplasm in each hepatocyte. The nucleus was ovoid and contained a prominent nucleolus. Hepatocytes displayed dark and slender mitochondria appeared with well-developed cristae and matrix. However, several abnormalities were observed in the livers of fish that received the high-fat diet. Hepatocytes exhibited many large, electron-dense fat droplets, some even larger than the nucleus. These extensive intracellular lipid droplets resulted in the displacement of the nucleus to the cell margin and a loss of cytoplasm. All the cellular membranes, including the mitochondrial and nuclear membranes, were ruptured and the nucleus was atrophic and appeared as a polygon. Due to extremely hydropic alterations, the mitochondria lost their cristae, matrix, and matrix density. However, this effect was reversed with feeding the supplemented diet.

3.6. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Serum Biochemical Parameters. In Table 7, fish fed on HFD show a higher trend ($P < 0.05$) in glucose, cortisol, AST, and ALT, while the opposite direction was found for TP. These parameters were all enhanced in the supplemented groups.

3.7. Effect of Dietary GA, Berberine, and Resveratrol Supplementation on Antioxidant Capacity Parameters. In Figure 2, SOD, CAT, and GPx were lower ($P < 0.05$) in fish fed on HFD than in the control group. However, supplementing HFD with GA, berberine, and resveratrol kept these parameters similar to the control with GA significantly higher in GPx than other supplementations.

3.8. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Immune Parameters. In Figure 3, fish fed on HFD were significantly lower ($P < 0.05$) in LYM, MPO, ACP, AKP activities, and IgM levels than the control. These parameters were all improved by feeding GA, berberine, and resveratrol.

3.9. Effects of Dietary GA, Berberine, and Resveratrol Supplementation on Immune Parameters. As seen in Figure 4, fish fed the HFD significantly upregulated ($P < 0.05$) the TNF-α, INF-γ, ILβ, XBP1, NF-κB, and iNOS gene expression compared to the control. However, these parameters were enhanced considerably with the supplements.

4. Discussion

The growth rate in this study was not significantly influenced by the rise in the dietary fat levels, despite a minor tendency towards slower growth in the HFD group. Although the

Table 3: Growth performance and feed utilization of juvenile channel catfish subjected to different dietary treatments[a].

	ND	HF	HF + R	HF + GA	HF + B
IBW[b] (g)	20.54 ± 0.10	20.50 ± 0.20	20.48 ± 0.15	20.50 ± 0.10	20.52 ± 0.19
SR[c] (%)	100	100	100	100	100
FBW[d] (g)	43.28 ± 0.57[c]	33.88 ± 0.73[d]	43.11 ± 0.61[c]	55.87 ± 0.65[a]	48.55 ± 59[b]
BWG[e] (%)	110.71 ± 0.64[c]	64.41 ± 0.54[d]	110.69 ± 0.66[c]	172.54 ± 0.0.73[a]	136.60 ± 0.48[b]
SGR[f] (% day^{-1})	1.66 ± 0.04[c]	1.14 ± 0.03[d]	1.68 ± 0.04[c]	2.23 ± 0.04[a]	1.93 ± 0.02[b]
TFI[g] (g/fish)	41.33 ± 0.41	43.08 ± 0.89	42.47 ± 0.98	39.50 ± 0.99	38.02 ± 0.77
FCR[h]	1.82 ± 0.07[bc]	3.18 ± 0.09[a]	1.87 ± 0.09[b]	1.12 ± 0.05[d]	1.35 ± 0.08[cd]

[a]Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly ($P < 0.05$) different. [b]Initial body weight. [c]Survival rate. [d]Final body weight. [e]Body weight gain. [f]Specific growth rate. [g]Total feed intake. [h]Feed conversion ratio. Survival rate (SR, %) = 100 × (Number of fish at the end/Number of fish at the start of the experiment). Body weight gain (BWG, %) = 100 × (Final body weight (g) – Initial body weight (g))/Initial body weight (g). Total feed intake (TFI, g fish-1) = Dry feed intake in each tank (g)/Number of fishes in the tank. Specific growth rate (SGR, % day^{-1}) = (Ln Final body weight – Ln Initial body weight) × 100/Duration of feeding (days). Feed conversion rate (FCR) = total intake (g)/(Final body weight–Initial body weight).

Table 4: Biometric parameters of channel catfish subjected to different dietary treatments[a].

	ND	HF	HF + R	HF + GA	HF + B
HSI[b] (%)	1.65 ± 0.15[b]	1.89 ± 0.17[a]	1.61 ± 0.20[bc]	1.49 ± 0.15[d]	1.59 ± 0.24[c]
VSI[c] (%)	10.02 ± 0.03[b]	11.48 ± 0.05[a]	10.00 ± 0.02[b]	9.37 ± 0.03[d]	9.48 ± 0.01[c]
K[d]	1.97 ± 0.15[ab]	1.93 ± 0.17[c]	1.96 ± 0.13[b]	1.99 ± 0.13[a]	1.96 ± 0.12[b]
MFI[e] (%)	1.18 ± 0.06[b]	1.76 ± 0.02[a]	1.17 ± 0.08[b]	1.05 ± 0.01[d]	1.11 ± 0.2[c]

[a]Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly ($P < 0.05$) different. [b]Hepatosomatic index (HSI, %) = Hepatopancreas weight (g) × 100/body weight (g). [c]Visceralsomatic index (VSI, %) = Visceral weight (g) × 100/body weight (g). [d]Condition factor (K, %) = Body weight (g) × 100/body length (cm)^3. [e]Mesenteric fat index (MFI, %) = Mesenteric fat weight (g) × 100/body weight (g).

Table 5: Proximate composition of channel catfish fed on GA, berberine, and resveratrol diets.

	ND	HF	HF + R	HF + GA	HF + B
Moisture%	72.85 ± 0.36	72.31 ± 0.27	72.80 ± 0.25	72.81 ± 0.21	72.86 ± 0.26
Crude protein%	16.69 ± 0.22[ab]	16.44 ± 0.14[b]	16.38 ± 19[ab]	17.11 ± 0.30[a]	16.94 ± 0.21[ab]
Ether extract%	7.62 ± 0.28[b]	8.57 ± 0.40[a]	7.72 ± 0.17[b]	6.77 ± 0.57[c]	7.13 ± 0.37b[c]
Ash%	2.82 ± 0.18	2.66 ± 0.11	3.08 ± 0.16	3.29 ± 0.19	3.05 ± 0.20

[a]Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly ($P < 0.05$) different.

Table 6: Liver lipid profile of channel catfish fed on GA, berberine, and resveratrol diet[a].

Liver	ND	HF	HF + R	HF + GA	HF + B
TC[b] (mg g^{-1})	1.15 ± 0.13[b]	2.05 ± 0.10[a]	1.17 ± 0.14[b]	1.06 ± 0.12[d]	1.11 ± 0.11[c]
TG[c] (mg g^{-1})	2.12 ± 0.22[b]	2.63 ± 0.26[a]	2.10 ± 0.23[b]	1.69 ± 0.20[d]	1.94 ± 0.21[c]
HDL[d](mg g^{-1})	2.07 ± 0.01[d]	1.38 ± 0.02[e]	2.30 ± 0.01[c]	2.76 ± 0.01[a]	2.53 ± 0.00[b]
LDL[e] (mg g^{-1})	1.26 ± 0.06[b]	1.89 ± 0.06[a]	1.25 ± 0.04[c]	1.08 ± 0.01[e]	1.18 ± 0.01[b]

[a]Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly ($P < 0.05$) different. [b]Total cholesterol. [c]Triglycerides. [d]High-density lipoprotein. [e]Low-density lipoprotein.

protein-sparing impact has been well documented in numerous fish species, including white sea bass (*Atractoscion nobilis*) [13], large rainbow trout (*Salmo gairdneri*) [51], salmon (*Salmo gairdneri*), and salmon (*Salmo salar*) [52], this effect was not observed in this study. That agrees with McGoogan and Gatlin [53] and Thoman et al. [54], who observed the absence of the protein-sparing effect of lipids in red drums (*Sciaenops ocellatus*). Diet lipids' lack of protein-sparing impact is possibly influenced by dietary protein and fat levels, certain fish species, and feeding schedules [55]. The majority of research links high dietary energy to metabolic imbalance reduced feed intake, growth-depressing effects, and excessive lipid accumulation in the liver and other visceral organs [56, 57]. In fact, in this study, VSI, MFI, and HSI were all higher in the HFD-fed group. Interestingly, HFD supplemented with GA, berberine, and resveratrol improved this parameter, suggesting the decisive role of these additives on growth performance and alleviation of fat mass in this species fed on HFD. In fact, studies have shown that resveratrol, berberine, and GA consist of natural phenolic compounds [31], benzylisoquinoline alkaloids [20], and 3β-monoglucuronyl-18β-glycyrrhetinic acid [32], respectively, which improve growth performance and reduce fat mass in fish.

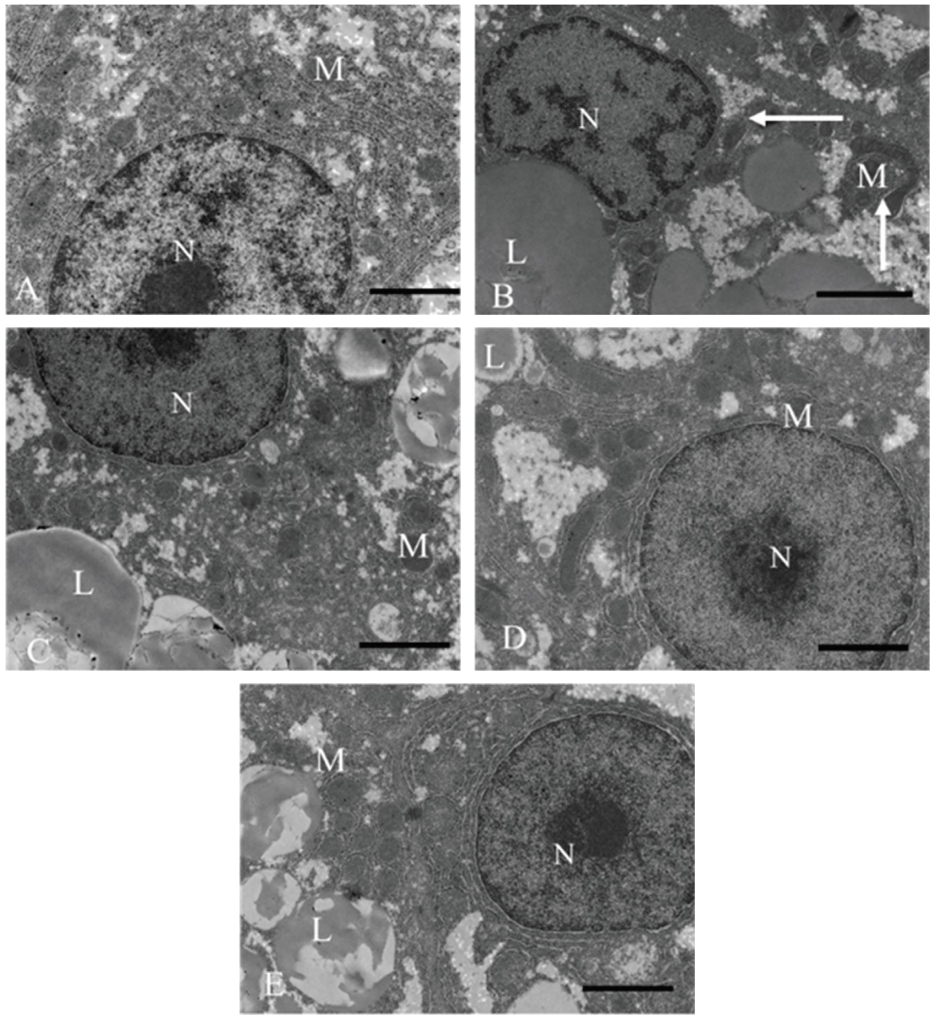

Figure 1: Transmission electron microscope images of hepatocyte, nucleus (N), (scale bar = 1 μm): (a) hepatocytes of a control channel catfish (6% fat diet), showing normal structure; (b) hepatocytes of a channel catfish that received the high-fat (11% fat) diet, presenting extensive intracellular lipid droplets (L); (c) hepatocytes of channel catfish fed on resveratrol diet showing normal structure; (d) hepatocytes of channel catfish fed on GA diet showing the normal structure and reduce lipid deposition; (e) hepatocytes of channel catfish fed on berberine diet showing the normal structure and less lipid deposition.

Table 7: Biochemical parameters of channel catfish fed on GA, berberine, and resveratrol diets[a].

	ND	HF	HF + R	HF + GA	HF + B
Glucose (g/L)	11.34 ± 0.20^{b}	14.23 ± 0.29^{a}	11.40 ± 0.21^{b}	8.63 ± 0.27^{d}	10.06 ± 0.23^{c}
Cortisol (μg/dL)	8.69 ± 0.16^{b}	11.51 ± 0.15^{a}	8.71 ± 0.16^{b}	6.60 ± 0.13^{d}	7.23 ± 0.12^{c}
TP[b] (g/L)	5.19 ± 0.19^{c}	3.21 ± 0.20^{d}	5.24 ± 0.18^{c}	7.43 ± 0.16^{a}	6.65 ± 0.12^{b}
ALB[c] (g/L)	2.82 ± 0.09	3.06 ± 0.10	2.69 ± 0.08	2.90 ± 0.9	2.73 ± 0.9
AST[d] (U/L)	20.12 ± 0.22^{b}	29.63 ± 0.26^{a}	22.10 ± 0.23^{b}	18.69 ± 0.20^{d}	19.94 ± 0.21^{c}
ALT[e] (U/L)	36.11 ± 0.01^{b}	44.31 ± 0.01^{a}	35.12 ± 0.01^{b}	29.32 ± 0.00^{b}	32.32 ± 0.00^{b}

[a]Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly ($P < 0.05$) different. [b]Total protein. [c]Albumin. [d]Aspartateamino transferase. [e]Alanineamino transferase.

In fish nutrition, the proximate composition is typically employed as an indicator to control the nutritional value [30]. In this investigation, nutritional therapy did not impact moisture and ash content. However, the HFD group's crude protein level was considerably lower than the control groups, although crude fat showed the reverse trend. With the supplemented diets, everything was turned around. These outcomes might be explained by the possibility that these additions influence fish protein synthesis and promote fatty acid oxidation. However, more research is required regarding this aspect.

According to this study, fish fed on HFD had significantly higher AST and ALT activity than the control group, indicating that intracellular enzymes were distributed into

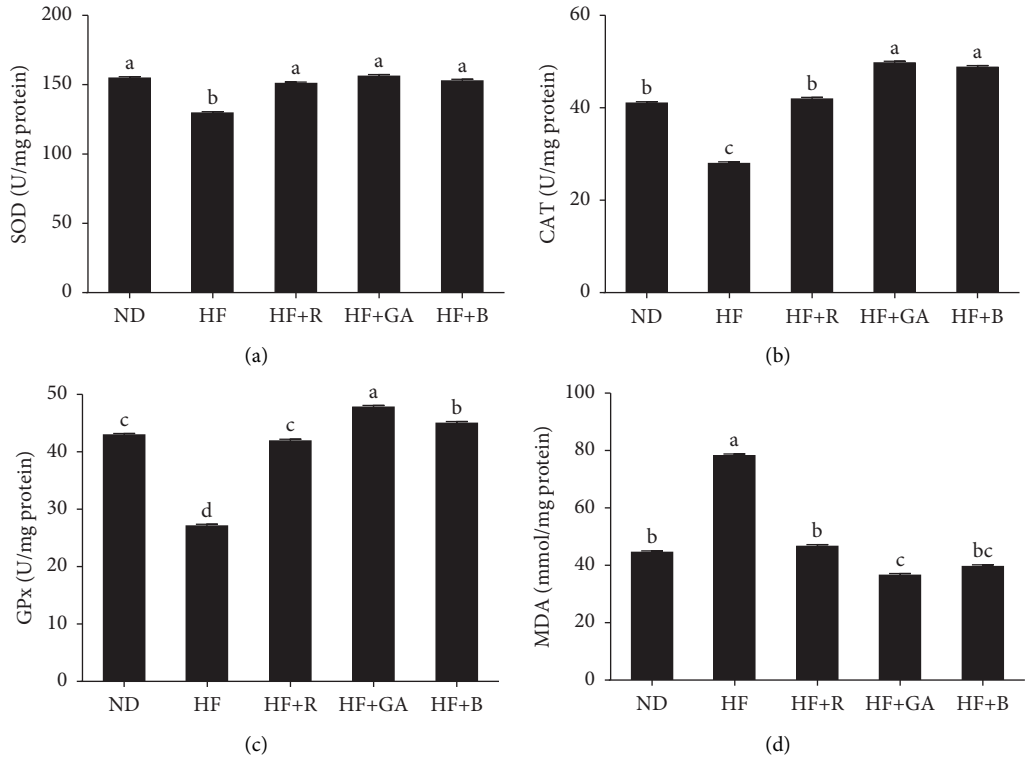

Figure 2: (a) Liver superoxide dismutase (SOD), (b) catalase (CAT), (c) glutathione peroxidase (GPx), and (d) malondialdehyde (MDA) activities, contents of channel catfish fed on HFD supplemented with GA, berberine, and resveratrol. Bars assigned with different superscripts are significantly different ($P < 0.05$). Data are represented as the mean values ± SEM of three replicates.

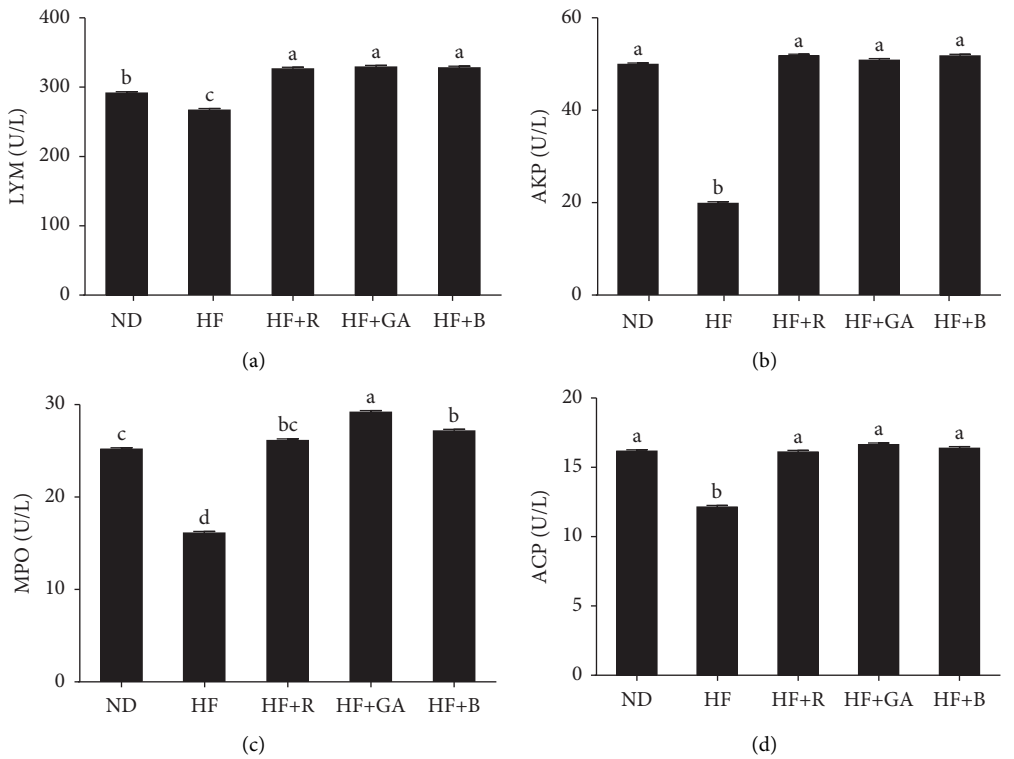

Figure 3: Continued.

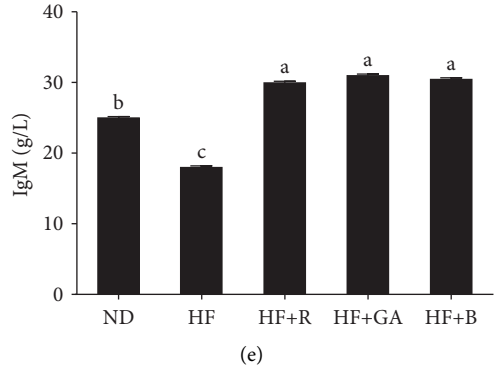

Figure 3: (a) Plasma lysozyme, (b) alkaline phosphatase (AKP), (c) myeloperoxidase (MPO), (d) acid phosphatase (ACP) activities, and (e) immunoglobulin M (IgM) levels of channel catfish fed on HFD supplemented with GA, berberine, and resveratrol. Bars assigned with different superscripts are significantly different ($P < 0.05$). Data are represented as the mean values ± SEM of three replicates.

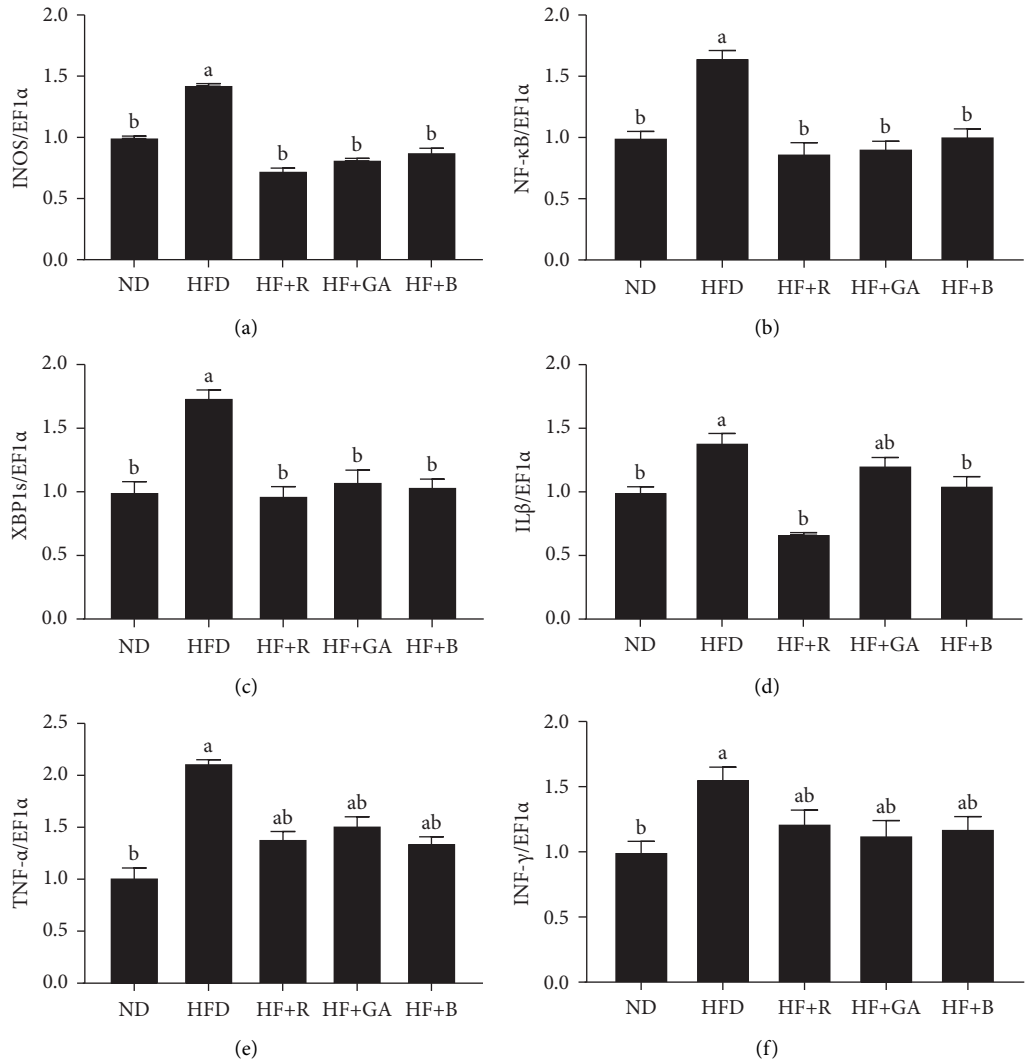

Figure 4: (a) iNOS, nitric oxide (NO); (b) NF-κB, nuclear factor-kappa B; (c) XBP1s, spliced X box-binding protein 1; (d) IL-1β, interleukin 1; (e) TNF-α, tumour necrosis factor-alpha; and (f) IFN-γ, interferon-gamma expression levels of channel catfish fed on HFD supplemented with GA, berberine, and resveratrol. Bars assigned with different superscripts are significantly different ($P < 0.05$). Data are represented as the mean values ± SEM of three replicates.

the blood [58]. This likely indicated that oxidative stress damaged hepatocyte membranes, leading to further lipid peroxidation. Indicators of fish's health status in this study included their levels of energy metabolites, i.e., TG, TC, HDL, and LDL [59]. Fish fed on HFD had higher plasma triglycerides, cholesterol, and LDL levels than control fish but lower HDL levels. According to Mensinger et al., abnormalities in lipid and lipoprotein metabolism, particularly liver dysfunction, can be indicated by high cholesterol levels [60]. Cholesterol has also been employed in numerous earlier research studies as a diagnostic tool for biologically monitoring the condition of farmed fish [61, 62]. Consequently, increased energetic metabolites, such as TG, TC, HDL, and LDL, may indicate lipids or lipoproteins metabolic problems or liver dysfunction [60, 63]. Additionally, higher glucose and cortisol levels were found in fish fed on HFD compared to control groups, indicating a stress response to high-fat diets in this fish species. That is because cortisol mainly mediates glucose production in fish by causing liver glucogenesis and gluconeogenesis when fish experience unfavorable or stressful circumstances [64].

A diet supplemented with these additions may reduce liver damage brought on by a high-fat diet, as seen by the reversed trend in the concentrations of liver TC, TG, plasma glucose, cortisol, AST, and ALT. Uncertainty persists regarding how resveratrol, berberine, and GA inhibit TC, TG, cortisol, AST, and ALT concentrations. However, most research has linked these additions to active substances, such as naturally occurring phenolic compounds and benzylisoquinoline alkaloids, which prevent the liver from producing extra fat [20, 31]. Studies have demonstrated that naturally occurring phenolic substances, such as the benzylisoquinoline alkaloids 3-monoglucuronyl-18-glycyrrhetinic, can neutralize free radicals, remove excess plasma lipids, TC, and TG from the blood, and then restore it in the liver, where it is metabolized into the blood [65]. As a result, it is feasible that a similar mechanism could be seen in diets that include resveratrol, berberine, and GA supplements. More research is therefore required regarding this aspect.

Fish fed on HFD significantly had greater MDA levels than the control group, although SOD, CAT, and GPx levels were the exact opposite. These findings show that channel catfish could experience hepatopancreatic oxidative damage due to HFD. Higher MDA concentration result in lipid peroxidation, which is brought on by free radicals resulting from a disorganized lipid metabolism [17]. According to this study, fishes fed on HFD also had increased liver lipids, TC, and TG. As a result, greater TC, TG, and liver lipid levels may be linked to higher MDA levels in fish fed on the HFD, which, in this study, resulted in oxidative stress. In other research, similar modifications were also noted [66].

In contrast, fish fed berberine, glycyrrhetinic acid, and resveratrol diets had higher liver SOD, CAT, and GPx activities with a lowered MDA concentration, indicating that these supplements may reduce MDA formation and boost the activities of antioxidant enzymes. This could be explained by the fact that resveratrol, glycyrrhetinic acid, and berberine are ester-linked phenolic acids. This phenolic substance either decomposes peroxide or absorbs and neutralizes free radicals [46]. In other investigations, similar findings were also reported. As an illustration, Zhang et al. reported that blunt-snout bream fed the resveratrol diet had considerably higher plasma and liver CAT and SOD activity than those fed the control diet [31]. Following feeding berberine combined with HFD, Chen et al. [20] and Singh and Mahajan [67] showed an improved plasma CAT, SOD, and decreased MDA content compared to the control. In light of these findings, it was determined that these additives could increase antioxidant capabilities in channel catfish fed HFD.

The nonspecific immune system, which is more important for fish than mammals, is the first line of defence against any broad spectrum of infections in fish [68–70]. According to earlier research, longer-term administration of HFD often has immune function-suppressing effects [71]. This study showed that the high-fat treatment fishes showed immunosuppression because all the immune response characteristics were lower in the high-fat diet group than in the control group. An earlier experiment on rats found that immunological suppression was linked to increased oxidative damage and mitochondrial dysfunction [72]. For instance, a prior study demonstrated that a high-fat diet could decrease immunological markers in fish. This was found in grass carp, blunt-snout bream, and common carp [16, 17, 20]. In the meantime, all plasma immunological parameters in HFD supplemented with these additives were enhanced. This proved that berberine, glycyrrhetinic acid, and resveratrol supplements might boost the immunological parameters of channel catfish fed high-fat diets [73–77]. That is because a fish's natural defence mechanism is an increase in these parameters [30].

According to numerous research studies, the features of berberine, glycyrrhetinic acid, and resveratrol's immune-stimulatory impact may be ascribed to the stimulating influence of phenolic compounds from plant extract, which may have immune-stimulatory capabilities [20, 31, 78]. Studies have shown that the active ingredient in this additive (phenolic compounds, benzylisoquinoline alkaloids, and 3β-monoglucuronyl-18β-glycyrrhetinic) could stimulate an immune response in a mammal. For example, Chen et al. reported an enhanced immune response after offering a berberine diet to blunt snout offered HFD [20]. Simultaneously, dietary supplementation with GA extracts improved the resistance to diseases and stress in *Ctenopharyngodon idellus* [79]. Our study is in line with these studies.

Recent research on mammals has established that a wide range of inflammatory illnesses are caused by the unchecked and persistent overproduction of inflammatory mediators [80]. Systemic inflammatory reaction syndrome can occasionally result in death and multiple organ failure [81]. Chronic inflammation is now understood to be a crucial mediator that may explain how excess dietary fat and HFD-induced liver damage are related [82]. According to this study's findings, HFD raised all cytokines gene responses (TNF-INF-IL-β), the inflammatory response (NF-KB and iNOS 2), and endoplasmic reticulum gene (XBP1), showing that fish fed HFD could cause an inflammatory response

[83, 84], causing chronic inflammation in these fish. The observation supports that those increased levels of the mentioned genes are linked to neutrophils, macrophage natural killer cells, and cell proliferation in fish [83, 85]. Studies have shown that HF feeding is connected to the upregulation of these genes [83]. Generally, IL-β activation-induced multiple inflammatory mediators' transcription levels, including TNF-α and INF-γ, cause autoimmune disorders [84], which is connected to higher expression of iNOS 2 and NF-KB resulting in higher expression of endoplasmic reticulum stress genes such as XBP1. However, the adverse effect was reversed when HFD was supplemented with glycyrrhetinic acid, berberine, and resveratrol.

The ability of these additives to reversely express these genes suggests that they may improve the health of channel catfish fed an HFD and reduce inflammation while enhancing the immune response. This study is the first to examine how GA affects immune-related genes in fish fed HFD. As a result, it seems impossible to relate the outcome to fish species. However, according to numerous research studies, this additive's natural components, such as *3-monoglucuronyl-18-glycyrrhetinic*, *isoquinoline alkaloid*, and *Polygonum cuspidatum*, could suppress fat mass. This active chemical may have been crucial in reducing inflammatory signals in this fish on a high-fat diet. Therefore, additional research is required regarding this point.

In conclusion, glycyrrhetinic acid, berberine, and resveratrol could reduce inflammation and enhance the immune response by mitigating high cholesterol in channel catfish fed HFD.

Data Availability

The data supporting this study are available from the corresponding author upon request.

Ethical Approval

Animals were managed, and the experimental manipulations were performed following the guidelines of the Animal Care and Use Committee in China. The Animal Research Ethics Committee has approved the current study at Nanjing Agricultural University, China (permit number: SYXK (Su) 2011-0036).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Hesham Eed Desouky was responsible for the whole experiment including feed formulation, culturing and applying treatments, performing different analysis, and writing the manuscript. Guang-zhen Jiang and Wen-bin Liu contributed to set up the final experiment and gave suggestions during the pretrial period. Yong-Jun Dai and Kenneth Prudence Abasubong collaborated in the formulating diets and laboratory analysis. Xiangyang Yuan and Kenneth Prudence Abasubong revised the statistical analysis. Jean-Jacques Y. Adjoumani and Kenneth Prudence Abasubong revised the manuscript and gave suggestions.

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