

Article

Effects of Dietary Carbohydrate Level on Growth Performance, Immune Response, and Antioxidant Capacity of the Male Giant Freshwater Prawn *Macrobrachium rosenbergii*

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Abstract

This study examined the effects of dietary carbohydrate levels on growth performance, immune response, and antioxidant capacity of male giant freshwater prawn *Macrobrachium rosenbergii*. Prawns with an initial body weight of 15.04 ± 0.08 g were fed for 9 weeks on five isonitrogenous, isolipidic diets formulated to contain 10%, 15%, 20%, 25%, and 30% carbohydrate (designated CHO10–CHO30). Weight gain rate and specific growth rate rose from CHO10 to CHO20 and then declined (peak in CHO20); broken-line regression of weight gain rate estimated the optimal dietary carbohydrate level at approximately 19.36% under the present experimental conditions. Moderate carbohydrate inclusion (15–20%) enhanced hepatopancreatic total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and catalase (CAT) activities together with the transcription of *cu/znsod*, *cat* and glutathione peroxidase (*gsh-px*), whereas levels $\geq 25\%$ lowered these defenses and raised hepatopancreatic malondialdehyde (MDA). High-carbohydrate diets also increased hemolymph glucose, triglyceride, total cholesterol and low-density lipoprotein cholesterol, progressively altered the mRNA expression of genes involved in the Toll-like receptor (TLR)/myeloid differentiation primary response 88 (MyD88) signaling pathway (upregulated *tlr*, *myd88*, *relish* and *tumor necrosis factor*; downregulated inhibitor of NF- κ B alpha (*I κ B- α*)), and altered the expression of apoptosis-related genes (downregulated B-cell lymphoma 2 (*bcl-2*), upregulated *caspase-3*). These results suggest that *M. rosenbergii* has an estimated optimal dietary carbohydrate level of approximately 19.36% under the present experimental conditions; intakes at or above 25% are associated with compromised hepatopancreatic health and growth, likely through the induction of oxidative stress, altered transcription of TLR/MyD88 pathway-related genes, and changes in apoptosis-related gene expression.

Keywords: male *Macrobrachium rosenbergii*; carbohydrate; growth performance; antioxidant; immune response

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Key Contributions: This study estimated the optimal dietary carbohydrate level for male giant freshwater prawn *Macrobrachium rosenbergii* at approximately 19.36% under the present experimental conditions, as determined by broken-line regression of growth performance. It further shows that dietary carbohydrate levels at or above 25% are associated with impaired hepatopancreatic health, involving oxidative stress, altered

transcription of TLR/MyD88 pathway-related genes, and changes in apoptosis-related gene expression.

1. Introduction

Carbohydrates rank among the most cost-effective sources of dietary energy and are consequently common ingredients in aquafeeds [1,2]. Appropriate dietary carbohydrate inclusion can exert a protein-sparing effect, thereby reducing feed costs [3,4]. However, excessive dietary carbohydrate intake is associated with a range of metabolic and physiological disturbances in aquatic animals, including persistent hyperglycemia, hepatic glycogen accumulation [5–7], reduced meat quality [8–10], immunosuppression [8,11,12], and reduced growth performance [13,14]. For example, dietary carbohydrate content exceeding 25% induces sustained hyperglycemia, suppressed growth, and marked deterioration of immune competence in rainbow trout (*Oncorhynchus mykiss*) [14]. Therefore, determining the optimal inclusion level is a critical priority in aquaculture nutrition research and commercial feed formulation, as it ensures both economic viability and optimal growth performance.

The giant freshwater prawn (*Macrobrachium rosenbergii*) ranks among the most economically valuable crustaceans in global aquaculture, characterized by rapid growth, high market value, and excellent meat quality [15,16]. According to the China Fishery Statistical Yearbook (2026), the national production of *M. rosenbergii* reached approximately 280,720 tons in 2025 [17], representing a year-on-year increase of 14.27%. To date, research on carbohydrate nutrition in this species has been limited. A few studies have examined the effects of carbohydrate sources (e.g., starch, glucose, dextrin) on growth and digestive enzyme activities in *M. rosenbergii*, with evidence suggesting that complex polysaccharides such as starch are utilized more efficiently than simple sugars [18]. However, systematic dose–response studies that comprehensively evaluate the effects of varying carbohydrate inclusion levels on growth, immunity, and antioxidant capacity remain scarce. In contrast, the carbohydrate requirements of other commercially farmed crustaceans have been thoroughly investigated. For example, for *Litopenaeus vannamei* reared in biofloc systems, a linear regression analysis based on growth performance determined the optimal carbohydrate level in the diet to be 31.44–31.77% [12]. However, the literature also documents species-specific responses to dietary carbohydrates; for example, in *Macrobrachium nipponense*, carbohydrate levels exceeding 15% have been shown to inhibit growth and digestive enzyme activity [19]. These findings indicate that the utilization of carbohydrates by crustaceans is species-dependent, highlighting the need to develop species-specific dietary recommendations for *M. rosenbergii*.

Moreover, the effects of dietary carbohydrates on immune and antioxidant systems of *M. rosenbergii* have received little attention. Innate immunity is the primary defense mechanism against pathogens in crustaceans [20], and the hepatopancreas plays a central role in both immune surveillance and antioxidant defense [21–23]. The Toll-like receptor (TLR)/myeloid differentiation primary response 88 (MyD88) signaling pathway is a key mediator of immune response [24,25], while antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), are critical for scavenging reactive oxygen species and maintaining redox homeostasis [26,27]. Recent evidence suggests that nutritional factors, including polysaccharides [24], tea tree oil [26], and protein levels [21], can modulate these pathways in *M. rosenbergii*; however, it remains unclear whether dietary carbohydrate levels affect the expression of immune and antioxidant-related genes and enzyme activities in *M. rosenbergii*.

To date, no comprehensive study has evaluated the dose-dependent effects of dietary carbohydrate on the growth performance, immune and antioxidant systems of *M. rosenbergii*, particularly at the molecular level. Given the economic importance of this species and the lack of systematic research on its carbohydrate nutrition, this study is of significant scientific and practical importance.

2. Materials and Methods

2.1. Experimental Diets

A 9-week feeding trial was conducted to evaluate the effects of different levels of dietary carbohydrate on male *M. rosenbergii*. Five diets were formulated with equal nitrogen (approximately 39% crude protein) and equal lipid (approximately 7.5% crude lipid) content, with carbohydrate levels of 10%, 15%, 20%, 25%, and 30% (named CHO10, CHO15, CHO20, CHO25, and CHO30, respectively). Dietary carbohydrate content was estimated based on the reducing sugar content determined using the 3,5-dinitrosalicylic acid (DNS) method following acid hydrolysis; the measured reducing sugar values were 10.33%, 16.02%, 20.89%, 25.92%, and 30.45%, respectively (as shown in Table 1).

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

Ingredients	CHO10	CHO15	CHO20	CHO25	CHO30
Fish meal	28	28	28	28	28
Shrimp meal	6	6	6	6	6
Squid paste	2	2	2	2	2
Soybean meal	8	8	8	8	8
Soy protein concentrate	16	16	16	16	16
Wheat starch	1	6	11	16	21
Soybean oil	1.75	1.75	1.75	1.75	1.75
Fish oil	1.75	1.75	1.75	1.75	1.75
Soybean lecithin	1	1	1	1	1
Choline chloride (50%)	1	1	1	1	1
Calcium dihydrogen phosphate	2	2	2	2	2
Vitamin premix ¹	1.5	1.5	1.5	1.5	1.5
Mineral premix ²	1.5	1.5	1.5	1.5	1.5
Microcrystalline cellulose	26	21	16	11	6
Bentonite	2	2	2	2	2
Cholesterol	0.4	0.4	0.4	0.4	0.4
Commercial antioxidant premix ³	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100
Proximate composition (% dry matter basis)					
Dry matter	89.67	90.05	88.92	89.45	88.79
Crude protein	39.47	38.94	39.81	38.96	38.67
Crude lipid	7.23	7.48	7.27	7.75	7.34
Ash	10.43	9.29	10.63	9.84	10.09
Reducing sugar ⁴	10.33	16.02	20.89	25.92	30.45

¹ Vitamin premix (per kg diet): vitamin A, 8000 IU; vitamin B₁, 15 mg; vitamin B₂, 17 mg; vitamin B₃, 50 mg; vitamin B₅, 40 mg; vitamin B₆, 15 mg; vitamin B₇, 0.25 mg; vitamin B₉, 5 mg; vitamin B₁₂, 0.1 mg; vitamin C, 140 mg; vitamin D₃, 2000 IU; vitamin E, 100 mg; vitamin K₃ 7.5 mg; inositol, 120 mg.

² Mineral premix (per kg diet): FeSO₄·7H₂O 40 mg; CuSO₄·5H₂O, 25 mg; MnSO₄·4H₂O, 10 mg; ZnSO₄·7H₂O, 100 mg; MgSO₄·7H₂O, 200 mg; CoSO₄·7H₂O, 0.35 mg; KCl, 0.05 mg; Na₂SeO₃, 0.3 mg. ³

Commercial antioxidant premix was provided by Weifang Jiayijia Biotechnology Co., Ltd. The

active ethoxyquin content in the premix does not exceed 10%. ⁴ Dietary carbohydrate level was estimated as reducing sugar content determined by the 3,5-dinitrosalicylic acid method [28].

All dry ingredients were ground and sieved through a 60-mesh screen, then mixed thoroughly. Soybean oil and fish oil were added, followed by distilled water (approximately 25–30% of the dry mixture weight). The mixture was blended until a uniform dough formed. Next, a laboratory-scale pelletizer (Shandong Saibainuo Machinery Co., Ltd.) was used to pelletize the dough into rod-shaped pellets measuring 1.5 mm in diameter and 3.0 mm in length. The pellets were dried at 40 °C for 24 h, then cooled and stored at −20 °C until use.

2.2. Rearing Management and Feeding

The feeding trial was conducted in an earthen pond at the Hubei Xunweitang Food Co., Ltd. (Wuhan, China). Prior to the feeding trial, all prawns were acclimated to the experimental conditions for 14 days. Then a total of 600 healthy male *M. rosenbergii* with a uniform initial body weight of 15.04 ± 0.08 g (mean $\pm$ SD) were randomly assigned to 15 net cages, with every three replicate cages constituting one experimental treatment group. The cages measured 70 cm $\times$ 50 cm $\times$ 70 cm, with 40 prawns stocked in each cage. During the culture period, the prawns were hand-fed to apparent satiation twice daily at 08:30 and 16:30. Dead prawns were weighed and counted during the feeding trial. Water quality parameters throughout the experiment were kept within the ranges below: dissolved oxygen > 8.0 mg/L, temperature 25–30 °C, pH 7.1–7.7, ammonia–nitrogen < 0.2 mg/L, and nitrite–nitrogen < 0.3 mg/L.

2.3. Sample Collection and Preparation

At the end of the feeding trial, all prawns were fasted for 24 h. After individual weighing, growth performance and survival rate (SR) were calculated. For biochemical and gene-expression analyses, six prawns were sampled from each cage. Samples collected from these six prawns per cage were pooled to constitute one biological replicate, with three replicate cages per treatment group ($n = 3$). Specifically, hemolymph was first collected from the pericardial cavity using a 1.5 mL syringe pre-loaded with an equal volume of pre-chilled anticoagulant solution (R1016, Solarbio, China). The hemolymph–anticoagulant mixture was centrifuged at $12,000 \times g$ for 20 min at 4 °C and stored at −80 °C for biochemical analysis. Following hemolymph collection, the hepatopancreas was dissected and weighed for hepatosomatic index (HSI) calculation. Hepatopancreas tissue samples from the same six sampled prawns per cage were stored at −80 °C for enzyme activity assays and qRT-PCR analysis. In addition, muscle tissue samples were collected for nutritional composition analysis.

2.4. Growth Performance and Feed Utilization Calculations

The growth performance and survival rate were calculated as follows:

$$\text{Weight gain rate (WGR, \%)} = 100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}.$$

$$\text{Specific growth rate (SGR, \% / d)} = [(\ln \text{ final body weight} - \ln \text{ initial body weight})] \times 100 / 63 \text{ days}.$$

$$\text{Apparent feed conversion ratio (Apparent FCR)} = \text{total feed consumed (g)} / [(\text{final total body weight of surviving prawns (g)} + \text{total body weight of dead prawns (g)}) - \text{initial total body weight (g)}].$$

Survival rate (SR, %) = $100 \times (\text{final prawn number} / \text{initial prawn number})$.

Hepatosomatic index (HSI, %) = $100 \times \text{hepatopancreas weight} / \text{final body weight}$.

2.5. Proximate Composition Analysis of Muscle

All nutritional analyses of experimental diets and prawn tissue samples were performed following the standard protocols established by the Association of Official Analytical Chemists (AOAC, 2005). Moisture content was measured by oven drying at 105 °C until a constant weight. Crude protein levels were determined via the Kjeldahl method ($N \times 6.25$) using a Kjeltec 8400 analyzer (FOSS, Denmark). Crude lipid was determined by the Soxhlet extraction method (SX-360, Opsis). Ash content was quantified by burning samples in a muffle furnace at 550 °C for 6 h.

2.6. Hemolymph Biochemical Parameters

Contents of glucose (GLU, A154-1-1), total cholesterol (TC, A111-1-1), triglycerides (TG, A110-1-1), high-density lipoprotein cholesterol (HDL-C, A112-1-1), low-density lipoprotein cholesterol (LDL-C, A113-1-1), glutamic-pyruvic transaminase (GPT, C009-2-1), glutamic-oxaloacetic transaminase (GOT, C010-2-1), acid phosphatase (ACP, A059-1-1), and alkaline phosphatase (AKP, A059-2-2) were quantified via commercial colorimetric assay kits supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.7. Immune and Antioxidant Parameters in Hepatopancreas

The indices of total antioxidant capacity (T-AOC) (A015-2-1), malondialdehyde (MDA, A003-1-1), hepatopancreatic antioxidant enzymes superoxide dismutase (SOD, A001-1) and catalase (CAT, A007-1-1) were quantified using commercial assay kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.8. RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from the hepatopancreas of *M. rosenbergii* using the RNAeasy™ Animal RNA Isolation Kit (Beyotime, Shanghai, China). First-strand cDNA was reverse-transcribed using the PrimeScript® RT Reagent Kit (Takara, Japan). The primer sequences and corresponding GenBank accession numbers are listed in Table 2. The qRT-PCR amplifications were performed in a 15 µL reaction system consisting of 7.9 µL 2× SYBR Green Master Mix, 0.3 µM each primer, 5.8 µL sterilized ddH₂O and 1 µL cDNA. The cycling protocol was as follows: 95 °C for 30 s; 40 cycles of 95 °C for 5 s; and 72 °C for 20 s. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method and standardized with CHO10, with β -actin and 18S rRNA as the dual internal reference genes.

Table 2. Primers for quantitative real-time PCR.

Genes	Primer Sequence (5'-3')	Accession No.
<i>cat</i>	F: ACTACAACCAGGAAAGTGCTCCCA	HQ668089.1
	R: TGGCGTTCCTCTTCGTTTCATGACT	
<i>cul/znsod</i>	F: GGAGCTAGATTGGCTTGCTG	MF678811.1
	R: AGAAAATCACTGCCCTCCT	
<i>gsh-px</i>	F: AGGGAAGGATCGTTCTTGTGGA	FJ670566.1
	R: TTACAGGGGAAAGCCAGGGA	
<i>tlr</i>	F: TTCGTGACTTGTCGGCTCTC	KX610955.1
	R: CGGAGTGGGAGTGAACAG	
<i>tnf</i>	F: GCAACTCCTCCAGTTCCTC	MW590714.1
	R: TTGGCTGTCTCCGAACGAC	

<i>IκB-α</i>	F: AATCATACCGGAAGGACGGCGTTA R: TCACGGGTCTGGTTAATTGGGTCA	HQ668091.1
<i>myd88</i>	F: GACCATGAGGTGACCTCTAA R: CTGCCCCAAAACGTGTGTAAG	XM_067119354.1
<i>relish</i>	F: GATGAGCCTTCAGTGCCAGA R: CCAGGTGACGCCATGTATCA	KR827675.1
<i>caspase-3</i>	F: AGGGGATAAAACCGATGGAGA R: CCCACATGACGAGCATATCA	KF878972.1
<i>bcl-2</i>	F: CGCCACAGTAGGAGAGAAGG R: TGAAAACGGCAATGGACATA	HG530759.1
<i>β-actin</i>	F: TCCGTAAGGACCTGTATGCC R: TCGGGAGGTGCGATGATTTT	AY651918.2
<i>18S</i>	F: TGTACGGGTGACGGA R: AATTACGCAGACTCGGAAGA	GQ131934.1

Note: *cat*, catalase; *cu/znsod*, copper/zinc superoxide dismutase; *gsh-px*, glutathione peroxidase; *tlr*, Toll-like receptor; *tnf*, tumour necrosis factor; *IκB-α*, inhibitor of nuclear factor kappa-B alpha; *myd88*, myeloid differentiation primary response 88; *caspase3*, caspase-3; *bcl-2*, B-cell lymphoma 2. *18S*, 18S ribosomal RNA.

2.9. Statistical Analysis

Statistical analyses were performed using SPSS 24 (IBM, Armonk, NY, USA). Group differences were tested by one-way ANOVA with Tukey's post hoc comparison at a significance level of $p < 0.05$. Broken-line regression was applied to estimate the optimal carbohydrate inclusion level. Data are expressed as mean $\pm$ SD. The cage was considered the independent replicate for all analyses ($n = 3$ replicate cages per treatment).

3. Results

3.1. Growth Performance and Feed Utilization

The FBW, WGR and SGR were significantly increased with dietary carbohydrate levels from CHO10 to CHO20 and then decreased ($p < 0.05$); the highest values for FBW (48.15 ± 2.48 g), WGR ($221.43 \pm 14.73\%$), and SGR ($1.30 \pm 0.06\%/d$) were observed in the CHO20 group. HSI increased with dietary carbohydrate levels from 10% to 25%, and then decreased in the CHO30 group ($p < 0.05$). The apparent FCR and SR had no significant difference ($p > 0.05$) (Table 3). Based on the broken-line regression analysis, the estimated optimal dietary carbohydrate level for male *M. rosenbergii* under the present experimental conditions was 19.36% (Figure 1).

Table 3. Effects of dietary carbohydrate levels on growth performance of *Macrobrachium rosenbergii*.

Parameters	Dietary Carbohydrate Levels (%)				
	CHO10	CHO15	CHO20	CHO25	CHO30
IBW (g)	15.02 ± 0.08	15.05 ± 0.07	14.98 ± 0.06	15.11 ± 0.09	15.02 ± 0.07
FBW (g)	39.10 ± 1.41^a	45.22 ± 2.55^c	48.15 ± 2.48^d	44.32 ± 1.52^c	42.65 ± 1.43^b
WGR (%)	160.31 ± 10.37^a	200.47 ± 13.55^c	221.43 ± 14.73^d	193.32 ± 10.12^c	183.97 ± 10.68^b
SGR (%/d)	1.52 ± 0.04^a	1.75 ± 0.06^c	1.85 ± 0.06^d	1.71 ± 0.04^{bc}	1.66 ± 0.04^b
Apparent FCR	1.63 ± 0.26	1.59 ± 0.15	1.67 ± 0.12	1.62 ± 0.19	1.58 ± 0.29
SR (%)	83.33 ± 2.89	86.67 ± 6.29	88.33 ± 1.44	84.17 ± 6.29	79.17 ± 3.82
HSI (%)	3.52 ± 0.15^a	3.78 ± 0.17^{ab}	4.21 ± 0.19^c	4.25 ± 0.21^c	3.93 ± 0.23^b

Note. Values (mean $\pm$ SD) ($n = 3$) with different superscripts in the same row were significantly different ($p < 0.05$). IBW, initial body weight; FBW, final body weight; WGR, weight gain rate; SGR, specific growth rate; apparent FCR, apparent feed conversion ratio; SR, survival rate; HSI, hepatosomatic index.

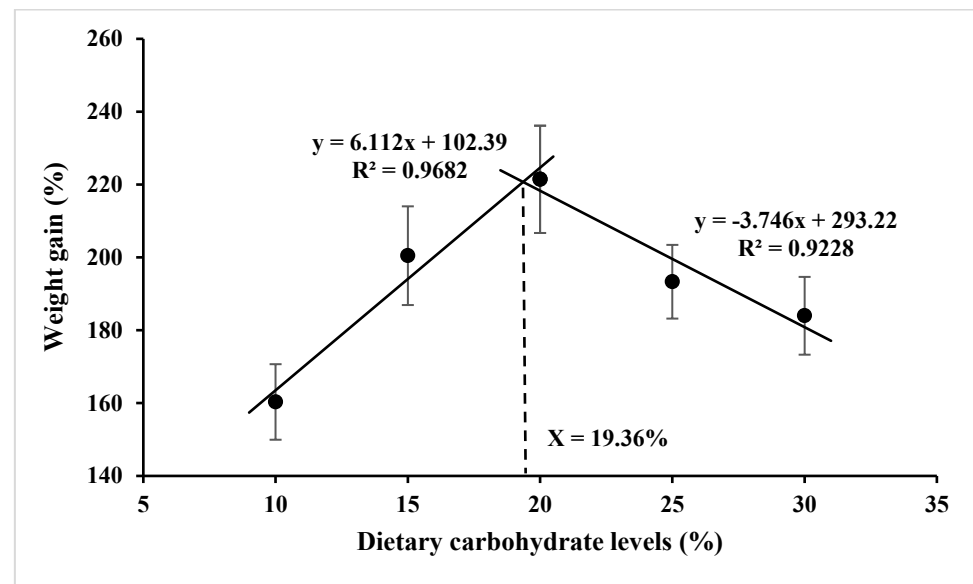


Figure 1. Estimated optimal dietary carbohydrate level for male *Macrobrachium rosenbergii* based on broken-line regression of weight gain rate.

3.2. Body Composition

Muscle crude lipid content was significantly elevated by increasing carbohydrate levels, reaching the maximum value at the highest carbohydrate inclusion ($p < 0.05$). Dietary carbohydrate supplementation exerted no significant effects on the moisture, crude protein, and ash contents in prawn muscle tissue ($p > 0.05$) (Table 4).

Table 4. Effects of dietary carbohydrate levels on muscle nutritional composition (% wet weight) of *Macrobrachium rosenbergii*.

Parameters	Dietary Carbohydrate Levels (%)				
	CHO10	CHO15	CHO20	CHO25	CHO30
Moisture (%)	73.12 ± 1.05	72.85 ± 0.92	72.53 ± 0.78	72.21 ± 0.85	71.96 ± 0.96
Crude protein (%)	17.55 ± 0.46	17.82 ± 0.52	17.93 ± 0.38	18.10 ± 0.44	17.68 ± 0.49
Crude lipid (%)	2.10 ± 0.08 ^a	2.48 ± 0.10 ^b	2.75 ± 0.12 ^c	2.82 ± 0.09 ^c	3.42 ± 0.11 ^d
Ash (%)	4.42 ± 0.23	4.58 ± 0.19	4.45 ± 0.21	4.33 ± 0.18	4.50 ± 0.22

Note. Values (mean ± SD) ($n = 3$) with different superscripts in the same row were significantly different ($p < 0.05$).

3.3. Biochemical Parameters in Hemolymph

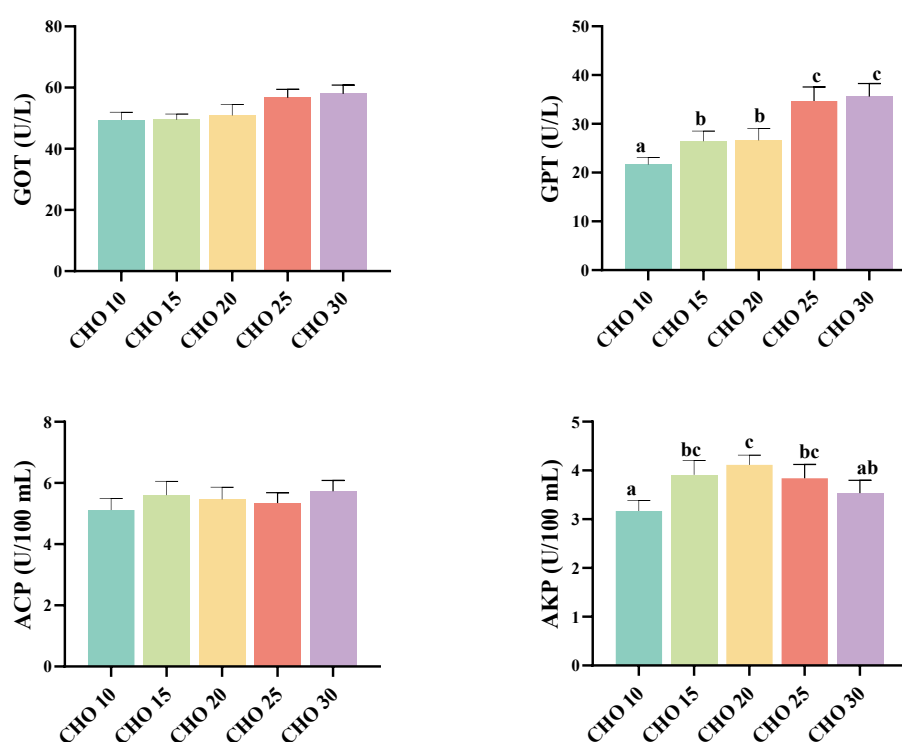
Increasing dietary carbohydrate levels markedly elevated hemolymph supernatant GLU and TC concentrations ($p < 0.05$), reaching the maximum value in the CHO30 group. TG concentrations increased with dietary carbohydrate from CHO10 up to CHO25, followed by a decline ($p < 0.05$). LDL-C levels in the CHO25 and CHO30 groups were significantly elevated compared to the CHO10 and CHO15 groups ($p < 0.05$). No significant differences in HDL-C concentration were detected among carbohydrate groups ($p > 0.05$) (Table 5).

Table 5. Effects of dietary carbohydrate levels on hemolymph biochemical parameters of *Macrobrachium rosenbergii*.

Parameters	Dietary Carbohydrate Levels (%)				
	CHO10	CHO15	CHO20	CHO25	CHO30
GLU (mmol/L)	0.49 ± 0.05 ^a	0.62 ± 0.04 ^{ab}	0.74 ± 0.03 ^{bc}	0.85 ± 0.04 ^{cd}	0.88 ± 0.07 ^d
TG (mmol/L)	0.33 ± 0.03 ^a	0.39 ± 0.04 ^a	0.50 ± 0.02 ^b	0.60 ± 0.03 ^c	0.54 ± 0.06 ^{bc}
TC (mmol/L)	0.38 ± 0.04 ^a	0.61 ± 0.09 ^{ab}	0.73 ± 0.05 ^{bc}	0.96 ± 0.07 ^{cd}	1.06 ± 0.22 ^d
HDL-C (mmol/L)	0.23 ± 0.03	0.22 ± 0.01	0.23 ± 0.04	0.27 ± 0.05	0.28 ± 0.04
LDL-C (mmol/L)	0.12 ± 0.03 ^a	0.15 ± 0.03 ^a	0.18 ± 0.02 ^{ab}	0.24 ± 0.04 ^{bc}	0.28 ± 0.03 ^c

Note. Values (mean ± SD) ($n = 3$) with different superscripts in the same row were significantly different ($p < 0.05$). GLU, glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Dietary carbohydrate inclusion had no significant impact on hemolymph GOT and ACP activities in *M. rosenbergii* ($p > 0.05$). GPT activity rose markedly as dietary carbohydrate levels increased ($p < 0.05$). AKP activity increased from CHO10 to CHO20 and subsequently declined ($p < 0.05$) (Figure 2).

**Figure 2.** Effects of dietary carbohydrate levels on immune and hepatic function parameters in hemolymph of *Macrobrachium rosenbergii*. GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; ACP, acid phosphatase; AKP, alkaline phosphatase; error bars represent the mean ± SD ($n = 3$). Bars with different superscript letters indicate significant differences among dietary groups ($p < 0.05$).

3.4. Antioxidative Parameters in Hepatopancreas

Hepatopancreatic T-AOC, SOD and CAT activities in *M. rosenbergii* increased significantly from CHO10 to CHO20, followed by a decline at higher carbohydrate levels ($p < 0.05$). The content of MDA decreased with dietary carbohydrate levels from CHO10 to CHO20, and then increased ($p < 0.05$) (Figure 3).

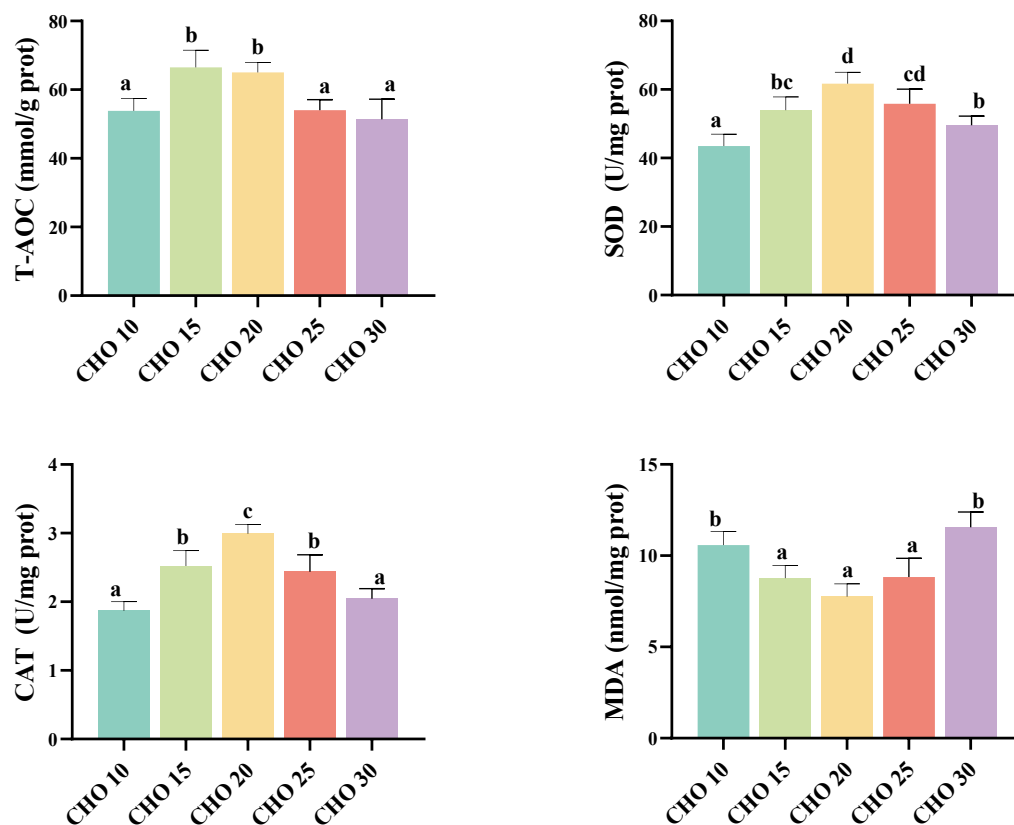


Figure 3. Effects of dietary carbohydrate levels on antioxidant parameters in hepatopancreas of *Macrobrachium rosenbergii*. T-AOC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; error bars represent the mean $\pm$ SD ($n = 3$). Bars with different superscript letters indicate significant differences among dietary groups ($p < 0.05$).

3.5. Expression of Antioxidation-Related Genes in Hepatopancreas

The mRNA levels of *cu/znsod*, *cat*, and *gsh-px* increased progressively with the dietary carbohydrate level rising from CHO10 to CHO20, peaking in the CHO20 group. Further increases to 25% and 30% resulted in varying degrees of reduction in the mRNA expression levels of these three genes ($p < 0.05$) (Figure 4).

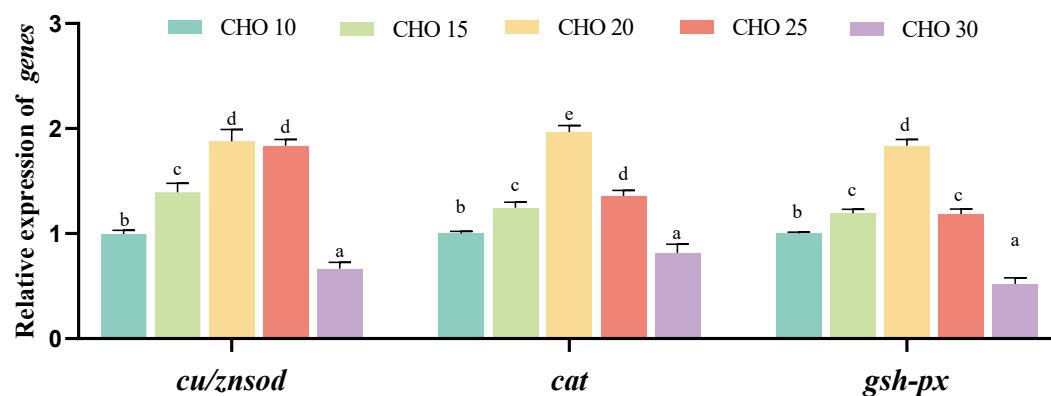


Figure 4. Effects of dietary carbohydrate levels on the expression of antioxidation-related genes in the hepatopancreas of *Macrobrachium rosenbergii*. Data are presented as mean $\pm$ SD ($n = 3$). Bars with different letters indicate significant differences among groups ($p < 0.05$).

3.6. Expression of Immune-Related Genes in Hepatopancreas

The gene expressions of *tlr*, *myd88*, *relish* and *tnf* were significantly upregulated from CHO10 to CHO30 ($p < 0.05$), whereas the expression of *IκB-α* was downregulated progressively from CHO10 to CHO30 ($p < 0.05$) (Figure 5).

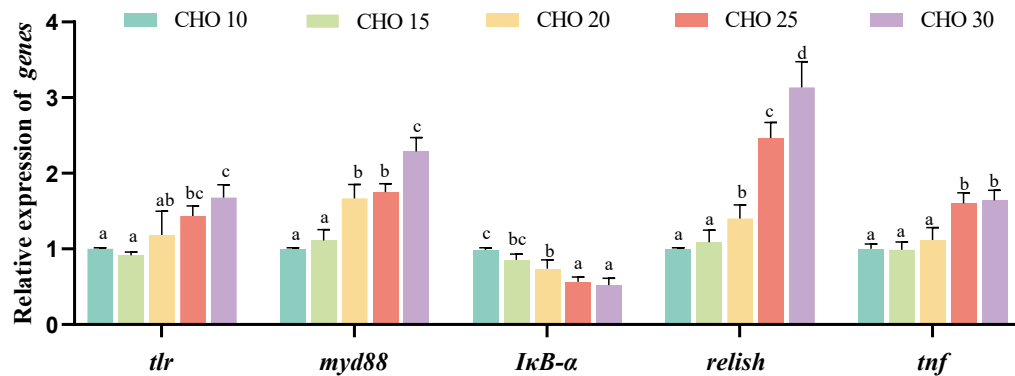


Figure 5. Effects of dietary carbohydrate levels on the expression of immune-related genes in the hepatopancreas of *Macrobrachium rosenbergii*. Data are presented as mean $\pm$ SD ($n = 3$). Bars with different letters indicate significant differences among groups ($p < 0.05$).

3.7. Expression of Apoptosis-Related Genes in Hepatopancreas

The mRNA expression of *bcl-2* was significantly downregulated, while that of *caspase-3* was upregulated as dietary carbohydrate levels increased ($p < 0.05$) (Figure 6).

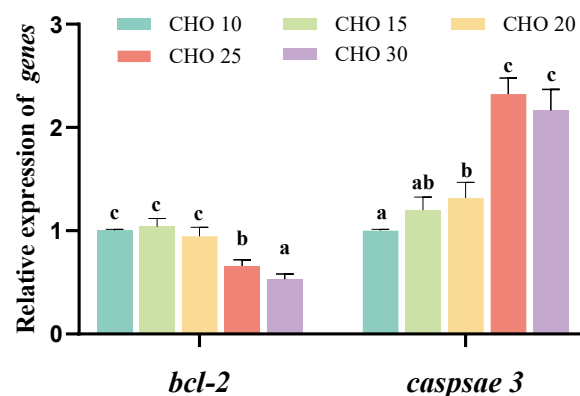


Figure 6. Effects of dietary carbohydrate levels on the expression of apoptosis-related genes in the hepatopancreas of *Macrobrachium rosenbergii*. Data are presented as mean $\pm$ SD ($n = 3$). Bars with different letters indicate significant differences among groups ($p < 0.05$).

4. Discussion

4.1. Growth Performance

Earlier investigations have demonstrated that adequate dietary carbohydrate levels can promote growth in aquatic animals. Representative examples include *O. mykiss* (optimal carbohydrate level: 12.62%), *Oncorhynchus kisutch* (14.15%), *Halotis discus hannai* (37.05%), *Schizopygopsis younghusbandi* (12%) [7,11,13,14]. In our current trial, according to broken-line regression of WGR, the estimated optimal dietary carbohydrate level for male *M. rosenbergii* under the present experimental conditions was 19.36%. High carbohydrate content in feed inhibits growth performance, which is consistent with observations in

other aquatic species [13,14]. This inhibitory effect may be attributed to crustaceans' limited ability to regulate glucose levels; excessive carbohydrate intake leads to chronic hyperglycemia, which disrupts metabolic homeostasis and redirects energy originally intended for growth toward stress responses and detoxification processes [29]. It should be noted that wheat starch and microcrystalline cellulose were substituted reciprocally to maintain isonitrogenous and isolipidic conditions across the five experimental diets. This substitution is a common practice in the formulation of isonitrogenous and isolipidic experimental diets [11–13]. However, the CHO10 diet contained both the lowest wheat starch level (1%) and the highest microcrystalline cellulose level (26%); therefore, the responses observed in this treatment—including lower growth performance, antioxidant enzyme activities, and immune parameters—cannot be attributed specifically to carbohydrate deficiency. High dietary fiber levels in crustacean feeds have been associated with reduced digestive enzyme activity, shortened gut transit time, and impaired nutrient absorption [30]. Therefore, the estimated optimal level should be interpreted with caution, as the reciprocal substitution of starch with cellulose may introduce confounding effects on digestibility.

The estimated optimal carbohydrate level obtained in this study differs notably from values reported in other crustaceans. In *L. vannamei* reared in biofloc systems, the optimal level was estimated at 31.44–31.77% [12], substantially higher than our finding. This difference may be due to the supplemental nutrients provided by the biofloc system that enhance carbohydrate utilization, whereas our pond cage system relies entirely on compound feed; alternatively, it may be that *L. vannamei* possesses stronger glycolytic capacity and higher carbohydrate tolerance than freshwater prawns [2]. In contrast, the closely related freshwater shrimp *M. nipponense* exhibits growth inhibition when the carbohydrate content in the diet exceeds 15% [19], a threshold lower than that observed in *M. rosenbergii*. These comparisons highlight the species-specific nature of carbohydrate nutrition in crustaceans.

This study was conducted exclusively on male *M. rosenbergii*. Given the significant sexual size dimorphism in this species—with males growing faster and larger than females [31]—and the potential for sex-specific differences in carbohydrate metabolism and energy allocation, the findings of this study may not be directly extrapolated to female prawns. Given that females allocate substantial energy to gonadal development, their carbohydrate requirement and tolerance may differ, warranting separate investigation.

4.2. Body Composition

In this study, the carbohydrate content in the diet significantly influenced the crude lipid content of muscle and hemolymph biochemical parameters of *M. rosenbergii*. As the carbohydrate content in the diet increased, the crude lipid content in muscle showed a gradual rise. This reflects the conversion of excess carbohydrates into lipids for storage—a metabolic strategy commonly observed in many aquatic animals when carbohydrate intake exceeds immediate energy requirements [8,10]. Excess glucose is directed toward lipid synthesis pathways rather than being fully oxidized [5]. Although moderate lipid deposition is physiologically normal, excessive lipid accumulation in muscle tissue may affect flesh quality and nutritional value, which are key considerations in commercial aquaculture production.

4.3. Hemolymph Biochemical Parameters

In this study, as the carbohydrate content in the diet increased, hemolymph glucose concentration increased continuously. This observation is consistent with the pattern reported in other crustaceans fed high-carbohydrate diets like *M. nipponense* [19] and *L. vannamei* [12], and suggests that high dietary carbohydrate intake may impair glucose

homeostasis in *M. rosenbergii*. Persistently elevated circulating glucose levels induce oxidative stress and tissue damage [29,32]. The increases in TG, TC, and LDL-C further indicate that lipid metabolism is disrupted under high-carbohydrate conditions, as excess glucose is converted into lipoproteins and transported to peripheral tissues for storage [33,34]. At excessively high carbohydrate levels (CHO30), the HSI decreased, together with elevated GPT, which may suggest altered hepatopancreatic function or cellular stress under chronic metabolic overload, although histological confirmation is needed.

4.4. Antioxidant Defense System

The antioxidant defense system is essential for protecting *M. rosenbergii* against oxidative stress by scavenging excess reactive oxygen species, and enzymes such as superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC) constitute the principal components of this protective mechanism [29]. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently decomposed by CAT, thereby limiting the accumulation of reactive intermediates that drive lipid peroxidation [35]. In the present study, hepatopancreatic T-AOC, SOD, and CAT activities increased significantly as dietary carbohydrate rose from 10% to 20%, and then declined at higher levels (25–30%) (Figure 3). This inverted-U pattern indicates that moderate carbohydrate levels (15–20%) were associated with higher antioxidant enzyme activities and lower oxidative damage, whereas lower (10%) or higher ($\geq 25\%$) levels were associated with reduced enzyme activities and increased lipid peroxidation. However, it is worth noting that elevated SOD and CAT activities can be interpreted in two ways: they may reflect an enhanced antioxidant capacity, or they may represent a compensatory response to increased reactive oxygen species (ROS) production. In the present study, the inverted-U pattern of SOD and CAT activities (peaking at CHO20) together with the lowest MDA level at CHO20 suggests that moderate carbohydrate levels support a favorable redox balance. At carbohydrate levels $\geq 25\%$, the decline in enzyme activities accompanied by elevated MDA suggests that sustained metabolic overload may eventually impair antioxidant defense [12]. This interpretation is reinforced by the transcript levels of the antioxidative genes *cu/znsod*, *cat*, which followed the same trajectory as their corresponding enzyme activities—both peaking at CHO20 and declining at CHO25–CHO30—indicating that the high-carbohydrate suppression of antioxidant capacity is manifested coherently at both the transcriptional and enzymatic levels [36]. These findings are consistent with previous studies in *M. rosenbergii* demonstrating that nutritional stressors modulate antioxidant capacity [37–39].

Malondialdehyde (MDA), a well-established indicator of oxidative stress, reflects the extent of lipid peroxidation and cellular membrane damage [40]. In this study, hepatopancreatic MDA content was lowest in the CHO20 group and increased progressively with further carbohydrate elevation, reaching the maximum in the CHO30 group. This result is consistent with increased lipid peroxidation and oxidative damage under high-carbohydrate conditions, suggesting that excessive dietary carbohydrate may induce lipid peroxidation in *M. rosenbergii* [41]. The inverse relationship between MDA and the SOD/CAT/T-AOC activities across the dietary gradient further corroborates that impairment of the antioxidant defense system is the proximate cause of the observed oxidative damage. These observations align with previous reports linking high dietary carbohydrate intake to compromised antioxidant defense and elevated oxidative stress in crustaceans [42]. Additionally, commercial antioxidant premix was supplemented to all experimental diets in this study as a synthetic antioxidant to prevent oxidative rancidity of unsaturated fatty acids (from fish oil and soybean oil). It is also worth noting that commercial antioxidant premix may have raised the baseline antioxidant status of all groups and affected the absolute values of SOD, CAT, T-AOC and MDA. As all treatment

groups received an identical concentration of commercial antioxidant premix, its potential antioxidant effects were balanced across groups and did not confound the relative comparisons of the effects of dietary carbohydrate levels.

4.5. Immune Response and TLR/MyD88 Pathway

ACP is a key lysosomal enzyme, and AKP is a membrane-bound phosphatase. Both play roles in breaking down pathogens and in cellular immunity of crustaceans [20,43]. In the present study, hemolymph AKP activity showed an initial increase under moderate carbohydrate supply, suggesting enhanced innate immunity—possibly via improved metabolic energy support for immune cells. By contrast, the decline in AKP at the highest carbohydrate levels may suggest a partial reduction in non-specific immune capacity, although ACP activity remained unchanged.

Dietary carbohydrate increments dose-dependently altered the mRNA expression of genes involved in the TLR/MyD88 signaling pathway, with *tlr*, *myd88*, *relish* and *tnf* mRNA upregulated, and *IκB-α* downregulated. This conserved cascade is central to crustacean inflammatory responses [25,43]. Normally, IκB sequesters NF-κB in an inactive state. When stressed or damaged cells release damage-associated molecular patterns, TLR-MyD88 signaling leads to IκB degradation, which frees NF-κB to enter the nucleus and induce pro-inflammatory cytokines such as TNF [24,44]. In animals on a high-carbohydrate diet, this coordinated expression pattern is consistent with enhanced inflammatory signaling at the transcriptional level, potentially reflecting a metabolic stress response.

The antioxidant and inflammatory responses do not operate in isolation. Antioxidant enzyme activities (e.g., SOD and CAT) and NF-κB-driven inflammation are reciprocally interconnected: efficient ROS scavenging by these enzymes can dampen NF-κB activation [45], whereas persistent NF-κB signaling may impair antioxidant enzyme function and aggravate oxidative injury [46]. In our high-carbohydrate feeding trials, downregulation of antioxidant enzyme activities (SOD and CAT) occurred alongside activation of the TLR/MyD88 inflammatory cascade—a pattern that would amplify rather than mitigate tissue damage [47,48]. The insufficient antioxidant capacity to counteract NF-κB activation likely accounts for why oxidative stress (elevated MDA) and inflammation (upregulated *tlr*, *myd88*, *relish*, *tnf*) both increased with rising carbohydrate levels from CHO25 to CHO30. This pattern is consistent with a proposed bidirectional disturbance in which oxidative stress and inflammation may reinforce each other, although the causal direction remains to be established by interventional studies [27,49].

4.6. Apoptosis Cascade

High dietary carbohydrate altered the expression of apoptosis-related genes in the hepatopancreas. In the present study, increasing dietary carbohydrate was accompanied by elevated lipid peroxidation (MDA), reduced antioxidant enzyme activities (T-AOC, SOD, CAT), and downregulated transcription of key antioxidative genes (*cu/znsod*, *cat*, *gsh-px*), together with upregulated transcription of TLR/MyD88 pathway-related genes. This combined oxidative and inflammatory milieu is a well-established trigger of apoptotic cell death in crustacean hepatopancreas [21,50]. The observed changes in apoptosis-related gene expression (Figure 6), reflected by downregulated *bcl-2* and upregulated *caspase-3* transcription, thus may be associated with unresolved metabolic and immune stress rather than an isolated molecular event. The downregulation of the anti-apoptotic gene *bcl-2* and upregulation of the pro-apoptotic gene *caspase-3* are consistent with a shift toward pro-apoptotic signaling, but these transcript-level changes do not directly demonstrate the occurrence of apoptosis; future work incorporating

protein or histological evidence is required to confirm the upstream triggers and the execution phase of the cascade.

5. Conclusions

Under the present experimental conditions, the CHO20 diet was associated with the best growth performance in male *M. rosenbergii*, and broken-line regression estimated the optimal dietary carbohydrate level at approximately 19.36%. Levels $\geq 25\%$ were associated with reduced growth, decreased hepatopancreatic antioxidant enzyme (SOD, CAT, T-AOC) activities and elevated MDA, altered transcription of TLR/MyD88-related genes (*tlr*, *myd88*, *relish*, *tnf*, *I κ B- α*), and altered expression of apoptosis-related genes (*bcl-2*, *caspase-3*). Excessive carbohydrate may affect hepatopancreatic health at the transcriptional level via oxidative stress, inflammatory signaling, and apoptosis-related pathways.

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References

1. Kamalam, B.S.; Medale, F.; Panserat, S. Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. *Aquaculture* **2017**, *467*, 3–27. <https://doi.org/10.1016/j.aquaculture.2016.02.007>.
2. Singha, K.P.; Sahu, N.P.; Sardar, P.; Shamna, N.; Kumar, V. A strategic roadmap for carbohydrate utilization in crustaceans feed. *Rev. Aquac.* **2024**, *16*, 674–705. <https://doi.org/10.1111/raq.12861>.
3. Maas, R.M.; Verdegem, M.C.J.; Wiegertjes, G.F.; Schrama, J.W. Carbohydrate utilisation by tilapia: A meta-analytical approach. *Rev. Aquac.* **2020**, *12*, 1851–1866. <https://doi.org/10.1111/raq.12413>.
4. Paredes, J.F.; Habte-Tsion, M.; Riche, M.; Mejri, S.; Bradshaw, D.; Chin, L.S.; Perricone, C.; Wills, P.S. Effects of different carbohydrates on growth, hepatic glucose metabolism, and gut microbiome of Florida pompano (*Trachinotus carolinus*). *Aquaculture* **2025**, *600*, 742237. <https://doi.org/10.1016/j.aquaculture.2025.742237>.
5. Boonanuntanasarn, S.; Jangprai, A.; Kumkhong, S.; Plagnes-Juan, E.; Veron, V.; Burel, C.; Marandel, L.; Panserat, S. Adaptation of Nile tilapia (*Oreochromis niloticus*) to different levels of dietary carbohydrates: New insights from a long term nutritional study. *Aquaculture* **2018**, *496*, 58–65. <https://doi.org/10.1016/j.aquaculture.2018.07.011>.
6. Qian, P.; Liu, Y.; Zhang, H.; Zhang, P.; Xie, Y.; Wu, C. Effects of five dietary carbohydrate sources on growth, glucose metabolism, antioxidant capacity and immunity of Largemouth Bass (*Micropterus salmoides*). *Animals* **2024**, *14*, 1492. <https://doi.org/10.3390/ani14101492>.

7. Guo, J.; Fu, Y.; Wu, Z.; Yu, X.; Guo, Y.; Liu, J.; Zhang, W.; Mai, K. Effects of dietary carbohydrate levels on growth performance, body composition, glucose/lipid metabolism and insulin signaling pathway in abalone *Haliotis discus hannai*. *Aquaculture* **2022**, *557*, 738284. <https://doi.org/10.1016/j.aquaculture.2022.738284>.
8. Han, Z.; Gong, Y.; Zhang, N.; Sun, Z.; Liu, S.; Huang, X.; Chen, N.; Li, S. Effects of dietary carbohydrate and lipid levels on growth performance, hepatic histology and antioxidant capacity and flesh texture of mandarin fish (*Siniperca chuatsi*). *Br. J. Nutr.* **2024**, *133*, 299–309. <https://doi.org/10.1017/s0007114524003003>.
9. Xiao, P.F.; Wu, Y.Y.; Sha, H.; Luo, X.Z.; Zou, G.W.; Liang, H.W. Effects of different carbohydrate levels in diets on growth performance and muscle nutritive value of Ying Carp and Scattered-Scaled Mirror Carp (*Cyprinus carpio*). *Aquac. Nutr.* **2025**, *2025*, 9966429. <https://doi.org/10.1155/anu/9966429>.
10. Wang, B.K.; Liu, W.B.; Xu, C.; Cao, X.F.; Zhong, X.Q.; Shi, H.J.; Li, X.F. Dietary carbohydrate levels and lipid sources modulate the growth performance, fatty acid profiles and intermediary metabolism of blunt snout bream *Megalobrama amblycephala* in an interactive pattern. *Aquaculture* **2017**, *481*, 140–153. <https://doi.org/10.1016/j.aquaculture.2017.08.034>.
11. Ye, T.; Luo, M.; Liao, Z.; Zhang, W.; Gu, X.; He, X.; Pu, H.; Li, X.; Zeng, B.; Niu, J. Effects of dietary carbohydrate levels on growth performance, antioxidant capacity, and hepatointestinal health in *Schizopygopsis younghusbandi*. *Fishes* **2025**, *10*, 489. <https://doi.org/10.3390/fishes10100489>.
12. Sun, Y.; Zhang, S.; Feng, W.; Zhang, Y.; Han, T.; Wang, J. Impact of dietary carbohydrate levels on growth performance, feed efficiency, and immune response in *Litopenaeus vannamei* cultured in biofloc systems. *Metabolites* **2025**, *15*, 409. <https://doi.org/10.3390/metabo15060409>.
13. Yu, H.; Li, L.; Yu, L.; Rahman, A.; Sattanathan, G. Effects of dietary carbohydrate level on growth performance, glucose metabolism, antioxidant capacity, immune response, and digestive function in coho salmon *Oncorhynchus kisutch* (Walbaum, 1792) fry. *Aquac. Rep.* **2025**, *43*, 102917. <https://doi.org/10.1016/j.aqrep.2025.102917>.
14. Zhao, W.; Wei, H.L.; Wang, Z.Q.; He, X.S.; Niu, J.; Kubilay, A. Effects of dietary carbohydrate levels on growth performance, body composition, antioxidant capacity, immunity, and liver morphology in *Oncorhynchus mykiss* under cage culture with flowing freshwater. *Aquac. Nutr.* **2022**, *2022*, 7820017. <https://doi.org/10.1155/2022/7820017>.
15. Jian, Y.H.; Song, J.X.; Chen, Z.Y.; Kong, Y.Q.; Qi, C.L.; Ding, Z.L.; Xu, Q.Y. Positive effects of a mixture of fish oil and soybean oil as a dietary lipid source on the ovarian development and health of female giant river prawn, *Macrobrachium rosenbergii* broodstock. *Aquac. Rep.* **2024**, *39*, 102407. <https://doi.org/10.1016/j.aqrep.2024.102407>.
16. Zhang, Y.; Tang, X.Z.; Ye, X.M.; Huan, H.H.; Miao, L.H.; Jiang, Q.; Gao, X.J.; Zhang, X.J. Impact of black soldier fly (*Hermetia illucens*) larvae meal on giant freshwater prawn (*Macrobrachium rosenbergii*): Antioxidant capacity, flesh quality, and myofiber development. *Aquaculture* **2026**, *612*, 743110. <https://doi.org/10.1016/j.aquaculture.2025.743110>.
17. Ministry of Agriculture and Rural Affairs and Bureau of Fisheries. *China Fishery Statistical Yearbook*; China Agriculture Press: Beijing, China, 2026.
18. Liming, H.; Min, L.; Huizan, Y.; Hui, G.; Xiaohua, L.; Zhid, R.; Zexing, W.; Huawei, M. Effects of different carbohydrate sources on growth performance and glycometabolism of *Macrobrachium rosenbergii*. *Chin. J. Anim. Nutr.* **2019**, *31*, 5635–5644.
19. Ding, Z.L.; Kong, Y.Q.; Li, J.F.; Cao, F.; Zhang, Y.X.; Du, Z.Y.; Ye, J.Y. Growth and metabolic responses of juvenile *Macrobrachium nipponense* to different dietary carbohydrate levels. *Aquac. Nutr.* **2017**, *23*, 1136–1144. <https://doi.org/10.1111/anu.12482>.
20. Huang, Z.; Aweya, J.J.; Zhu, C.; Tuan, T.N.; Zhang, Y. Modulation of crustacean innate immune response by amino acids and their metabolites: Inferences from other species. *Front. Immunol.* **2020**, *11*, 574721. <https://doi.org/10.3389/fimmu.2020.574721>.
21. Fu, Y.; Wang, A.; Luo, D.; Miao, S.; Chang, E.; Guo, H.; Zhang, X. Effects of dietary protein levels on *Macrobrachium rosenbergii* reared under a high-density culture model: Growth, immune response, hepatopancreatic health, and antistress capacity. *Aquac. Nutr.* **2025**, *2025*, 8880975. <https://doi.org/10.1155/anu/8880975>.
22. Xu, Z.; Liu, A.; Li, S.; Wang, G.; Ye, H. Hepatopancreas immune response during molt cycle in the mud crab, *Scylla paramamosain*. *Sci. Rep.* **2020**, *10*, 13102. <https://doi.org/10.1038/s41598-020-70139-2>.
23. Duan, Y.; Xiao, M.; Wang, Y.; Huang, J.; Yang, Y.; Li, H. The high temperature stress responses in the hepatopancreas of *Litopenaeus vannamei*: From immune dysfunction to metabolic remodeling cascade. *Front. Immunol.* **2025**, *16*, 1631655. <https://doi.org/10.3389/fimmu.2025.1631655>.
24. Sun, S.; Bian, C.; Zhou, N.; Shen, Z.; Yu, M. Dietary Astragalus polysaccharides improve the growth and innate immune response of giant freshwater prawn *Macrobrachium rosenbergii*: Insights from the brain-gut axis. *Int. J. Biol. Macromol. Struct. Funct. Interact.* **2023**, *243*, 125158. <https://doi.org/10.1016/j.ijbiomac.2023.125158>.
25. Li, C.Z.; Wang, S.; He, J.G. The two NF- κ B pathways regulating Bacterial and WSSV infection of shrimp. *Front. Immunol.* **2019**, *10*, 1785. <https://doi.org/10.3389/fimmu.2019.01785>.

26. Liu, M.; Zheng, X.; Sun, C.; Zhou, Q.; Liu, B.; Xu, P. Tea tree oil mediates antioxidant factors Relish and Nrf2-Autophagy axis regulating the lipid metabolism of *Macrobrachium rosenbergii*. *Antioxidants* **2022**, *11*, 2260. <https://doi.org/10.3390/antiox11112260>.
27. Pu, C.; Liu, Y.; Cheng, Y.; Wang, B.; Wang, A.; Zhang, C. The effects of Myo-inositol on antioxidant capacity and immunity in freshwater crayfish. *Aquac. Int.* **2025**, *33*, 263. <https://doi.org/10.1007/s10499-025-01949-2>.
28. Zhang, X.L.; Cai, Y.L.; Zhang, H.; Zhang, Z.G.; Ding, N.S. Study on the detection method of starch in feed. *Feed Res.* **2021**, *44*, 106–109. <https://doi.org/10.13557/j.cnki.issn1002-2813.2021.18.024>.
29. Wang, X.; Li, E.; Chen, L. A Review of Carbohydrate Nutrition and Metabolism in Crustaceans. *N. Am. J. Aquac.* **2016**, *78*, 178–187. <https://doi.org/10.1080/15222055.2016.1141129>.
30. del Carmen González-Peña, M.; Gomes, S.Z.; Moreira, G.S. Effects of dietary fiber on growth and gastric emptying time of the freshwater prawn *Macrobrachium rosenbergii* (De man, 1879). *J. World Aquacult. Soc.* **2002**, *33*, 441–447.
31. Liu, D.; Hong, Z.; Gui, L.; Zhao, L.; Zhou, W.; Wang, Y.; Sun, S.; Li, M. Full-length transcriptomes of brain and ganglia and sex-based differentially-expressed genes involved in growth dimorphism of giant river prawn *Macrobrachium rosenbergii*. *Authorea* **2022**, *3*, 1–26. <https://doi.org/10.22541/au.166998431.12387161/v1>.
32. Ao, S.; Xu, W.; Ding, Q.; Gao, X.; Zhang, X.; Jiang, Q. Regulation of glucose homeostasis in *Macrobrachium rosenbergii*: Effects of glucose load on glucose metabolism and hormonal response. *Aquaculture* **2023**, *577*, 11. <https://doi.org/10.1016/j.aquaculture.2023.739902>.
33. Zhao, W.; Jin, M.; Zhu, T.; Xie, S.; Yang, Y.; Li, X.; Zhou, Q. Dietary starch level regulates the growth and glycolipid metabolism through the insulin-PI3K-AKT signaling pathway of juvenile mud crab (*Scylla paramamosain*). *Aquac. Rep.* **2024**, *36*, 102124. <https://doi.org/10.1016/j.aqrep.2024.102124>.
34. Xiao, K.; Jia, X.; Qiang, W.; Chang, L.; Liu, W.; Zhang, D. Tryptophan supplements in high-carbohydrate diets by improving insulin response and glucose transport through PI3K-AKT-GLUT2 pathways in blunt snout bream (*Megalobrama amblycephala*). *J. Nutr. Biochem.* **2024**, *134*, 109715. <https://doi.org/10.1016/j.jnutbio.2024.109715>.
35. Maciel, F.E.; Geihs, M.A.; Monserrat, J.M.; Nery, L.E.M. Antioxidant defense system rhythms in crustaceans and possible roles for melatonin. *Front. Biosci. (Elite Ed.)* **2010**, *2*, 1448–1459. <https://doi.org/10.2741/E204>.
36. Li, S.; Wang, A.; Li, Z.; Zhang, J.; Sang, C.; Chen, N. Antioxidant defenses and non-specific immunity at enzymatic and transcriptional levels in response to dietary carbohydrate in a typical carnivorous fish, hybrid grouper (*Epinephelus fuscoguttatus* ♀×*E. lanceolatus* ♂). *Fish Shellfish Immunol.* **2020**, *100*, 109–116. <https://doi.org/10.1016/j.fsi.2020.03.015>.
37. Asaikkutti, A.; Bhavan, P.S.; Vimala, K. Effects of different levels of dietary folic acid on the growth performance, muscle composition, immune response and antioxidant capacity of freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture* **2016**, *464*, 136–144. <https://doi.org/10.1016/j.aquaculture.2016.06.014>.
38. Zheng, X.; Liu, Y.; Zhu, L.; Sun, C.; Wang, A.; Chen, J.; Jing, X.; Liu, B.; Chen, L. Dietary Protein and Lipid Levels Affect Growth Performance, Antioxidant Capacity, and Metabolism in Postlarval *Macrobrachium rosenbergii*. *Aquac. Nutr.* **2026**, *2026*, 9480329. <https://doi.org/10.1155/anu/9480329>.
39. Wang, L.; Yang, C.; Huang, F.; Liu, C.; Xu, J.; Chen, L.; Wu, Y.; Wang, J.; Liu, Y.; Gu, Z. Effects of dietary 5-hydroxytryptophan on growth characteristic, hepatopancreas health, antioxidant defenses, immune status, and reproductive potential of *Macrobrachium rosenbergii* broodstock. *Anim. Nutr.* **2026**. <https://doi.org/10.1016/j.aninu.2026.04.003>.
40. Yu, H.; Liang, H.; Ren, M.; Ji, K.; Yang, Q.; Ge, X.; Xi, B.; Pan, L. Effects of dietary fenugreek seed extracts on growth performance, plasma biochemical parameters, lipid metabolism, Nrf2 antioxidant capacity and immune response of juvenile blunt snout bream (*Megalobrama amblycephala*). *Fish Shellfish Immunol.* **2019**, *94*, 211–219. <https://doi.org/10.1016/j.fsi.2019.09.018>.
41. Wei, S.; Liu, T.; Zhao, Y.; Xiao, Y.; Zhou, D.; Zheng, J.; Zhou, D.; Ding, Z.; Xu, Q.; Limbu, S.M. Combined effects of dietary carbohydrate levels and ammonia stress on growth, antioxidant capacity and glucose metabolism in juvenile oriental river prawn (*Macrobrachium nipponense*). *J. Exp. Zool. Part A Ecol. Integr. Physiol.* **2023**, *339*, 978–993. <https://doi.org/10.1002/jez.2747>.
42. Wen, C.; Jia, X.; Zhu, C.; Fang, X.; Tian, H.; Ma, S.; Wang, A.; Jiang, W.; Liu, W.; Zhang, D. Berberine supplementation enhances antioxidant defense and glucose metabolism in crayfish (*Procambarus clarkii*) fed a high-carbohydrate diet. *Anim. Adv.* **2025**, *2*, e020. <https://doi.org/10.48130/animadv-0025-0018>.
43. Sun, L.; Lin, F.; Sun, B.; Qin, Z.; Chen, K.; Zhao, L.; Li, J.; Zhang, Y.; Lin, L. Scutellaria polysaccharide mediates the immunity and antioxidant capacity of giant freshwater prawn (*Macrobrachium rosenbergii*). *Dev. Comp. Immunol.* **2023**, *143*, 104678. <https://doi.org/10.1016/j.dci.2023.104678>.
44. Wanvimonsuk, S.; Jaree, P.; Kawai, T.; Somboonwivat, K. Prx4 acts as DAMP in shrimp, enhancing bacterial resistance via the toll pathway and prophenoloxidase activation. *iScience* **2023**, *26*, 105793. <https://doi.org/10.1016/j.isci.2022.105793>.

45. Wardyn, J.; Ponsford, A.; Sanderson, C. Dissecting molecular cross-talk between Nrf2 and NF- κ B response pathways. *Biochem. Soc. Trans.* **2015**, *43*, 621–626. <https://doi.org/10.1042/BST20150014>.
46. Guo, X.; Hong, S.; He, H.; Zeng, Y.; Chen, Y.; Mo, X.; Li, J.; Li, L.; Steinmetz, R.; Liu, Q. NF κ B promotes oxidative stress-induced necrosis and ischemia/reperfusion injury by inhibiting Nrf2-ARE pathway *Free Radic. Biol. Med.* **2020**, *159*, 125–135. <https://doi.org/10.1016/j.freeradbiomed.2020.07.031>.
47. He, X.; Chen, B.; Chen, Y.; Chen, A.; Lin, S.; Guo, Y.; Liao, Z.; Cui, X.; Niu, J. Astaxanthin regulates high-carbohydrate diet-induced oxidative stress, inflammation and apoptosis in an organ-specific manner in largemouth bass (*Micropterus salmoides*). *Aquaculture* **2025**, *594*, 741400. <https://doi.org/10.1016/j.aquaculture.2024.741400>.
48. Song, K.; Shen, L.; Li, X.; Kang, H.; Shi, L.; Liu, Q.; Du, X.; Tahir, R.; Yang, S.; Zhao, L. Silybin modulates hepato-intestinal redox/immune homeostasis and lipid turnover via AMPK/Nrf2/NF- κ B crosstalk in juvenile largemouth bass fed high-energy diets. *Fish Shellfish Immunol.* **2025**, *166*, 110622. <https://doi.org/10.1016/j.fsi.2025.110622>.
49. Guo, Y.; Huang, P.; Wang, W.; Wu, J.; Du, J.; Li, J.; Gao, J.; Zhu, H.; Gao, J.; Zheng, Y. Positive effects of allicin on cytotoxicity, antioxidative status, and immunity in “*Eriocheir sinensis*” hepatopancreatic cells against oxidative stress-induced injury. *Antioxidants* **2026**, *15*, 93. <https://doi.org/10.3390/antiox15010093>.
50. Li, Y.; Du, X.; Li, W.; Jiang, Q.; Ye, Y.; Yang, Y.; Liu, X.; Zhao, Y.; Che, X. Two genes related to apoptosis in the hepatopancreas of juvenile prawn, *Macrobrachium nipponense*: Molecular characterization and transcriptional response to nanoplastic exposure. *Sci. Total Environ.* **2023**, *877*, 162863. <https://doi.org/10.1016/j.scitotenv.2023.162863>.

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