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Original Article

Effect of emodin on growth performance, antioxidant status, intestinal morphology and heat shock protein gene expressions of freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract
The purpose of this study was to assess the effect of emodin on biochemical variables, antioxidant activity, heat shock protein gene expression and prawn intestinal morphology (*Macrobrachium rosenbergii*). Three feeds containing 0 mg kg^{-1} (T1, control group), 25 mg kg^{-1} (T2) and 50 mg kg^{-1} (T3) of emodin were formulated and fed for eight weeks. Alanine aminotransferase, total cholesterol and triglyceride contents were significantly lowered although glucose tended to increase following the emodin remedy. Superoxide dismutase variables in haemolymph were dramatically upgraded, whilst malondialdehyde and superoxide anion situations were significantly lowered when compared to control. Emodin supplementation had no influence on glutathione peroxidase contents. Emodin remedy dropped the expression of HSP60 and HSP90 genes in comparison to the control. In T2, the intestinal epithelial cells were complete, well-organised and tightly affixed to the basement membrane, in discrepancy to the slightly disunited basement membrane in the control group. The emodin-treated prawn individuals had a thicker and full intestinal morphology than the control groups. Supplementation with emodin at dosages of 25 mg kg^{-1} improved growth performance, blood biochemical, antioxidant capacity and immune gene expression.

Keywords: antioxidant; emodin; heat shock protein; intestinal epithelial cells; *Macrobrachium rosenbergii*

1 | INTRODUCTION

Amongst farmed crustaceans, the giant freshwater prawn (*Macrobrachium rosenbergii*) is a tropical freshwater crustacean species set up in tropical and subtropical localities

across the world (Wangari *et al.* 2021). Its blistering growth rate, carcass trait and profitable return on investment form have been widely appreciated by stakeholders (Wangari *et al.* 2021). Likewise, owing to its tastiness and nutritive value, the prawn is a cherished food item among customers (Yu *et al.* 2018). Freshwater prawn performance, on the different aspect, is hindered by a multiplicity of biotic and abiotic stresses, resulting in a drop in profitable asset, earnings characteristics and mortality prevalence (Adisu *et al.* 2020; Wangari *et al.* 2021). Antibiotic treatment against complication circumstance has long been seen as a gratuitous choice (Ranjit Kumar *et al.* 2013; Nadella *et al.* 2018). The majority of aquatic species, still, have acquired disease resistance, directing in the buildup of end products in animal tissue and the environment (Ranjit Kumar *et al.* 2013; Wangari *et al.* 2021). In the worst-case script, the development of new antibiotic-resistant genes able of causing huge mortalities has caused stakeholders to demand for a global execration on the application of antibiotics in aquaculture (Van Boeckel *et al.* 2015).

Medicinal herbs contain a dense of promising bioactive compounds such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils (Citarasu 2010). These compounds may act as an anti-stress agent in freshwater prawn culture (Liu *et al.* 2016a), growth protagonist (Vaseeharan and Thaya 2014), appetite stimulation (Lans *et al.* 2018; Sahebkar-Khorasani *et al.* 2019), immunostimulant (Rufchaei *et al.* 2017), disease resistance (Liu *et al.* 2010; van Wyk and Prinsloo 2020) and antimicrobial agents (AftabUddin *et al.* 2017). Emodin (1, 3, 8-trihydroxy-6-methyl-anthraqui-none) is an anthraquinone compound that is naturally present and is extracted from the roots and rhizomes of *Rheum palmate* L. (He *et al.* 2012; Zhao *et al.* 2017a; Adisu *et al.* 2020) that has been shown to have functional conditioning suchlike as antioxidant (Yen *et al.* 2000; Cui *et al.* 2014), immune encouragement (Giri *et al.* 2016), anti-cancer (Pecere *et al.* 2000; Guo *et al.* 2007; Ahirwar and Jain 2011; Hsu and Chung 2012) and anti-inflammatory (Park *et al.* 2009; Hu *et al.* 2014). In giant freshwater prawn, study indicated that 0.1 – 0.2% supplementation of emodin ameliorated the adverse effect of high temperature stress, reduced feed conversion ratio, stimulated non-specific immunity, enhanced growth performance and gene expression of HSP70, with much lower level of 0.05% improved the haemolymph lysozyme activity and total anti-oxidative capacity (Liu *et al.* 2010). Similarly, treatment of hepatic cell by emodin resulted reduction of the superoxide dismutase (SOD) activities which intended to protect oxidative stress in grass carp (*Megalobrama amblycephala*) (Cui *et al.* 2014). Other study on the same species corroborated that supplementation of emodin enhanced HSP70 mRNA levels, antioxidant status, coping up to crowding stress and growth (Liu *et al.* 2014). Apart from the report of emodin under stress challenges, studies elucidating the effect of emodin in *M. rosenbergii* under stress free challenges have not been reported. Therefore, this study aimed to evaluate the effect of emodin on growth performance, blood biochemical, intestinal morphology, antioxidant status and heat shock protein gene expressions of freshwater prawn (*M. rosenbergii*).

2 | METHODOLOGY

2.1 Experimental set up and diet

The proximate compositions and formulations of the trial feeds were ruled after containing feed factors from different sources (Table 1). Emodin uprooted from rhubarb was acquired at a concentration of 99.9 from Jubang Plant Material Co., Ltd. (Xi'an, China). There were three trial diets created two with emodin attention of 25 and 50 mg kg^{-1} and one with no emodin at all.

The emodin powder was precisely associated with the vitamin and mineral premix before being unified with the fresh components. Vegetable oil and 30 % water were appended to guarantee the dough's unity and consistency. Pellet diets for prawns at various growth stands were fitted using a laboratory pelletiser with diet compasses of 1.5 and 2 mm. The essay diets were kept at –20°C after dehydrating the pellets in the open air for 24 hours.

2.2 Proximate composition analysis of the diets

By applying procedures consigned by the Association of Official Analytical Chemists, the proximate compositions of dietary crude protein, fat, and moisture were adjudged. The crude protein content (N6.25) was determined employing a protein analyser and the Dumas burning approach (FP-528, LECO, USA). A petroleum ether extraction system and a Soxtec System HT were used to arbitrate the crude lipid content of the trial diets (SX360, OPSIS, Sweden).

2.3 Experimental conditions

The experiment was conducted at the Zhejiang South Tai Lake Freshwater Aquatic Seed Co. Ltd experimental base (Huzhou, China). The prawns were provided by Zhejiang South Tai Lake Freshwater Aquatic Seed Breeding Co. Ltd. Before the start of the experiment, the prawns were acclimatized for 14 days to the experimental facilities and conditions. After acclimatisation, prawns at the initial body weight of 0.19 ± 0.5 g were randomly distributed into nine cement tanks (2.0 × 1.5 × 0.65 m, water depth: 0.5 m). Triplicate tanks were used for each treatment with 50 prawn individuals in each tank. The trial was carried out in an indoor flow-through water system. All prawn individuals were hand-fed three times per day at 7:00, 12:00, and 17:30 hours for eight weeks at the feeding rate of 2 – 5% of body weight. Uneaten feed and faeces were collected by siphoning the tanks before the morning feeding. Throughout the feeding trial, the nor-

mal temperature in the water was (30 ± 0.4) °C; continuous air circulation was provided to each tank to maintain the level of the average dissolved oxygen at 6.0 mg L^{-1}; pH was 7.6 – 8.0, and absolute alkali nitrogen level was under 0.02 mg L^{-1}. The other parameters, apart from temperature, were measured weekly.

TABLE 1 Formulation and proximate composition of the basal diet.

Ingredients	Concentration (%)
Fish meal[1]	35.00
Soybean meal[2]	20.00
Rape seed meal[1]	10.00
Shrimp meal[1]	8.00
Squid extract[1]	3.00
Soybean oil[1]	1.50
Fish oil[1]	1.50
α-starch[1]	14.00
Lecithin powder[1]	1.00
Cholesero[l]	0.30
Ecdysone[1]	0.20
Monocalcium phosphate[1]	2.00
Vitamin premix[3]	1.00
Mineral premix[4]	1.00
Choline chloride	1.00
Bentonite	0.5
Proximate analysis (%)	
Crude protein	40.91
Crude lipid	9.76
Moisture	10.40

[1]Obtained from Jiangsu Fuyuda Food Products Co., Ltd., Yangzhou, China; [2]Obtained from Hulunbeier Sanyuan Milk Co., Ltd., Inner Mongolia, China; [3]Vitamin premix provided (per kg of diet): vitamin A 1500 mg, vitamin B1 1300 mg, vitamin B2 2000 mg, vitamin B6 2400 mg, vitamin B12 800 mg, vitamin C 40000 mg, vitamin D3 500 mg, vitamin K3 3500 mg, nicotinic acid 3600 mg, D-calcium pantothenate 3300 mg, folic acid 400 mg, D-biotin 800 mg, inositol 12500 mg. [4]Mineral premixes provided (per kg of diet): rice chaff powder 267400 mg, $CuSO_4 \cdot 5H_2O$ 10000 mg, $FeSO_4 \cdot 7H_2O$ 66700 mg, $MnSO_4 \cdot 4H_2O$ 9400 mg, $ZnSO_4 \cdot 7H_2O$ 34800 mg, $MgSO_4 \cdot 7H_2O$ 150000 mg, KCl 23600 mg, Na_2SeO_3 4500 mg, $CaH_4I_2O_6$ 6500 mg, $CoSO_4 \cdot 7H_2O$ 1700 mg, zeolite powder 352800 mg.

2.4 Sample collection

After the feeding trial concluded, the prawn individuals were fasted for 24 hours. Also, nine individuals were randomly named from each treatment group (three prawn individuals per tank) for the appraisal of body indicators. For investigation, respective intestinal samples were collected and kept at –80 °C. Nine haemolymph samples from each group were collected using a 1 ml syringe and kept at 4°C. Alsever's solution was appended as an anticoagulant to avert the haemolymph from clotting. Alsever's solution (anticoagulant) was adulterated 11 with haemolymph (Rodriguez *et al.* 1995). The samples were centrifuged for 10 minutes at 4°C and 4000 rpm, the treated hemolymph samples were frozen in liquid nitrogen at a temperature of – 80°C until further analysis.

2.5. Growth performance

The feed conversion ratio (FCR), specific growth rate (SGR), survival rate and weight gains (WG) were calculated as follows:

The SGR and survival rate were calculated according to Lugert *et al.* (2016) and Khan *et al.* (1998) respectively. Formulas used for other parameters considered were

Condition factor (CF) = (body weight in g) / (body length in cm))^3) × 100

WG (%) = [(final body weight − initial body weight) / initial body weight] × 100

FCR = feed consumed (g) / weight gain (g).

Hepatosomatic index, HSI (%) = (liver weight (g) / body weight (g)) × 100.

2.6 Haemolymph biochemical parameter assay

Plasma biochemical assay of glucose (GLU-HK), alanine aminotransferase (ALT), triglyceride (TG) and total cholesterol (TC) was carried out coinciding to the manufacturer's instructions and analysed employing a Mindray BS-400 automated biochemical analyser (Mindray BS-400, China). All assays were conveyed out utilising entire test kits attained from Shenzhen Mindray Bio-Medical Electronics Co. Ltd. (Shenzhen, China). The TG, TC, GLU, and alanine aminotransferase (ALT) metabolites were ruled analytically harnessing an automated biochemical analyser (Mindray, BS-400, Shenzhen, China).

2.7 Antioxidant enzymes analysis

The antioxidant enzyme labels in hemolymph, like as superoxide dismutase (SOD), malondialdehyde (MDA), superoxide anion and glutathione peroxidase (GPx), were adjudged corresponding to the approaches delineated by (Wang *et al.* 1999) and spectrophotometric measures were accomplished employing devices from Bio Tek Instruments, Inc., USA, using marketable kits supplied by Nanjing Jiancheng Bioengineer.

2.8 Total RNA isolation and cDNA synthesis

Total RNA was uprooted from the intestine employing the TRIzol reagent (Dalian Takara Co.Ltd., China), and RNA samples were also shielded against genomic DNA amplification applying RQ1 RNase-Free DNase (Dalian Takara Co. Limited, China). Thermo Scientific Nano Drop 2000 (USA) was applied to lay RNA quality and amount, with cleansed RNA owning an OD260 / OD280 ratio of 1.80 – 2.00. Also, applying a Prime Script TM RT reagent kit (Takara, Dalian, P.R. China), cDNA was synthesised from 500 ng DNase-acted RNA agreeing to the manufacturer's instructions.

0.25 µL Ex Script TM RTase (200 U μL^{-1}), 0.50 µL dT-AP Primer (50 mM), 2 µL 5x Buffer, 500 ng RNA and DEPC H_2O up to a last volume of 10 µL form up the rear transcription PCR reaction solution. For PCR reaction conditions, adjustable temperatures matching as 42°C for 40 minutes, 90°C for 2 minutes and 4°C after that were utilised.

2.8.1 Real-time qPCR: Primer's peculiar to HSP60 and HSP90, as well as β-actin genes, were attained from *M. rosenbergii* genome database of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences' aquatic disease and feed laboratory (Table 2). Harnessing the *M. rosenbergii* transcriptome study, specific primers for the target genes were evolved in line with the partial cDNA sequences of the target genes. The primers were 100 – 150 bp lengthy and fabricated by Shanghai Biocolor, Bioscience and Technology Company (Shanghai, China). HSP60 and HSP90 expression situations were quantified utilising RT-PCR on the ABI 7500 Real-time PCR System with SYBR® Premix Ex TaqTM II (TliRNase Plus) Kit under the succeeding contingencies 95°C for 10 seconds, succeeded by 45 cycles of 95°C for 5 seconds, 62°C for 15 seconds, 72°C for 10 seconds, plate reading and a terminal move of 72°C for 3 minutes (Zhao *et al.* 2017b). The trials were conveyed out in a 20 µL volume consisting 10 µL SYBR® premix Ex TaqTM (2), 0.4 µL Rox Reference Dye, 0.8 µL PCR Forward Primer (10 µM), 0.8 µL PCR Reverse Primer (10 µM), 2 µL RT reaction blend (cDNA solution) and 6 µL DEPC water. The samples were tried in duplicate. The slopes and regression curves of the standard curves were reckoned to ascertain the effectiveness of the PCR reactions (E) harnessing the equation E = 10 (−1/slope) –1 at least with seven dilutions of the template (109 – 103 copies / µL). Due to the usage of a melting curve, alone one PCR product was amplified, and primer-dimer artifacts were blowing off. The findings were standardised to β-actin (acc. No. KC195970), which exhibited minimum variability in our experimental arrangement (lower than 0.25 CT). The 2 − ΔΔCT system was applied to see the comparative fluctuations in gene (Livak and Schmittgen 2001).

TABLE 2 Details of the primers used for real-time qPCR study.

Target gene	Accession number	Primer sequences (5′-3′)	Length (bp)
β-actin	AY651918.2	TCCGTAAGGACCTGTATGCC	20
		TCGGGAGGTGCGATGATTTT	20
HSP60	KC521465	CGACGCCAACGGAATCCTAAAT	20
		CTTTGCTCAGTCTGCCCTTGT	21
HSP90	KC521466	TCTGCTATGTGGCTCTTGACTTCG	20

2.8.2 RNA isolation and RT-qPCR assay: RNA iso Plus (Takara Co. Ltd, Japan) was exploited to isolate total RNA from nine *M. rosenbergii* gut from each group, which was latterly purified with RNase-Free DNase (Takara Co. Ltd, Japan) to shuffle genomic DNA amplification. A Nanodrop was applied to adjudge the purity and concentration of RNA (DN-1000, Thermo Scientific, USA). After homogenising the concentration of the RNA samples, cDNA was generated from 300 ng DNase- treated RNA using the Ex-Script TM RT-PCR kit according to the manufacturer's instructions (Takara Co. Ltd, Japan). The cDNA samples were tried using the SYBR Green II Fluorescence Kit on a real-time quantitative detector (BIO-RAD, USA) (Takara Co. Ltd, Japan). The fluorescent qPCR response result included 10L SYBR® premix Ex Taq^{TM}, 0.4 µL ROX Reference Dye II, 0.4 µL PCR forward primer (10 µM), 0.4 µL PCR reverse primer (10 µM), 2.0 µL RT reaction (cDNA solution) and 6.8 µL dH2O. Table 2 lists all of the RT-qPCR primers that were created operating the Primer 5 program. The thermal profile was 95°C for 30 seconds, succeeded by 40 cycles of 95°C for the 5 seconds and 60°C for the 30 seconds, succeeded by a melt curve study of 15 seconds from 95 to 60°C, 60 seconds for 60°C and also up to 95°C for 15 seconds. To estimate the extent of genomic DNA impurity, control reactions were administered employing on-turn around transcribed RNA. Genomic DNA impurity was minimum in all cases. Because of its harmonious expression in the current disquisition, actin was labelled as the housekeeping gene to homogenise our samples. Each sample was subordinated to the reaction three times. The CT values from the acted and control tissue templates were compared and the $2^{-\triangle\triangle CT}$ approach was employed to compute relative quantification. (Livak and Schmittgen 2001).

2.9 Histopathological assay

For histological test, intestinal specimens from three shrimps per tank were contained and kept up in 10% formalin for 24 hours. The specimens were also dehydrated in a succession of alcohols (50 – 95%) before being bedded in paraffin. Using an Olympus CKX41 microscope (Tokyo, Japan), tissue cross sections of the specimens (5 µm thick) were stained with hematoxylin and eosin to analyse the intestinal structure

2.10 Data analysis

All data were analysed using SPSS version 25 (IBM, SPSS). The mean ± standard error (SE) was worked out and their

variation across and between treatments was examined applying a one-way analysis of variance (ANOVA). The significance position was chosen at $p < 0.05$. Duncan's Multiple Range Test was suited to differentiate means that displayed variance among treatments.

3 | RESULTS

3.1 Growth performance

Final body weight (FBW), WG and SGR of prawn individuals treated with emodin were considerably higher compared to the control treatment ($p < 0.05$; Table 3). There was a significant difference in FCR ($p < 0.05$) and the lowest value was found in the group fed with 25 mg kg^{-1} diet. The survival rate exhibited no significant difference amongst groups. But statically 25 mg kg^{-1} containing emodin group showed the higher survival rate compared to the remaining groups. No significant differences in CF and HSI were observed across treatments ($p > 0.05$).

TABLE 3 Growth performance, feed utilisation and somatic indices of *Macrobrachium rosenbergii* fed the experimental diets.

Index	Experimental diet (mg kg^{-1})		
	0	25	50
IBW	0.39±0.00	0.39±00	0.38±00
FBW	12.95±0.46^{b}	14.6±0.28^{a}	14.16±0.47^{a}
WG	3172.29±125.96^{b}	3644.73±88.59^{a}	3548.07±85.91^{a}
SGR	12.29±0.10^{b}	12.64±0.03^{a}	12.57±0.07^{a}
FCR	1.89±0.11^{a}	1.54±0.04^{b}	1.64±0.08^{ab}
SR	76.66±2.40	82±3.05	80.66±1.76
CF	0.02±0.00	0.02±00	0.02±0
HIS	5.83±0.28	5.84±0.20	6.53±0.36

Data are mean values of three replicates expressed as mean ± standard error of mean (M ± SE). Mean values in the same column with different superscripts were significantly different ($p < 0.05$). IBW, initial body weight; FBW, final body weight WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate; CF, condition factor; HIS, Hepatosomatic index.

3.2 Plasma biochemical parameter analysis

Plasma biochemical indices of *M. rosenbergii* are described in Figure 1. Dietary emodin levels significantly modified the serum profile of *M. rosenbergii* (Figure 1). Plasma biochemical modules containing Alanine aminotransferase (ALT), triglyceride (TG) total cholesterol (TC) of the prawns were considerably dropped compared to the control group, whereas there was an upgrading in glucose (GLU-HK) succeeding the smallest emodin content in the diet ($p < 0.05$).

3.3 Antioxidant enzymes

The effects of feeding emodin on haemolymph antioxidant enzymes of *M. rosenbergii* were described in Figure 2. The haemolymph of SOD was raised while MDA and superoxide anion were reduced in emodin acted groups compared to control ($p < 0.05$). No significant fluctuations in the GPx were detected with any of the parameters of interest ($p > 0.05$).

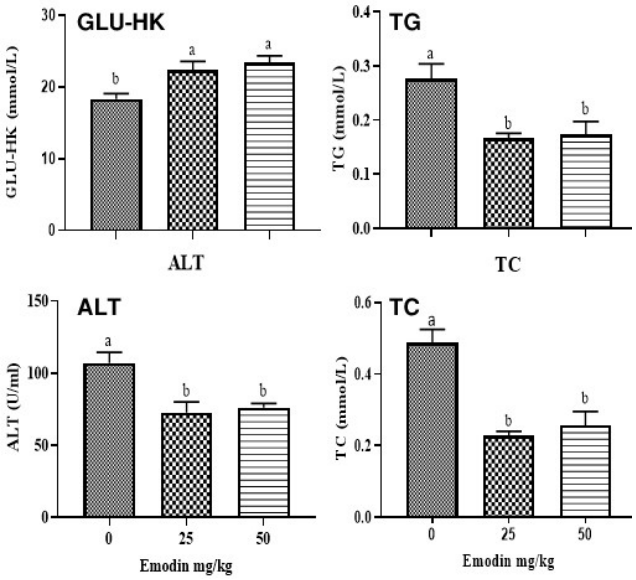

FIGURE 1 Effect of dietary emodin on haemolymph plasma Glucose (GLU-HK), Alanine aminotransferase (ALT), triglyceride (TG) total cholesterol (TC) of *Macrobrachium rosenbergii*. Data are mean values of three replicates expressed as mean ± SE. Data with different superscripts show significant differences ($p < 0.05$) between groups.

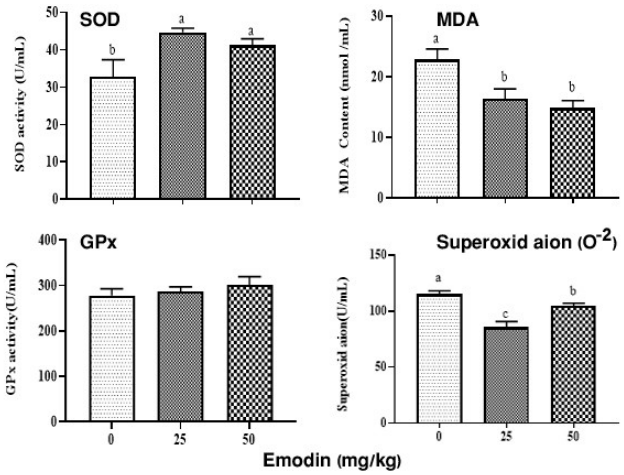

FIGURE 2 Effect of dietary emodin on haemolymph antioxidant activity Superoxide dismutase (SOD) malondialdehyde (MDA), glutathione peroxidase (GPx) and Superoxide anion of *Macrobrachium rosenbergii*. Note: Data are mean values of three replicates expressed as mean ± SE. Legends are the same as in Figure 1.

3.4 HSP60 and HSP90 gene expressions

The HSP60 and HSP90 gene expressions were vastly down-regulated directly with the reducing quantities of dietary emodin compared to the control group (Figure 3).

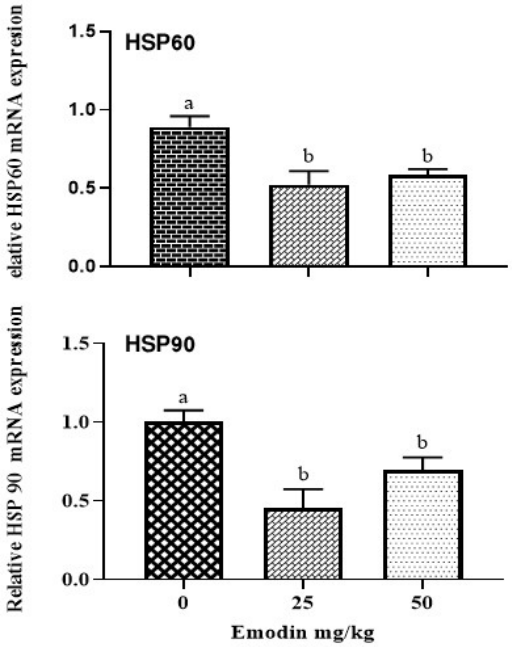

FIGURE 3 Effects of dietary emodin on HSP60 and HSP90 gene mRNA expression levels in intestine of *Macrobrachium rosenbergii*. Data are mean values of three replicates expressed as mean ± SE. Legends are the same as in Figure 1.

3.5 Histopathological assay

Figure 4 depicts fluctuations in the intestinal morphology of *M. rosenbergii* as an aftermath of emodin treatment at the arrestment of an eight-week feeding trial. Among the three treatments, 25 mg kg^{-1} emodin redounded in closely connected and fixed intestinal epithelial cells to the basement membrane (Figure 4B), whereas 50 mg kg^{-1} tended to produce a clear gap, performing in the separation of the intestinal epithelial cells from the basement membrane compared to the 25 mg kg^{-1} emodin group. Likewise, as compared to the control, the 50 mg kg^{-1} emodin group had a fairly thick and full intestinal morphology (Figure 4C).

4 | DISCUSSION

Herbal plants might be a viable substitute to mitigate the negative effects of antibiotics and enhance the immune responses of aquatic species, particularly freshwater prawns, without producing chemical residues in aquatic systems. The current study depicted that final body weight, weight gain, specific growth rate and feed conversion ratio were improved in groups supplemented by 25 mg kg^{-1} and 50 mg kg^{-1} of emodin, implying that emodin induced efficient utilisation of dietary nutrients than the control group (Zhang *et al.* 2014; Giri *et al.* 2016). Corresponding to the this, 30 mg kg^{-1} of emodin for 30 – 60 days of feeding duration (Giri *et al.* 2016) and the same level for four weeks on *Megalobrama amblycephala* diet (Zhang *et al.* 2014) and 60 mg kg^{-1} of emodin supplemented to the diet of *M. amblycephala* (Ming *et al.* 2012; Liu *et al.* 2014) improved the growth performance than the control diet. These results imply that there may be further advantages to using herbal plants.

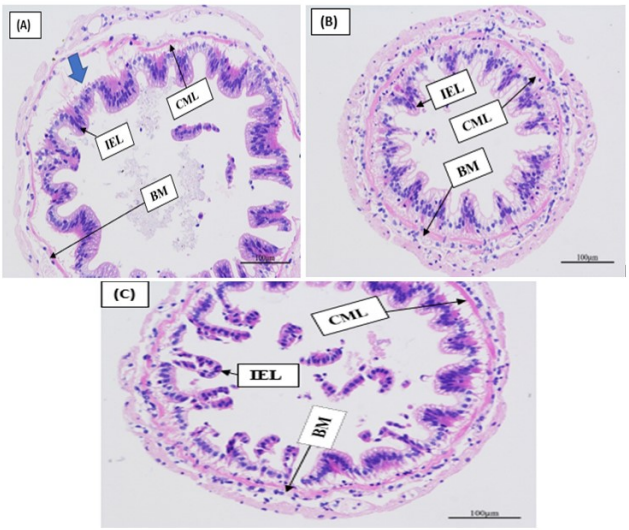

FIGURE 4 Histological analysis of intestine of *Macrobrachium rosenbergii* fed from 8 weeks with the control diet (A), with a diet containing emodin 25 mg kg^{-1} (B) and with a diet containing emodin 50 mg kg^{-1} (C). BM, basement membrane; CML, circular muscle layers; IEL, intestinal epithelial layer.

Researches indicated that emodin's similar favourable benefits may be credited for the host's improved digestive capacity by enhancing vitamin and enzyme activities, which consequently promote weight growth (Liu *et al.* 2016a, 2016b). Serum biochemical profiles are applied as an indirect marker for metabolic diseases, physiological anomalies and pathological conditions in cells and tissues of farmed creatures, including freshwater prawns (Hussein *et al.* 2018; Salisu *et al.* 2018). A drop in serum triglycerides, total cholesterol and alanine aminotransferase levels while augmenting glucose levels in the emodin supplemented groups is a signal of a managing strategy in the emodin supplemented groups compared to the control groups. As preliminarily reported in mice (Ming *et al.* 2012) and Wuchang bream (Wang *et al.* 2012). Except for GPx, the hemolymph SOD, MDA and superoxide anion levels were mainly altered in the present exploration, indicating that emodin supplementation defended against oxidative damage by boosting SOD levels and lowering MDA and superoxide anion levels. The SOD was stimulated by emodin to dimutate greatly reactive ($^{-}$O2) to lower reactive H2O2, resulting in a low level of ($^{-}$O2), which is substantial for oxidative stress mitigation. Also, emodin may have supported in the reaction to MDA, panning out in a drop to a safe position to keep up cell structure and function from intoxication (Giri *et al.* 2016). The findings of this investigation are coherent with those of Song *et al.* (2017 and 2019) in *M. amblycephala*, Cui *et al.* (2014) in

hepatic cells of grass carp (*Ctenopharyngodon idella*) and Devi *et al.* (2019) in the head kidney leucocytes of *Labeo rohita*. Likewise, the addition of emodin in the diet of *Caenorhabditis elegans* has been shown to downgrade oxidative stress (Zhao *et al.* 2017b).

HSP60 and HSP90, which differ in molecular weight, position, and functions, are up- regulated as an aftermath of heat shock liability in fish, denoting that these may be harnessed as a stress marker (Rigg *et al.* 2018). Still, emodin administration redounded in a drop in HSP60 and HSP90 gene expression compared to control, pointing that supplemented emodin levels of 25 and 50 mg kg^{-1} could not initiate HSP expression under free stress trials, resulting in *M. rosenbergii* failing to maintain cellular hemostasis. The mechanism behind emodin's failure to spark heat shock genes has to be delved further. In discrepancy to this outcome, Liu *et al.* (2016b) found that 150 mg kg^{-1} of emodin helped yellow catfish cope with acute crowding stress by boosting HSP70 expression at 12 and 24 hours after the onset of crowding stress compared to before crowding stress. In *M. amblycephala*, hepatic HSP70 mRNA levels were expounded by 60 mg kg^{-1} emodin 12 and 24 hours after stress compared to control (Liu *et al.* 2014). Research found that during high temperature stress, all groups' expression situations of liver HSP70 mRNA dropped at first, also elevated toper-stress levels, hinting that emodin permitted systems to deal with temperature stress (Ming *et al.* 2012).

Likewise, Liu *et al.* (2012) found that emodin concentrations of 5 and 25 lg ml^{-1} tended to up-regulate hepatic mRNA expression in grass carp (*C. idella*) exposed to hyperthermic stress. Also, before and after 6 hours of *A. hydrophila* infection, adding 0.1% anthraquinone extract accelerated HSP70 mRNA expression compared to control. In a former study, the same authors found that 0.1% anthraquinone extract and 0.2% anthraquinone extract redounded increased significantly HSP70 expression in *M. rosenbergii* for 6 or 8 weeks and 8 weeks, independently, when compared to the control group (Liu *et al.* 2010).

The qualitative assay of the histological partitions of the intestinal tract of freshwater prawn exhibited that the intestinal epithelial cells were intact, well-organised and closely fixed to the basement membrane in 25 mg kg^{-1} emodin group compared to the incompletely detached basement membrane in control group. Still, the intestinal epithelial cells were fairly separated in the 50 mg kg^{-1} emodin group. The intestinal morphology was more dense and complete in emodin treated groups than control. The changes spotted in the epithelial cells imply that the emodin treatment had no bodacious effect compared to the control as delineated for the normal penaeid shrimp (Bell and Lightner 1988)

5 | CONCLUSIONS

This study demonstrated that dietary emodin might boost immune system and antioxidant defenses of *M. rosenbergii* while also enhancing growth performance. Feeding diets supplemented with 25 mg kg^{-1} of emodin is the best level. This may provide additional information on how emodin is used as an immunostimulant in aquaculture.

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ETHICAL STATEMENT

The Nanjing Agricultural University located in Nanjing, China has its own guidelines for the care and use of laboratory animals when researchers and scientists should strictly follow and obey while conducting animal experiments. Accordingly, we have properly followed this guideline and the University's Animal Care and Use Committee approved the research (permit number: SYXK (Su) 2011-0036).

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

DAT conceptualisation, formal analysis, investigation; GT software, writing – original draft, writing – review & editing; MT data curation, writing – original draft, writing – review & editing; LT formal analysis, writing – original draft, writing – review & editing; FG formal analysis, writing – original draft, writing – review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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