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Original Article

Deciphering the phyto-immunomodulatory potential of orange (*Citrus sinensis*) peel in goldfish (*Carassius auratus*) diet against *Aeromonas hydrophila*

Ng Chinglembi Devi[1] • Cheryl Antony[2] • Elangovan Prabu[3] • Suraj Kumar[4] • Supratim Malla[4] • S. Selvaraj[1] • A. Uma[3] • R. Somu Sunder Lingam[5] • David Waikhom[4] • Kumaresan M.[6]

[1] *Tamil Nadu Dr. J. Jayalalithaa Fisheries University - Dr M.G.R Fisheries College and Research Institute, Ponneri, Tiruvallur – 601 204, Tamil Nadu, India*
[2] *Directorate of Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam – 611 002, Tamil Nadu, India*
[3] *Directorate of Incubation and Vocational Training in Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Muttukadu – 603 112, Tamil Nadu, India*
[4] *College of Fisheries, Central Agricultural University (Imphal), Lembucherra – 799 210, Tripura, India*
[5] *Krishnagiri Barur Centre for Sustainable Aquaculture, Tamil Nadu Dr J. Jayalalithaa Fisheries University, Barur, Krishnagiri – 635 201, Tamil Nadu, India*
[6] *Shenglong Biotech India Pvt. Ltd., Gummudipoondi, Thiruvallur, – 601 201, Tamil Nadu, India*

Correspondence
Elangovan Prabu; Directorate of Incubation and Vocational Training in Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Muttukadu – 603 112, Tamil Nadu, India
prabu@tnfu.ac.in

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Abstract
The increasing demand for sustainable and functional feed additives in aquaculture has driven interest in agro-industrial by-products such as orange (*Citrus sinensis*) peel, which are rich in bioactive compounds. This study investigated the effects of dietary orange peel supplementation on growth performance, digestibility, physiological responses, and disease resistance in goldfish *Carassius auratus*. Fish were fed diets containing 0% (control), 2.5%, 5%, and 7.5% orange peel for 60 days, followed by analyses of growth, digestive enzymes, haematological, biochemical, immunological parameters, and a pathogen challenge test. Results showed that the 2.5% inclusion level significantly enhanced growth, with approximately two-fold higher weight gain and improved feed conversion ratio (ANOVA: $p < 0.05$). Protein digestibility increased by ~20% in 2.5% and 5% groups, while digestive enzyme activities, particularly amylase (~120% increase) and protease (~25% increase), were markedly elevated. Haematological indices (RBC, HB, HT) improved significantly ($p < 0.001$), while glucose, triglycerides, and cholesterol were reduced in higher inclusion groups ($p < 0.001$). Antioxidant (SOD, CAT) and immune responses (NBT, MPO) were significantly enhanced ($p < 0.001$), indicating improved oxidative stress resistance and immune competence. Carotenoid deposition increased significantly in muscle (~24%) and skin (~23%). Following *Aeromonas hydrophila* challenge, survival improved up to 1.7-fold in supplemented groups ($p < 0.05$). Overall, the results demonstrate that orange peel, particularly at 2.5% inclusion, serves as an effective, sustainable functional feed additive that enhances growth, physiological health, and disease resistance, offering practical implications for improving aquaculture productivity and resilience.

Keywords: agricultural waste; bioactive compounds; colouration; fruit peels; orange peel

1 | INTRODUCTION

Sustainable intensification of aquaculture is increasingly recognized as essential for balancing production efficiency with environmental stewardship and public health (FAO 2022). However, intensification has concurrently escalated disease prevalence and dependence on antibiotics, accelerating concerns over antimicrobial resistance, ecological disruption, and reduced therapeutic efficacy (Choudhury and Kamilya 2019; Ahmad *et al.* 2020). These challenges have shifted emphasis toward nutritional strategies capable of enhancing endogenous immunity and resilience rather than relying solely on chemotherapeutic control (Rohani *et al.* 2022). Within this paradigm, functional feed additives derived from sustainable resources are gaining strategic importance (Laktuka *et al.* 2023).

Among freshwater bacterial pathogens, *Aeromonas hydrophila* remains one of the most pervasive and economically significant threats, causing hemorrhagic septicemia, ulcerative lesions, and systemic infections across cultured species (Pattanayak *et al.* 2020; Lavanya *et al.* 2021). Recurrent outbreaks despite antibiotic availability highlight the limitations of reactive treatment approaches and underscore the need for preventive immunomodulatory interventions that strengthen innate defense mechanisms and oxidative balance. Agricultural by-products have emerged as promising candidates within circular bioeconomy frameworks, simultaneously addressing waste management and nutritional functionality (Stevens *et al.* 2018; Kari *et al.* 2023). Orange (*Citrus sinensis*), a globally cultivated fruit crop, generates substantial quantities of peel waste during juice processing (Lv *et al.* 2015). Improper disposal of citrus residues poses environmental concerns due to their acidity and essential oil content, which can disrupt microbial ecosystems and impede composting processes (Chavan *et al.* 2018; Russo *et al.* 2021; Lucia *et al.* 2022). Nevertheless, citrus peel represents a concentrated source of bioactive compounds, including flavonoids, limonoids, carotenoids, phenolic acids, alkaloids, and volatile essential oils, with demonstrated antimicrobial and antioxidant properties (Panwar *et al.* 2021; Ortiz-Sanchez *et al.* 2024; Bratovcic 2026). These phytochemicals are known to modulate redox homeostasis and innate immune activity, key determinants of host resistance to opportunistic pathogens. Elevated polyphenolic content in the peel further supports its antimicrobial potential (Wedamulla *et al.* 2022). Additionally, orange peel provides dietary fibre, vitamin C, and bioactive antioxidants that have been shown to enhance growth efficiency, immune competence, and stress tolerance in Nile tilapia (*Oreochromis niloticus*) (Salem *et al.* 2018), bagrid catfish (*Mystus nemurus*) (See *et al.* 2024), and common carp (*Cyprinus carpio*) (Hosseini *et al.* 2020).

The ornamental aquaculture sector, valued at USD 15–30 billion annually and involving nearly two million stakeholders globally (Domínguez and Botella 2014; FAO 2021), presents a unique context where growth, pigmentation quality, and disease resistance are equally critical. Goldfish (*Carassius auratus*), a commercially prominent cyprinid species, combines economic importance with well-characterized physiology and immunological responsiveness (Camplin 2019; Kon *et al.* 2020; Filice *et al.* 2022). Given that pigmentation in ornamental fish is closely linked to carotenoid deposition and oxidative stability, phytochemical-rich feed additives may simultaneously influence aesthetic and immunological outcomes.

Despite increasing interest in phytogenic additives, most studies have evaluated isolated parameters, typically growth or antioxidant responses, without integrating digestive efficiency, pigmentation, immune modulation, and pathogen challenge within a single experimental framework. In particular, the comprehensive role of *C. sinensis* peel as a phyto-immunomodulatory agent against *A. hydrophila* in ornamental species such as *C. auratus* remains inadequately characterized. This lack of integrative assessment limits the scientific validation and translational application of citrus-processing waste as a functional aquafeed ingredient.

Therefore, the present study investigated the effects of dietary *C. sinensis* peel supplementation on growth performance, pigmentation, digestive enzyme activity, innate immune responses, and survival following *A. hydrophila* challenge in goldfish. We hypothesized that citrus peel-derived phytochemicals would enhance nutrient utilization and modulate innate immune defenses, thereby improving both ornamental quality and pathogen resistance. By aligning agro-industrial waste valorization with functional nutrition, this work advances sustainable disease management strategies in ornamental aquaculture.

2 | METHODOLOGY

2.1 Experimental fish and acclimatization

Goldfish fry (initial body weight: 0.15±0.02 g) were procured from Kolathur, Chennai, India, a recognized ornamental fish production hub, and transported to the laboratory under aerated conditions. Upon arrival, fish were stocked in 500 L fiber-reinforced plastic (FRP) tanks containing dechlorinated freshwater and acclimated for 15 days in a clear-water rearing system.

During acclimatization, water quality parameters were maintained within optimal ranges for goldfish culture (temperature: 26–28°C; dissolved oxygen: >5.0

mgL^{-1}; pH: 7.0–7.5), with continuous aeration and partial water exchange performed daily to maintain system stability. Photoperiod was maintained at 1 : 1 h (light : dark). Throughout the acclimation period, fish were fed a formulated control diet containing 35% crude protein (Table 1) at 10% of body weight per day, divided into two equal feedings at 09:30 and 16:30 h (IST). Feed ration was adjusted periodically based on biomass estimation to ensure consistent nutritional input.

2.2 Collection of orange peel, preparation of experimental diets, and proximate analysis

Fresh orange peels were collected from fruit vendors in Ponneri, Tamil Nadu, India. Peels were washed with potable water, oven-dried at 45°C to constant weight, ground into powder, sieved (0.3 mm mesh), and stored in airtight containers at 4°C until use. Experimental diets were formulated using feed ingredients procured from the Directorate of Incubation and Vocational Training in Aquaculture (DIVA), TNJFU, Muthukadu, Tamil Nadu, India. Four iso-nitrogenous diets (35% crude protein) were prepared with graded inclusion levels of orange peel powder: 0% (Control), 2.5% (T1), 5% (T2), and 7.5% (T3) (Table 1). All dry ingredients were finely ground, sieved (0.3 mm), and mixed thoroughly. Water was added to obtain uniform dough, which was steam-cooked at atmospheric pressure for 15 min. After cooling, heat-sensitive ingredients (orange peel powder, fish oil, soy lecithin, chromic oxide, vitamin and mineral premixes) were incorporated and blended uniformly. The mixture was pelleted (2 mm diameter), air-dried for 24 h, oven-dried at 45°C to <10% moisture, and stored at –4°C until use. Proximate composition (moisture, crude protein, crude lipid, ash, and crude fibre) was determined according to AOAC (1995) methods. Ingredient formulation and nutrient composition are presented in Table 1.

TABLE 1 Ingredients and proximate composition of the experimental diets (g kg^{-1}) fed during the experimental trial to goldfish (*Carassius auratus*).

Feed ingredients	Control	Orange peel		
		2.5%	5.0%	7.5%
Fish meal	150	150	150	150
Soybean meal	220	220	220	220
Corn gluten meal	140	140	140	140
Wheat	180	180	180	180
Corn flour	200	175	150	125
Casava flour	75	75	75	75
Fish oil	10	10	10	10
Soy lecithin	10	10	10	10
Vitamin and mineral premix	10	10	10	10
Chromic oxide	5	5	5	5
Orange Peel	0	25	50	75
Total	1000	1000	1000	1000
Proximate composition (%)				
Crude protein	35.02	35.01	35	34.99
Crude lipid	4.54	4.49	4.43	4.37
Crude fibre	2.38	2.64	2.91	3.17
Ash	2.59	2.61	2.63	2.65

Composition of vitamin premix (quantity per kg): Vit. A - 1,00,00,000 IU, Vit. B1-5,000 mg, Vit. B2 -5,000 mg, Vit. B3 - 6,000 mg, Vit. B5 - 6,000 mg, Vit. B6 - 6,000 mg, Vit. C - 60,000 mg, Vit. D3 - 20,00,000 IU, Vit. E - 10,000 EU, Vit. H – 200 mg.

Composition of mineral premix (quantity per kg): Magnesium – 2,800 mg, Iodine – 7.4 mg, Iron – 7,400 mg, Copper – 1,200 mg, Manganese – 11,600 mg, Zinc – 9,800 mg, Chlorides Cobalt – 4 mg, Potassium – 100 mg, Selenium – 4 mg, Calcium Carbonate – 27.25%, Phosphorous – 7.45 mg, Sulphur – 0.7 mg, Sodium – 6 mg, Calpan – 200 mg, Aluminium – 1,500 mg and Choline Chloride – 10,000 mg

2.3 Feeding experiments

A 60-day feeding trial was conducted using 12 fibre-reinforced plastic (FRP) tanks (500 L capacity) maintained at a working volume of 350 L. Goldfish fry (0.15±0.02 g) were stocked at a density of 20 fish m^{-2} and randomly distributed into four dietary treatments, each in triplicate, following a completely randomized design (CRD). Prior to stocking, tanks were disinfected with 5 ppm potassium permanganate, rinsed thoroughly, and filled with aerated freshwater. Continuous aeration was maintained throughout the experimental period. Tanks were siphoned on alternate days to remove uneaten feed and faecal matter, and water volume was restored to 350 L after cleaning. Fish were fed their respective experi-

mental diets at 10% of tank biomass per day, divided into two equal feedings at 09:30 and 16:30 h (IST), seven days per week, and feed rations were adjusted periodically based on biomass estimation.

2.4 Sample collection and analysis

2.4.1 Growth performance: At the end of the 60-day feeding trial, the body weight and total length of the fish (*n* = 3 per tank) were measured using an electronic balance and a measuring scale, respectively. The following parameters were then calculated to evaluate the growth performance of the fish.

Mean weight gain (g) = Final weight (g) – Initial weight (g)

Specific growth rate (SGR % d^{-1}) = [ln (Final weight) – ln (Initial weight)] / (Experimental period) × 100

Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g)

Feed efficiency ratio (FER) = Weight gain (g) / Feed intake (g)

Protein efficiency ratio (FER) = Weight gain (g) / Protein intake (g)

Survival rate (%) = (Total number of harvested fish / Total number of initial stock) × 100

Fulton's condition factor (K) = [Final weight (g) / Final length (cm^3)] × 100

2.4.2 Digestibility: Fecal collection commenced one week after the start of feeding to allow clearance of residual feed. One hour post-feeding, uneaten feed was removed by siphoning, and after two hours, intact fecal matter was carefully collected to avoid contamination. Samples were rinsed with distilled water, dried at 40°C on filter paper, and pooled daily for analysis. Chromium oxide (Cr_2O_3) was incorporated into the diets at 0.5% as an inert marker. Chromium content in diet and fecal samples was determined following the method of Furukawa and Tsukahara (1966). The apparent digestibility coefficients (ADC) of crude protein and crude lipid were calculated using the indicator method described by Pond *et al.* (1995) as using the following formulas:

ADC_{DIET} (%) = 100 – [(% indicator in diet / % indicator in faeces) × (% nutrient in faeces / % nutrient in diet) × 100]

2.4.3 Digestive enzyme analysis: Muscle and intestinal tissues were excised on ice, weighed, and homogenized (5% w/v) in chilled 0.25 M sucrose. The homogenates were centrifuged at 5,000 rpm for 20 min at 4°C, and the supernatants were collected and stored at –20°C for subsequent analysis. Total protein content was determined at 660 nm following Lowry *et al.* (1951), using bovine serum albumin (BSA) as the standard. Protease activity was measured using the casein digestion method (Drapeau 1976). The reaction mixture containing 1% casein in 0.05 M Tris–HCl buffer (pH 7.8) was incubated at 37°C for 10 min and terminated with 10% trichloroacetic acid (TCA). One unit of protease activity was defined as the amount causing an increase of 0.001 absorbance at 280 nm per minute. Lipase activity was determined by the titrimetric method (Cherry and Crandell 1932). The reaction mixture containing olive oil emulsion in phosphate buffer (pH 7.0) was incubated with the enzyme extract at 4°C for 24 h. The liberated fatty acids were titrated against 0.05 N NaOH using phenolphthalein as an indicator, and the amylase activity was estimated using the dinitrosalicylic acid (DNS) method (Rick and Stegbauer 1974). Samples were incubated with 2% starch substrate (pH 7.0) at 37°C for 30 min. The reaction was terminated by boiling with DNS reagent for 5 min, and absorbance was recorded at 540 nm. Enzyme activity was expressed as µmol maltose released min^{-1} g $protein^{-1}$.

2.4.4 Sampling: At the end of the feeding trial, three fish per tank (nine fish per treatment) were randomly sampled and anaesthetised using clove oil at 50 µL/L (Devi and Kamilya 2019). Approximately 1 mL of blood was collected from the caudal vein using a 1 mL syringe fitted with a 26-G needle. Blood was divided into two portions, one for haematological analysis and the other centrifuged at 3,000 rpm for 5 min to obtain serum, which was stored at –20°C for biochemical and immunological assays.

Haematological analysis: Haematological parameters including Red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and haematocrit (Ht) were measured using an automated haematological analyser (Cell Tech 380, Alpha Technologies, India). Erythrocyte indices were calculated according to Wintrobe (1934) as follows:

MCH (pg) =Hb/RBC×10

MCHC (g/dL) =Hb/Hct×100

MCV (fL) =Hct/RBC×10

Biochemical assay: Serum biochemical parameters, including total protein, albumin, glucose, total cholesterol, and triglycerides, were determined using an automated biochemical analyser (100i, Alpha Technologies, India) following standard methods as described by Biuret (Gornall *et al.* 1949), BCG (Doumas *et al.* 1971), GOD/POD (Trinder 1969), CHOD-PAP (Allain *et al.* 1974), and GPO/PAP (Fossati and Prencipe 1982). Globulin was calculated as the difference between total protein and albumin, and the albumin/globulin ratio was subsequently derived.

Immunological parameters: Respiratory burst activity (RBA) was assessed by the NBT reduction assay (Anderson

and Siwicki 1995). Superoxide dismutase (SOD) activity was determined based on inhibition of epinephrine auto-oxidation (Misra and Fridovich 1972), catalase (CAT) activity by monitoring H_2O_2 decomposition at 240 nm (Takahara *et al.* 1960), and myeloperoxidase (MPO) activity using the TMB-H_2O_2 method (Kaur *et al.* 2018). Enzyme activities were expressed per mg protein where applicable.

Tissue carotenoid analysis: Carotenoid content in muscle and skin tissues was determined following Olson (1979). Approximately 1 g of tissue was homogenized with 2.5 g of anhydrous sodium sulfate and extracted with 5 mL chloroform at 0°C overnight. After phase separation, 0.3 mL of the chloroform extract was diluted to 3 mL with absolute ethanol, and absorbance was recorded at 380, 450, 475, and 500 nm using a spectrophotometer. Total carotenoid content was calculated at the wavelength of maximum absorbance and expressed as μg g^{-1} wet tissue.

2.4.5 Challenge study: At the end of the 60-day feeding trial, fish were subjected to a bacterial challenge using a virulent strain of *A. hydrophila* (ATCC 7966). The bacterium was cultured in tryptic soy broth (TSB; HiMedia, India) for 24 h at room temperature, harvested by centrifugation at 5,000 rpm for 10 min, and re-suspended in sterile phosphate-buffered saline (PBS; pH 7.4). Fish were intraperitoneally injected with 100 μL of the bacterial suspension at the median lethal dose (LD50; 1.5×10^8 CFU mL^{-1}) using a sterile 1 mL syringe. Following injection, fish were maintained under the same rearing conditions and fed a basal diet. Mortality was monitored daily for 14 days, and dead fish were removed immediately. Infection was confirmed by re-isolation of the pathogen from moribund individuals.

2.5 Data analysis

Data were tested for normality using the Shapiro-Wilk test. Variables meeting normality assumptions were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify significant differences among groups. For data that did not meet normality assumptions, the Kruskal-Wallis test was applied, followed by Dunn's post hoc test with Bonferroni correction for multiple comparisons. Results are presented as mean ± standard error (SE), and statistical significance was set at $p < 0.05$. All experiments were conducted in triplicate and analyzed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Graphs were generated using GraphPad Prism (10.4.1) and Origin (9.0) software.

3 | RESULTS

3.1 Growth performance

Dietary inclusion of orange peel significantly affected growth performance parameters in *Carassius auratus* ($p < 0.05$; Table 2). Final weight and weight gain were markedly improved in all treatment groups compared to the control, with the 2.5% inclusion level exhibiting the highest response, showing approximately a two-fold increase over the control. The 7.5% and 5% groups also showed significant improvements, though to a lesser extent than the 2.5% group ($p < 0.05$). Specific growth rate (SGR) was significantly elevated in all supplemented groups relative to the control ($p < 0.05$), with the 2.5% group showing the greatest enhancement, followed by 7.5% and 5% inclusion levels. Feed conversion ratio (FCR) was significantly reduced in all treatment groups compared to the control ($p < 0.05$), indicating improved feed utilization efficiency, with the most pronounced reduction observed at 2.5% inclusion ($p < 0.05$). Condition factor varied significantly among treatments ($p < 0.05$), with the 2.5% and 5% groups showing higher values compared to the control, whereas a decline ($p < 0.05$) was observed at the highest inclusion level (7.5%). No significant differences were observed among treatments for feed efficiency ratio (FER), protein efficiency ratio (PER), and survival rate ($p > 0.05$).

TABLE 2 Growth parameters of goldfish (*Carassius auratus*) fed with varying levels of orange peels in the formulated diets.

Parameters	Dietary inclusion level of orange peels				ANOVA results		
	Control	2.5%	5%	7.5%	*df*	*F*-value	*p*-value
Final weight (g)	1.03 ± 0.02^a	2.07 ± 0.04^d	1.52 ± 0.03^b	1.83 ± 0.03^c	3, 8	783.216	<0.05
Weight gain (g)	0.88 ± 0.02^a	1.92 ± 0.04^d	1.37 ± 0.03^b	1.68 ± 0.03^c	3, 8	783.216	<0.05
SGR (% day^{-1})	3.21 ± 0.02^a	4.38 ± 0.03^d	3.86 ± 0.04^b	4.17 ± 0.02^c	3, 8	982.883	<0.05
FCR	1.34 ± 0.01^c	1.08 ± 0.01^a	1.23 ± 0.02^b	1.22 ± 0.02^b	3, 8	204.983	<0.05
FER	0.62 ± 0.04	0.85 ± 0.17	0.68 ± 0.09	0.74 ± 0.08	3, 8	2.375	0.146
PER	1.77 ± 0.10	2.42 ± 0.49	1.95 ± 0.27	2.11 ± 0.24	3, 8	2.437	0.14
Survival rate (%)	75.00 ± 6.25	79.17 ± 7.22	77.08 ± 3.61	75.00 ± 0.58	3, 8	0.458	0.719
Condition factor	2.28 ± 0.03^b	2.55 ± 0.08^d	2.42 ± 0.08^c	2.02 ± 0.02^a	3, 8	46.563	<0.05

Values are presented as means ± SD ($n = 3$). SGR, specific growth rate; FCR, feed conversion ratio; FER, feed efficiency ratio; PER, protein efficiency ratio. Values with different letters within a row differ significantly ($p < 0.05$).

Principal component analysis (Figure 1) revealed that the first principal component (PC1) explained 99.99% of the total variance (eigenvalue = 3.99977), whereas PC2 accounted for only 0.01% (eigenvalue = 2.04×10^{-4}), indicating that variability in the dataset was almost entirely driven by a single dominant axis. All growth-related parameters exhibited strong and negative loadings on PC1, including feed efficiency ratio (FER; –0.79569), feed conversion ratio (FCR; –0.75798), weight gain (WG; –0.73989), final weight (FW; –0.72852), protein efficiency ratio (PER; –0.69435), condition factor (CF; –0.67506), and specific growth rate (SGR; –0.55491), indicating a high degree of correlation among these variables. In contrast, survival showed a markedly higher positive loading (4.94639) on PC1, suggesting a distinct scaling relative to other parameters. The contribution of PC2 was negligible, with all variables showing near-zero loadings (ranging from –0.01677 to 0.0206), confirming that this component did not meaningfully explain variation in the dataset. Treatment-wise, all groups exhibited nearly identical contributions along PC1 (coefficients ≈ 0.50), indicating uniform distribution along the primary axis of variation. However, separation among treatments was observed along PC2, where the control group showed the highest positive coefficient (0.76205), while T1 (2.5%) (–0.45904) and T3 (5%) (–0.43668) exhibited negative loadings, and T2 (7.5%) showed minimal deviation (0.13369), suggesting minor differentiation among treatments along the secondary axis.

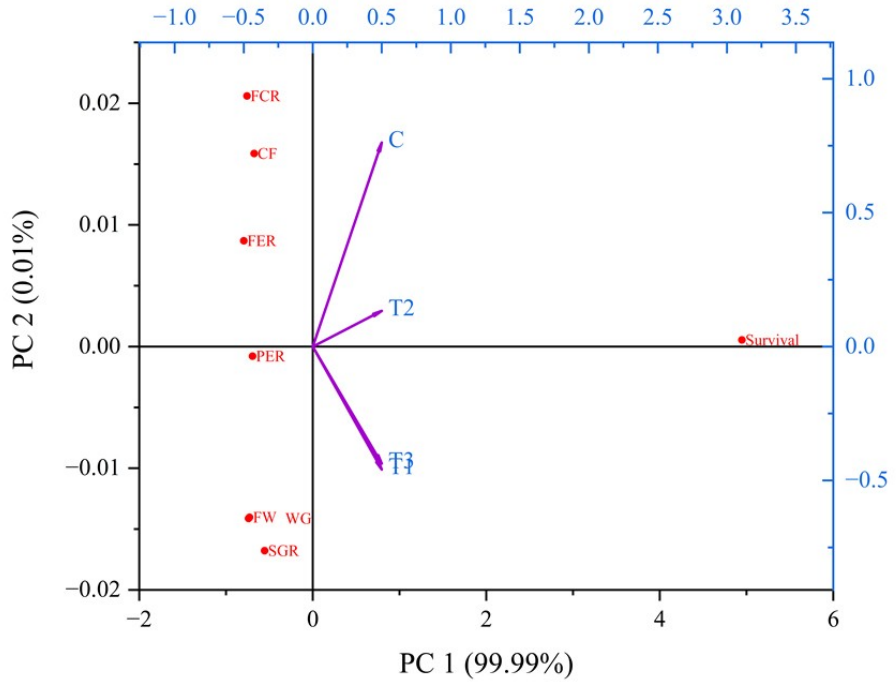

FIGURE 1 PCA biplot showing the distribution of treatments and loadings of growth performance and survival parameters in *Carassius auratus* fed diets supplemented with Citrus sinensis peel (CSP). PC1 and PC2 explained 99.99% and 0.01% of the total variance, respectively. Vectors represent variable loadings (FW, WG, SGR, FCR, FER, PER, CF, survival), and points denote treatment groups: C (Control), T1 (2.5% CSP), T2 (5% CSP), and T3 (7.5% CSP).

3.2 Apparent digestibility

Dietary inclusion of orange peel significantly influenced APD (Figure 2; $p < 0.05$). Protein digestibility was significantly higher in all supplemented groups compared to the control, with the most pronounced improvement observed in T1 and T3 ($p < 0.001$), showing approximately ~20% higher digestibility relative to the control. The T2 group also exhibited a moderate but significant increase (~7%) compared to the control ($p = 0.023$). However, T1 showed significantly higher protein digestibility than T2 (~12% increase; $p = 0.007$), while no significant difference was observed between T1 and T3 ($p > 0.05$), indicating comparable efficiency at these inclusion levels. In contrast, T3 exhibited significantly higher digestibility than T2 (~11% increase; $p = 0.011$). Apparent lipid digestibility (ALD) was also significantly affected by dietary treatments (Figure 2; $p < 0.05$). Lipid digestibility was significantly enhanced in T1 and T3 compared to the control ($p < 0.001$), with T1 showing the greatest improvement (~12%) and T3 a moderate increase (~8%). However, no significant difference was observed between the control and T2.

3.3 Digestive enzyme activity

Dietary supplementation with orange peel significantly influenced digestive enzyme activities in *C. auratus* (Figure 3; $p < 0.05$). Protease activity showed a significant increase in T2 and T3 compared to the control ($p < 0.001$), with T2 exhibiting the highest activity (~25% increase), followed by T3 (~8% increase). No significant difference was observed between the control and T1 ($p > 0.05$). Among treatments, T2 showed significantly higher activity than both T1 (~22% increase; $p < 0.001$) and T3 (~16% increase; $p < 0.001$), while T3 was also significantly higher

than T1 (~5% increase; p = 0.003). Amylase activity was significantly elevated in all treatment groups compared to the control (p < 0.001). The highest activity was observed in T2, showing approximately a ~120% increase over the control, followed by T1 (~50%) and T3 (~37%). Significant differences were also observed among all treatments (p < 0.001), with T2 > T1 > T3. Lipase activity varied significantly among treatments (p < 0.05). T2 showed a marked increase (~75%) compared to the control (p = 0.002), while T1 exhibited a moderate increase (~34%; p = 0.029). No significant difference was observed between the control and T3 (p > 0.05). Among treatments, T2 had significantly higher activity than both T1 (~31% increase; p = 0.010) and T3 (~89% increase; p = 0.001), whereas T1 was also significantly higher than T3 (~44% increase; p = 0.010).

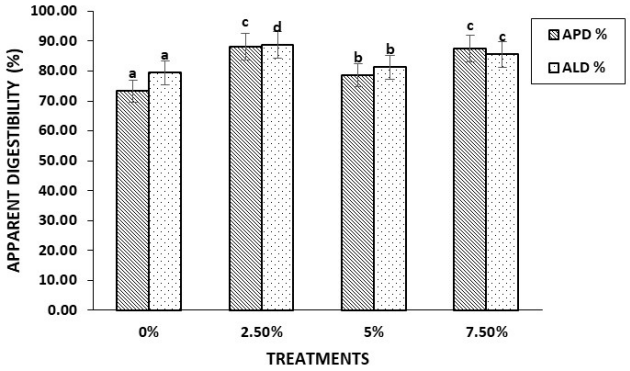

FIGURE 2 Apparent protein digestibility (APD) and apparent lipid digestibility (ALD) in goldfish (*Carassius auratus*) fed diets containing 0%, 2.5%, 5%, and 7.5% orange peel for 60 days (Mean±SE; n = 3). Different letters within the same bar pattern indicate significant differences among treatments (p < 0.05).

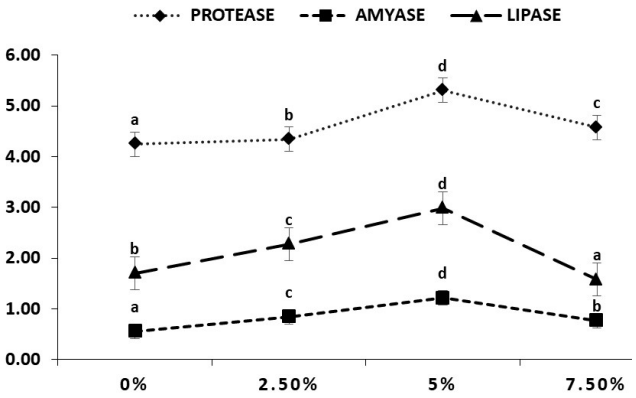

FIGURE 3 Protease, amylase, and lipase activities in goldfish, expressed as U mg^{-1} protein, fed diets containing 0%, 2.5%, 5%, and 7.5% orange peel for 60 days (Mean±SE, n = 3). Different letters along the same line indicate significant differences among treatments (p < 0.05).

3.4 Haematological, biochemical and immunological parameters

The effects of varying levels of dietary orange peel on haematological, and biochemical parameters at the end of the experiment are presented in Table 3. RBC count showed significant increases in T1 and T2 compared to control (p < 0.001), while no difference was observed between control and T3 (p = 0.818). Among treatments, T2 was significantly higher than T1 (p = 0.024), whereas both T1 and T2 were significantly higher than T3 (p < 0.001). In contrast, WBC count did not differ significantly among any groups (p > 0.05), indicating no treatment effect. Haemoglobin (HB) levels were significantly elevated in all treatment groups compared to control (p < 0.001). However, no difference was observed between T1 and T2 (p = 0.721), while both were significantly higher than T3 (p < 0.001). Similarly, haematocrit (HT) was significantly higher in T1 and T2 compared to control (p < 0.001), with no difference between control and T3 (p = 0.084). T2 was significantly higher than T1 (p = 0.002), and both were markedly higher than T3 (p < 0.001). For erythrocyte indices, MCH showed a significant increase in T2 compared to control (p = 0.033), while T3 was significantly lower than control (p = 0.004). T3 was also significantly lower than both T1 (p = 0.001) and T2 (p = 0.001). MCHC exhibited a slight but significant increase in T1 compared to control (p = 0.032), and was also higher in T1 than T2 (p = 0.033) and T3 (p = 0.011), while other comparisons remained non-significant. MCV was significantly reduced in T1 (p = 0.014) and T2 (p = 0.005) compared to control, whereas no difference was observed between control and T3 (p = 0.325). Additionally, T3 showed significantly higher values compared to both T1 (p = 0.001) and T2 (p = 0.006).

Glucose levels showed no difference between control and T1 (p > 0.999), whereas significant reductions were observed in T2 and T3 compared to control (p < 0.001). All inter-treatment comparisons were also highly significant (p < 0.001). TGA were significantly reduced in all treatment groups compared to control (p < 0.001). Additionally, significant differences were observed among all treatment groups (p < 0.001), demonstrating a consistent decreasing trend with increasing supplementation. In contrast, cholesterol levels did not differ between control and T1 (p = 0.674), but were significantly lower in T2 and T3 compared to both control and T1 (p < 0.001). A significant difference was also observed between T2 and T3 (p < 0.001). Total protein, albumin and globulin exhibited significant differences across all groups (p < 0.001), with all pairwise comparisons being significant, including between T2 and T3 (p = 0.009), indicating subtle but consistent variation among treatments. Similarly, the albumin: globulin (A : G) ratio also differed significantly among all groups (p < 0.001), with a consistent declining trend from control to higher inclusion levels.

The immunological parameters of fish fed diets exhibited significant variations among the control and treatment groups, as revealed by Tukey's multiple comparison test (Table 4). Superoxide dismutase (SOD) activity was significantly elevated in all treatment groups compared to the control (C vs. T1: $p < 0.001$; C vs. T2: $p < 0.001$; C vs. T3: $p < 0.001$). Among treatments, T1 showed the highest SOD activity, followed by T3 and T2, with all intergroup differences also being highly significant ($p < 0.001$). Similarly, catalase activity was significantly higher in all supplemented groups than in the control (C vs. T1, T2, T3: $p < 0.001$). A progressive increase was observed from T1 to T3, with all pairwise comparisons among treatment groups remaining significant ($p < 0.001$). NBT activity showed significant differences between control and treatments (C vs. T1: $p < 0.001$; C vs. T2: $p < 0.001$; C vs. T3: $p = 0.010$). However, no significant difference was observed between T1 and T2 ($p = 0.062$), while T3 differed significantly from both T1 and T2 ($p < 0.001$). Myeloperoxidase (MPO) activity was significantly enhanced in all treatment groups compared to control ($p < 0.001$). Additionally, all pairwise comparisons among treatments (T1 vs. T2, T1 vs. T3, T2 vs. T3) were also statistically significant ($p < 0.001$).

TABLE 3 Haematological and biochemical parameters of goldfish (*Carassius auratus*) fed with varying levels of orange peels in the formulated diets.

Parameters	Dietary inclusion level of orange peels				ANOVA results		
	Control	2.5%	5%	7.5%	*df*	*F*-value	*p*-value
RBC (x 10^6 μL^{-1})	0.67 ± 0.03^a	0.88 ± 0.02^b	0.93 ± 0.01^c	0.66 ± 0.01^a	3, 8	167.135	<0.05
WBC (x 10^4 μL^{-1})	2.06 ± 0.04	2.03 ± 0.03	2.07 ± 0.05	2.06 ± 0.05	3, 8	0.383	0.768
Hb (g dL^{-1})	6.90 ± 0.10^a	8.70 ± 0.10^c	8.60 ± 0.10^c	7.77 ± 0.15^b	3, 8	158.562	<0.05
Ht (%)	25.20 ± 0.36^a	30.07 ± 0.02^c	31.40 ± 0.10^d	25.66 ± 0.14^b	3, 8	724.578	<0.05
MCH (pg)	102.65 ± 5.87^b	99.27 ± 2.87^b	92.14 ± 0.57^a	117.70 ± 3.38^c	3, 8	25.545	<0.05
MCHC (g dL^{-1})	27.39 ± 0.78^a	28.94 ± 0.31^b	27.39 ± 0.41^a	27.03 ± 0.55^a	3, 8	7.371	<0.05
MCV (fL)	374.70 ± 15.32^a	343.04 ± 6.25^a	336.47 ± 5.15^b	388.92 ± 7.72^b	3, 8	21.018	<0.05
Glucose (mg dL^{-1})	88.62 ± 0.04^c	88.62 ± 0.03^c	88.15 ± 0.04^b	81.02 ± 0.03^a	3, 8	3.03	<0.05
Triglycerides (mg dl^{-1})	161.32 ± 0.16^d	112.09 ± 0.06^c	104.95 ± 0.04^b	103.61 ± 0.02^a	3, 8	2.823	<0.05
Cholesterol (mg dL^{-1})	194.17 ± 0.09^c	195.52 ± 0.14^c	123.49 ± 2.87^a	175.02 ± 0.20^b	3, 8	1.638	<0.05
Total Protein (g dL^{-1})	5.86 ± 0.003^c	6.32 ± 0.003^d	5.15 ± 0.003^b	5.14 ± 0.002^a	3, 8	1.038	<0.05
Albumin (g dL^{-1})	2.32 ± 0.001^b	1.98 ± 0.001^a	2.49 ± 0.001^c	2.99 ± 0.002^d	3, 8	2.523	<0.05
Globulin (g dL^{-1})	3.53 ± 0.005^c	4.34 ± 0.002^d	2.66 ± 0.003^b	2.15 ± 0.001^a	3, 8	3.324	<0.05

Values are presented as means ± SD ($n = 3$). RBC, red blood cells; WBC, white blood cells; Hb, haemoglobin; Ht, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume. Values with different letters within a row differ significantly ($p < 0.05$).

TABLE 4 Immunological parameters of goldfish (*Carassius auratus*) fed with varying levels of orange peels in the formulated diets.

Parameters	Dietary inclusion level of orange peels				ANOVA results		
	Control	2.5%	5%	7.5%	df	F-value	*p*-value
SOD (U mg^{-1} protein)	4.25 ± 0.03^a	6.32 ± 0.03^d	4.77 ± 0.04^b	5.42 ± 0.06^c	3, 8	1.319	<0.05
CAT (U mg^{-1} protein)	3.25 ± 0.02^a	4.02 ± 0.02^b	4.76 ± 0.04^c	6.08 ± 0.07^d	3, 8	2.559	<0.05
NBT (540 nm)	0.123 ± 0.002^c	0.109 ± 0.002^b	0.105 ± 0.002^a	0.129 ± 0.001^d	3, 8	151.516	<0.05
MPO (U mg^{-1} protein)	0.025 ± 0.01^d	0.042 ± 0.02^b	0.045 ± 0.03^c	0.032 ± 0.02^a	3, 8	2.528	<0.05

Values are presented as means ± SD ($n = 3$). SOD, superoxide dismutase; CAT, catalase; NBT, nitro blue tetrazolium; MPO, Myeloperoxidase. Values with different letters within a row differ significantly ($p < 0.05$).

Pearson correlation analysis (Figure 4) indicated several strong and significant associations ($p < 0.05$) between the studied parameters. Among haematological indices, RBC showed a very strong positive correlation with HT ($r = 0.996$, $p < 0.01$) and HB ($r = 0.875$), while exhibiting a strong negative correlation with MCV ($r = –0.979$, $p < 0.05$) and NBT ($r = –0.982$, $p < 0.05$). RBC was also strongly positively associated with MPO ($r = 0.941$). Similarly, HB and HT were strongly correlated with MPO ($r = 0.976$ and 0.967, $p < 0.05$) and negatively correlated with NBT ($r = –0.776$ and –0.961). However, for erythrocyte indices, MCV exhibited an almost perfect positive correlation with NBT ($r = 0.999$, $p < 0.001$) and a strong negative association with RBC and HT ($p < 0.05$).

For biochemical parameters, total protein was strongly positively correlated with globulin ($r = 0.978$, $p <$

0.05) and negatively with albumin (r = –0.876), while albumin and globulin were strongly negatively correlated (r = –0.958, $p < 0.05$). Glucose showed strong negative correlations with MCH (r = –0.889) and CAT (r = –0.884), and a positive correlation with globulin (r = 0.740). In the antioxidant and immune profile, SOD was positively correlated with MCHC (r = 0.732) and HB (r = 0.677), but negatively with WBC (r = –0.822). CAT showed a positive correlation with albumin (r = 0.823) and a negative correlation with globulin (r = –0.790). However, NBT and MPO displayed contrasting trends, with NBT positively correlated with MCV (r = 0.999, $p < 0.001$) and negatively with RBC and HT, whereas MPO was strongly positively associated with HB (r = 0.976) and HT (r = 0.967) but negatively with NBT (r = –0.861).

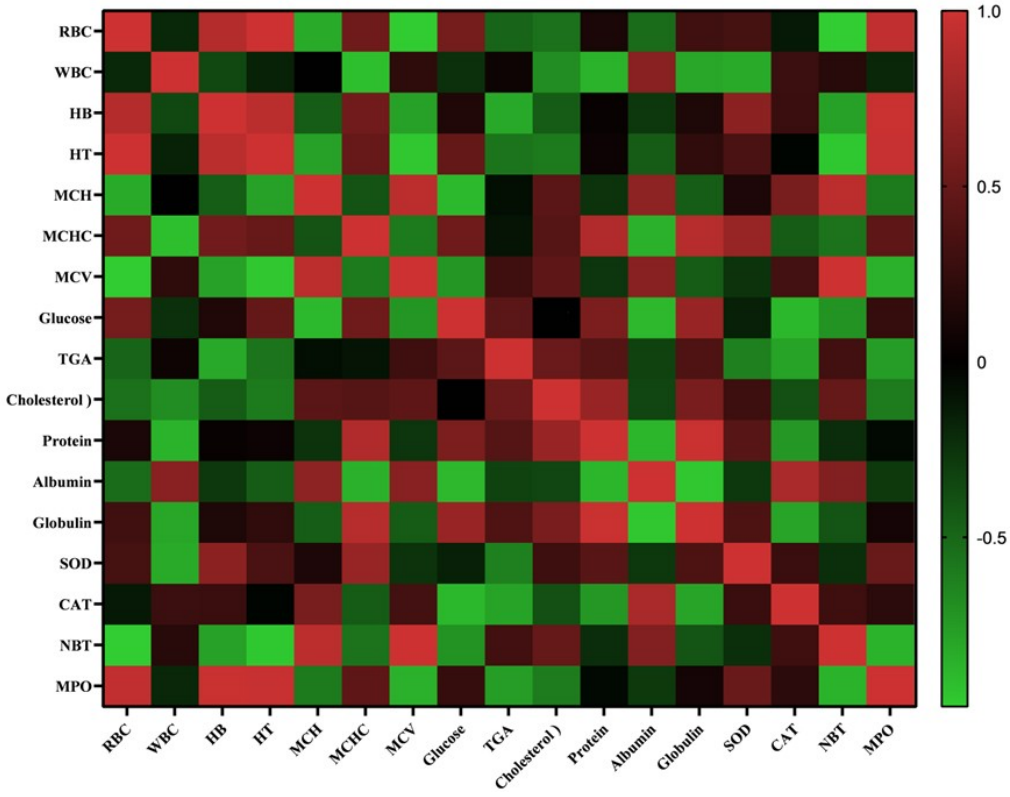

FIGURE 4 Pearson correlation matrix showing relationships among haematological (RBC, WBC, HB, HT, MCV, MCH, MCHC), biochemical (glucose, triglycerides, cholesterol, protein, albumin, globulin), and immunological/antioxidant parameters (SOD, CAT, NBT, MPO) in *Carassius auratus*. Correlation coefficients (r) indicate the strength and direction of associations, with significance determined at $p < 0.05$.

3.5 Pigmentation deposition

Dietary inclusion of orange peel significantly influenced carotenoid deposition in both muscle and skin tissues of *C. auratus* (Table 5; $p < 0.05$). Muscle carotenoid content showed a moderate but significant increase with increasing inclusion levels. A significant enhancement was observed in T2 (~16% increase; p = 0.041) and T3 (~24% increase; p = 0.004) compared to the control, whereas no significant difference was found between the control and T1 ($p > 0.05$). Among treatments, T3 exhibited significantly higher carotenoid deposition than T1 (~18% increase; p = 0.018), while differences between T1 vs. T2 and T2 vs. T3 were not statistically significant ($p > 0.05$). Skin carotenoid content was markedly influenced by dietary treatments, with all supplemented groups showing highly significant increases compared to the control ($p < 0.001$). The highest deposition was observed in T3, exhibiting approximately a ~23% increase over the control, followed by T1 (~13%) and T2 (~12%). Significant differences were also observed among all treatment groups ($p < 0.001$), with a clear hierarchy of T3 > T1 > T2.

TABLE 5 Deposition of muscle and skin carotenoids in *Carassius auratus* across different experimental groups: Control, T1 (2.5% CSP), T2 (5.0% CSP), and T3 (7.5% CSP).

Treatment group	Muscle Carotenoids (µg/g)	Skin Carotenoids (µg/g)
Control	0.71±0.01^{a}	14.12±0.00^{a}
T1 (2.5%)	0.75±0.01^{ab}	15.90±0.00^{c}
T2 (5.0%)	0.83±0.01^{bc}	15.79±0.00^{b}
T3 (7.5%)	0.89±0.05^{c}	17.37±0.00^{d}
df	3, 8	3, 8
F-value	10.276	6.318

Values are presented as means ± SD (n = 3). Different superscript letters are significantly different ($p < 0.05$).

3.6 Challenge study

Following *A. hydrophila* challenge, survival analysis revealed a statistically significant improvement in survivability among treatment groups compared to the control ($p < 0.05$). The highest survival was observed in the T1 group, showing approximately 1.7-fold higher survival

relative to the control, followed by the T2 (~1.3-fold) and T3 (~1.1-fold) groups (Figure 5). Survival curve comparison using the Log-rank (Mantel-Cox) test indicated significant differences among groups (χ^2 = 1.227, *df* = 3, *p* = 0.049). Similarly, the Gehan-Breslow-Wilcoxon test confirmed significant variation (χ^2 = 1.617, *df* = 3, *p* = 0.046), supporting differences in early survival responses and treatment-dependent survival response.

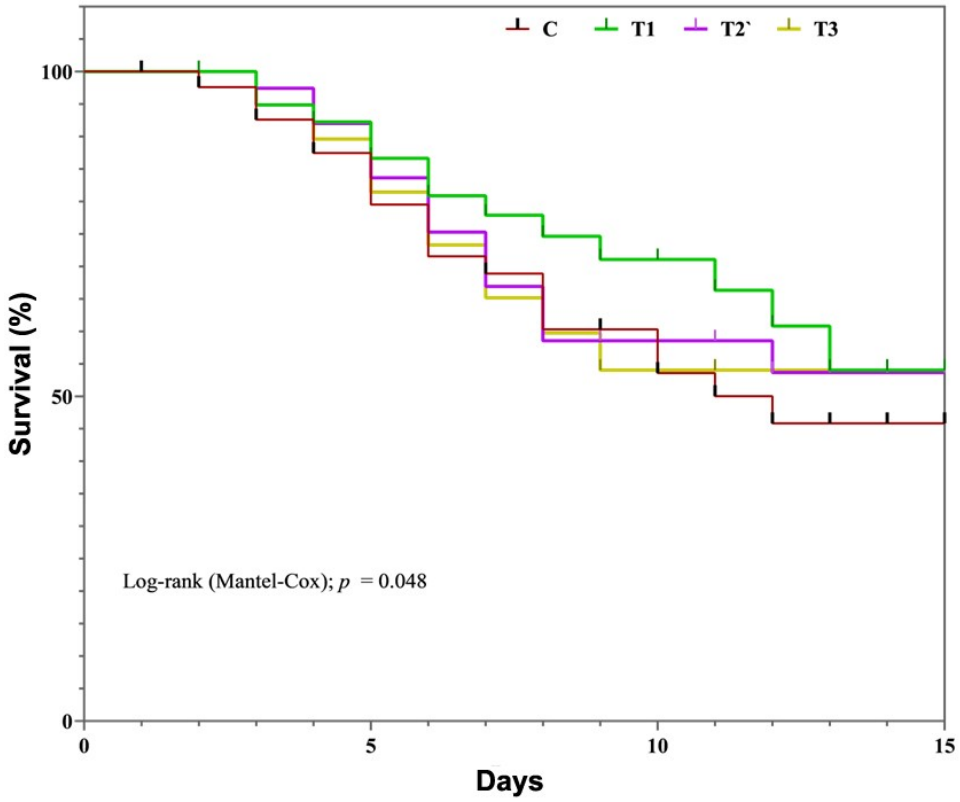

FIGURE 5 Kaplan–Meier survival curves of *Carassius auratus* following challenge with Aeromonas hydrophila (ATCC 7966).

4. DISCUSSION

4.1 Growth parameters

The present study demonstrates that dietary inclusion of orange (*C. sinensis*) peel positively influenced growth performance in fish, while survival remained unaffected across all treatments. Among the tested inclusion levels, the 2.5% supplementation consistently produced the most favorable outcomes. The improvement in growth parameters may be attributed to the rich composition of bioactive compounds in orange peel, including flavonoids, essential oils, and antioxidants, which are known to enhance digestive efficiency, nutrient utilization, and metabolic activity. At optimal levels, these compounds likely stimulate enzyme activity and gut health, thereby improving feed conversion and protein utilization (Athanasiadis *et al.* 2022). However, the reduced performance observed at higher inclusion levels (5% and 7.5%) may be due to the presence of anti-nutritional factors or excessive fiber content, which can limit nutrient absorption when included beyond optimal concentrations (Panwar *et al.* 2023).

These findings are in agreement with previous studies. See *et al.* (2024) reported improved growth performance in juvenile bagrid catfish (*Mystus nemurus*) fed diets supplemented with sweet orange peel, while survivability remained unaffected. Similarly, Hussein *et al.* (2022) found no significant effect on survival in mono-sex Nile tilapia fry, confirming the safety of orange peel supplementation. Salem and Abdel-Ghany (2018) also observed that lower inclusion levels resulted in superior growth performance in Nile tilapia fingerlings, supporting the present observation that moderate supplementation yields the best results. Collectively, these studies reinforce the role of citrus by-products as functional feed additives that enhance growth without compromising fish health.

Despite these promising outcomes, certain limitations should be acknowledged. The study was conducted over a relatively short duration and under controlled conditions, which may not fully reflect long-term or field-scale aquaculture systems. Additionally, the analysis did not include detailed digestive enzyme profiling or gut microbiota assessment, which could provide deeper insights into the mechanisms underlying growth enhancement. Future research should focus on long-term feeding trials, dose optimization across different species, and molecular or microbiome-based investigations to better understand the physiological effects of orange peel supplementation.

4.2 Digestibility indices

Digestive enzymes play a critical role in nutrient breakdown and absorption, making their activity a reliable indicator of feed digestion and nutrient utilisation (Zuo *et al.* 2019). Assessing intestinal enzyme activity in fish provides an indirect measure of the species' capacity to digest and metabolise feed (Hu *et al.* 2023). In this study, the effects of diets supplemented with varying concentrations of orange peel on nutrient digestion were evaluated by measuring intestinal protease, amylase, and lipase activities. Dietary supplementation with orange peel enhanced the activities of protease, amylase, and lipase in goldfish compared to the control group, indicating improved nutrient digestion. Similar results were reported by Shabana *et al.* (2019) in *Catla catla* fed diets containing 2–10 g kg^{-1} *Citrus sinensis*. The observed enhancement in enzyme activity may be attributed to bioactive compounds in plant by-products, which are known to stimulate digestive enzymes such as lipase, trypsin, alkaline phosphatase, protease, and amylase, thereby facilitating the digestion and absorption of proteins, lipids, and carbohydrates (Platel *et al.* 2002; Silva-Brito *et al.* 2021; Oh *et al.* 2023). Consequently, this led to improved protein and lipid digestibility in fish fed orange peel-supplemented diets compared to the control.

4.3 Haematological, biochemical and immunological parameters

The present study demonstrates that dietary supplementation of orange (*C. sinensis*) peel markedly improved haematological, biochemical, and immunological profiles of fish, indicating enhanced physiological status, metabolic efficiency, and immune competence compared to the control group. Haematological indices provide important insights into physiological status, stress response, and overall health in fish. Dietary orange peel supplementation produced measurable changes in blood profiles, with fish fed the 5% inclusion level showing the most pronounced improvement in red blood cell (RBC) count, haemoglobin, haematocrit, and MCH. These enhancements suggest improved erythropoiesis and oxygen-carrying capacity, likely due to improved nutrient availability and bioactive compounds in orange peel. Flavonoids and other phenolic compounds present in citrus peels are known to stabilize erythrocyte membranes and reduce oxidative damage, which may explain the observed improvements in haematological indices. In contrast, the 7.5% group did not show additional benefits over moderate supplementation, indicating a possible saturation or counteracting effect at higher inclusion levels.

Biochemical indices further supported the health-promoting effects of orange peel. Blood glucose levels remained within the physiological range across treatments, indicating the absence of metabolic stress and stable energy homeostasis (Sopinka *et al.* 2016). The significant reduction in triglyceride levels in supplemented groups suggests improved lipid metabolism, possibly due to the presence of bioactive compounds such as flavonoids and dietary fibre that enhance lipid utilization and reduce fat deposition (Ademosun *et al.* 2025). Variations in cholesterol levels further indicate dietary modulation of sterol metabolism (Feng *et al.* 2020). Such lipid-lowering effects have also been reported in fish fed plant-derived additives, supporting the role of citrus by-products in regulating energy balance and metabolic efficiency (Lee *et al.* 2024).

Serum protein profiles provided additional insight into the physiological and immune status of fish (Parthasarathy and Ravi 2011). In the present study, total protein and albumin levels were markedly elevated in the 2.5% supplementation group compared to the control and higher inclusion levels, suggesting that moderate incorporation of orange peel more effectively enhances protein synthesis and overall nutritional status. These findings are in agreement with previous studies where dietary plant-based additives improved serum protein profiles in fish. For instance, Attalla *et al.* (2021) reported increased total protein and globulin levels in Nile tilapia (*O. niloticus*) fed phytogenic supplements, indicating enhanced immune competence. Likewise, Lee *et al.* (2024) and Yun *et al.* (2025) observed improved serum protein fractions in Korean rockfish (*S. schlegelii*) following dietary inclusion of functional plant extracts, while See *et al.* (2024) documented similar enhancements in Asian redtail catfish (*Mystus nemurus*). Comparable improvements have also been reported in *Cyprinus carpio* (Hosseini *et al.* 2020) and when fed orange peel-derived additives. However, unlike some studies where higher inclusion levels resulted in a continuous increase in protein fractions, the present findings suggest that excessive supplementation may not yield proportional benefits, possibly due to reduced palatability or metabolic limitations at higher doses. Thus, the comparatively superior response at lower inclusion levels in this study highlights the importance of optimizing dosage for maximizing physiological and immunological benefits.

The observed enhancement in antioxidant enzyme activities further highlights the protective role of orange peel. In the present study, SOD and CAT activities were significantly higher in all supplemented groups compared to the control. This enhancement suggests a strengthened capacity to mitigate oxidative stress through efficient scavenging of reactive oxygen species and prevention of cellular damage (Hoseinifar *et al.* 2020). The improvement in antioxidant status can be attributed to the presence of phenolic compounds, flavonoids, and vitamin C in citrus peels, which are known to stimulate endogenous antioxidant systems (Sir Elkhatim *et al.* 2018). Similar trends have been consistently reported in other fish species receiving plant-based or citrus-derived supple-

ments. For instance, Yun *et al.* (2025) and Lee *et al.* (2024) documented significant increases in SOD and CAT activities in *S. schlegelii*, while Hosseini *et al.* (2020) reported enhanced antioxidant enzyme responses in common carp (*C. carpio*). Likewise, Salem *et al.* (2019) observed improved antioxidant status in gilthead seabream (*Sparus aurata*) following dietary phytogenic supplementation. The present findings align closely in demonstrating enhanced antioxidant capacity; however, the variation in magnitude of response across supplementation levels suggests that optimal dosing is critical for maximizing physiological benefits.

Immunological parameters serve as vital indicators of fish health and immune competence (Nayak 2010). In the present study, these parameters were significantly influenced by dietary supplementation, with clear differences observed between the control and treatment groups. Respiratory burst activity, as indicated by NBT reduction, increased with higher inclusion levels of orange peel, demonstrating enhanced phagocytic activity and improved innate immune response compared to the control (Chi *et al.* 2014). This suggests that dietary supplementation effectively stimulated cellular defence mechanisms, particularly at elevated inclusion levels. In contrast, MPO activity was reduced in all supplemented groups relative to the control. This decline may indicate a more regulated inflammatory response, where excessive production of reactive intermediates is minimized while still maintaining effective pathogen defence (Rahman *et al.* 2019). Such modulation reflects a balanced immune response rather than suppression, which is crucial for preventing tissue damage associated with prolonged oxidative stress. Similar immunomodulatory effects have been reported in other fish species receiving plant-based or citrus-derived supplements. Harikrishnan *et al.* (2020) documented enhanced phagocytic and immune activities in *Labeo rohita*, while Rahman *et al.* (2019) observed increased NBT and MPO activities in *O. niloticus* and *Clarias gariepinus*. However, unlike these studies where MPO activity increased alongside NBT, the present findings demonstrate a reduction in MPO activity despite elevated respiratory burst activity. This contrast suggests that orange peel supplementation may promote a more controlled and efficient immune response by enhancing pathogen clearance while limiting excessive inflammatory reactions. Further investigations focusing on molecular immune markers, cytokine profiling, and gene expression analyses are needed to elucidate the underlying mechanisms of this immunomodulation.

4.4 Pigmentation

In ornamental fish, carotenoids are the primary pigments responsible for yellow, red, and related hues (Rana *et al.* 2023). Pigmentation is influenced by carotenoid source, chemical structure, dietary lipid levels, species characteristics, feeding duration, and environmental factors (Lee *et al.* 2010). In this study, total carotenoid content in goldfish muscle and skin increased with higher levels of dietary orange peel, indicating enhanced carotenoid deposition. These findings are consistent with Hussein *et al.* (2022), who reported increased carotenoid content in mono-sex Nile tilapia fed diets supplemented with orange peel. Similar effects were observed in goldfish using citrus peel as a carotenoid source (Abbas *et al.* 2020). The enhancement is likely due to the high carotenoid content in orange peel, which acts as a potent antioxidant, protecting cellular membranes and lipoproteins from reactive oxygen species (Stahl and Sies 2003).

4.5 Challenge study

The fish immune system comprises both innate and adaptive components that provide protection against pathogens (Magnadottir 2010). In the present study, the *A. hydrophila* challenge test demonstrated improved survivability in orange peel-supplemented groups compared to the control, indicating enhanced disease resistance. The relatively better performance at lower inclusion levels suggests that moderate supplementation may be more effective than higher doses. This enhanced resistance can be attributed to bioactive phytochemicals in orange peel, such as phenolics, flavonoids, tannins, and saponins, which are known to stimulate immune responses and exhibit antimicrobial properties (Abdelazem *et al.* 2021). These compounds likely contributed to improved innate immune functions, thereby increasing the ability of fish to withstand pathogenic infection.

Similar protective effects have been reported in other studies using citrus and fruit peel-based additives. Harikrishnan *et al.* (2020) demonstrated that a dried lemon peel-enriched diet enhanced immune responses and significantly improved resistance of *L. rohita* against *Aeromonas* infection. Similarly, Yun *et al.* (2025) reported that supplementation with *C. sinensis* by-product extract improved immune status and conferred higher disease resistance against *V. harveyi* in *S. schlegelii*. In addition, See *et al.* (2024) showed that dietary inclusion of sweet orange peel waste enhanced survival and provided protective effects against *A. hydrophila* infection in *M. nemurus*. These findings are consistent with the present study and further support the role of citrus peel-derived bioactives in enhancing disease resistance and immune protection in aquaculture species.

5 | CONCLUSIONS

The present study demonstrates that dietary supplementation with orange peel effectively enhances growth performance, physiological stability, antioxidant capacity, immune response, and disease resistance in fish, thereby directly addressing the central research objective of evaluating its functional role as a feed additive. Collectively,

the findings indicate that moderate inclusion levels, particularly at 2.5%, yield the most consistent improvements across biochemical, immunological, and challenge parameters, highlighting the importance of dose optimization rather than maximal inclusion. These findings advance current knowledge by highlighting citrus by-products as sustainable, cost-effective alternatives to synthetic additives. Additionally, dietary incorporation of orange peel resulted in a modest reduction in feed preparation cost, decreasing from ₹84.09 kg^{-1} for the control diet to ₹82.84, ₹81.59, and ₹80.34 kg^{-1} for the 2.5%, 5%, and 7.5% supplementation levels, respectively. Considering that orange peel is an abundant agro-industrial by-product readily available from fruit processing industries and local markets, its utilization offers an economically attractive and environmentally sustainable approach for aquafeed formulation. Improvements in lipid metabolism, serum protein profiles, antioxidant enzymes, and immune modulation indicate enhanced physiological resilience and pathogen resistance, supporting reduced dependence on antibiotics in aquaculture.

Practically, orange peel can be incorporated into feed formulations to improve fish health and survival. However, limitations such as short experimental duration and controlled conditions may restrict broader applicability. Future research should focus on long-term field validation, molecular mechanisms, and economic feasibility to strengthen its commercial potential.

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ETHICAL APPROVAL

The study was performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU, 2022), Nagapattinam, Tamil Nadu, India, has also approved the study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Ng Chinglembi Devi: Investigation, Writing-Original manuscript, Data Curation, formal analysis; Cheryl Antony: Conceptualization, methodology, formal analysis, writing – review and editing, Supervision; Elangovan Prabu: writing – review and editing, Supervision; Suraj Kumar- Statistical analysis; Supratim Malla- Writing, reviewing and editing; S. Selvaraj: writing – review and editing, Supervision; A. Uma: Providing facilities, writing – review and editing, Supervision; R. Somu Sunder Lingam: Data Curation, formal analysis; David Waikhom: Writing-editing and review manuscript and Kumaresan M: Supervision

AI USE DECLARATION

In the preparation of this manuscript, generative AI tools (such as ChatGPT developed by OpenAI) were used only to assist in language editing, grammar correction, and improving the clarity of expression. The use of AI was limited to linguistic refinement and did not involve any data generation, data interpretation, or content creation that would affect the scientific integrity of the work. The authors take full responsibility for the content, accuracy, and originality of the manuscript.

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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