Journal of Animal & Plant Sciences, 35(4): 2025, Page: 981-990
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.4.0084

EFFECTS OF PYRROLOQUINOLINE QUINONE ON GROWTH PERFORMANCE, ANTIOXIDANT ACTIVITY, AND IMMUNITY IN *PLECOGLOSSUSALTIVELIS*

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ABSTRACT

The rapid growth of the aquaculture industry has necessitated the exploration of novel feed additives to enhance fish production performance and health status. This study evaluates the effects of pyrroloquinolinequinone (PQQ) on the production performance, antioxidative capacity, and nonspecific immune response of *Plecoglossusaltivelis*, with a focus on elucidating its functional mechanisms. Through precise control of PQQ supplementation dosages(0.2, 0.4, and 0.6 mg/kg in the diet), the study analyzed its impact on growth metrics, serum biochemical parameters, antioxidant enzyme activities, and intestinal digestive enzyme activities in *Plecoglossusaltivelis*. Results indicated that PQQ supplementation markedly improved the growth performance of *Plecoglossusaltivelis*, including final body weight, weight gain rate, specific growth rate, and condition factor (P<0.05). Additionally, PQQ reduced serum triglyceride concentrations (P<0.05) and enhanced antioxidant enzyme activities, including catalase and superoxide dismutase (P<0.05), as well as total antioxidant capacity (P<0.05). Regarding nonspecific immunity, PQQ elevated serum immunoglobulin M levels, lysozyme activity, and complement C3 content (P<0.05). Furthermore, PQQ stimulated intestinal protease activity (P<0.05).These findings demonstrate that PQQ effectively enhances the production performance, antioxidative capacity, and nonspecific immune response of *Plecoglossusaltivelis*.

Keywords: Pyrroloquinolinequinone (PQQ);*Plecoglossusaltivelis*; production performance; antioxidative capacity; nonspecific immune response.

Published first online June 10, 2025 Published final July 29, 2025

INTRODUCTION

In recent years, aquaculture has experienced rapid growth and has become a key component of the global food supply chain, attracting extensive attention. Renowned for its delicate flesh and rich nutritional profile, *Plecoglossusaltivelis* has become a prominent species in East Asian aquaculture and culinary traditions. However, with the intensification of aquaculture practices, challenges such as deteriorating farming environments, increased pathogen resistance, and inefficient feed utilization have become increasingly prevalent, impeding the sustainable development of *Plecoglossusaltivelis* aquaculture (Watanabe *et al.* 2014).

Within this framework, increasing attention is being directed toward investigating novel feed supplements to enhance the growth performance and health of *Plecoglossusaltivelis*. Pyrroloquinolinequinone (PQQ)is a redox cofactor initially discovered in bacteria and later found to play significant roles in eukaryotic organisms. It has attracted attention due to its notable functions in energy metabolism, antioxidation, and immunomodulation (Jonscher *et al.* 2021).PQQ acts as a cofactor for several enzymes involved in the oxidation of alcohols and sugars, and it has been shown to enhance mitochondrial function and reduce oxidative stress. These properties make PQQ a promising candidate for improving growth performance and health in aquaculture species, such as *Plecoglossusaltivelis*.Existingresearch demonstrates that PQQ can play a crucial role across various organisms by enhancing mitochondrial function and mitigating oxidative stress, thereby promoting overall health and growth. For instance, studies in poultry and livestock have shown that PQQ improves growth performance and antioxidative status, suggesting its potential applicability in aquaculture (Liu *et al.* 2020;Ming *et al.* 2021). In aquaculture, the potential benefits of PQQ are gradually being recognized. Research indicates that PQQ can increase growth rates in fish, improve feed conversion efficiency, and potentially strengthen disease resistance (Gopika *et al.* 2022;Li *et al.* 2023;Shi *et al.* 2022). Nonetheless, systematic and in-depth research examining the specific effects of PQQ on the growth performance, antioxidative properties, and immune functionality of *Plecoglossusaltivelis* remains sparse.

This study evaluates the effects of dietary PQQ supplementation on the production performance, antioxidative capacity, and nonspecific immunity of *Plecoglossusaltivelis*, with a focus on elucidating its mechanistic basis.

MATERIALS AND METHODS

This experiment was formally approved by the Animal Experimental Ethics Committee of Liaodong University. All procedures and protocols strictly adhered to the guidelines on animal welfare issued by the Chinese Association for Laboratory Animal Science.

Experimental Materials and Design: The PQQ· Na_2used in this experiment was a commercially available product with a purity of over 98%, appearing as a red powder (ZhuchengHaotian Pharmaceutical Co., Ltd.). The feeding trial was conducted in the indoor aquaculture facility of the Liaodong University Aquaculture Base. Juvenile *Plecoglossusaltivelis* were purchased from the market and acclimated in a recirculating aquaculture system for two weeks. After a 24-hour fasting period, 480 healthy juvenile *Plecoglossusaltivelis* with an initial body weight of (1.26±0.01)g were selected and randomly divided into four groups, each with three replicates of 40 fish.The control group (CON) was fed a basal diet without PQQ·Na_2. The experimental groups were fed a basal diet supplemented with 0.2 mg/kg PQQ·Na_2 (low PQQ, LP), 0.4 mg/kg PQQ·Na_2 (middle PQQ, MP), and 0.6 mg/kg PQQ·Na_2 (high PQQ, HP) respectively, for a period of eight weeks. Each component was finely milled to achieve passage through an 80-mesh sieve, then gradually combined and mixed with oil and ultrapure water (with or without PQQ·Na_2). The mixture was then processed through a noodle machine with a 1 mm diameter and passed through a 36-mesh screen. In the final step, the test feeds were dried using air, securely sealed, and kept at -20C until they were utilized. The dietary compositions and their nutritional contents are detailed in Table 1.

Feeding Management: The experimental fish were divided into four groups and housed in twelve net cages, each measuring 1.0 m × 1.0 m × 1.2 m, which were suspended in three indoor concrete tanks of dimensions 5.0 m × 3.0 m × 1.2 m, all under natural light conditions. Throughout the rearing period, they were fed thrice daily at 08:00, 13:00, and 18:00, continuing until the majority had ceased active feeding. Daily records of feed intake and fish health status were maintained. The rearing period lasted for eight weeks, during which the tanks were inspected every morning and evening to ensure the proper functioning of the inflow and outflow water systems and the air pumps. Feeding was conducted following the four determinations principle: fixed time, fixed quantity, fixed location, and fixed quality. All net cages operated in a semi-open recirculating system using well-aerated tap water with a flow rate of 1.5 L/min. The aeration system operated continuously to ensure that dissolved oxygen concentrations remained above 5.0 mg/L while ammonia nitrogen levels were kept below 0.2 mg/L. The water temperature was controlled at 24±1°C throughout the experiment. Given the high water quality requirements of *Plecoglossusaltivelis*, siphoning was performed every morning and evening to clean the tanks, followed by replenishment with pre-aerated water to the original level, ensuring fresh water quality. For the first two weeks of rearing, the photoperiod was maintained at 16 hours per day; thereafter, continuous artificial lighting was used to delay sexual maturity.

Sample Collection: Upon completing the feeding trial, the fish were subjected to a 24-hour fasting period. Subsequently, the fish in each net cage were individually weighed and counted. Ten fish were randomly selected from each cage and then anesthetized. Subsequently, three individuals per replicate were selected to measure their length and weight. Blood was collected from the caudal vein and incubated at 4° C for 24 hours prior to centrifugation at 3,287.27 × g for 10 minutes to isolate the serum. The separated serum was then preserved at -80°C for later analysis of antioxidant parameters. Following the blood extraction, the fish's viscera and liver were dissected and precisely weighed.Additionally, four fish from each replicate were dissected in a sterile environment to collect intestines, removing mesenteric fat and gut contents to obtain intact anterior intestines for the determination of intestinal digestive enzyme activities.

Measurement Indices and Methods

Growth Performance: The growth performance metrics were assessed and calculated using the methodologies outlined by (Khieokhajonkhet *et al.* 2022 and Mozanzadeh *et al.* 2021). The growth performance indices were calculated using the following formulas:

Survival Rate (SR, %) $=100*N_t/N_g$;

Weight Gain Rate (WGR, %) $=100*(W_t-W_s)/W_s$;

Specific Growth Rate (SGR, %/d) $=100*(\ln W_t - \ln W_s)/N$;

Feed Conversion Ratio (FCR) $=W_f/(W_t-W_s)$;

Hepatosomatic Index (HSI, %) $=100*W_h/W_b$;

Condition Factor (CF, g/cm^3) $=100*W_b/L^3$.

Where $N_t$is the final number of fish per net cage; $N_s$is the initial number of fish per net cage; W_t is the final body weight (g); W_s is the initial body weight (g); N is the number of days in the rearing period; W_f is the feed intake (g); W_h is the liver weight (g); W_b is the body weight (g); and L is the body length (cm).

Serum Biochemical and Antioxidative Indices: Serum biochemical and antioxidant indices were measured following the methodologies described by (Chen *et al.* 2022). Serum total protein (TP), total cholesterol (TC),

triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels, as well as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activities, total antioxidative capacity (T-AOC), and malondialdehyde (MDA) content were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute.

Intestinal Digestive Enzyme Activities: The anterior intestine samples were homogenized with nine times the volume of physiological saline, and the homogenate was centrifuged at 2,739.40 × g for 10 minutes at 4°C. The supernatant was collected, and the activities of amylase, lipase, and protease were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute (Yuan *et al.* 2021).

Nonspecific Immune Indices: Commercial kits were used to measure immune indices, including alkaline phosphatase (AKP), acid phosphatase (ACP), complement C3 (C3), immunoglobulin M (IgM), and lysozyme (LZM). The lysozyme detection kit was purchased from Shanghai Sangon Biotech Co., Ltd., and the remaining kits were purchased from Nanjing Jiancheng Bioengineering Institute.

Data Analysis: The experiment followed a completely randomized design with four PQQ levels (0, 0.1, 0.5, 1.0 mg/kg), each with three replicates. One-way analysis of variance was conducted on the experimental data utilizing SPSS version 25.0. The bar graphs were generated using GraphPad PRISM 9.0. When ANOVA indicated significant differences ($P < 0.05$), Duncan's Multiple Range test was employed to assess group variations. Data are reported as mean ± standard error of the mean (Mean ± SEM).

RESULTS

Effects of PQQ on growth performance and serum biochemical parameters of *Plecoglossusaltivelis*:Dietary supplementation with PQQ markedly improved the growth performance of *Plecoglossusaltivelis*. As shown in Table 2, fish in the LP, MP, and HP groups exhibited significantly higher FBW, WGR, SGR, and CF compared with the control group ($P < 0.05$). These findings suggest that PQQ can effectively promote somatic growth, consistent with previous reports in other aquaculture species. Furthermore, serum biochemical analysis (Figure 1) revealed a significant reduction in TG levels in the experimental groups ($P< 0.05$), implying that PQQ may favorably modulate lipid metabolism and energy homeostasis.

Effects of PQQ on antioxidant and non-specific immune capacity in the serum of *Plecoglossusaltivelis*: PQQ supplementation markedly improved the antioxidant status and non-specific immune responses of *Plecoglossusaltivelis*. As summarized in Table 3, the MDA content was significantly decreased in the LP, MP, and HP groups ($P< 0.05$), indicating reduced oxidative stress. Concurrently, CAT activity was significantly enhanced in the MP and HP groups ($P < 0.05$), while significant improvements were also noted in SOD and GSH-Px activities. Moreover, non-specific immune parameters (Table 4) demonstrated that IgM levels were significantly elevated across the LP, MP, and HP groups ($P< 0.05$); LZM activity was increased in the MP and HP groups, and the complement component C3 level was significantly higher in the HP group ($P< 0.05$). These findings collectively suggest that PQQ not only mitigates oxidative damage but also fortifies the innate immune defense mechanisms.

Effects of PQQ on digestive enzyme activity in the intestine of *Plecoglossusaltivelis*: In terms of digestive efficiency, dietary PQQ also exerted a positive influence. Figure 2 illustrates that the protease activity in the anterior intestine was significantly increased in the LP, MP, and HP groups compared to the control group ($P < 0.05$). This enhancement in digestive enzyme activity may contribute to more efficient nutrient utilization, which in turn could support the observed improvements in growth performance.

Table 1. Ingredient and chemical composition of the diets (%).

Item	Content %
Feeds Ingredients	
Fish Meal	51.36
Soybean Meal	20.55
Wheat Flour	21.34
Spirulina	1.00
Fish Oil	1.00
Soybean Oil	1.60
Soybean Lecithin	1.00
Multi-Minerals Premix[1]	1.00
Multi-Vitamins Premix[2]	0.20
Salt	0.30
Chrome Trioxide	0.50
Choline Chloride	0.15
Nutritional Composition Analysis	
Crude Protein	49.12
Crude Fat	7.15
Moisture	6.08
Ash	11.83
Total Energy (kJ/g)	18.95
Essential Amino Acids Composition	
Histidine	1.45
Threonine	1.92
Arginine	2.69
Valine	2.44
Methionine	0.92
Phenylalanine	1.99

Isoleucine	2.10
Leucine	3.46
Lysine	3.47

Note:

1. Mineral Premix (mg/kg feed): Sodium chloride 260, Magnesium sulfate 300, Sodium dihydrogen phosphate 6 000, Potassium dihydrogen phosphate 8 500, Ferric citrate 800, Zinc sulfate 80, Manganese sulfate 30, Copper sulfate 5, Cobalt 0.25, Potassium iodide 0.80.
2. Vitamin Premix (per kilogram of feed): vitamin A 4,000 IU, vitamin B_2 20 mg, vitamin C 500 mg, vitamin D 1,500 IU, vitamin E 200 IU, vitamin K 20 IU, Niacin 150mg, Riboflavin 40mg, Inositol 300mg, Pyridoxine 30mg, Biotin 1mg, Folic Acid 10mg.
3. The total energy is calculated based on the average calorific values of protein, fat, and carbohydrates, which are 23.6, 39.5, and 17.2 kJ/g, respectively. Carbohydrates = 100 - Water - Crude Protein - Crude Fat - Ash.

Table 1. Effects of PQQ on growth performance of *Plecoglossusaltivelis*.

Items	Groups				SEM	*P*-value
	CON	LP	MP	HP		
IBW/(g)	1.26	1.26	1.25	1.26	0.001	0.467
FBW/(g)	8.08^{c}	8.68^{b}	9.27^{a}	9.43^{a}	0.052	<0.001
SR/(%)	92.50	94.17	94.17	93.33	0.625	0.752
WGR/(%)	540.98^{c}	588.54^{b}	636.22^{a}	648.48^{a}	4.210	<0.001
SGR/(%)	3.30^{c}	3.44^{b}	3.55^{a}	3.59^{a}	0.111	<0.001
FCR	1.53	1.51	1.47	1.47	0.012	0.249
CF	0.67^{c}	0.70^{b}	0.71^{a}	0.71^{ab}	0.002	<0.001
HSI	0.68	0.68	0.68	0.69	0.012	0.709

Values in the same row with different letter superscripts mean significant difference(P<0.05), the same as below.
IBW: Initial Body Weight; **FBW**: Final Body Weight; **SR**: Specific Growth Rate; **WGR**: Weight Gain Rate; **SGR**: Specific Growth Rate; **FCR**: Feed Conversion Ratio; **CF**: Condition Factor; **HSI**: Harvesting Stock Index

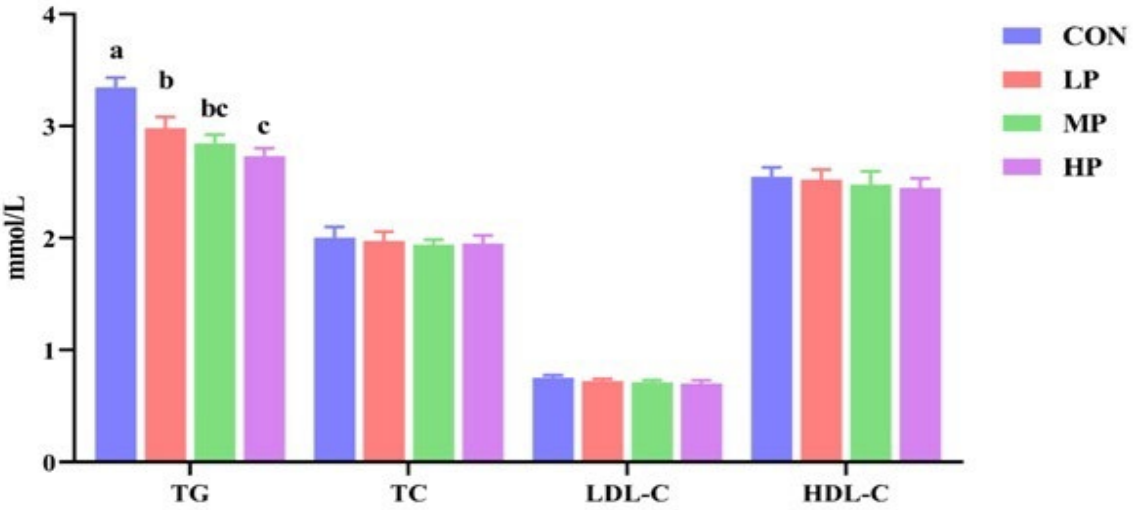

Figure 1. Effects of PQQ on serum biochemical parameters of *Plecoglossusaltivelis*.
The different superscript letters indicate significant differences for each row (P < 0.05).The same as below.

Table 3. Effects of PQQ on antioxidant capacity in the serum of *Plecoglossusaltivelis*.

Items	Groups				SEM	*P*-value
	CON	LP	MP	HP		
CAT/(U/mL)	15.51^{c}	16.23^{c}	20.48^{b}	24.52^{a}	0.665	<0.001
SOD/(U/mL)	243.31^{b}	256.23^{b}	262.86^{b}	295.61^{a}	5.189	<0.001
T-AOC/(mM)	47.50^{b}	48.69^{b}	51.21^{ab}	54.33^{a}	1.017	0.041
MDA/(nmol/mL)	4.25^{a}	3.59^{b}	3.25^{bc}	3.13^{c}	0.093	<0.001
GSH-Px/(U/mL)	282.81^{b}	288.53^{b}	305.91^{b}	308.08^{a}	6.603	<0.001

CAT:Catalase Activity**; SOD**:Superoxide Dismutase; **T-AOC**:Total Antioxidant Capacity; **MDA**:Malondialdehyde;**GSH-Px**:Glutathione Peroxidase.
^{abcd}The different superscript letters indicate significant differences for each row (P < 0.05). .

Table 4. Effects of PQQ on the non-specific immune capacity in the serum of *Plecoglossusaltivelis*.

Items	Groups				SEM	*P*-value
	CON	LP	MP	HP		
ACP/(U/mg)	131.55	133.70	135.50	133.74	2.253	0.948
ALP/(U/mg)	42.72	41.90	42.81	43.89	0.713	0.818
LZM/(U/mL)	450.81^{b}	483.69^{ab}	501.37^{a}	517.15^{a}	8.458	0.027
C3/(μg/mL)	255.79^{b}	258.69^{b}	275.09^{ab}	305.93^{a}	5.213	0.002
IgM/(mg/mL)	1 129.39^{b}	1 359.73^{a}	1 428.20^{a}	1 484.99^{a}	30.517	<0.001
TP/(mg/mL)	30.01	32.49	32.21	32.96	0.636	0.378

ACP: Acid Phosphatase; **ALP:** Alkaline Phosphatase; **LZM:** Lysozyme; **C3:** Complement Component 3; **IgM:** Immunoglobulin M; **TP:** Total Protein

^{ab}The different superscript letters indicate significant differences for each row ($P < 0.05$).

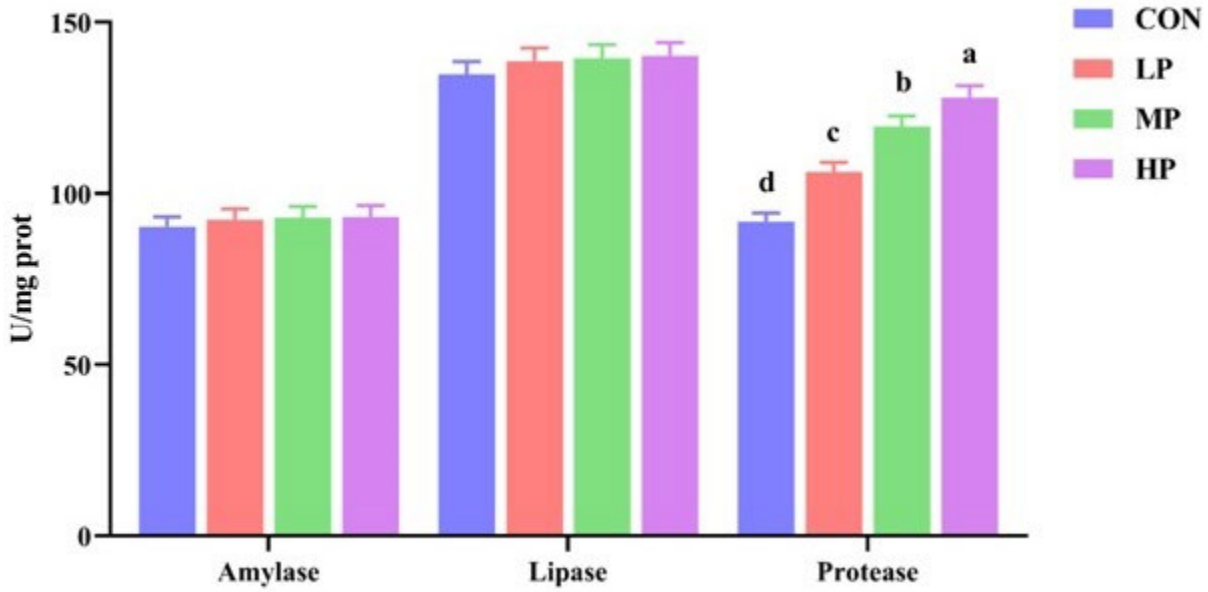

Figure 2. Effects of PQQ on digestive enzyme activity in the intestine of *Plecoglossusaltivelis*.

DISCUSSION

Impact of PQQ on the Growth Dynamics of *Plecoglossusaltivelis*: This investigation elucidates the substantial impact of various concentrations of PQQ supplementation in the diet, which markedly enhanced the FBW, WGR, SGR, and CF of *Plecoglossusaltivelis*, with statistical significance. These empirical findings underscore the potent augmentative influence of PQQ on the growth performance of *Plecoglossusaltivelis*.PQQ, a pivotal redox cofactor, serves an essential function in the regulation of energy metabolism within biological entities. Prior studies have substantiated that PQQ augments mitochondrial functionality and bolsters antioxidant defenses, thereby facilitating enhanced metabolic activity and growth(Liu *et al.* 2021). Moreover, PQQ's role in modulating key metabolic pathways, such as the AMPK pathway, further contributes to improved energy utilization and growth efficiency. The present results indicate that PQQ significantly boosts the nutrient absorption efficiency and growth potential of *Plecoglossusaltivelis*. The observed enhancements in WGR and SGR are indicative of PQQ's positive modulation of energy metabolism within the species. Furthermore, the observed improvements in CF demonstrate PQQ's role in fostering healthy growth in aquatic organisms.Additionally, PQQ may influence anabolic processes by upregulating muscle protein synthesis and reducing protein catabolism, thereby contributing to increased muscle mass and overall body condition. Supporting literature corroborates the potential of PQQ in enhancing animal growth metrics. For example, one particular study highlighted that dietary inclusion of PQQ substantially improved the growth indices of weaned piglets by stimulating mitochondrial biogenesis and amplifying antioxidant capacities, thus ameliorating overall health status (Yin *et al.* 2019). Similarly, dietary supplementation with PQQ

significantly elevated WGR and feed conversion efficiency in poultry, indicating its prospective applications within avian agriculture (Samuel *et al.* 2015).These studies collectively emphasize PQQ's versatility in promoting growth across different species, likely through conserved biochemical pathways that enhance energy metabolism and cellular resilience.

The underlying mechanism by which PQQ elevates the growth performance of *Plecoglossusaltivelis* appears potentially linked to its capacity to enhance cellular mitochondrial functionality. While these observations align with prior studies in non-aquatic models, the absence of direct molecular analyses (e.g., mitochondrial biogenesis markers or proteomic profiling) in this study limits mechanistic certainty. Scientific inquiry reveals that PQQ may potentiate mitochondrial genesis and function, thereby optimizing energy supply efficiency, a critical factor for the accelerated growth observed in *Plecoglossusaltivelis* (Charrier *et al.* 2024). Additionally, PQQ's potent antioxidant properties enable it to neutralize reactive oxygen species, diminish cellular injury caused by oxidative stress, and sustain normal metabolic functions (Saihara *et al.* 2017).This antioxidative action not only protects cellular structures but also preserves the integrity of metabolic enzymes, ensuring consistent and efficient metabolic processes essential for growth.

Effects of PQQ on serum biochemical parameters of *Plecoglossusaltivelis*: PQQ significantly impacts lipid metabolism in organisms due to its strong antioxidant properties and its capacity to enhance mitochondrial function (Liu *et al.* 2021). In this study, PQQ markedly reduced serum TG levels in *Plecoglossusaltivelis*, aligning with previous research findings. Evidence suggests that PQQ can lower serum TG levels by reducing lipid peroxide formation. Additionally, PQQ may affect the regulation of genes associated with the lipid production and transport, thereby contributing to its effects in reducing lipid levels. This mechanism has been validated across various organisms. For instance, (Qu *et al.* 2022)reported that PQQ supplementation significantly decreased TG levels in mice serum, likely due to PQQ's enhancement of fatty acid oxidation. Additionally, PQQ may upregulate key enzymes involved in lipid metabolism, further contributing to the reduction of TG levels. Similarly, studies on other aquatic species have demonstrated PQQ's capacity to improve lipid metabolism. (Shi *et al.* 2022)found that dietary PQQ supplementation in grass carp resulted in reduced serum TG and cholesterol levels in juvenile yellow catfish, and elevated HDL-C levels, suggesting PQQ's role in modulating lipid metabolism.Moreover, the rise in HDL-C levels suggests an improvement in reverse cholesterol transport, a vital mechanism for extracting surplus cholesterol from tissues and offering protection against atherosclerosis.

Despite PQQ's significant efficacy in reducing TG levels, no substantial changes were observed in other serum biochemical indices. This observation indicates that PQQ's effects may be more targeted towards specific aspects of lipid metabolism rather than causing a broad-spectrum alteration of all lipid parameters. It is also possible that the duration of the study was insufficient to observe changes in other biochemical markers, or that the baseline levels of these parameters were already within optimal ranges. This lack of effect could be attributed to the dosage of PQQ, its mechanism of action, and the specific metabolic pathways within *Plecoglossusaltivelis*. Research indicates that the metabolic effects of varying PQQ dosages can differ across species (Harris *et al.* 2013).Dose-response studies are essential to determine the optimal PQQ concentration that maximizes therapeutic benefits while minimizing potential side effects. Furthermore, it is possible that *Plecoglossusaltivelis* possesses unique metabolic regulatory mechanisms that respond differently to PQQ supplementation compared to other species. Genetic variations and species-specific enzyme activities might influence how PQQ is metabolized and utilized within different organisms. Understanding these species-specific responses is crucial for optimizing PQQ supplementation strategies in aquaculture.

Effects of PQQ on antioxidant capacity in the serum of *Plecoglossusaltivelis*: This research thoroughly investigates the impact of PQQ on the antioxidative capacity of *Plecoglossusaltivelis* serum. As shown in Table 4, the addition of PQQ significantly impacts the antioxidative indices of *Plecoglossusaltivelis* serum. The observed reduction in MDA levels suggests that PQQ enhances the antioxidative defense mechanisms of *Plecoglossusaltivelis*, thereby mitigating free radical-induced cellular membrane damage. This finding corroborates previous studies, such as those by(Jonscher *et al.* 2021), which have highlighted the antioxidative properties of PQQ in alleviating oxidative stress-induced damage in organisms. The marked increase in CAT activity may indicate the activation of the antioxidative enzyme system in *Plecoglossusaltivelis* by PQQ. As a crucial enzyme in hydrogen peroxide detoxification, the enhanced CAT activity helps reduce intracellular hydrogen peroxide accumulation, thereby attenuating oxidative stress levels (Yamada *et al.* 2020).

Furthermore, the significant increases in SOD, T-AOC,and GSH-Px activities further substantiate the enhanced antioxidative capability in *Plecoglossusaltivelis* serum attributed to PQQ. SOD is essential for the dismutation of superoxide radicals, while GSH-Px plays a key role in reducing lipid hydroperoxides. T-AOC serves as a vital indicator of the organism's overall antioxidative potential (L. Ma *et al.* 2024). The enhancement of these

parameters suggests that PQQ may boost the antioxidative capacity of *Plecoglossusaltivelis* through multiple pathways. These findings imply that the augmentation of antioxidative defense by PQQ supplementation may provide effective protection, thus maintaining the health status of the fish and potentially improving their growth performance. The enhancement of antioxidative capacity not only mitigates oxidative stress-induced cellular damage but may also improve the physiological state of the fish, thereby enhancing their productive performance(Nakano *et al.* 2015;Zhang *et al.* 2024).

At the molecular level, speculative mechanisms such as PQQ's potential activation of the Nrf2/ARE signaling pathway could contribute to the observed effects, though future studies quantifying Nrf2 nuclear translocation or ARE-binding activity are needed to confirm this hypothesis (Xue *et al.* 2024). PQQ might promote the movement of Nrf2 into the nucleus and its subsequent attachment to ARE, thereby triggering the expression of antioxidative enzyme genes. Moreover, PQQ might hinder the interaction between KEAP1 and Nrf2, preventing the degradation of Nrf2 and boosting its transcriptional activity (Huang *et al.* 2021). Moreover, the modulation of gut microbiota by PQQ could be an important indirect route through which it affects the antioxidative status of *Plecoglossusaltivelis*. The gut microbiota not only participates in nutrient metabolism but also interacts with the host immune system, influencing oxidative stress levels. PQQ might influence the gut microbiome by boosting the population of short-chain fatty acid-producing probiotics. This, in turn, enhances gut barrier function and diminishes the production of gut-derived free radicals (Wang *et al.* 2020).

Effects of PQQ on the non-specific immune capacity in the serum of *Plecoglossusaltivelis*: This study demonstrates that dietary PQQ supplementation markedly affects the nonspecific immune parameters in the serum of *Plecoglossusaltivelis*.As a potent antioxidant and immunomodulator, PQQ not only enhances the organism's antioxidative capacity but also fortifies nonspecific immune functions through diverse mechanisms. Research indicates that PQQ can activate immune cells, augmenting their activity and functionality, thereby bolstering the immune response of the organism. For instance, PQQ is known to modulate immune cell signaling pathways, thereby increasing the synthesis and secretion of immunoglobulins and complements (X. Ma *et al.* 2024). Our results demonstrated a marked elevation in IgM concentrations within the serum of *Plecoglossusaltivelis*, indicating that PQQ supplementation may enhance the humoral immune system.IgM is a crucial component of the early immune response, capable of effectively neutralizing pathogens and toxins, thus enhancing the organism's resistance.

Moreover, the notable elevation of LZM activity in the medium and high dosage groups suggests that PQQ can enhance lysozyme activity. As an important antimicrobial protein, lysozyme disrupts bacterial cell walls, playing a pivotal role in infection defense (El-Deep *et al.* 2020). PQQ may boost the expression and activity of lysozyme, thereby amplifying the antibacterial capacity of *Plecoglossusaltivelis*. In the high-dose group, a pronounced increase in the complement component C3 was observed, further supporting PQQ's role in enhancing immune responses. Complement C3 is pivotal in the complement system, capable of being activated through classical, alternative, and lectin pathways, thereby enhancing phagocytic activity and pathogen lysis. PQQ potentially modulates the activity of the complement system, increasing the synthesis and secretion of C3, thus fortifying the immune defense of *Plecoglossusaltivelis* (Jonscher *et al.* 2021).

Additional research supports the efficacy of PQQ in enhancing nonspecific immune functions. For example, a study found that PQQ significantly elevated IgM and IgG levels in mice, enhancing their humoral immune response (Hoque *et al.* 2023). Additionally, the application of PQQ in poultry demonstrated an enhancement in immune function, markedly increasing lysozyme activity and complement levels in chickens (Zheng *et al.* 2020). These findings align with the present study, further substantiating the efficacy of PQQ in bolstering nonspecific immune capabilities.We believe that PQQ enhances the activity and proliferative capacity of immune cells through the modulation of their metabolism and function. PQQ might modulate the regulation of genes linked to immunity, thus influencing the strength and duration of the immune response. Furthermore, the antioxidative properties of PQQ contribute to maintaining the optimal functionality of immune cells, as oxidative stress is known to impair immune system efficacy(Huang *et al.* 2022).

Effects of PQQ on digestive enzyme activity in the intestine of *Plecoglossusaltivelis*: Our research indicates that dietary supplementation with PQQ markedly influences the activity of digestive enzymes within the intestines of *Plecoglossusaltivelis*. As a crucial redox cofactor, PQQ is pivotal in regulating energy metabolism and antioxidative functions. It also enhances digestive enzyme functions, thereby promoting the breakdown and uptake of nutrients. Furthermore, PQQ's antioxidant properties help mitigate oxidative stress in intestinal cells, creating a more favorable environment for enzyme activity. Research suggests that PQQ can modulate the intestinal microenvironment, stimulating the production and release of digestive enzymes. This subsequently improves the efficiency of intestinal digestion. For instance, PQQ might improve mitochondrial function,

increasing cellular energy metabolism efficiency, which promotes the synthesis of more digestive enzymes by intestinal cells (Wang *et al.* 2020).In this study, PQQ significantly enhanced the protease activity within the intestines of *Plecoglossusaltivelis*, indicating an enhanced capacity for protein digestion. Proteases are essential enzymes for protein digestion, and their increased activity implies that *Plecoglossusaltivelis* can more efficiently break down feed proteins, gaining more amino acids and peptide chains, thus supporting growth and development. This improvement in protein digestion not only supports growth but may also enhance overall metabolic health and disease resistance in the fish. This finding aligns with the mechanism by which PQQ regulates the gut microbiota to enhance digestive enzyme activity (Zhang *et al.* 2020).

Additional studies support PQQ's potential to elevate digestive enzyme activity. One study found that PQQ significantly boosted trypsin and lipase activity in juvenile yellow catfish intestines, thereby enhancing nutrient digestion and absorption (Shi *et al.* 2022). Consistently, PQQ supplementation has been shown to improve gut barrier function, which may further facilitate nutrient uptake and protect against pathogenic microorganisms. Furthermore, the application of PQQ in poultry has shown beneficial effects in enhancing intestinal digestive function, markedly increasing digestive enzyme activity and feed conversion efficiency in chickens (Shao *et al.* 2023). These results align with our current study, providing additional evidence for the efficacy of PQQ in enhancing digestive enzyme activity.While preliminary evidence from experimental models has not identified significant adverse effects associated with prolonged PQQ supplementation, comprehensive clinical evaluations are still required to fully assess its long-term safety profile(Ikemoto *et al.* 2024;Nakano *et al.* 2015;Yan *et al.* 2024).

Conclusion: This study has demonstrated that dietary supplementation with PQQ at various concentrations (0.2, 0.4, and 0.6 mg/kg) positively influences the production performance, antioxidative capacity, and nonspecific immune response of *Plecoglossusaltivelis*. The 0.4 mg/kg PQQ·Na_2 dosage proved to be the most effective in enhancing growth performance, as evidenced by significant increases in FBW,WGR, SGR, and CF. Additionally, this dosage led to notable improvements in serum biochemical parameters, antioxidant enzyme activities, and nonspecific immune indices, suggesting a comprehensive enhancement of fish health and resilience.

Author Contributions: WX: conceptualization. HY: investigation. ZJ: visualization. WX, HY and ZJ: writing—original draft. WX: writing—review and editing.

Funding: This work was supported by Liaoning Provincial Natural Science Foundation of China (Grant No. 2022-NLTS-15-04).

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