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# EFFECT OF ORANGE *(Citrus sinensis)* PEEL POWDER SUPPLEMENTATION ON GROWTH, SURVIVAL AND IMMUNE RESPONSE AGAINST *Aeromonas hydrophila* IN ROHU (*Labeo rohita)*

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**ABSTRACT**

The present study aimed to evaluate the effect of orange *(Citrus sinensis)* peel powder supplementation on growth, survival and immune response against *Aeromonas hydrophila* in Rohu (*Labeo rohita)* at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. The experiment included five treatments; a control group and four experimental groups supplemented with 0.25%, 0.50%, 0.75% and 1.00% orange peel powder (OPP), respectively. The experimental design was completely randomized design with four replications. The mean weight, mean weight gain, FCR, SGR, PER, FCE and survival of the experimental fish (*Labeo rohita*) were recorded fortnightly throughout the experimental period. The results of the present study showed that the highest weight was found in 1.00% orange peel powder (5.10 ± 0.03 g), followed by 0.75% (4.73 ± 0.03 g), 0.50% (4.50 ± 0.05 g), 0.25% (4.16 ± 0.04 g) and the control group (T0) (3.75 ± 0.05 g). The SGR, FCE, FCR, PER and survival rate were also higher in the 1.00% OPP supplemented group. To determine how the diets affected the immune system, total erythrocyte count (TEC), total leucocyte count (TLC), nitroblue tetrazolium test (NBT), lysozyme activity and myeloperoxidase activity (MPO) was measured and 1 % OPP is effective. Disease resistance to *Aeromonas hydrophila* was assessed through a challenge test after 60 days of the experimental period, revealing that the 1.00% OPP had a higher survival rate than all other treatments as well as the control group. In conclusion, the findings of this study demonstrate that 1.00% supplementation of orange peel powder in the diet of Rohu fish (*Labeo rohita*) positively impacts their growth, feed utilization, immune parameters and disease resistance.

***Keywords*** **:** Orange *(Citrus sinensis)* peel, Immune response, *Aeromonas hydrophila,* Rohu (*Labeo rohita*).

## Introduction

Fisheries and aquaculture are among the fastest-growing food-producing sectors in the world and play an important role in ensuring food and nutritional security. With the rapid increase in the global population, there is a growing demand for high-quality protein sources, making the development of sustainable aquaculture practices essential. According to the Food and Agriculture Organization (FAO, 2025), global fisheries and aquaculture production reached a record 228 million tonnes in 2023, including 189 million tonnes of aquatic animals and 39 million tonnes of algae. India is currently the second-largest fish-producing country in the world, contributing around 8% of global fish production, with an estimated production of 19.77 million metric tonnes during 2023–24 (DOF, 2025). Among freshwater aquaculture species, Indian major carps such as *Labeo rohita*, catla and mrigal are highly preferred due to their rapid growth, consumer acceptance and compatibility in polyculture systems. Rohu alone contributes nearly 35% of India's major carp production and is valued for its excellent taste, high nutritional value and adaptability to formulated feeds (Chagli *et al*., 2021).

In recent years, the use of natural feed additives in aquaculture has gained considerable attention as an eco-friendly alternative to antibiotics and synthetic growth promoters. Plant-derived phytobiotics obtained from fruits, vegetables, herbs and medicinal plants are rich in bioactive compounds such as flavonoids, phenolics, alkaloids and carotenoids, which possess antimicrobial, antioxidant and immunostimulatory properties (Rachwal and Gustaw, 2025). Among these, sweet orange (*Citrus sinensis)* peel has emerged as a valuable agro-industrial by-product due to its high content of vitamin C, flavonoids, pectin and essential oils such as D-limonene. Orange peel exhibits strong antioxidant, antibacterial and immune-enhancing properties and can improve digestive enzyme activity, nutrient utilization and disease resistance in fish (Singh *et al*., 2020; Nedaei *et al*., 2019). Utilization of orange peel powder in aquafeeds also supports waste valorization and sustainable aquaculture practices by converting low-value citrus waste into a cost-effective functional feed additive.

Intensive aquaculture systems often expose fish to stress conditions, making them more susceptible to infectious diseases. Among bacterial pathogens, *Aeromonas hydrophila* infection caused by *Aeromonas hydrophila* is one of the major diseases affecting freshwater fish, leading to haemorrhage, ulceration, tissue necrosis and high mortality rates (Yardimci and Aydin, 2011). Enhancing the innate immune response of fish through dietary supplementation of natural immunostimulants has therefore become an important strategy in disease management. Orange peel powder, due to its rich bioactive composition may enhance immune parameters such as lysozyme activity, peroxidase activity and mucus immunity, thereby increasing resistance against bacterial infections. Hence, the present study entitled "Effect of orange *(Citrus sinensis)* peel powder supplementation on growth, survival and immune response against *Aeromonas hydrophila* in Rohu *(Labeo rohita)*".

## Materials and Methods

The feeding experiment was conducted for 60 days (October–December 2025) at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. Fingerling rohu *(Labeo rohita)* with an average initial weight of 1.75 ± 0.007 g were acclimatized for seven days and randomly stocked into 20 rectangular plastic tanks (50 L capacity) following a Completely Randomized Design with five treatments and four replicates each. Five isonitrogenous diets (30% crude protein) were formulated by incorporating orange peel powder at different inclusion levels. Orange peel was washed, sun-dried and powdered before feed preparation.

**Table 1:** Composition of Experimental Diets

| Ingredients (%) | Diet (30% Protein) | | | | |
|---|---|---|---|---|---|
| | T0 (Control) | T1 (0.25 %) | T2 (0.50%) | T3 (0.75%) | T4 (1.00%) |
| Orange peel powder (OPP) | 0.00 | 0.25 | 0.50 | 0.75 | 1.00 |
| Sterilized Fish meal | 38.00 | 38.00 | 38.00 | 38.00 | 38.00 |
| GNOC | 28.00 | 28.00 | 28.00 | 28.00 | 28.00 |
| Wheat flour | 17.00 | 16.75 | 16.50 | 16.25 | 16.00 |
| Tapioca powder | 11.00 | 11.00 | 11.00 | 11.00 | 11.00 |
| Fish oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Sun flower oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Vitamin and minerals premix | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

Fish were fed twice daily at 3% of body weight with feeding rates adjusted fortnightly. Continuous aeration and daily partial water exchange were maintained and water quality parameters were monitored regularly. Growth performance parameters including weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and survival rate were recorded at 15 days' intervals. The immune response of *Labeo rohita* fingerlings supplemented with orange peel powder was evaluated using hematological and non-specific immune parameters such as total erythrocyte count (TEC), total leucocyte count (TLC), respiratory burst activity, lysozyme activity and myeloperoxidase (MPO) activity following standard methods. TEC and TLC were estimated using a hemocytometer after dilution of blood samples with Hayem's fluid and Turk's fluid, respectively. Respiratory burst activity was assessed through the nitro blue tetrazolium (NBT) reduction assay, while lysozyme activity was determined using *Micrococcus sp.* culture based on bacterial lysis. MPO activity was

estimated spectrophotometrically using hydrogen peroxide and TMB substrate. Disease resistance against *Aeromonas hydrophila* infection was also evaluated through an antibacterial assay and challenge test by monitoring survival and health condition of fish after bacterial infection. Completely Randomised Design (CRD) with four replications was used for each treatment.

## Result and Discussion

The present study confirmed that plant-based orange peel supplementation significantly improved growth performance, feed utilization, survival and immune response in rohu, with the effects varying according to the dietary inclusion level.

**Table 2:** Mean weight (g) of *L. rohita* fed with diets containing orange peel powder during the experimental period (Mean±SE)

| Treatment | Experimental Period | | | | |
|---|---|---|---|---|---|
| | 0 DAYS | 15 DAYS | 30 DAYS | 45 DAYS | 60 DAYS |
| T0 | 1.74±0.02$^{a}$ | 2.08±0.02$^{c}$ | 2.63±0.04$^{d}$ | 3.17±0.03$^{d}$ | 3.75±0.05$^{e}$ |
| T1 (0.25% OPP) | 1.76±0.02$^{a}$ | 2.26±0.03$^{b}$ | 2.90±0.03$^{c}$ | 3.55±0.05$^{c}$ | 4.16±0.04$^{d}$ |
| T2 (0.50% OPP) | 1.76±0.03$^{a}$ | 2.31±0.05$^{b}$ | 3.14±0.05$^{b}$ | 3.78±0.01$^{b}$ | 4.50±0.05$^{c}$ |
| T3 (0.75% OPP) | 1.75±0.01$^{a}$ | 2.39±0.06$^{ab}$ | 3.20±0.04$^{b}$ | 3.85±0.06$^{b}$ | 4.73±0.03$^{b}$ |
| T4 (1.00% OPP) | 1.76±0.08$^{a}$ | 2.50±0.01$^{a}$ | 3.37±0.03$^{a}$ | 4.17±0.02$^{a}$ | 5.10±0.03$^{a}$ |

In the present study, the mean weight of *L. rohita* was highest in treatment T4 (5.10±0.03 g) and lowest in treatment T0 (3.75±0.05 g). Maximum growth was observed in fish fed 1.00% orange peel powder supplementation, indicating that this inclusion level provided an optimum balance of vitamins, antioxidants and palatability. Similar findings were reported by Lee *et al*. (2024) and Gultepe *et al*. (2018), who observed enhanced growth in fish fed diets containing orange peel products. The improved growth may be attributed to bioactive compounds such as flavonoids, phenols and terpenes present in orange peel, which enhance digestive enzyme activity, nutrient utilization and feed absorption efficiency.

**Table 3:** Growth parameters after a diet with orange peel powder (OPP) in *L. rohita* during the experimental period (Mean±SE)

| Parameter | $T_0$ Control | $T_1$ (0.25% OPP) | $T_2$ (0.50% OPP) | $T_3$ (0.75% OPP) | $T_4$ (1.00% OPP) |
|---|---|---|---|---|---|
| MWG (g) | 2.01±0.07 | 2.39±0.04 | 2.74±0.05 | 2.97±0.02 | 3.35±0.02 |
| SGR (%) | 1.28±0.04 | 1.43±0.02 | 1.56±0.02 | 1.65±0.01 | 1.78±0.01 |
| Survival (%) | 79.99±2.72 | 83.33±1.92 | 86.66±2.72 | 90.00±1.92 | 96.67±1.92 |
| FCR (%) | 2.75±0.045 | 2.44±0.023 | 2.32±0.015 | 2.23±0.051 | 2.08±0.022 |
| FCE | 0.36±0.012 | 0.40±0.004 | 0.43±0.003 | 0.45±0.010 | 0.48±0.006 |
| PER (%) | 1.21±0.050 | 1.37±0.026 | 1.46±0.020 | 1.49±0.047 | 1.60±0.037 |

In the present study, the mean weight gain of *Labeo rohita* was highest in treatment T4 (3.35±0.02 g) and lowest in treatment T0 (2.01±0.07 g). The highest weight gain was recorded in treatment T4, indicating that supplementation with 1.00% orange peel powder had the most positive effect on the growth performance of *L. rohita*. Comparable results were also reported by Lee *et al*. (2024) and Gultepe *et al*. (2018), who found improved growth in fish fed diets supplemented with orange peel products.

Specific growth rate (SGR) varied significantly among the treatments, with the highest value observed in T4 (1.00% orange peel powder supplementation), followed by T3, T2 and T1, while the lowest SGR was recorded in the control group (T0). Similar observations have been reported by Gultepe *et al*. (2018), Acar *et al*. (2015), Lee *et al*. (2024) and Yun *et al*. (2025), who also found enhanced growth in fish fed diets supplemented with orange peel products. Similarly, the highest survival rate was recorded in T4, followed by T3, T2 and T1, whereas T0 showed the lowest survival. However, the differences among treatments were not statistically significant ($p > 0.05$). The improved survival trend observed in the supplemented groups may be attributed to the beneficial effects of orange peel bioactive compounds on fish health and immunity. These findings are in agreement with Acar *et al*. (2015), who reported enhanced survival and immune status in fish receiving citrus-based dietary supplements.

Dietary supplementation with orange peel powder significantly improved feed utilization in *Labeo rohita.*

The most efficient feed utilization was observed in treatment T4, which recorded the lowest feed conversion ratio (FCR) and the highest feed conversion efficiency (FCE) and protein efficiency ratio (PER), while the least efficient performance was recorded in the control treatment T0. These outcomes are consistent with Lee *et al*. (2024), who used 1% orange peel powder in Black Rockfish and found an improved feeding efficiency during their experiment. The present findings are consistent with those of Gultepe *et al*. (2018), who reported that rainbow trout fed a diet supplemented with 1% orange peel essential oil exhibited improved protein efficiency ratio (PER) compared to the control group.

The improved growth performance observed in fish fed diets supplemented with orange peel may be attributed to the presence of various bioactive compounds such as flavonoids, phenols, alkaloids, saponins and terpenes. These phytochemicals are known to enhance digestive processes by stimulating digestive enzyme activity, improving nutrient digestibility and increasing feed absorption efficiency. As a result, fish are able to utilize nutrients more effectively, leading to enhanced weight gain, better feed conversion and overall growth performance (Salem and Abdel-Ghany, 2018).

**Immune Response**

**Table 4:** Haematological parameters and non-specific immune parameters of *Labeo rohita* during the experimental period (Mean±SE)

| Parameter | $T_0$ Control | $T_1$ (0.25% OPP) | $T_2$ (0.50% OPP) | $T_3$ (0.75% OPP) | $T_4$ (1.00% OPP) |
|---|---|---|---|---|---|
| **TEC** | 1.17±0.045 | 1.32±0.021 | 1.40±0.042 | 1.62±0.022 | 1.78±0.023 |
| **TLC** | 40.88±0.63 | 42.63±0.31 | 44.27±0.59 | 47.52±0.83 | 54.12±0.51 |
| **NBT** | 0.21±0.010 | 0.24±0.004 | 0.26±0.010 | 0.29±0.006 | 0.33±0.009 |
| **Lysozyme** | 4.18±0.19 | 4.58±0.19 | 5.08±0.16 | 5.98±0.30 | 6.78±0.25 |
| **MPO** | 0.19±0.004 | 0.22±0.009 | 0.27±0.007 | 0.34±0.011 | 0.41±0.010 |
| **Disease resistance (Survival rate (%) at the end of $7^{th}$ days)** | 50 | 60 | 70 | 70 | 80 |

At the end of the 60 days experiment, the total erythrocyte count (TEC) of *Labeo rohita* was highest in treatment T4 (1.78 ± 0.023), while the lowest value was observed in T0 (1.17 ± 0.045). Similar findings were reported by Acar *et al*. (2015), who observed increased TEC values in tilapia fed citrus essential oil-supplemented diets. The highest TLC was recorded in T4 (54.12±0.51), while the lowest value was observed in T0 (40.88±0.63). Similar findings were reported by Vicente *et al*. (2019) in Nile tilapia fed orange peel supplemented diets. The enhanced TLC may be attributed to bioactive compounds such as flavonoids and essential oils present in orange peel, which stimulate immune cell activity and improve disease resistance. The highest respiratory burst activity was recorded in treatment T4 (0.33 ± 0.009), while the lowest value was observed in the control group T0 (0.21 ± 0.010). The results indicate that respiratory burst activity increased with increasing levels of orange peel powder supplementation.

The highest lysozyme activity was recorded in treatment T4 (6.78±0.25) and lowest observed in T0 (4.18±0.19). Similar findings were reported by Lee *et al*. (2024), who observed increased lysozyme activity in fish fed diets supplemented with orange peel waste. The enhanced lysozyme activity may be due to the presence of bioactive compounds such as flavonoids, phenolics and vitamin C in orange peel, which stimulate the non-specific immune system and improve antibacterial defense mechanisms. These compounds activate immune pathways and promote the production of antimicrobial enzymes, thereby enhancing bacterial lysis and disease resistance in fish (Yun *et al*., 2025). The highest MPO activity was recorded in treatment T4 (0.41±0.010) and lowest observed in T0 (0.19±0.004). Similar results were reported by Acar *et al*. (2015), who observed enhanced MPO activity in tilapia fed diets supplemented with sweet orange peel essential oil. The increased MPO activity in the present study indicates that higher levels of orange peel powder strengthened the innate immune response of fish and improved their defense mechanism against pathogens.

After the 60 days experimental period, a challenge test was conducted. Ten fish from each treatment group were randomly selected and injected intramuscularly with *Aeromonas hydrophila* at a concentration of $1 \times 10^6$ cells/ml to minimize handling stress. Mortality in each group was then observed and recorded over a period of seven days. The inclusion of orange peel powder in the diet enhanced the survival rate of fish following the *A. hydrophila* challenge compared to the

control group. At the end of the seven days observation period, the control group showed a significantly lower survival rate of 50%, whereas the highest survival rate of 80% was observed in treatment T4 (1.00% Orange peel powder).

## Conclusion

In conclusion, the findings of this study demonstrate that supplementing the diet of Rohu fish (*Labeo rohita*) with orange peel powder has a positive impact on their overall performance. Fish that received a 1.00 % inclusion of orange peel powder in their feed exhibited significant improvements in growth performance and survival rate. Moreover, orange peel powder supplementation also boosted the fish immune response, leading to greater resistance against disease challenges. These outcomes suggest that incorporating 1.00% orange peel powder into the diet of rohu can serve as an effective strategy for promoting sustainable aquaculture practices by improving growth, survival and disease resistance without relying on antibiotics or chemical additives.

## References


Acar, U., Kesbiç, O. S., Yilmaz, S., Gultepe, N., & Turker, A. (2015). Evaluation of the effects of essential oil extracted from sweet orange peel *(Citrus sinensis)* on growth rate of tilapia *(Oreochromis mossambicus)* and possible disease resistance against *Streptococcus iniae*. *Aquaculture*, **437**, 282-286.

Chagli, M. K., Vadher, K. H., & Patel, M. R. (2021). Effect of partial replacement of fish meal with moringa leaf meal in the diet of *Labeo rohita* FRY. *The Pharma Innovation Journal*, **10**(12), 1645-1648.

Department of Fisheries (2025). *Annual report 2024-25.* Ministry of Fisheries, Animal Husbandary and Dairying, Government of India. p. 204

FAO. (2025). *The state of world fisheries and aquaculture*. blue transformation in action. Rome. p. 418

Gultepe, N. (2018). How the use of orange *(Citrus sinensis)* peel essential oil affected the growth performance of rainbow trout *(Oncorhynchus mykiss*). *Bulletin UASVM Animal Science and Biotechnologies* **75**(1), 16-20.

LAL, S. S. (2010). Hematological changes in *Tinca tinca* after exposure to lethal and sublethal doses of Mercury, Cadmium and Lead.

Lee, T. H., Kim, K. T., Oh, H. Y., Park, S. Y., Lee, G. J., Kim, H. S., & Kim, H. S. (2024). Effect of Blood Orange (*Citrus sinensis* L. Osbeck) Peel Waste as a Feed Additive on the Growth Performance, Digestive Enzyme Activity, Antioxidant Capacity, and Immune Response in Juvenile Black Rockfish *(Sebastes schlegelii)*. *Antioxidants*, **13**(12), 1452.

Nedaei, S., Noori, A., Valipour, A., Khanipour, A. A., & Hoseinifar, S. H. (2019). Effects of dietary galactooligosaccharide enriched commercial prebiotic on growth performance, innate immune response, stress resistance, intestinal microbiota and digestive enzyme activity in Narrow clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823). *Aquaculture*, **499**, 80-89.

Rachwal, K., & Gustaw, K. (2025). Plant-Derived Phytobiotics as Emerging Alternatives to Antibiotics Against Foodborne Pathogens. *Applied Sciences*, **15**(12), 6774.

Salem, M., & Abdel-Ghany, H. M. (2018). Effects of dietary orange peel on growth performance of Nile tilapia *(Oreochromis niloticus)* fingerlings. *Aquaculture studies*, **18**(2), 127-134.9

Secombes, C. J., & Fletcher, T. C. (1992). The role of the respiratory burst in the immunological response of fish. *Fish & Shellfish Immunology*, **2**(5), 431-447.

Shah, S. L., & Altindag, A. (2004). Hematological Parameters of Tench (*Tinca tinca* L.) after Acute and Chronic Exposure to Lethal and Sublethal Mercury Treatments. *Bulletin of Environmental Contamination & Toxicology*, **73**(5).

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2020). Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Research International*, **132**, 1-22.

Snedecor, W. G., & Cochran, G. W. (1971). Statistical methods 10the ed, Lowa state.

Stasiak, K., & Baumann, R. (1996). Modifications of the nitroblue tetrazolium assay for the detection of superoxide production in fish phagocytes. *Journal of Fish Diseases*, **19**(5), 393-397.

Vicente, I. S., Fleuri, L. F., Carvalho, P. L., Guimarães, M. G., Naliato, R. F., Müller, H. D. C., ... & Barros, M. M. (2019). Orange peel fragment improves antioxidant capacity and haematological profile of Nile tilapia subjected to heat/dissolved oxygen‐induced stress. *Aquaculture Research*, **50**(1), 80-92.

Yardimci, B., & Aydin, Y. (2011). Pathological findings of experimental Aeromonas hydrophila infection in Nile tilapia *(Oreochromis niloticus). Ankara Üniversitesi Veteriner Faults Dergisi*, **58**(1), 47-54.

Yun, A., Oh, H. Y., Lee, T. H., Kang, D. Y., Kim, K. T., Kim, H. S., & Kim, H. S. (2025). Blood Orange (*Citrus sinensis* L. Osbeck) Juice By-Product Extract as a Functional Feed Additive: Effects on Growth Performance, Digestive Enzyme Activity, Antioxidant Status, Immune Parameters, and Disease Resistance Against *Vibrio harveyi* in Juvenile Black Rockfish *(Sebastes schlegelii). Antioxidants*, **14**(6), 745.