



## Effect of Grape Seed (*Vitis vinifera*) Extract on Growth and Blood Parameters of *Ctenopharyngodon idella* (Grass carp) and *Carassius auratus* (Goldfish)

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Received: 27.09.2025

Accepted: 13.11.2025

Published: 25.12.2025

**How to Cite:** Nazir et al. (2025). Effect of Grape Seed (*Vitis vinifera*) Extract on Growth and Blood Parameters of *Ctenopharyngodon idella* (Grass carp) and *Carassius auratus* (Goldfish). *VZS*, 1(2), 172–184.

**DOI:** <https://doi.org/10.64614/vzs-18>

**Abstract:** Proanthocyanidin found in the grape seed (*Vitis vinifera*) have an ameliorating effect for fish health. *Ctenopharyngodon idella* (grass carp) is an important species of aquaculture having significant economic value, and *Carassius auratus* (goldfish) is a potential aquarium fish species. This research work was conducted to study the growth and blood parameters of *C. idella* and *C. auratus* after administration of grape seed extract (GSE) for about 60 days. Total 150 fingerlings of *C. idella* and *C. auratus* were taken and divided into two groups, each group with 25 specimens, randomly placed for unbiased results. Experimental groups were fed with *V. vinifera* extract at 2.75% body weight of fish, and the control group with 100% commercial basal diet. The length and weight of fishes were noted throughout the trial period, keeping the physicochemical parameters of water in optimum ranges. The results showed an increase in values between groups as survival rate (96%), increase in fork length (1.17, 3.67 cm), weight gain (4.9, 11.8 g), FCR (0.0227, 0.0093), SGR (8.88, 9.44), and the improvement in the count of RBCs (10.5,  $7 \times 10^6/\mu\text{L}$ ), WBCs (46.8,  $4.6 \times 10^3/\mu\text{L}$ ) and Hb (9.25, 6.5 g/dL) for the grass carp and goldfish, respectively. Statistical analysis under CRD showed that Grape Seed extract had a significant effect on these parameters, as the p-value was less than alpha (0.05%). Thus, grape seed extract (GSE) proved to be a potential growth and immunity enhancer for fish.

**Keywords:** Aquaculture, feed additive, grass carp, goldfish, grape seed extract, proanthocyanidin

### INTRODUCTION

Aquaculture is rapidly expanding in the world, which provides foodstuff. It is growing and developing in all parts of the world (Subasinghe et al., 2009). Ornamental fish are traded worldwide each year (Stickney and Gatlin, 2022). One of the fundamental sources of animal protein is fish, which provides about 26.2% animal protein (Hassan et al., 2021). There are 193 species of fish that inhabit freshwater in Pakistan. Fish is a source

of protein and minerals (Rafique and Khan, 2012). Aquaculture is the cultivation of marine plants or animals that has been developed continuously since 1970, and the fish that it produced at that time was 3.9 %. More than 90% of aquaculture takes place in developing countries, and it is a source of diet for the poor people there. According to Food and Agricultural Organization (FAO), fish production from farming in Asia is about 84% of the total production (FAO, 2022). Aquaculture is anticipated to produce fifty percent of the world's food in the next 2 decades (Pullin, 2006). In 2016, countries in Asia made for roughly 89% of the world's output. Despite continuing to be the top producers until the 1970s, the majority of the wealthy nations have contributed very little to the blue revolution (Garlock et al., 2020).

Aquatic specie *Ctenopharyngodon idella*, locally known as grass carp, is a chief specie of aquaculture that have much economic value (Cudmore and Mandrak, 2004). Grass carp is a herbivorous fish and is capable of controlling aquatic vegetation, providing potential benefit to the aquatic system. A large amount of information has been issued on the grass carp, as it was used to control aquatic plants globally, as in Arkansas, after 10 years, the use of *C. idella* in the management operation kicked off the vegetative burden, as more than 50 genera of macrophytes and algae are consumed by *C. idella* (Dibble and Kovalenko, 2009). The grass carp, as an economically significant herbivorous fish, has a wider global distribution and is becoming more widely recognized for its ecological contributions discussed (Li et al., 2023).

The Goldfish, *Carassius auratus* (Cypriniformes: Cyprinidae), is one of the ornamental fish species that is traded the most, internationally. It is native to Asian rivers and lakes, were most likely brought to Japan in 1502 by way of China. *C. auratus* is highly sought after ornamental cyprinid fish that is marketed in over 100 countries. At least 2000 years ago, these were first kept in China, where they were mainly reared for food (Brown et al., 2018). Goldfish normally grow to be 15 to 20 cm in length and have a lifespan of six to seven years. The ideal pH range for them is 5.5 to 7.0 (Blanco et al., 2018).

In addition to being a great source of vitamins and fibre, proanthocyanidins, a type of polyphenol, are abundantly found in the grape seeds (*Vitis vinifera*). These compounds can be employed as functional ingredients to treat a variety of health conditions by enhancing the body's natural bioprocesses. Since grape seeds are a byproduct of wine firms, they are readily available commercially. Grape seed extract (GSE) is richest in polyphenolic chemicals and it has anti-inflammatory, antibacterial effects, and it also

obstruct the lipid oxidation, an important perspective for food safety (Gupta et al., 2020). Due to the presence of proanthocyanidin in the grape seed extract it has gained the attention of the user and it influenced the health of organisms also. Proanthocyanidin has an antioxidant effect and it regulates the immune response with decreased platelet aggregation (Kwatra, 2020).

Grape seed extract or *V. vinifera* is a flavonoid polymer and polyphenolic in nature (Nazima et al., 2015), an antioxidant that kills viruses, bacteria, and inhibits programmed cell death or apoptosis (Gao et al., 2014). Phytochemicals have been used as healing additives in aquaculture, and they perform physiological function in aquatic animals (Zhu, 2020). In the winery and grape juice industry, *V. vinifera* production takes place. Phenolic compounds in *V. vinifera* include lipids, fatty acids, carbohydrates, amino acid, proanthocyanidin, and polymerized oligomers, etc. GSE also protects fish against DNA damage and excess free radicals formation. Additionally, it has to increase body weight, reduced immune-mediated injury, as well as oxidative spleen damage in fish. GSE supplemented diets in fish enhance antioxidant capacity and growth rate (Jahanbakhshi et al., 2023).

GSE proved to be a potent growth enhancer that improves basically the feed efficiency in fish and other livestock animals if appropriate dosages are incorporated in diets of animals (Mehrinakhi et al., 2021). Proanthocyanidins found in it has strong antioxidant, anti-inflammatory and it may act as digestive enzymes booster that ultimately leads to better growth and health of fish. Many studies proved an elevation in red blood cell count, haemoglobin, and other blood parameters after low-to-moderate GSE administration (Jahanbakhshi et al., 2023; Abdulrahman et al., 2013; Al-Atbee and Al-Niaeem, 2021)

Researchers have found *V. vinifera* potential in enhancing fish physiology and digestive capabilities, accessing the beneficial role of proanthocyanidins. But specifically, the effect of grape seed extract on the growth and immunity of *C. idella* and *C. auratus* in a system is not well studied before.

## **MATERIAL AND METHOD**

### ***Location***

The present research was accomplished at Fisheries Research Farm located at Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad in a two month span period from 19-12-23 to 20-02-24.

### ***Investigatory Species***

Grass Carp (*Ctenopharyngodon idella*) and Goldfish (*Carassius auratus*) was the experimental specimens used. Local Ethics Committee principles have been followed with ethical approval number 3301-04 dated 16/02/2024, University of Agriculture, Faisalabad.

### ***Experimental Trial***

150 fingerlings of both species *C. idella* and *C. auratus* with an average weight of 4.25-6.5g were taken from Fisheries Research Farm, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, and were acclimatized for 48 hours. Two groups of Grass Carp and two groups of Goldfish (experimental and control) were made with randomized aquarium placement of fishes for unbiased results in triplicates. Aquariums were provided with full aeration, and other water quality parameters were maintained throughout the experiment period.

### ***Trial Diets***

Grape seed extract was used as the experimental diet, according to 2.75% of the body weight of fishes, and 100% commercial basal diet was given to the control group of fishes. Commercially available grape seed extract powder (95% proanthocyanidins) was obtained from an e-commerce platform, Daraz, with brand name Scitonics, and added to the fish diet. Typical nutrient composition of GSE available is mentioned below in Table 1.

$$\text{Amount of feed/Day in grams} = \frac{\text{Total weight of fish samples} \times \text{Feeding rate at 2\% body weight}}{100} = 2.7$$

**Table 1.** Grape seed extract (GSE) nutrient composition

<b>Parameter</b>	<b>Typical range (per 100g GSE powder)</b>
Total polyphenols	70-90g gallic acid equivalent (GAE)
Proanthocyanidins extract	50-80g catechin equivalent
Moisture	3-8%
Ash	1-3%
Total sugars	<5
Proteins	<2
Lipids	<1

### ***Blood Collection and Haematological Parameters***

Blood samples from the caudal vein of fish were taken as samples at the end of the trial, kept in sterile tubes, and labeled accordingly for analysis. A hemocytometer was

used for total RBCs and WBCs counts with the standard protocol mentioned by Parida et al. (2011). One portion of the sample, treated with EDTA, was used to determine hematological parameters, and differential staining was performed to count leukocytes. The other portion was clotted, centrifuged, and stored for measuring total plasma protein and albumin. The other hematological parameters, such as MCV, MCHC, and MCH, were analyzed using the method followed by Saravanan et al. (2011).

### ***Growth Parameters***

Growth parameters such as length gain, weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) were calculated.

### ***Weight Gain (g) and Length Gain (cm) Calculation***

Weight gain was calculated by subtracting the initial weight from the final weight, and Length was calculated by subtracting the initial length from the final length.

### ***FCR and SGR Calculation***

Feed conversion ratio (FCR) and Specific Growth Rate (SGR) was calculated by using the following formulas:

Feed Conversion Ratio = Feed given (dry weight)/ body weight gain (wet weight)

% SGR =  $\ln(\text{final weight}) - \ln(\text{initial weight}) \div \text{time duration in days} \times 100$

### ***Physicochemical Parameters of Water***

Since physicochemical parameters play an important role in the survival of fish, all those major indicators were regulated and noted regularly during the whole period.

For the determination of pH, a Microprocessor pH meter (HANNA-HI-8424) was used after setting its range at the pH point. Water temperature and dissolved oxygen (DO) was recorded by using a microprocessor meter (HANNA-HI, 9146). The sensor of the meter was dipped into the water surface to directly measure the concentration of dissolved oxygen (DO).

### ***Statistical Analysis***

The data was subjected to statistical interference using Minitab software at the end of the research analysis. The results were compared to get the final interpretation by using two factor factorial in Completely Randomized Design (CRD)

## **RESULTS**

### ***Physio-chemical Parameters***

Estimation of physio-chemical parameters of water quality is an essential indicator of fish health, as fish is an ectothermic chordate and is dependent on its

environment, i.e., water. Thus, the values for pH, DO, temperature, total hardness (bicarbonates and carbonates), and total alkalinity of water under T<sub>0</sub> and T<sub>1</sub> were noted throughout the period, and the average mean±SD was calculated and is shown in Table 2.

**Table 2.** Mean±SD of different physicochemical parameters

	<b>pH</b>	<b>DO (mgL<sup>-1</sup>)</b>	<b>Temperature (°C)</b>	<b>Carbonates (mgL<sup>-1</sup>)</b>	<b>Bicarbonates (mgL<sup>-1</sup>)</b>	<b>Total Alkalinity (mgL<sup>-1</sup>)</b>
<b>T<sub>0</sub></b>	7.79±0.3	7.61±2.1	28.11±2.8	115.7±16.8	120±9.5	145.4±7.9
<b>T<sub>1</sub></b>	7.2±0.4	6.0±0.5	27.0±1.5	70.0±14	79.0±6.9	120.0±6.6

±: Standard Deviation

### ***Fish Growth Studies***

Growth metrics of Grass Carp (*C. idella*) and Goldfish (*C. auratus*) fed with Grape seed extract (*V. vinifera*) was examined by accessing the following parameters, and are shown in Table 3 and Table 4.

#### ***Increase in the Weight (g) and the Fork Length (cm)***

The increase in weight in Goldfish was recorded as 11.8 grams and 4.9 grams in grass carp (experimental group). At the start of the experiment, the average fork length of the *C. idella* was 2 cm, and at the end of the trial period, it was 3.17 cm, so the increase in fork length of *C. idella* was 1.17 cm. The average fork length of the *C. auratus* at the start of the experiment was 3 cm, and at the end of the trial period, it was 6.67 cm, so the increase in the fork length of the *C. auratus* was 3.67 cm.

#### ***Feed Conversion Ratio (FCR)***

Feed conversion ratio evaluated the best performance of fingerlings in experimental group S2 (1.21±0.005) as compared to its performance in the control group of S2 (1.857 ±0.005). S1 also exhibited significant results in terms of FCR, as shown in Table 3.

#### ***Specific Growth Ratio (SGR) (%)***

The specific growth rate (SGR) under the control group (T<sub>0</sub>), S2 exhibited the lowest SGR (1.27±0.005), while S1 showed a comparatively higher value (1.72±0.007). In contrast, the experimental treatment (T<sub>1</sub>) resulted in a marked increase in percentage

SGR for both species, reaching  $4.03 \pm 1.73$  in S1 and  $4.81 \pm 2.04$  in S2, as shown in Table 3.

### ***Survival Rate***

Survival Rate Percentage for *C. idella* and *C. auratus* was about 96 percent. Fish mortality for both species remained lowered throughout the trial period, specifically in the experimental group than in the control, as shown in Table 3.

**Table 3.** Mean $\pm$ SD for growth performance parameters the *C. idella* (S<sub>1</sub>) and *C. auratus* (S<sub>2</sub>)

Parameters	T <sub>0</sub>		T <sub>1</sub>	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>
<b>Weight Gain</b>	1.065 $\pm$ 0.005	5.425 $\pm$ 0.100	4.822 $\pm$ 0.080	11.75 $\pm$ 0.129
<b>Fork Length Gain</b>	0.625 $\pm$ 0.129	1.708 $\pm$ 0.228	1.025 $\pm$ 0.115	3.585 $\pm$ 0.081
<b>FCR</b>	2.022 $\pm$ 0.016	1.857 $\pm$ 0.005	1.703 $\pm$ 0.002	1.211 $\pm$ 0.005
<b>SGR (%)</b>	1.72 $\pm$ 0.007	1.27 $\pm$ 0.005	4.03 $\pm$ 1.73	4.81 $\pm$ 2.04
<b>Survival Rate (%)</b>	82	85	95	96.5

FCR: Feed conversion ratio, SGR: Specific growth rate;  $\pm$ : Standard Deviation

**Table 4.** Analysis of growth performance parameters of experimental treatments under CRD

Source	DF	Weight (g)	Fork Length (cm)	FCR	SGR
<b>Specie</b>	1	14540.58 ( $<0.001^*$ )	894.51 ( $<0.001^*$ )	81266.74 ( $<0.001^*$ )	0.48 (0.497 <sup>ns</sup> )
<b>Treatments</b>	1	11612.62 ( $<0.001^*$ )	135.84 ( $<0.001^*$ )	62754.81 ( $<0.001^*$ )	22.13 ( $<0.001^*$ )
<b>Specie*Treatments</b>	1	751.20 ( $<0.001^*$ )	135.84 ( $<0.001^*$ )	71452.83 ( $<0.001^*$ )	0.44 (0.517 <sup>ns</sup> )
<b>Error (residual sum of square)</b>	12				
<b>Total (N-1)</b>	15				

Values represent F-statistics with corresponding p-values in parentheses. Significant effects are observed at  $p < 0.05$ .  $\pm$ : Standard Deviation; \* = significant, ns = not-significant

### ***Blood and Immunological Parameters***

Immunological parameters like red blood cell (RBCs) count, white blood cell (WBCs) count, haemoglobin, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), lymphocyte, monocyte, granulocyte, and PLT were accessed and are shown in Table 5.

**Table 5.** Mean±SD for the Blood Parameters and Immunity of *C. idella* (S<sub>1</sub>) and *C. auratus* (S<sub>2</sub>)

Parameters	T <sub>0</sub>		T <sub>1</sub>	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>
<b>RBCs Count (RBCs×10<sup>6</sup> μL)</b>	1.25±0.25	2.85±0.05	11.75±1.639	9.8125±0.205
<b>Hb Count (g/dl)</b>	1.317±0.157	1.458±0.334	10.2916±0.293	8.041±0.271
<b>MCV Level (fl)</b>	109.75±0.75	87.036±0.128	149.33±0.781	105.11±0.071
<b>MCH (pg)</b>	29.83±0.463	26.193±0.351	39.95±0.513	29.8±0.014
<b>MCHC (g/dL)</b>	25.79±0.624	25.643±0.01	34.616±0.722	36.43±0.018
<b>Lymphocytes %</b>	69.85±0.256	65±1.29	75.66±0.092	79.41±0.552
<b>WBCs Count (WBCs×10<sup>3</sup>μL)</b>	132.83±0.303	4.866±0.268	180.23±0.466	9.85±0.479
<b>Monocytes%</b>	2.15±0.189	3.11±0.206	3.75±0.095	5.0±0.224
<b>Granulocytes %</b>	28.03±0.149	26.01±0.226	30.83±0.395	33.1±0.071
<b>PLT Count (mcL)</b>	10.133±0.767	17.0±1.82	93.0±3.15	95.5±0.958

RBCs count was  $10.5 \times 10^6$  and  $7 \times 10^6$  for the *C. auratus* and *C. idella*, respectively. There was a significant difference in the WBCs count between the control group and the experimental group. WBCs count for *C. idella* was  $46.8 \times 10^3$  and  $4.6 \times 10^3$  for the *C. auratus*.

Hemoglobin (Hb) concentration for the *C. idella* was 1.5 g/dL initially and 10.75 g/dL at the end of the experiment, so the improvement in hemoglobin concentration was 9.25 g /dL. Similarly, hemoglobin concentration for *C. auratus* was 2 g/dL initially and 8.5 g/dL at the end of the trial period, so improvement in the hemoglobin concentration in the *C. auratus* was 6.5 g/dL. MCHC value 8.4 g/dL of the *C. idella*, and its value was 10.8 g / dL for the *C. auratus* after 90 days of trial period.

MCV level was 109 fl at the start for the *C. idella* and 149 fl after 90 days of experiment so improvement in the MCV level for the *C. idella* was 40 fl. For the *C. auratus* MCV level was 87.09 fl at the start and 105.33 fl at the end of trial period, so this improvement was 18.24 fl. The value of MCH was 8.4 g/dL for the *C. idella* and 11.94 g/dL for the *C. auratus* at the end of the experimental period. The HCT percentage was 7 % for the *C. idella* and 10.5% for the *C. auratus*, and MCH level was 13.6 pg for the *C. idella* and 3.72 pg for the *C. auratus* at the end of the trial period.

Similarly, lymphocytes, monocytes and platelets counts were higher in the experimental group of the *C. idella* and *C. auratus* that were fed with grape seed extract

in comparison to the control group that was fed with the commercial diet. Lymphocytes percentage was improved 5.7% in the *C. idella* and 15% in the *C. auratus*. Monocytes percentage was also improved 1.8% for the *C. idella* and 2% for the *C. auratus*. Improvement in the granulocytes percentage was 3.1% and 7% for the *C. idella* and *C. auratus*, respectively. Improvement was also seen in the platelets count, 79 mcL and 80 mcL for the *C. idella* and *C. auratus*, respectively.

## DISCUSSION

This study was conducted to evaluate the effect of grape seed extract on growth and blood parameter of *Ctenopharyngodon idella* and *Carassius auratus*. Fish immunological responses, disease resistance, and growth performance could all be considerably improved by the use of *V. vinifera* extract as previously showed by Mehrinakhi et al. (2021). Mohammadi et al. (2021) also conducted a study using *V. vinifera* extract as fish food and resulted in increased growth performance in the experimental group of Common carp as in present study grape seed extract increased the growth performance of *C. idella* (4.9 g increase in weight) and *C. auratus* (11.8 g increase in weight), similar to the previous research findings.

Zhai et al. (2014) also documented improvements in growth metrics in *Oreochromis niloticus* fed with meals containing *V. vinifera* at varying concentrations for a 49 day period. The results are compatible with the current study of grape seed extract as it enhanced the growth (FCR, SGR, total length, fork length, etc.) of the *C. idella* and *C. auratus*, and it was shown that FCR, SGR, and fork length of the *C. idella* and *C. auratus* was significantly improved than the control group (T<sub>0</sub>). Similarly, according to Huang et al. (2012), diets containing *V. vinifera* significantly enhanced growth characteristics in Crucian Carp (*Carassius carassius*).

Grape seed extract improved the immunity and blood parameters of the *C. idella* and *C. auratus*. Similar results were obtained by *Onchorhynchus mykiss* and *O. niloticus*. Arslan et al. (2018) and Mousavi et al. (2020) elucidated that when given meals containing *V. vinifera* or grape seed extract, Rainbow Trout (*O. mykiss*) and Nile Tilapia (*O. niloticus*) showed comparable outcomes. The effective technique for drawing out various polyphenols from *V. vinifera* is of higher significance for many investigators due to the advantageous influence of phenolic compounds on health enhancement and illness control in living organisms, as stated by Nowshetri et al. (2015).

The incorporation of grape seed extract in the animal dietary regimen offers nourishment with elevated levels of different food compounds such as protein, carbohydrate, and fat, and also a source of pharmacologically active compounds (Shiel et al. 2017). Despite the fact that growth improving impact of different substances in the grape seed (*V. vinifera*) extract is not fully committed, increased growth efficiency might take place by various processes. The amplification of digestive enzyme function after the use of different supplements might lead to improved growth capability in the *O. mykiss*. The fact is, grape seed (*V. Vinifera*) extract could enhance the functionality of some intestinal or digestive enzymes, as shown by Xie et al. (2012) and Laurent et al. (2007).

## CONCLUSION

In conclusion, grape seed (*Vitis vinifera*) extract proved to be efficient in improving the growth metrics and blood parameters of two economically important carp species: *Ctenopharyngodon idella* (grass carp) and *Carassius auratus* (goldfish). Therefore, it is recommended to ameliorate the growth and immunity of the fish using easily accessible natural extracts found in food materials. But further studies are required to optimize the use of *V. Vinifera* extract on other fish species and make it accessible as a grape seed extract-supplemented diet to get the full potential of the aquaculture sector.

## Conflict of interest

There is no conflict of interest stated by the authors.

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