



Dietary Effect of *Withania somnifera* (Ashwagandha) Root Powder on Growth and Blood Parameters of *Cyprinus carpio* (Common Carp)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MSJ designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author PC managed the analyses of the study and performed the statistical analysis. Author GG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study evaluated the effect of different levels of *Withania somnifera* root powder in fish formulated diets on the growth performance of economically important freshwater fish species in Asia *Cyprinus carpio* and compare the hematological changes occurred in fishes fed with control feed and herbal formulated diet.

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Study Design: The experiment was planned with the size group of 30.0 ± 1.0 g. Fish of the respective size group of *Cyprinus carpio* were subjected to different herbal formulated diets at a concentration 0%, 0.5%, 1.0%, 1.5%, 2.0% of *Withania somnifera* root powder extract through their diet, while the control group received a standard diet without any supplementation.

Duration of Study: The study was conducted over a period of 4 weeks.

Methodology: During which growth parameters such as Average Daily Growth, Specific Growth Rate and Absolute Growth Rate were monitored at regular (10 days) intervals. The blood samples were collected at 4th week from each group to assess hematological parameters including Red Blood Cell count, White Blood Cell count, Hemoglobin, Total Cholesterol and Total serum protein levels. The overall results revealed that the inclusion of *Withania somnifera* extract in the diet of *Cyprinus carpio* significantly influenced both growth and blood parameters.

Results: Fish exposed to *Withania somnifera* extract exhibited enhanced growth performance compared to the control group, with increased Average Daily Growth, Specific Growth Rate and Absolute Growth Rate. Moreover, hematological analysis revealed positive effects on the blood parameters of treated fish, including improvements in Red Blood Cell count, White Blood Cell count, Hemoglobin, Total Cholesterol and Total serum protein levels.

Conclusion: The overall results revealed that the inclusion of *Withania somnifera* extract in the diet of *Cyprinus carpio* significantly influenced both growth and blood parameters.

Keywords: Immunomodulant; growth; blood parameters; Ashwagandha; *Cyprinus carpio*.

1. INTRODUCTION

Fish is a major source of animal protein source and an essential food item in the diet of many people in the world. Fish is also a good source of thiamine, riboflavin, vitamin A and D, phosphorus, calcium and iron. It is also rich in polyunsaturated fatty acids, which are important in lowering blood cholesterol levels. Therefore, it is suitable as a supplement for high carbohydrate diets specific to low-income individuals (Pradeepkiran, 2019). The growth of fish production is scornfully affected by high cost of fish feed. Olomola reiterated that the cost of fish feed is recognized as a major factor in the development and expansion of aquaculture business in developing countries (Olomola 1990). Reducing feed costs is therefore a major challenge in aquaculture nutrition. According to Falaye, feed costs account for approximately 60% of farm operating costs (Falaye 1992). This minimizes profit margins for fish farmers and destroys the economic viability and sustainability of the fishing industry. Pathmasethy also report that feed alone accounts for his estimated 40–70% of the cost of intensive aquaculture (Pathmasothy 1983). All these emphasize the importance and need for development of economic nutrient-complete diets for continuing expansion of aquaculture industry supported this by stating that rapid expansion and success of commercial fish culture depends largely on availability of good quality and cheap feed. To fish farmer, in orders to minimize running cost, it is important to use cheaper alternative feed ingredients that are locally available to produce

good quality, suitable and palatable fish feeds (Roques et al. 2020, Rombenso 2022). The use of non-conventional feeds and by-products in aquarium feeds reduces the unit cost of fish production. By switching to alternative raw materials, we not only pursue economic efficiency, but also improve the environmental friendliness of waste (unused agricultural waste). However, the increasing prohibitive cost of this commodity as a result of its many competing uses has made it necessary to evaluate other ingredients to replace with other cheaper carbohydrates and protein (Olurin et al. 2006).

Fish should be fed with a balanced diet as nutritional deficiency can have an adverse impact on disease resistance (Harikrishnan et al. 2009). Many plants have been used to prevent disease by incorporating them into fish diets. About 10 herbs are commonly used in China to treat ailments such as enteritis, gill rot, baldness, and white mouth disease (Rath 2000). The HIRM (Herbal immune regulation Mixture) is a combination of several traditional Chinese medicines that can effectively activate the immune system and therefore have immense potential as immunomodulators (Jian and Wu 2004, Tzianabos 2000). and stabilizing the improved immune status. Recently, extracts from *Azadirachta indica* leaves have been used to protect blood parameters and boost immunity in fish *A. invadans* (Harikrishnan et al. 2005, Harikrishnan et al. 2009). *Cyprinus carpio* has the ability to survive in a wide variety of climatic conditions and have proven to be the most suitable for many agricultural systems. *Withania*

somnifera has long been recognized as an excellent rejuvenator, general health tonic, and remedy for various health ailments. It is a neuroleptic, diuretic, anti-inflammatory and generally respected for increasing energy, toleration, and acts as an-nutraceutical that exerts a strong immunostimulatory and anxiolytic agent (Umadevi et al. 2012).

The nutrient status of an animal depends on the provision of sufficient nutritive substances and good utilization of these nutrients. On the contrary poor status of nutrition may be caused by consuming inadequate amount of food, kind or due to failure in digestion and metabolic utilization of nutrients from the digested food. Intensive production of fish relies upon many factors, one of which is a nutritionally complete diet (Adrian and Shim 1997). However, there are constraints experienced by the nutritionists and by the fish farmers with respect to non-availability of supplementary ingredients and high cost of fish feed. The knowledge of nutritional requirements of culturable species is a prerequisite in designing and fabricating nutritionally efficient and economically viable feeds. Artificial feeding allows fish to grow quickly by stimulating fish cultural management and greater emphasis is focused on maximizing the fish production by artificial feeding with minimal feed cost. In order to increase the deficiency of fish production, one must take into account both the nutrition cost and feed cost.

Supplying adequate nutrition for various species of fishes involves different formulation and proper management. The bioavailability of nutrients, diet palatability and acceptability, diet stability, feed manufactures' storage methods and chemical contaminations may have profound effect on fish performance (Sethuramalingam et al. 1996). Good quality of feeds could be prepared from good quality ingredients. When protein levels are stated, it should contain proper balance of amino acids. The absolute amount of protein, fatty acid, vitamin and minerals ingested depend to a large extent on energy intake. So dietary balance is more critical than that of absolute levels of specific nutrients.

2. MATERIALS AND METHODS

2.1 Experimental Animal and Management

The common carp, *Cyprinus carpio* were purchased from Fish Farmer's Development

Agency, Manimuthar. They were acclimated to the laboratory condition with aerated water for 2 weeks to assess their disease free health status. During the acclimation period, fishes were provided with adequate aeration and fed daily with the control diet without supplementation of the plant extract at 5% of their body weight twice a day (Plate 1).

2.2 Screening of Herbs

The immunostimulant herb such as *Withania somnifera* (root), were selected based on the previous literature of biological importance (Jaya et al. 2003). The roots were collected from the plants and washed thoroughly with tap water to rid them of dirt. After washing, the roots were dried in the shade to make them suitable for crushing. The dried plant roots were grounded in a mechanical grinder and sieved. The resulting powder was stored in an airtight container for further use.

2.3 Preparation of Standard Diet

The prepared standard feed (g/kg) was composed of fish meal (35%), groundnut oil cake (15%), soya flour (15%), rice bran (20%), Wheat flour (10%) and 5% vitamin and mineral mix (w/v) with an approximate composition of 35% protein. The pellets were dried in an oven at 30°C for 18 h, packed and stored in a freezer at -20°C until used. The supplemented diets were prepared by adding herbal root powder of *Withania somnifera* at a concentration of 0.5%, 1.0%, 1.5%, 2.0% to standard feed (Plate 1).

2.4 Experimental Design

The experiment was planned with the size group of (30.0±1.0g). Fish of the respective size group of *Cyprinus carpio* were subjected to different herbal formulated diets at a concentration of 0%, 0.5%, 1.0%, 1.5%, 2.0%. All experiments were performed in triplicate at room temperature. Healthy fishes were selected and the wet weight of the experimental fishes was determined at the beginning of the experiment. All these experiments lasted for 30 days. For every 10 days, the wet weight of the fishes was measured. At the termination of the experiment, the final wet weight of the individual fish of each experimental set up was taken. Average Daily Growth and Specific Growth Rate were analyzed for the experimental fishes using standard formula (Bulut et al. 2014). Total RBC, Total WBC, Hemoglobin (Hb), Serum cholesterol and serum protein were analysed using blood samples which

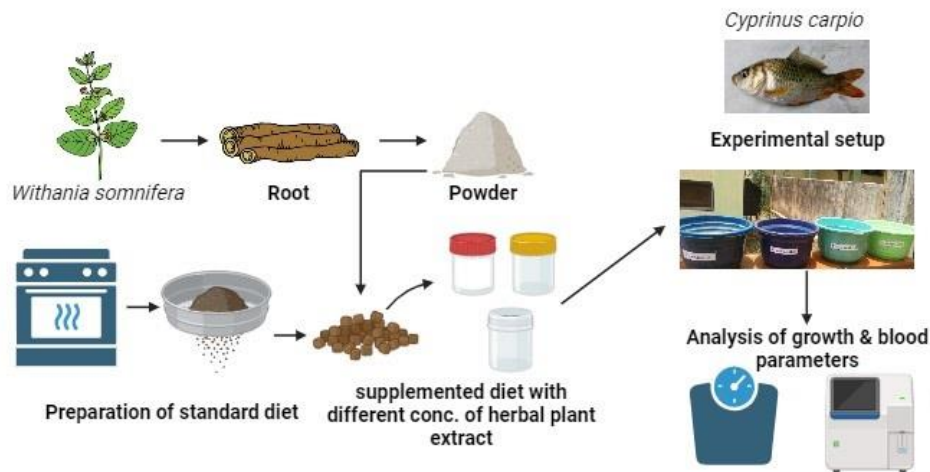


Plate 1. Protocol for the growth and blood parameters of *Cyprinus carpio*

was obtained from the caudal vein randomly chosen fish from each replicate by using a 1 ml heparinized syringe (Henry 1964, Goldenfarb et al. 1971). The blood was then transferred to an Eppendorf tube containing heparin solution. Shaken gently and stored in refrigerator at 40C. For serum, blood samples were collected without heparin and allowed to clot for 2h at room temperature. The supernatant serum was separated after centrifugation at 2500 rpm for 15 minutes and then kept in freezer for further analysis.

3. RESULTS AND DISCUSSION

3.1 Growth

Several herbal growth promoters have already been used in the diet of *Cirrhinus mrigala* with success (Dalveer et al. 2004). The values of weight gain, specific growth rate and food conversion rates reflected the best utilization of diet containing *Moringa* leaf meal at 10% inclusion level for *Cirrhinus mrigala*. The specific growth rate of *O. mossambicus* fed with four herbal formulated feed *Cyanadon dactylon*, *Aegle marmelos*, *Withania somnifera* and *Zingiber officinale* was significantly greater than control diet feeds fish (Immanuel et al. 2009). Turan found that administration of red clover extract in *Tilapia Oreochromis aureus* improved growth rate and feed efficiency (Turan 2006). Ahilan observed that in *Phyllanthus niruri* fed group, the highest mean weight was 1.769 g and specific growth rate (0.662) at 1.0% concentration in the fishes (Ahilan et al. 2010). In our present study, specific growth rate was

obtained as $3.6 \pm 0.52\%$ in 2.0% concentration of *Withania somnifera* diet fed fishes having the initial weight of $30 \pm 0.5\text{g}$. Sudarmathi observed that maximum ADG recorded in 30% protein diet (Sudarmathi 2005). In our present investigation in the maximum ADG observed in 2% conc of *Withania somnifera* diet fed fishes was $38.3 \pm 0.14\%$ (Table 1 & Fig. 1). It could be concluded that the addition of herbs in common carp diets improved growth performances, average daily growth and specific growth rate. The different concentrations of herbs used in the present study were effective in stimulating the growth performance.

3.2 Hematology

The observed results showed the elevation of total RBC, WBC, Hb, total protein and serum cholesterol, in *Cyprinus carpio* after feeding the fishes for 30 days revealed the positive role played by the herbal diet. In the present investigation the Hb content was $4.52 \pm 0.15\text{gms}\%$ in control fishes, but maximum level of Hb $7.37 \pm 0.22\text{gms}\%$ in *Withania somnifera* formulated diet fed fishes (Table 2). This proved that the treatment with herbal drugs significantly increased the hemoglobin level compared to control fish. The black seed cake *N. sativa* fed diet to the *Cyprinus carpio* showed the highest value of hemoglobin (8.9 gm) packed cell volume (26.81%) and red blood cell count (24.22×10^5 cell / mm^3) (Al-Shawi et al. 2009). Total protein level also increased in diets containing 0.5% and 1.0% of *Cotinus coggyria* (tetra) an ornamental bush plant than control (Bilen et al. 2011). These results are in accordance with our

Table 1. Effect of different concentration of *Withania somnifera* herbal diet on the Average Daily Growth (%), Specific Growth Rate (%) and Absolute Growth Rate (gm/wt/day) of *C. carpio* (mean $\pm$ SD)

Conc. of the Herbs %	10th Day			20th Day			30th Day		
	ADG	SGR	AGR	ADG	SGR	AGR	ADG	SGR	AGR
Control	3.0 $\pm$ 0.06	3.2 $\pm$ 0.08	0.03 $\pm$ 0.01	6.5 $\pm$ 0.12	3.2 $\pm$ 0.09	0.03 $\pm$ 0.01	13.0 $\pm$ 0.24	3.3 $\pm$ 0.52	0.13 $\pm$ 0.05
0.5	8.0 $\pm$ 0.13	3.2 $\pm$ 0.19	0.08 $\pm$ 0.01	9.0 $\pm$ 0.09	3.2 $\pm$ 0.14	0.09 $\pm$ 0.02	15.0 $\pm$ 0.14	3.3 $\pm$ 0.17	0.15 $\pm$ 0.04
1	10.0 $\pm$ 0.02	3.2 $\pm$ 0.21	0.10 $\pm$ 0.04	22.5 $\pm$ 0.15	3.3 $\pm$ 0.24	0.22 $\pm$ 0.08	22.0 $\pm$ 0.23	3.4 $\pm$ 0.29	0.22 $\pm$ 0.06
1.5	18.0 $\pm$ 0.31	3.2 $\pm$ 0.19	0.18 $\pm$ 0.01	19.0 $\pm$ 0.17	3.3 $\pm$ 0.22	0.19 $\pm$ 0.04	26.0 $\pm$ 0.08	3.5 $\pm$ 0.40	0.26 $\pm$ 0.07
2	23.0 $\pm$ 0.05	3.2 $\pm$ 0.64	0.23 $\pm$ 0.02	32.5 $\pm$ 0.18	3.4 $\pm$ 0.72	0.32 $\pm$ 0.07	38.3 $\pm$ 0.14	3.6 $\pm$ 0.52	0.38 $\pm$ 0.09

Table 2. Effect of different concentration of *W.somnifera* herbal diet on the blood parameters of *C.carpio* (mean $\pm$ SD)

Conc. of the Herbs %	RBC $\times$ 106 million/cumm	WBC $\times$ 104 cells / cumm	Haemoglobin (gms %)	Total Protein (gms %)	Total Cholesterol (mgs / dl)
Control	1.50 $\pm$ 0.08	2.90 $\pm$ 0.15	4.52 $\pm$ 0.15	3.91 $\pm$ 0.92	45.51 $\pm$ 0.15
0.5	1.80 $\pm$ 0.11	3.95 $\pm$ 0.17	5.43 $\pm$ 0.21	4.32 $\pm$ 0.85	55.05 $\pm$ 0.19
1	2.03 $\pm$ 0.17	3.57 $\pm$ 0.18	6.15 $\pm$ 0.22	4.55 $\pm$ 0.33	55.29 $\pm$ 0.21
1.5	2.25 $\pm$ 0.14	5.76 $\pm$ 0.21	6.82 $\pm$ 0.15	5.17 $\pm$ 0.15	82.08 $\pm$ 0.13
2	2.43 $\pm$ 0.12	4.94 $\pm$ 0.32	7.37 $\pm$ 0.22	5.27 $\pm$ 0.25	68.45 $\pm$ 0.15

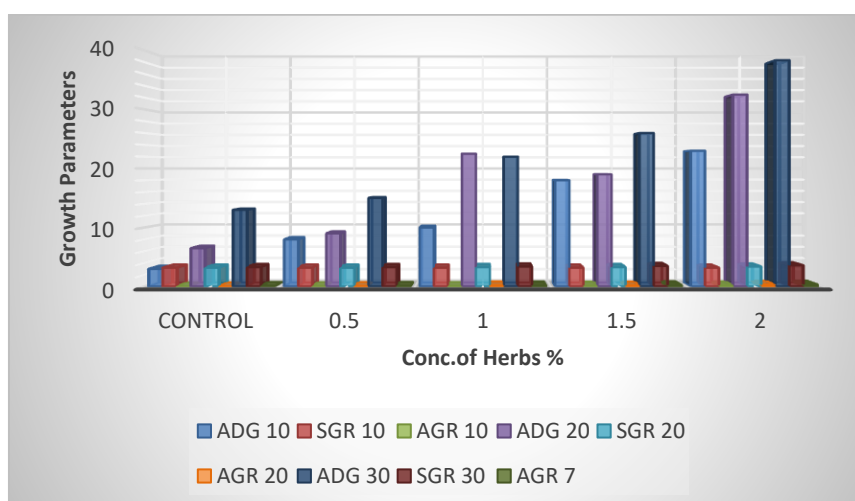


Fig. 1. Effect of different concentration of *W.somnifera* herbal diet on the Average Daily Growth (%), Specific Growth Rate (%) and Absolute Growth Rate (gm/wt/day) of *C.carpio*

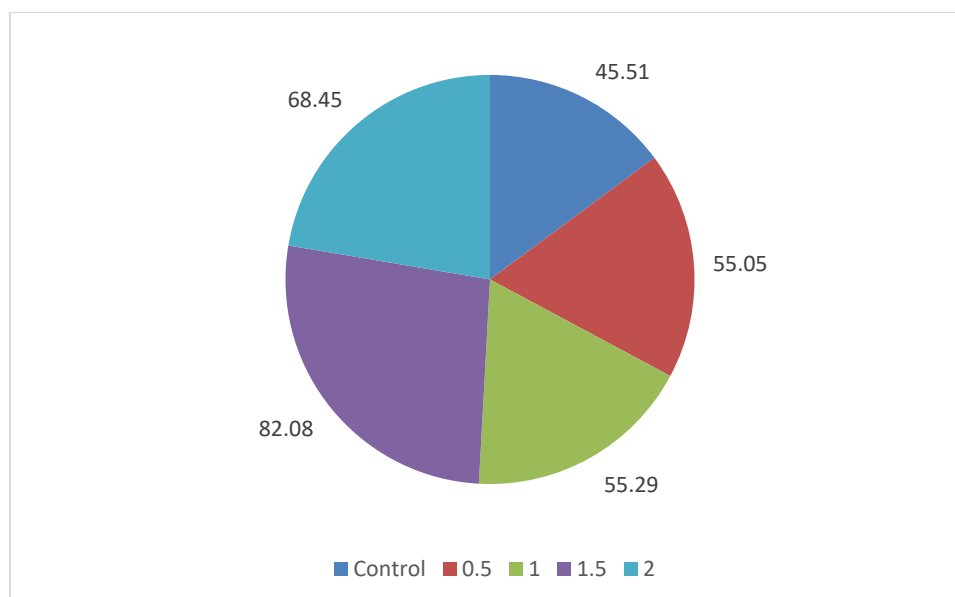


Fig. 2. Effect of different concentration of *Withania somnifera* herbal diet on the total cholesterol of *C. carpio*

data and showed that the herbal formulated diet fed fishes showed an increase of total protein content. Similar findings were reported by Misra with β glucan as immunostimulant which maintained globulin level significantly higher as compared to control (Misra et al. 2005). *Withania somnifera* exhibits properties that reduce lipid peroxidation and lipid metabolism during stress in higher vertebrates (Qarawi et al. 2000). *Withania somnifera* was evaluated for its proven multi-functional potential such as anti-stress, aphrodisiac, growth promoting and immune stimulative in shrimps as well as fin fish (Sivaram

et al. 2004). Pratheepa reported that the fish fed with leaf extract of *Aegle marmelos* incorporated into feed significantly enhanced the red blood cell count, hemoglobin, phagocytic activity and lysozyme (Pratheepa et al. 2010). Similarly, in the present investigation also, subjecting the experimental animals to different concentration of herbal diets increased the RBC count as 2.43 ± 0.12 at 2.0% concentration *Withania somnifera* (Table 2). In the overall results confirmed that the herbal formulated diet fed fishes has showed an increase in the hematological parameters of the RBC, WBC, HB,

serum cholesterol and total protein (Table 2 & Fig. 2).

4. CONCLUSION

In the present investigation, the potential value of *Withania somnifera* at 2% conc of feed enhanced the growth performance and hematological parameters such as RBC, Hb and total protein. Further research is necessary to explore the potential benefits of *Withania somnifera* and their derivatives for application in prevention and management of diseases in fresh water fishes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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