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Effect of Dietary *Solanum nigrum* Extract Supplementation on Growth Performance, Nutrient Digestibility, and Anti-Oxidant Activity in *Labeo rohita* Fingerlings

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Key words
***Labeo rohita, Solanum nigrum* extract (SNE), Growth, Nutrient digestibility, Hematological parameters, Aquaculture**

ABSTRACT

This study aimed to investigate the effect of *Solanum nigrum* extract (SNE) supplementation on the growth performance, nutrient digestibility, and antioxidant status of *Labeo rohita* fingerlings fed soybean meal diet. The fish were fed a formulated diet with varying concentrations of plant extract in the following percentages: T_0 (0.00%), T_1 (0.5%), T_2 (0.1%), T_3 (1.5%), T_4 (2%), and T_5 (2.5%) for 70 days. According to the findings of the research conducted, fingerlings that were fed a diet that consisted of 1.5% (15 g/kg) SNE showed significantly ($p<0.05$) higher weight gain (25.09g), weight gain percentage (274.11%), and a lower feed conversion ratio (1.65) compared to other groups. The highest apparent digestibility coefficient (ADC) for crude protein (CP) (70.86%), ether extract (EE) (72.23%), and gross energy (GE) (71.52%) were observed in fish fed 1.5% SNE-supplemented diet. There was a correlation between the level of SNE and a reduction in the level of oxidation percentage. The fish that had been given the control diet had the greatest oxidation percentage. The rate of oxidation decreased as the SNE level increased. The least amount of oxidation (37.18%) was found at a concentration of 2.5 % (25 g/kg). In conclusion, SNE is a suitable feed supplement for *L. rohita*, as it improves fish growth, nutrient digestibility, and antioxidant status of fish.

EINTRODUCTION

Aquaculture plays an important role in global protein production and reduces the fishing pressure on natural aquatic resources. The profitability of fish farms is closely linked to sustainable aquaculture practices, which can be optimized by minimizing disease losses and promoting growth rate (Lieke *et al*., 2020).

Plant extracts have been found to possess various active compounds, including flavonoids, saponins, and phenolics, which are known to exhibit growth-promoting, appetite-stimulating, immunostimulatory, and anti-pathogenic properties in fish aquaculture (Awad and Awaad, 2017). Plant extracts in aquaculture could reduce production costs and are ecologically beneficial because they are more biodegradable than pharmaceutical products. Because there are so many different kinds of bioactive compounds in plant extracts, they can be used to treat a wide range of diseases (Reverter *et al*., 2014).

African nightshade (*Solanum nigrum*) is a highly nutritious plant, containing dietary fiber, vitamins E and C, minerals (zinc, calcium, and iron), and β-carotene. Several improved varieties of *S. nigrum* have been produced, having high yields, low amounts of alkaloids, and abundant in nutrients. Because it contains glycoproteins, polyphenols, glycoalkaloids, and polysaccharides, this plant is effective against a wide variety of diseases. Studies have demonstrated that black nightshades have in vitro anti-monoamine-oxidase, anticholinesterase, and antioxidant properties, which enhance the effectiveness of black nightshades in the fight against neurodegenerative disorders. *S. nigrum* is also used as a food when it is

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prepared as a soup or cooked as vegetable (Rayner *et al*., 2001). In the 15th century, the mature berries of the *S. nigrum* plant were reportedly used as a food source in China due to its sweet and salty flavor. The plant's leaves and berries are frequently consumed as cooked food or vegetable in India (Wang *et al*., 2017). Research findings indicate that the fruit juice obtained from fully grown *S. nigrum* berries have antibacterial properties that are effective against several bacterial varieties, including Gram-negative as well as Gram-positive (Kusuma *et al*., 2019).

S. nigrum is known for its therapeutic properties and is characterized by its bitter flavor, cool nature, and low level of toxicity. In both traditional Chinese medicine (TCM) as well as traditional Chinese folk medicine, the usage of *S. nigrum* has been the subject of the large number of clinical research and investigation. *S. nigrum* has been used traditionally for the treatment of cancer sores, prostate, chronic bronchitis, skin dermatitis, bacterial dysentery, and urinary tract infections (Chen *et al*., 2022). *S. nigrum* is frequently used in combination with other medications for the treatment of different cancers, including lung, breast, stomach, liver, esophageal, bladder, and cervical cancers in contemporary clinical practice.

Labeo rohita, commonly known as rohu, holds significant importance among Indian major carps. This fish is best known for its rapid growth rate, greater disease resistance, and tasty flesh quality (Anand *et al*., 2018). The Rohu (*L. rohita*) is often considered to be the most valuable species of freshwater fish due to its popularity among consumers and strong market demand (Swain *et al*., 2019). *L. rohita*, an important aquaculture fish species in South Asia, is frequently subjected to nutritional and oxidative stress, which could negatively affect its growth and health (Debnath *et al*., 2018). The current study was carried out to investigate the effect of SNE on growth performance, nutrient digestibility, antioxidant function, and hematology in *L. rohita* fingerlings.

MATERIALS AND METHODS

This research was carried out in Fish Nutrition Laboratory, Government College University, Faisalabad, Pakistan.

Fish and experimental conditions

Fingerlings of the *L. rohita* were collected from the Government fish nursery and fish seed nurseries from the Pirmahal city of Pakistan, District, Toba Tek Singh. Fingerlings were shifted to cement tanks for two weeks for acclimatization, fed on basal diet once daily. After that, fingerlings were placed in v-shaped tanks (having 70L water capacity) that were made exclusively for excrement collection. Before the experiment, *L. rohita* fingerlings were fed a basal diet once-daily until apparent satiety (Allan and Rowland, 1992). Using a DO meter (Jenway 970) and a pH meter, the dissolved oxygen concentration and pH, respectively, were measured (model number: Jenway 3510). All experimental tanks received 24-h aeration through a capillary system.

Extract preparation

Fresh leaves and berries of the whole plant of *S. nigrum* were gathered from Kot Sultan, a city of the Layyah District in the Punjab Province of Pakistan. The authenticity of the plants was determined by the Department of Zoology at the Government College University in Faisalabad. The collected plant material was washed to remove dust and muck, and any rotting or damaged portions were discarded. The plant materials were dried in the shade for ten days and then ground using a commercially available grinder. The powdered *S. nigrum* was placed in a thimble or extraction chamber and subsequently inserted into the Soxhlet apparatus. The extract was obtained using a Soxhlet apparatus and subsequently solidified using a rotary apparatus. The extract was then transferred to a proper container, tightly sealed, and stored in a cool and dark location for further use in experimental trials (Rasul, 2011).

Feed components and experimental diet formulation

Ingredients of test diets were crushed to pass through a 0.50 mm filter. A basal diet was formulated with specific nutritional composition, including protein (35-38%), fat (4-8%), fiber (4-7%), ash (7-11%), phosphorus (1-1.5%), and moisture (5-11%). The basal diet was supplemented with different levels of extract (0%, 0.5%, 1%, 1.5%, 2%, and 2.50% per kilogram of diet) denoted as T_0, T_1, T_2, T_3, T_4 and T_5, as presented in Table I. Subsequently, the extracts were added and mixed thoroughly with the paste. Through a pelleting machine, the dough was converted into feed pellets.

Feeding and sample collection

L. rohita fingerlings were twice daily fed 5% of their body weight. Diets were made in triplicate for each trial, and in each replicate tank, 15 fingerlings were kept. After a two-hour feeding time, the remaining food in each tank was drained by opening the tank's valves. Following a comprehensive cleaning process, the tanks were completely removed from any residual feed particles and subsequently replenished with fresh water. Fecal samples were collected from each tank using valves. To minimize nutrient loss, attention was made to prevent the rupture of thin fecal strings. Each repeated treatment's material

Table I. The percentage composition of ingredients in the experimental diets.

Ingredients	Test Diet-I (Control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
SNE levels %	0	0.5	1	1.5	2	2.5
Soybean meal	55	55	55	55	55	55
Fish meal	16	16	16	16	16	16
Wheat flour*	11	10.5	10	9.5	9	8.5
Corn gluten	8	8	8	8	8	8
Fish oil	6	6	6	6	6	6
Vitamin premix	1	1	1	1	1	1
Mineral mixture	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
Total	100	100	100	100	100	100

*Plant extracts were supplemented at the cost of wheat flour.

was oven-dried, ground, and kept for chemical analysis. Experiments lasted for seventy days.

Chemical analyses of feed and feces

Feed for the experiment and a sample of feces were homogenized using motor and pestle-feed ingredients and then evaluated according to the usual technique (AOAC, 2005). The micro-kjedahl apparatus was used to determine the amount of protein that was present in each of the samples (N×6.25). The moisture content was determined through a 12-hour drying process in an oven set at 105°C. Using the petroleum ether extraction technique, the crude fat content was determined. The ash content was determined through the ignition of the samples in an electric furnace (Eyela-TMF 3100) at 650°C for 12 h. The total energy was determined using the oxygen bomb calorimeter.

Growth study

Fingerlings from each group were weighed in bulk at the start and end of the trial to determine the growth. The following formulas were used to estimate the feed conversion ratio (FCR) and weight gain (WG) of fingerlings:

$$\text{Weight gain \%} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

Digestibility studies

The following standard formula was used to determine the digestibility coefficients (%) of the experimental diets (NRC, 2011).

$$\text{ADC (\%)} = 100 - 100 \times \frac{\text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker in feces} \times \text{Percent nutrient in diet}}$$

Antioxidant function

The antioxidant activity was evaluated using the methods outlined by Hussain *et al*. (2011), with some modifications. Fish samples from each group were collected, dried, and ground. The ground samples were then transferred to separate test tubes. To prepare the hexane fraction, 1g of ground sample was mixed with 10 ml of n-hexane in each test tube. After gentle mixing, 200 µl from each test tube was transferred to new tubes. Equal volumes of a 35% ferrous chloride solution and a 30% aqueous ammonium thiocyanate solution, each measuring 200 µl, were added to separate tubes. The spectrophotometer was used to measure the absorbance at 500 nm following the addition of 10 ml of 95% ethanol to each test tube. The percentage of inhibition of oxidation was determined using the following formulas:

$$\text{Percent inhibition} = [(A0 - As) / A0] \times 100$$

$$\text{Oxidation (\%)} = 100 - 100 \times (A0 - As / A0)$$

A0 and As represent the sample's absorbance from 0 to 5 minutes in the formula.

Statistical analysis

One-way ANOVA was used to analyze growth performance, nutrient digestibility, and antioxidant status. Tukey's honest significant difference test (Snedecor and Cochran, 1989) was used to determine mean differences at $p<0.05$. For the statistical analysis, Co-Stat (Version 6.303, PMB 320, Monterey, CA 93940 USA) was used.

RESULTS

Growth performance

The results of growth performance of *L. rohita* fingerlings fed soybean meal-based test diets supplemented with SNE (solanum nigrum extracts) are presented in (Fig. 1). Initial weights of all the fingerlings were comparable, but significant differences in final weight were found across all treatments. Fish supplemented with 1.5% *S. nigrum* gained the most weight (25.09g), followed (24.35g) by fish fed at 2% *S. nigrum* supplemented diet. These results were statistically different (P <0.05) from the fish fed the control diet (0%) and other diets. The WG of *L. rohita* fingerlings was significantly influenced by *S. nigrum* at various doses. Up to 1.5% SNE supplementation, there was an increase in WG; however, no further increase in WG was seen above this level. FCR was found to be highest when fingerlings were fed control diet. The lowest FCR values were seen in fish fed diets with 1.5% SNE supplemented diet.

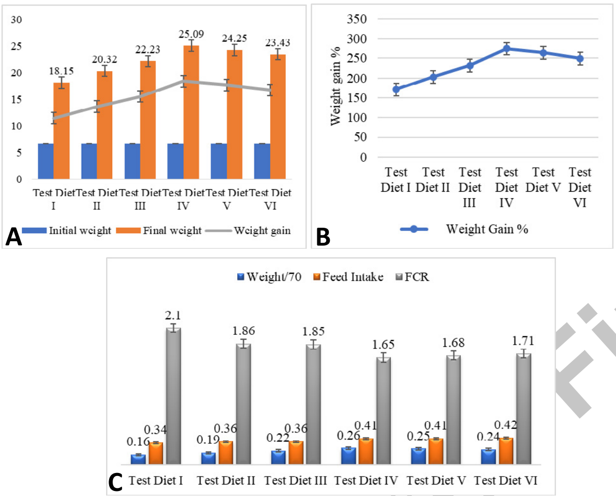

Fig. 1. Growth parameters of *L. rohita* fingerlings fed on *S. nigrum* extract supplementation with soybean meal-based diets. (A) Initial, final weights and weight gain, (B) weight gain% and (C) weight gain/day, feed intake and FCR.

Digestibility of nutrients

The nutritional composition of the analyzed diet and feces, including crude fat, gross energy, and crude protein is shown in Tables II and III, respectively. These findings suggested that a diet supplemented with 1.5% SNE significantly improved the digestibility of the nutrients in *L. rohita* fingerlings. Nutrient digestibility was found to increase up to level IV (1.5% SNE), but was reduced with further increase in SNE supplementation. The current study found that the digestibility values of crude protein (70.68%), crude fat (71.52%), and gross energy (72.23%) were highest in fish fed 1.5 SNE supplemented diet and significantly (P <0.05) different from all other test diets (Fig. 2). The control diet had the lowest digestibility values of CF, CP and GE as 60.22%, 58.11%, and 54.29%, respectively. These findings indicated that supplementing the diet of *L. rohita* fingerlings with 1.5% of SNE is optimal for maximizing the digestibility of nutrients.

Table II. Analyzed compositions (%) of apparent CP, EE and GE in feed of *L. rohita* fingerlings fed on soybean meal-based diet with solanum nigrum extracts (SNE) supplemented test diets.

Experimental diets	SNE levels (%)	CP (%)	EE (%)	GE (kcalg^{-1})
TD-1	0	32.48±0.01^{ab}	6.56±0.01^{a}	4.68±0.02^{a}
TD-2	0.5	32.44±0.01^{b}	6.55±0.02^{a}	4.64±0.01^{a}
TD-3	1	32.50±0.01^{a}	6.55±0.02^{a}	4.61±0.05^{a}
TD-4	1.5	32.51±0.02^{a}	6.58±0.01^{a}	4.60±0.02^{a}
TD-5	2	32.50±0.02^{ab}	6.57±0.02^{a}	4.61±0.01^{a}
TD-6	2.5	32.49±0.03^{ab}	6.56±0.02^{a}	4.62±0.01^{a}

Means within rows having different superscripts are significantly different at $p<0.05$. Data are means of three replicates. CP, crude protein; EE, ether extract; GE, gross energy; SNE, solanum nigrum extracts.

Table III. Analyzed compositions (%) of apparent CP, EE and GE in feces of *L. rohita* fingerlings fed on soybean meal-based diet with SNE supplemented test diets.

Experimental diets	SNE levels (%)	CP (%)	EE (%)	GE (kcalg^{-1})
TD-1	0	14.11±0.03^{a}	2.95±0.03^{a}	2.41±0.03^{a}
TD-2	0.5	12.19±0.05^{c}	2.73±0.04^{b}	1.96±0.03^{b}
TD-3	1	11.67±0.04^{d}	2.33±0.05^{c}	1.79±0.01^{c}
TD-4	1.5	10.72±0.14^{e}	2.07±0.02^{e}	1.47±0.03^{e}
TD-5	2	12.33±0.12^{c}	2.23±0.03^{d}	1.66±0.01^{d}
TD-6	2.5	12.76±0.06^{b}	2.33±0.03^{c}	1.70±0.03^{d}

For details of abbreviations and statistics see Table II.

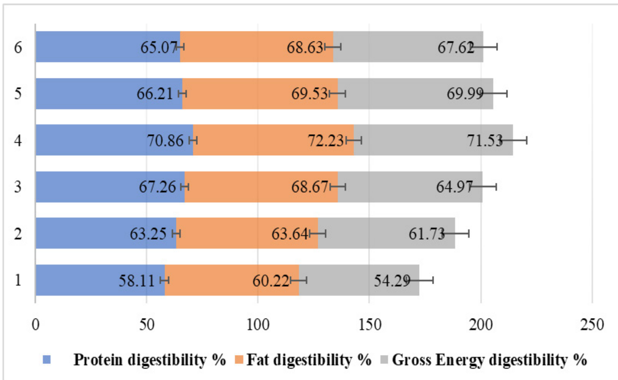

Fig. 2. Nutrient digestibility (%) of *L. rohita* fingerlings fed on *S. nigrum* supplementation with soybean meal-based diets.

Antioxidant activity

The results of antioxidant activity of SNE added to a soybean meal-based diet at different levels are shown in (Fig. 3). As a measure of the effect of SNE in each diet, the percentage of oxidation was used as a parameter. Oxidation was shown to be decreasing when SNE levels increased in all fish. Our experiment revealed that a test diet containing 2.5% SNE performed best as an antioxidant (lowest oxidation 37.18%), indicating that SNE has a role in increasing antioxidant activity.

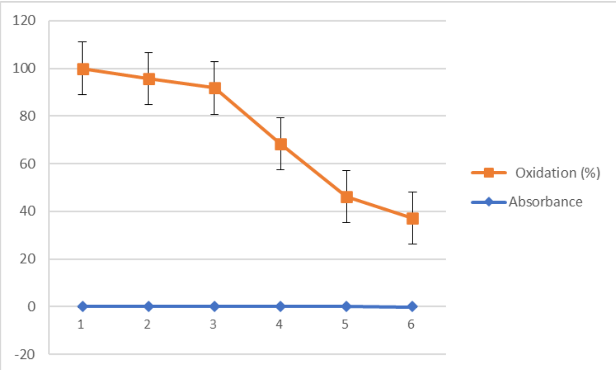

Fig. 3. Antioxidant activity of *L. rohita* fingerlings fed on *S. nigrum* supplementation with soybean meal-based diets.

DISCUSSION

In this study, the impact of SNE on growth performance, antioxidant status, and nutritional digestibility was investigated. According to the findings of the current research, supplementation of SNE (1.5 %/kg) in the feed of *L. rohita* improves overall growth performance. Similarly, several studies have documented the potential effect of herbal extracts on growth performance. For example, a rhubarb extract (1-2%) rich in anthraquinone increased growth rates in common carp (Xie *et al*., 2008). Growth of Nile tilapia (*O. niloticus*) was observed to improve upon dietary supplementation of 1% of ginseng (*Panax notoginseng*) extract (Van Doan et al., 2019). The potential of various herbal products to stimulate growth in aquatic animals has been investigated. Supplementation of dietary *Quillaja saponin* improved the growth performance of *C. carpio* (Francis *et al*., 2002). It was found that adding ginger to the diet has a beneficial impact on the growth rate of rainbow trout and *Huso huso* (Gholipour *et al*., 2014). Similar results were observed using medicinal herbs 1% (*Cnidium officinale, Artemisia capillaries, Massa medicate fermentata, and Crataegi fructus)* mixture in Japanese flounder (Ji *et al*., 2007).

Fish digestibility is directly linked to the availability of nutrients. The current study discovered that the digestibility values were highest at the 1.5% level and significantly different from all of the other test diets. The digestibility values of CP (71.23%), CF (59.98), and GE (68.83%) were found to be the highest and significantly (p<0.05) different from all of the other diets. *S. nigrum* improves the digestive process and nutritional absorption in the human gut. Additionally, it also increases antioxidant activity by boosting flavonoids and phenolic substances (Hur *et al*., 2014). Various processing and preservation methods of *S. nigrum* enhance dietary diversity, give a wide choice of products, improve nutrient bioavailability, eliminate potentially toxic compounds, and improve texture, aroma, and flavor. It also provides probiotics for the GIT health and improves the micronutrient bioavailability, and digestibility (Traoré *et al*., 2017). Fermentation of *S. nigrum* makes it more feasible for the body to digest a wide range of foods (Kaprasob *et al*., 2018).

The results of this research indicate that an increase in SNE levels led to a reduction in oxidation across all fish. Inclusion of 2.5% SNE in a test diet resulted in the highest level of antioxidant activity (37.18%). Therefore, *S. nigrum* serves as a significant feed supplement that improves antioxidant activity. Leaves of *S. nigrum* (black nightshade) are rich in nutrients, β-carotene, and minerals. The nutritional content of these vegetables is impacted by postharvest processes. It is believed that black nightshade may have potential benefits as a food source for humans (Grubben *et al*., 2014). *S. nigrum* comprises polyphenolic compounds, including steroids and flavonoids, as well as other constituents such as riboflavin, oils, vitamin C, nicotinic acid, β-carotene, and citric acid. Antioxidant activity is attributed due to its vitamin C, β-carotene, and polyphenolic compounds (Lin *et al*., 2008). *S. nigrum* extract is rich in polyphenols, which exhibit antitumor and antioxidant properties (Zakaria *et al*., 2006). As in previous research, leaves extract of the *S. nigrum* induced anti-oxidant status in rat hepatic tissues (Zaidi *et al*., 2019).

CONCLUSION

In summary, the inclusion of S. nigrum extract into the diet of *L. rohita* fingerlings resulted in enhanced growth performance, improved nutritional digestibility, and increased antioxidant activity. Therefore, this plant extract is recommended for use as a dietary supplement in aquaculture fish feed.

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Ethical statement

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Statement of conflict of interest

The authors have declared no conflict of interest.

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