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Effect of an ayurvedic drug "Amalaki Rasayan" as potent enhancer of growth and survival in Rohu (*L. rohita*)

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Abstract
The efficacy of Amalaki Rasayan (AR) as a dietary supplement in *Labeo rohita* fry was evaluated over a 60-day period. Amalaki (Indian gooseberry) is renowned for its high vitamin C content and its therapeutic efficacy is enhanced when processed into the Ayurvedic preparation AR. It serves as a potent antioxidant, even at concentrations far lower than standard Amalaki powder. The present study aims to determine the optimal dosage of AR to enhanced growth function in *L. rohita* fry. AR was incorporated into the diet at levels of 0% (T_0), 0.5% (T_1), 1% (T_2), 1.5% (T_3), and 2% (T_4) (equivalent to 5 g, 10 g, 15 g, and 20 g per kg of feed, respectively). Growth metrics such as mean weight, total weight gain, percentage weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) were measured. Results indicated that treatment T_4 yielded significantly superior mean weight and weight gain, while treatments T_3 and T_4 both demonstrated significantly higher percentage weight gain and SGR. FCR values were notably lower in treatments T_3 and T_4. No significant differences in survival rates were observed among the treatments. Regression analysis indicates strong linear relationships between AR inclusion and final body weight ($R^2 = 0.908$), SGR ($R^2 = 0.883$), and FCR ($R^2 = 0.856$). Correlation analysis confirmed significant associations between AR dose and growth parameters. The inclusion of AR at a dosage of 2% (20 g/kg of feed) significantly enhances overall health of *L. rohita* fry.

Keywords: *Labeo rohita*, Amalaki Rasayan, growth, vitamin C

Introduction

Fisheries and aquaculture play a vital role in ensuring food security and supporting the livelihoods of countless individuals globally. The growing need for seafood has been consistently addressed through a substantial rise in the production of aquaculture. The primary producing industry with the quickest growth rate is aquaculture, which has had very rapid expansion over the past 20 years. Total fish production in India has reached at 162.48 lakhs tonnes with 121.21 lakhs tonnes of inland fish production and 41.27 lakhs tonnes of marine fish production. With this, annual growth rate of fish production reached to 10.34% recently. Also, it contributed to about 8% in total fish production in the world (FAO, 2022) [11].

India stands second in largest aquaculture nation in the world after China (FAO, 2022) [11]. Freshwater fish make up about 89 percent of farmed fish, with Indian major carps (IMC) (Shridhar, 2021) [25]. Among IMC, rohu is popular due to its taste and preference by people. Also, it can grow very well within a year to about 800-1000gm (Jhingran and Pullin, 1988) [16]. Aquaculture has increased its output and productivity, but it is also plagued by a number of problems, including disease, low growth rate, low seed quality, high input costs, access to institutional credit and insurance, environmental sustainability, etc. For proper growth of fish, well-nourished feed with appropriate level of all macro and micronutrients should be added.

Vitamins are indispensable nutrients for ideal growth of cells and it also helps in proper functioning and development. They are integral to vital biological processes, including proper digestion, elevates immune response and also improves metabolism (Dewangan and Bhatia., 2023) [9].

A traditional Indian herb, Indian gooseberry or Amla (*Phyllanthus emblica*), belong to family Euphorbiaceae. It is also known for its richest source for vitamin C or ascorbic acid. It also contains many phytochemicals like alkaloids which include Phyllantidine and phyllantine, phyllaemblic compounds, ellagic acid, hydrolysable tannins (Emblicanin A and B), gallic acid, quercetin, flavonoids (kaempferol), pectin. Various phytochemicals present in Amla acts as antioxidants, anticarcinogenic, immunostimulatory, anti-inflammatory, etc.

Amalaki Rasayana (AR) is an herbal formulation, formulated by levigating amla in its own liquid. Ascorbic acid is increased by three times during the ayurvedic process of making AR (Layeeq, *et al*., 2014) [18]. It can reduce the quantity of additives in feed due to its high potency. By boosting immune responses, promoting growth, and reducing oxidative stress, this natural formulation offers a holistic approach to enhance health and well-being of fish. However, no work has been carried out on the use of AR as a fish feed. In view of all these facts, the present study was conducted with objective to study the effect of AR as a feed additive on its growth and survival of fresh water Indian major carp, Rohu (*Labeo rohita*).

Materials and Methods

Experimental Setup

The research was carried out in the Department of aquaculture, Collage of Fisheries Science, Kamdhenu University, Veraval for a period of 60 days. Healthy *L. rohita* were brought from a commercial hatchery to the experiment site and acclimatized in a 500-litre FRP tank with a constant aeration for a period of 2 to 3 days. All the required ingredients were procured and its proximate analysis was conducted by standard methods (AOAC 2000) [2].

Experiment feed preparations

The experimental diet was prepared in accordance with the Table 1. A total of five diets were prepared from T_0 to T_4 with 30% protein level. T_0 was kept as control without adding AR, and the remaining four diets were incorporated with AR at 0.5% (T_1), 1% (T_2), 1.5% (T_3), and 2% (T_4).

Table 1: Treatment details of formulated feed in all treatments with its inclusion level.

Sr. No.	Ingredients	T_0	T_1	T_2	T_3	T_4
1	A.R in%	0%	0.5%	1%	1.5%	2%
2	GNOC	79gm	79 gm	79 gm	79 gm	79 gm
3	Wheat flour	10 gm	10 gm	10 gm	10 gm	10 gm
4	Rice Bran	10 gm	10 gm	10 gm	10 gm	10 gm
5	Vitamin & mineral	1 gm	1 gm	1 gm	1 gm	1 gm

All feed ingredients were precisely weighed and homogenized to ensure uniform distribution. Water was added incrementally to achieve the desired dough consistency. The dough underwent thermal processing via steam sterilization in an autoclave set at 121 °C and 15 psi for a duration of 15 minutes to ensure proper gelatinization and pathogen elimination. Post-autoclaving, a premix of vitamins and minerals was incorporated into the dough, followed by the inclusion of AR, which was meticulously blended to guarantee homogenous integration of the additive. The resultant mixture was subsequently extruded through a mechanical pelletizer to obtain pellets of consistent diameter and uniform geometry. These pellets were systematically distributed across stainless steel drying trays and subjected to controlled dehydration in a batch dryer at 50-60 °C, ensuring a reduction in moisture content to approximately 10%. Once adequately dried, the pellets were sealed in airtight containers and stored under ambient conditions to preserve their nutritional integrity and prevent oxidative degradation until utilization.

Experimental procedure

Experiment was conducted in a 2×1×1 ft tank of 50 litres. A total of 15 tanks were used in this experiment with five different treatments and three replicas for each. The fish were then allocated to different experimental tanks using a completely randomized design (CRD). A total 225 fish with average weight of 0.86 gm were randomly distributed in all the treatment tanks, with 15 fish in each tank. Filtered and disinfected freshwater was filled in all the tanks, and continuous aeration was provided. Feeding was done twice a day to *L. rohita* at morning and evening. It was done at the rate of 4% of body weight during the experiment. Feed was prepared by keeping 30% protein level in each treatment diet. Feed required was weighted before feeding the fish. Water quality parameters such as temperature, pH, dissolved oxygen, alkalinity and hardness were measured by the standard analytic methods (APHA, 2017) [3]. Growth parameters like weight gain, SGR, FCR and survival rate was measured at 15-day intervals using standard methods (Manivannan and Saravanan, 2012 and El-Sayed, 1998) [19, 10].

Statistical Analysis

Data analysis was conducted using the one-way Analysis of Variance (ANOVA) to identify significant differences among the treatments, which were further assessed using Duncan's Multiple Range Test (DMRT). Statistical analysis was carried out using IBM SPSS version 22 software, Pearson correlation analysis was performed to evaluate the strength and direction of relationships between AR inclusion levels and growth parameters. Linear regression models were used to examine dose-response relationships between AR percentage and final weight, SGR, and FCR. A significance level of ($p<0.05$) was used in all tests.

Results

Among treatments, final body weight and net weight gain were highest in treatment T_4 compared to T_3, while T_2, T_1, and T_0 demonstrated markedly lower growth rates. Specifically, percentage weight gain increased by 0.4%, 6.41%, 27.01%, and 32.92% in treatments T_1, T_2, T_3, and T_4, respectively, relative to the control. Similarly, specific growth rate (SGR) rose by 0.2%, 4.10%, 16.35%, and 18.50% in T_1, T_2, T_3, and T_4, respectively, compared to the control. Survival rates were considerably higher in T_3 and T_4 than in other treatments. Feed conversion ratio (FCR) also significantly improved ($p\leq0.05$) with AR-supplemented diets, particularly at inclusion levels of 1.5% and 2%. These results underscore that even at lower doses, AR supplementation can effectively enhance growth, productivity, and FCR, affirming its value as a feed additive for aquaculture. The inclusion of AR in the diet of *L. rohita* notably improved growth parameters compared to the control group. The various treatments exhibited a statistically significant impact on the growth performance and survival rates of *L. rohita* fry, as evidenced in Table 2.

Correlation analysis revealed a strong and statistically significant positive relationship between Amalaki Rasayan (AR) inclusion levels and all key growth parameters of *L. rohita*. Final body weight ($r = 0.953$, $p = 0.012$), net weight gain ($r = 0.951$, $p = 0.013$), percentage weight gain ($r = 0.942$, $p = 0.017$), and specific growth rate (SGR) ($r = 0.940$, $p = 0.018$) all showed strong positive correlations with increasing AR levels (Figure-1). A notable positive correlation was also observed between AR inclusion and survival rate ($r = 0.904$, $p = 0.035$), indicating enhanced fish resilience. Additionally, a significant negative correlation was found between AR supplementation and feed conversion ratio (FCR) ($r = -0.925$, $p = 0.024$), demonstrating improved feed utilization efficiency with higher AR concentrations.

Regression analysis was conducted to evaluate the dose-dependent impact AR inclusion on the growth and feed performance of *L. rohita* fry. A strong positive linear relationship was observed between AR dosage and final body weight, with a regression equation of $FWG = 2.574 + 0.242 \times AR\%$ and a coefficient of determination ($R^2 = 0.908$). This indicates that 90.8% of the variation in final weight can be attributed to the AR level in the diet. Similarly, the specific growth rate (SGR) showed a significant positive trend with increasing AR inclusion, described by the regression equation $SGR = 1.18 + 0.13 \times AR\%$ ($R^2 = 0.883$), confirming the growth-promoting role of AR. In contrast, a significant negative linear relationship was observed between AR levels and feed conversion ratio (FCR), described by the equation $FCR = 2.38-0.17 \times AR\%$ ($R^2 = 0.856$), indicating that feed efficiency improved with higher AR supplementation. These findings are graphically represented in (Figure 2), which shows the regression lines for FWG, SGR, and FCR plotted against varying levels of AR in the diet. The strong regression relationships demonstrated by high R^2 values validate that AR inclusion has a predictable and positive impact on growth performance and feed utilization efficiency in *L. rohita* fry.

Table 2: Growth parameters of rohu (*L. rohita*) in different inclusion level of AR in feed.

Parameters	Treatments				
	T_0	T_1	T_2	T_3	T_4
Initial body weight (BW_i) (gm)	0.86±0.015^a	0.86±0.026^a	0.85±0.011^a	0.81±0.039^a	0.82±0.039^a
Final body weight (BW_f) (gm)	2.64±0.026^c	2.66±0.015^c	2.73±0.030^c	2.95±0.033^b	3.10±0.024^a
Net weight gain (NWG) (gm)	1.78±0.016^c	1.79±0.038^c	1.88±0.020^c	2.14±0.064^b	2.27±0.028^a
Percentage weight gain (PWG) (gm)	207.86±3.56^b	208.78±11.11^b (+ 0.4%)	221.20±1.54^b (+ 6.41%)	264.00±20.64^a (+ 27.01%)	276.29±12.58^a (+ 32.92%)
Specific growth rate (SGR) (%)	1.22±0.022^b	1.22±0.056^b (+ 0.2%)	1.27±0.012^{ab} (+ 4.10%)	1.42±0.088^a (+ 16.35%)	1.45±0.057^a (+ 18.50%)
Feed conversion ratio (FCR)	2.32±0.032^a	2.34±0.047^a	2.28±0.012^a	2.09±0.073^b	2.02±0.039^b
Survival	91.11±2.222^a	95.55±2.222^a	95.55±2.222^a	97.77±2.222^a	97.77±2.222^a

Values (Mean ± S.E.) with identical superscripts within a row are non-significance ($P \geq 0.05$).

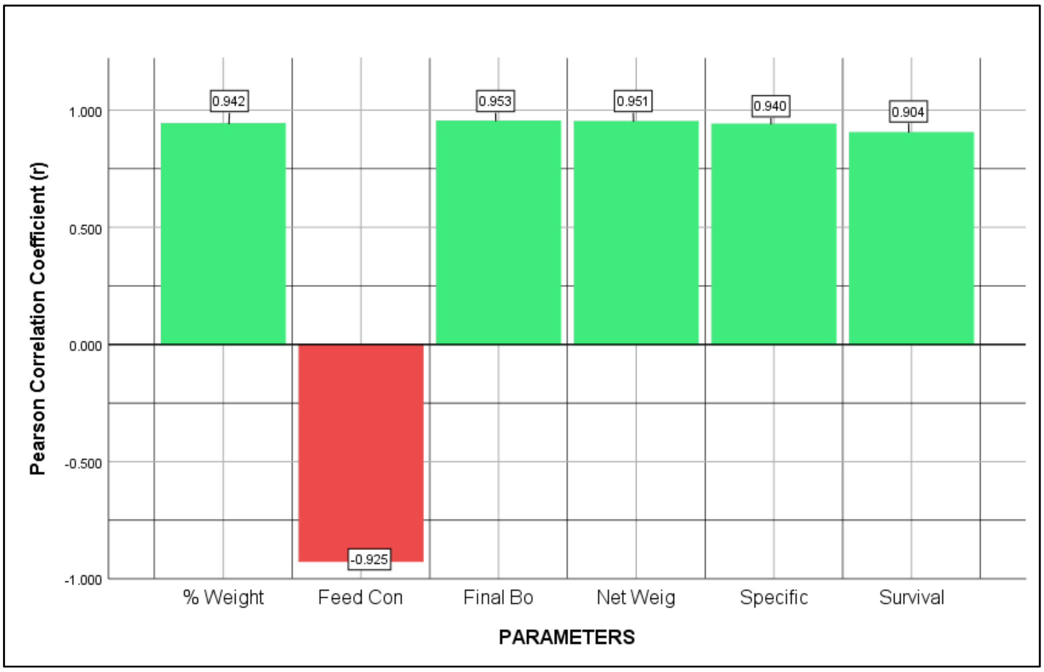

Fig 1: Pearson-correlation coefficients(r) between AR inclusion levels and key growth and feed performance parameters in *L. rohita*.

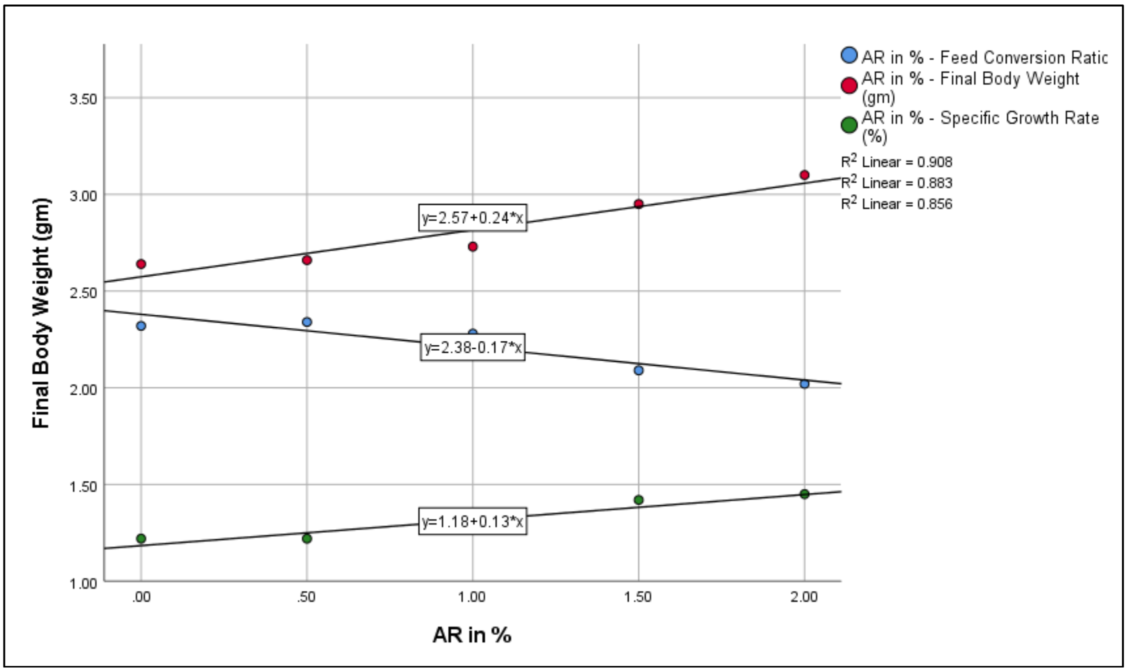

Fig 2: Regression lines for FWG, SGR, and FCR plotted against varying levels of AR in the diet of *L. rohita*.

Water quality parameters were analyzed throughout the experiment period on weekly basis. Water temperature observed during the study was in the range of 25-28 °C among all the treatment and differences among them was insignificant (p>0.05). The pH value recorded during the study was in the range of 6.5-7.9 and differences among them was insignificant (p>0.05). Recorded dissolved oxygen content throughout the study was in the range of 5.7-6.7 ppm. The recorded total hardness content throughout the study was in the range of 150-260 ppm during the study period (Table 3).

Table 3: Physio-chemical water parameters during the experiment.

Parameters	T_0	T_1	T_2	T_3	T_4
Water Temperature (°C)	26.16±0.166^a	26.37±0.182^a	26.16±0.140^a	26.16±0.154^a	26.20±0.107^a
pH	7.36±0.046^a	7.34±0.056^a	7.32±0.053^a	7.35±0.036^a	7.20±0.053^a
DO (ppm)	5.84±0.104^a	5.64±0.060^a	5.66±0.072^a	5.79±0.132^a	5.55±0.052^a
Total Hardness (ppm)	167.00±3.62^a	166.29±1.91^a	168.91±2.69^a	167.95±1.38^a	168.08±1.86^a

Values (Mean ± S.E.) with identical superscripts within a row are non-significance (P ≥ 0.05).

Discussions

This study sought to evaluate the impact of AR supplementation on growth, and survival of *L. rohita*. This experiment displayed the enhancement of growth in *L. rohita* fed with AR compared to control group. This can be due to its biological properties of which helps to promote better digestion and also increase the absorption which boost the health and functioning of liver, which ultimately improves the overall health of fish (Muzaffar *et al*., 2021) [21]. Dietary ascorbic acid inclusion in Japanese seabass on the growth, survival, tissue concentration, and immune response was found to be higher compared to control treatment (Ai *et al*., 2004) [1]. Also, at 3% of inclusion level of amla fruit powder (AFP) showed better growth and FCR in *L. rohita* (Srivastava *et al*., 2019) [26]. Similarly, Van Doan *et al*. (2022) [29] conducted a study using amla fruit extract (AFE) in the diet of *Oreochromis niloticus* to analyze growth, concluded that 20 mg/kg of AFE resulted in the best growth in *O. niloticus*. This implies that adding amla extract (AR) could decrease fish feed requirements and production costs by boosting nutrient absorption. Prior studies attribute these advantages to bioactive compounds in amla, such as phenolic compounds (tannins, phenolic acids, and flavonoids), alkaloids, phytosterols, terpenoids, organic acids, amino acids, and vitamins (Prananda *et al*., 2023) [22]. These compounds may expand the intestinal absorption area in fish, thereby improving nutrient utilization and supporting growth in *L. rohita* fed with AR-supplemented diets.

The present study demonstrated that dietary supplementation of AR has a significant and positive impact on the growth performance and feed utilization efficiency of *L. rohita* fry. Both regression and correlation analyses confirmed that AR inclusion levels were strongly associated with improvements in final body weight, net and percentage weight gain, specific growth rate (SGR), and survival, while feed conversion ratio (FCR) declined significantly.

Regression analysis revealed highly significant linear relationships between AR dosage and key performance parameters. The regression equation for final body weight (FWG = 2.574 + 0.242x, R^2 = 0.908) indicated that 90.8% of the variance in final weight could be explained by AR inclusion, confirming its predictable role in promoting somatic growth. Likewise, the positive regression relationship between AR and SGR (R^2 = 0.883) suggests that AR not only enhances growth but also accelerates metabolic efficiency. In contrast, the negative regression of FCR (FCR = 2.38-0.17x, R^2 = 0.856) clearly reflects improved feed utilization with increasing AR concentration. These findings demonstrate a dose-dependent response and validate AR as a functional growth promoter.

Pearson correlation analysis further supported these trends. Final body weight (r = 0.953), net weight gain (r = 0.951), percentage weight gain (r = 0.942), and SGR (r = 0.940) were all strongly and significantly correlated with AR inclusion, indicating that even small increments in AR level can have substantial effects on growth outcomes. A strong negative correlation between FCR and AR (r =-0.925) confirms that as AR level increases, less feed is required per unit of weight gain. Additionally, survival also showed a moderately strong positive correlation with AR (r = 0.904), suggesting possible immune-modulatory or health-enhancing effects of the herbal formulation.

The observed improvements may be attributed to the bioactive components of AR, particularly its high content of ascorbic acid (vitamin C), polyphenols, and natural antioxidants, which are known to enhance appetite, nutrient absorption, and immunity in fish (Sahu *et al*., 2007; Giri *et al*., 2010) [23, 13]. Similar results have been reported in herbal-supplemented diets for *L. rohita*, where growth performance, feed efficiency, and survival improved with the inclusion of *Emblica officinalis* (Giri *et al*., 2011) [12], *Withania somnifera* (Kumar *et al*., 2013) [17], and *Triphala* (Dawood *et al*., 2019) [8].

The positive correlation with survival rate may also be linked to the immunostimulatory properties of *Emblica officinalis*, a major component of AR, which is reported to enhance resistance against common bacterial infections such as *Aeromonas hydrophila* (Das *et al*., 2009; Harikrishnan *et al*., 2011) [7, 14].

Physio-chemical water parameters

The carps can thrive in temperatures ranging from 18 to 37 °C (Jhingran 1991) [15], while the optimal temperature range for their best growth is 25 to 32 °C. Importantly, the water temperature consistently remained within the optimal range throughout the entire study duration, providing suitable conditions for the experimental animals. Swingle (1961) [28] and Boyd and Pillai (1984) indicate that optimal fish production occurs when water pH levels are between 6.5 and 9.0. pH levels above 9.5 or below 4.5 are generally unsuitable for most aquatic life. Notably, the pH levels remained within the optimal range throughout the entire study duration, ensuring suitable and consistent conditions for the experiment. To ensure optimal productivity of carps, the dissolved oxygen (D.O.) level in ponds should be maintained above 5.0 mg/l throughout the culture period (Swingle 1961) [28]. D.O. levels below 3.0 mg/l are lethal for carps. Additionally, D.O. levels below the optimal range can cause several negative effects on fish, including stress, weakened immunity, increased susceptibility to disease, poor feed conversion ratio (FCR), stunted growth due to reduced feeding, and even death. Importantly, the dissolved oxygen content remained within the optimum range throughout the entire study period, ensuring favorable conditions for the aquatic environment. The recommended hardness range for fish culture is 30-180 mg $CaCO_3$/l (Santhosh and Singh 2007) [24], and it should never be below 20 mg $CaCO_3$/l (Swann, 1997) [27]. A total hardness above 40 mg $CaCO_3$/l protects fish from the harmful effects of pH fluctuations and metal ions (Ayyappan *et al*. 2011). According to Bhatnagar *et al*. (2004), hardness values below 20 mg $CaCO_3$/l cause stress, 75-150 mg $CaCO_3$/l is optimal for fish culture, and levels above 300 mg $CaCO_3$/l are lethal as they increase pH and affect nutrient availability. The total hardness content was within the optimum range throughout the study period.

Conclusion

The present study demonstrated that incorporating the Ayurvedic supplement amalaki rasayan (AR) at a 2% inclusion rate in feed positively impacted growth performance and survival rates in *L. rohita* fry. The presence of ascorbic acid in AR three times greater than in amla powder contributed to these effects. With increasing AR concentrations, its antioxidant properties also intensified, promoting better growth and survival throughout the experiment. These findings highlight AR's potential as a valuable Ayurvedic alternative to synthetic drugs, promoting the overall health, growth, and survival of *L. rohita* at an optimal dose of 2% (20 g/kg) in feed.

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