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Dietary Supplementation of Chlorophytum borivilianum root powder on the reproductive performance of Jayanti rohu

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Title

Dietary Supplementation of *Chlorophytum borivilianum* root powder on the reproductive performance of Jayanti rohu

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Abstract

The present experimental setup was designed to determine the effect of supplementation with Safed Musli (*Chlorophytum borivilianum*) root powder (SMRP) on the reproductive parameters of Jayanti rohu (*Labeo rohita*). In the present study, broodstocks were collected from earthen ponds at the Instructional Fish Farm, COF, G.B.P.U&T, Pantnagar, Uttarakhand. Diet C (control) received no SMRP supplementation, whereas diets CB1, CB2, and CB3 received 0.25 g, 0.5 g/kg and 0.75 g SMRP/kg feed, respectively. For quality parameters, milt was obtained from fully mature and vigorous *L. rohita* brooders (n = 10) after it had accumulated, and the milt was preserved at 4°C. The mean milt volume of Jayanti rohu was 9.4±0.34 ml, the pH was 8.26±0.26, the sperm motility was 90.0±0.57, the sperm motility duration was 118.0±1.15, the spermatocrit level was 73.0±0.57, and the maximum sperm count was 3.10±0.02 $\times 10^9$/ml in the diet observed with 0.5 g SMRP/kg. Taken together, the above trial results indicate that SMRP at 0.5g/kg feed significantly enhances different milt quality parameters, such as the viability, motility and survival of sperm. SMRP supplemented with 0.5 g/kg feed also acted as an immunostimulant and promoted the growth of the broodstock. The inclusion of SMRP at a rate of up to 0.5g per kilogram of feed during the initial rearing of broodstock has been found to enhance fertility in Jayanti rohu. This study's findings could prove helpful in developing strategies for mass seed production in carp.

Keywords- SMRP**,** Immunostimulant, Nutraceutical, Jayanti rohu and Milt.

1.0 Introduction

Jayanti rohu, *L. rohita*, is a commonly cultivated, high-growing and extremely saleable species in Asia. Among the IMC and EMC, rohu has become the third most important cultured fish in the world and is likely to be the most famous cultured fish according to the 21st Century **FAO (2022**). Owing to its rapid growth, high nutritive value, relatively low food conversion ratio, disease resistance, easy breeding and consumer acceptance, rohu is an appropriate fish for culture (**Gjedrem, 2012).** The maximum population consists of animal protein such as fish, meat and eggs; in these cases, all the fish are eaten by a fourth part of the world population, approximately 300 million (**Barik, 2016**). Approximately 16 million tons of fish are expected to be required to feed the rising population in 2025, almost double the present production. This is a vast task associated with the present challenges of resources related to water deficiency, climate transformation, increasing disease outbreaks, and numerous additional ecological and community issues (**Pritchard, 2023).** Among all IMCs, rohu stores the highest percentage of protein

Tran *et al*. (2023) and is also a good source of calcium and vitamin A (**Roos *et al.,* 2007**). After 5 generations of selective breeding, Jayanti rohu exhibited a 17% increase in growth rate according to **Bagchi and Jha (2011) and Nandanpawar *et al.* (2023)**. When females lack nutrients (malnutrition), they frequently experience further serious reproductive harm, such as the reserve of yolk formation process *i.e* vitellogenesis, gonadal maturation and spawning, declined sperm volume and diminished milt fluidity; these conditions negatively affect the success of egg fertilization (**Volkoff and London, 2018).** In breeding season, a higher number of males is required compared to females to safeguard adequate milt production. To meet this demand, it is necessary to augment brood quality, predominantly in terms of milt quality. Thus, a feeding strategy that uses the least amount of feed to increase economic income has the potential to lower assembly costs by decreasing the amount of diet taken to get a kilogram of fish (**Marimuthu, 2010).**

Ayurvedic plants contain a wide variety of nutrients and can be incorporated into entire plants and parts of plants, such as roots, pods, leaves, bark and seeds. For nutraceuticals and pharmaceuticals, the use of medicinal plants and plant materials has slowly increased worldwide. Medicinal herbs contain many categories of phytochemicals, including alkaloids, glycosides, flavonoids, steroids and saponins, which are used for fish growth and health. **Citarasu (2010)** discusses how medicinal plants can reduce feed costs and act as eco-friendly therapeutics, and the increasing industrial demand is linked to the development of various drugs and chemotherapeutics. *C. borivilianum* (*Santapau & Fernandez*), also known as safed musli, is a traditional herbal plant found in the ayurvedic genus by **Sharma and Chandrul (2017).** According to **Thakur *et al.* (2009**), improving male potency in safed musli has grown in popularity in recent years, and it is now considered an alternative to 'Viagra'. The proximate composition of the musli roots comprised 42 per cent carbohydrates, 8–9 per cent crude proteins, 3–4 per cent fibres, as well as 2–17% saponin. *C. borivilianum* generates the most roots of all the Chlorophytum species found in India and has the highest concentration of saponin, one of the major phytochemical elements.

The CB diet and its phytochemicals like glycosides, alkaloids, and saponins, which enhance the semen quality of Jayanti rohu, a fast-growing and highly demanded improved species. The objective was to improve Jayanti rohu milt quality, anticipating better seed production. Additionally, the research aimed to boost the reproductive potential and strengthen the immune system of Jayanti rohu by using safed musli as a feed additive.

2.0 Materials and Methodology

2.1 Procurement of Trial Fishes

The trial animals (fish) were obtained from the COF, Instructional Fish Farm, GBPUA&T Pantnagar, Uttarakhand, India. The trial brooders were kept by the institutional facility with formal institutional approval and consent were approved to use these stocks. The fish was adapted for 15 days and nourished through a basal diet (MOC+Rice bran). The crude protein component in the feed was the soybean oil cake (SOC) mentioned in Table 1. Earlier to handling, hormone applying and blood collection of experimental fishes were sedated with clove oil at a concentration of 40 µl/l for reduce handling stress. In the last part of the trial, fish designated for gonadal sampling were gently euthanized using an overdose immersion bath of clove oil more than 100 µl/l sustained until total opercular arrest was detected for at least 10 minutes, subsequent ordinary AVMA procedures for fishes.

2.2 Collection of *C. borivilianum* (Safed Musli)

The rhizomes of Safed musli were obtained from the Medicinal Plant Research and Development Centre Haldi, Uttarakhand. The plant material was taxonomically authenticated and confirmed by Medicinal Plant Research and Development Centre and this specimen has been deposited in the herbarium of Medicinal Plant Herbarium, MPRDC, Pantnagar, Uttarakhand. The freshly collected roots, after proper washing, were placed in the open air to dry to eliminate all moisture. The dried roots were finely ground in the grinder, and powder was made. Afterwards, SMRP was retained in a sealed plastic jar for the forthcoming practice.

2.3 Planning of Trial feed

For the preparation of trial feed, we mixed a weighted quantity of different feed material (like MOC, SOC, RB, Maize) with three different quantities of safed musli root powder. To harvest an acceptable dough uniformity, the ingredients were homogenised with a suitable amount of water. The pelletized feed was prepared at COF's Pantnagar Feed Mill; all the raw feed ingredients were crushed and properly homogenized in a pulveriser before being extruded. After pellets of a specific size (2 mm) were obtained from the extruder, they were dried and conditioned in the dryer chamber. The final diet had 28 % (crude protein), 7.0% (crude fat), and 42.00% (carbohydrate) (Table 1). Proximate compositions of the investigational diet were determined in triplicate following standard AOAC (2005) methods.

2.4 Phytochemical Analysis of Safed Musli and Preparation of Extract

SMRP was mined by joining it through methanol (1:10 ratio) in a conical flask and employing it on a shaker at room temperature for 2 days. The assortment was filtered through Whatman No. 1 filter paper. To confirm systematic extraction, the remaining leaf mass was managed a second time for 24 hours with extra solvent. The mutual filtrate

was then evaporated to dryness in a 40°C water bath and kept at 4°C. Normal qualitative analyses were cast off to classify key phytochemicals: Mayer's test for alkaloids, H2SO4 test for flavonoids, Salkowski test for terpenoids and glycosides, lead acetate test for phenols, frothing test for saponins, and ferric chloride test for tannins. Spectrophotometric methods were employed for quantitative analysis (Table 3).

2.5 Water Quality Parameters

The SOP for water quality analysis, APHA, 2017, was used to analyse all the experimental ponds' water quality throughout the experiment (Table 2).

2.6 Experimental Pond Preparation

The trial was carried out in 12 experimental earthen ponds, with each of the four dietary treatments assessed in triplicate (3 replicate for each treatment). Each pond had an area of 800 square metres. Initially, the ponds were drained and dried, followed by disinfection and pH balancing of the soil and water using slaked lime stone [$Ca(OH)_2$] at a rate of 250 kg/ha. During the culture phase, all experimental ponds were filled with bore well water, and the water level in the ponds was maintained at 1.25 m. To maintain plankton and nutrient status, each treatment pond was manured with raw cattle dung @10,000kg/ha as a prestocking dosage and then with raw cattle dung @800 kg/ha at monthly intervals.

2.7 Fish Stocking and Research Plan

The trial was conducted across twelve earthen ponds arranged in with 4 treatments and 3 replications per treatment. The fish stocking number was 100 fish per pond by an average IBW and IBL (Initial Body Weight & Initial Body Length) 830.45± 0.18g and 23.65±0.82cm. It was given @ 3 per cent of their total body weight twice a day, 10.00 am (IST) and 6.00 pm (IST). The fish group weight from all ponds was measured on a monthly basis. The total experimental set-up duration was 180 days. Water quality parameters were examined athwart replicate ponds for each treatment. Growth parameters were estimated at 30 day intervals by sampling fish from each replicate pond, and reproductive and hematological (n=10 each pond) parameters were assessed using mature male brooders sampled across the replicate ponds for each treatment group.

2.8 Growth parameters

The growth parameters of the experimental fish (*L. rohita*) were examined at 30-day intervals. On sampling day, 10 fish collected randomly in the experimental pond were caught with a net; every fish was weighed on an electronic

balance, and the weight was recorded in grams. The following formula was used to compute growth metrics: WG (Weight Gain), PWG (Per cent Weight Gain), SGR, FCR, and GCE.

2.8.1 WG (g) = FW (g) – IW (g)

Where; FW= Final Weight

IW = Initial Weight

2.8.2 Specific growth rate (SGR) % per day

$$\text{Specific Growth Rate \% per day} = \frac{(\ln Wt - \ln Wo)}{D} X100$$

Where

Ln = Natural log

W0 = Initial fish weight (g)

Wt = Final fish weight (g)

D = Duration of feeding (days)

2.8.3 Feed conversion ratio (FCR)

$$FCR = \frac{\text{Feed given (g)}}{\text{Total Weight gain (g)}}$$

2.8.4 Gross conversion efficiency (GCE)

$$GCE = \frac{\text{Weight gained (g)}}{\text{Food given (g)}}$$

2.8.5 Condition factor (K)

The length-weight frequencies of a sample of the fish population were used to determine the K value, which was used to measure the stoutness of the biomass of that sample.

$$\text{Condition factor (K)} = \frac{W}{L^3} \times 100$$

Where;

W is actual weight (g)

L is the actual length (cm)

2.9 Reproductive profile of Jayanti rohu

The entire body measurement of the fish, including total weight and length, was calculated before the dissection of the fish. Then, using a piercing blade and bone cutter, an opening was made on the ventral side of the

belly from the surface to the inside at the farthest ends of the fish. The testes were carefully removed, and the gonad weight was recorded before the samples were preserved at -20°C.

2.9.1 Gonadosomatic index (GSI)

The coefficient of gonadal maturity, or GSI, was computed using **Hopkins (1979).**

$$\text{GSI} = \frac{\text{Gonadal weight of fish(g)}}{\text{Weight of fish (g)}} \times 100$$

2.9.2 Milt Collection

Mature Jayanti rohu (*L. rohita*) male broodstock were chosen randomly from the control and treatment groups and acclimatised for 4-6 hours to evaluate semen or milt quality parameters. The semen donor fish were fasted for 48 hours. A single intraperitoneal dose of Gonopro-FH-inducing hormone was controlled @ of 0.2 ml/kg body weight. Jayanti rohu (*L. rohita*) male broodstock was anaesthetised with clove oil solution 40 µl/l) after 4–5 hours of hormone administration, and milt samples were taken by manual abdominal stripping. In ice-cold, cleaned test tubes, semen samples were obtained. More attention was given to the collection of semen since sperm can be studied; the milt was free of water, blood, urine, faeces, and scales. The tubes were given enough headspace and were immediately transferred to ice to keep the temperature of the collected milt at 4°C till further study.

2.9.3 Volume of Milt

Semen was collected in 50-ml calibrated plastic tubes, with the volume of measured semen in millilitres. The semen yield was measured in ml per kg body weight of fish.

2.9.4 Milt pH

A digital pen-type pH meter was used to determine the pH of the seminal plasma immediately (model of pH Meter - 0HQ 40d HACH).

2.9.5 Measurement of sperm motility (%)

Sperm motility was determined in freshly obtained samples of milt according to **Bozkurt *et al.* (2009).**

Calculation

The number of motile sperm cells (MCs) was measured according to the following formula (**Canyurt *et al.*, 2008)**:

$$\text{MC (per cent)} = \frac{MC}{WSC} \times 100$$

Where

WSC: Whole sperm cell count (activation stopped)

2.9.6 Seminal plasma osmolality (mOsm/kg)

The initiation of sperm motility was verified across various NaCl osmolalities using the method outlined by **Tiersch and Yang (2012)**. The sperm collected from mature Jayanti rohu brooders were preserved in an ice pack box until use. Nine fish were sacrificed for the experiment, with two replications for each extender. Ten graded dilutions of NaCl (ranging from 0.1% to 1%) were prepared, corresponding to osmotic pressures ranging from 80 to 384 mOsmol/kg. Osmolalities were measured using an osmometer (Model 7280, Modern Instruments lit). For sperm motility examination, 1-2 microliters of sperm suspension was placed on a glass slide, and 20 microliters of NaCl solution was added to activate the sperm. The sperm motility was promptly assessed using a microscope.

2.9.7 Motility duration

A digital stopwatch (1/3600 s) was activated immediately with the inclusion of the activation solution into the sample to measure the duration of spermatozoa movement. Nearly 90% of the sperm stopped moving forward after a certain amount of time had passed. The time required for motile sperm to move was measured in seconds.

2.9.8 Estimation of spermatocrit (%)

The analysis for spermatocrit (%) was done by the Microhaematocrit capillary tubes (75 mm in length with 1.2 mm in diameter), and these were packed with milt around 75 per cent to assess the spermatocrit of the Jayanti rohu broodstock. Nonabsorbent clay was used to seal one end of each tube. The loaded capillary tube was transferred to a microhematocrit centrifuge for tube centrifugation. The collected samples were centrifuged for 5 minutes at 10,000 rpm. The proportion of (PSC) packed sperm cells in the semen sample were determined by the amount of sediment in the capillary. For every sample, samples were recorded in triplicate using a reading device (Reading scale), and the mean of the 3 readings was used to calculate the results. Milt sample was measured soon after semen collection to avoid erroneous observations caused by CO_2 release-induced cellular swelling (**Wedemeyer and Yasutake, 1977**).

2.9.9 Measurement of spermatozoa concentration

Due to the large amount of sperm in undiluted milt, counting sperm is quite challenging. Sperm concentration was determined in this method. The density of spermatozoa was evaluated using the hemocytometer technique. For the analysis of spermatozoa concentration, Hayem's solution was used for diluting fluid. Hayem solution was cast off to thin the sperm at a part of 1:1000. A light microscope was used to count a drop of thinned

milt on a hemocytometer slide through a coverslip. At a magnification of x200, the mean spermatozoa calculation was assessed after three replicate samples for each fish. Before cell counting, the calculating compartments were retained in a moist atmosphere for 10 min. The value is given as x 10^9 per millilitre (**Bozkurt *et al*., 2009)**.

2.10 Hematological parameter analysis

Fish blood component analysis is crucial for diagnosing the impact of the experimental diet. Hematological tests, including Hb, TEC, TLC, PCV, MCV, MCH, and MCHC, were employed to assess stress resistance, pathological conditions, and specific and nonspecific immunity. Hematology sample was strained alone from 10 randomly selected male fish (n = 10 fish/treatment) with the help caudal vein puncture using heparinized syringes to determine haematological parameters.

2.11 Statistical analysis

The statistically analysed were using SPSS version 16.0 and one-way ANOVA, through meaningful difference at $P < 0.05$. Duncan multiple range testing was utilised to evaluate differences in water parameters, growth, blood profiles, and reproductive prospects among various food treatments.

3.0 Results

3.1 Physicochemical properties of water

The differences in water quality parameters, such as temperature (27.2-30.6°C), pH (7.3–8.4), D.O (5.3-6.2 mg L^{-1}), CO_2 (0-0.4 mg L^{-1}), alkalinity (76.0-210 mg L^{-1}), salinity (0.0-0.20 mg $^{L-1}$), and TDS (147.5-323.5 mg L^{-1}), were inside the appropriate variety for culture.

3.2 Phytochemical analysis of Safed Musli

The present study on *C. borivilianum* showed the incidence of active phytochemical constituents. The phytochemical energetic complexes of *C. borivilianum* were qualitatively analysed from roots, and the results are shown in Table 3. The current trial showed the occurrence of alkaloids, flavonoids, phenols, saponins and terpenoids.

3.2 Nutrient Utilisation and Growth Parameters

The maximum net weight gain (NWG), net length gain (NLG), percent weight gain (% WG), percent length gain (% LG) SGR, and GCE values were noted in the CB2 group, followed by the CB1 and CB3 groups (Table 4), and there was meaningful difference ($P > 0.05$) between CB2 and CB1 and CB3. The best k value was found in treatment CB2

after treatment CB1. The FCR was significantly lower in the CB2, CB1 and CB3 treatments. FCR values presented a contradictory movement, with a significantly lower value noted in the CB2 group, followed by CB3. There was no mortality noted in any of the trial groups during feeding. Feed conversion ratio (FCR) values showed significant variation among diets, with the lowest value recorded in the CB2 group (3.84±0.001), followed by CB1 (4.80±0.001), CB3 (4.82 ±0.0057), and Control (4.86± 0.0057).

3.3 Reproductive Profile of the Jayanti Rohu Brood Stock

The reproductive prospects of the Jayanti rohu brood stock in terms of the GSI were measured on a monthly basis.

3.3.1 Gonadosomatic index (GSI)

With respect to the exposure levels, a meaningful intensification in the GSI was noticed in treatment CB2 (Fig. 1), despite the nonsignificant change observed in treatments CB1 and CB3.

3.3.2 Quality parameters of the Jayanti rohu milt

During the milt collection period, the male brooders (n = 10) exhibited a mean body weight of 830.45 ± 0.18g and a length of 23.65 ± 0.82cm. The milt collected from Jayanti rohu in all treatments displayed distinctive milky-white colouration. The spermatological limitations, such as the mean milt volume (ml/kg body weight), milt pH, sperm motility (%), motility duration (sec), spermatocrit (%) and sperm concentration (no×109/ml), are presented in Table 5. The maximum mean semen volume was found in the CB2 treatment group (9.4±0.23 ml/kg BW) in July, followed by the CB3 and CB1 treatments. The maximum mean milt pH (8.26 ±0.26), sperm motility (90.0 ±0.57%), motility duration (118.0 ±1.15), spermatocrit (73.0 ±0.57 %) and sperm concentration (3.10 ±0.02 $\times 10^9$/ml) were found in treatment CB2, followed by treatment CB1 and CB3. Milt quality parameters had a significant relationship with treatment CB2 and CB1, and treatment CB and CB3 had no significant relationship during the trial.

The osmolality of seminal plasma plays a crucial role in regulating Jayanti rohu sperm motility across various breeding months. Osmolality values measured from June to August ranged from 240.4 ± 0.02 mOsm/kg, with the highest mean of 288.6 ± 4.81 mOsm/kg observed in treatment CB2 in July, and noticeable differences in osmolality levels were detected in the seminal fluid of Jayanti rohu throughout the spawning season.

3.3.3 Haematological parameter analysis

Haematological parameters, which are presented in Table 6, were examined to evaluate the health status of Jayanti rohu fed a diet supplemented with SMRP. The total erythrocyte count and haemoglobin (Hb) concentration in the

blood were significantly improved by the combined application of SMRP through the diet, and the highest values after July were recorded in the CB2 fish (2.05±0.02 mm TEC; 21600±57 mm TLC; 10.5±0.11 g L−1 haemoglobin). In contrast, TLC (21600±57 ×103/µl) was detected in the CB2 treatment group, and the maximum TLC (22300±99×103/µl) was detected in the CB3 treatment group. A greater amount of TLC indicated a greater level of stress.

4.0 Discussion

The primary goal of every farming system is to maximize yield per unit. The goal of aquaculture is to achieve the highest feasible productivity per unit area. Several technical interventions have been introduced in the recent past to increase the output of aquaculture species. A study of the effects of dietary Safed Musli (*C. borivilianum*) on the milt appearances and biological alignment of the seminal plasma of Jayanti rohu (*Labeo rohita*) and, in the present study, GSI showed large differences among the different treatments. **Linhart and Billard (1995) and Christ *et al.* (1996)** reported seasonal changes in the quality of male gametes in Indian major carp. Various factors that play important roles in variations in sperm quality have been reported **(Rurangwa *et al.* (2004); Rana 1995)**, and these variations include genomic changes among fish, rearing environments, brood management, semen collection methods, and sperm activation environments. Water quality is a meaningful ecological feature of aquaculture because it influences the maturity and survival of fish. The growth of fish is naturally related to fish gonad development because most of the development of potential brooders is attributable to gonad building rather than to dimensional growth. The consequent growth rate decreases substantially during the breeding months (April-July) due to gonad expansion and maturation (**Khan *et al.*, 2010**). In rainbow trout and Atlantic salmon, *Salmo Salar*, **Sylven and Elvingson (1991)** reported a negative linear association between growth rate and maturity age.

In the present study, the CB2 treatment group consistently displayed the greatest increase in growth parameters (NWG, NLG, SGR and GCE), while the CB3 treatment group consistently exhibited the lowest values. The notable increase in fish weight could be attributed to the presence of glycosides and glycosides, indicating known functions, including growth regulation. **Mohamed (2021)** and **Dada (2019)** studied the use of herbal diets as growth promoters, which are responsible for decreasing the FCR and enhancing the growth rate of fish. **Garg *et al.* (2019)** used a *Houttuynia cordata* leaf meal-based diet. The differences in the gross conversion efficiency (GCEs) between the treatments and the control were a meaningful variation ($p<0.05$). The findings of this study were sustained by some other works that revealed herbal substances, including herbs, as growth promoters in supplementary diets

(**Kumar *et al.,* 2007; Sinha *et al.,* 2017**). **Yadav *et al.* (2019)** concluded that papain affects fish length and weight. **Patil and Singh (2014)** studied the effect of a diet supplemented with papain on the growth and survival of postlarvae of *Macrobrachium rosenbergii* and found that the effects were positively correlated. When the value of the condition factor is greater than one, the fish are considered to be in good condition, and when it is less than one, the fish are considered to be in poor condition compared with an average individual of the same length (**Ahmed *et al.*, 2020).** In the present study, all treatments had good k values (K = 1.4-1.7). The average milt volume of *L. rohita* fed with medicinal plant root powder increased to a maximum of 8.3 ml in the rohu Mountains, and a minimum of 0.30 ml was found in common carp, according to **Chang *et al.* (2013) and Kumkar *et al.* (2021)**. **Bibi *et al.* (2021)** assessed extenders for refrigerated preservation of *L. rohita* milt and reported that the mean volume of *L. rohita* milt was 8.02 ± 0.12 ml. This study supports our results. **Verma *et al.* (2009)** reported similar mean pH values (7.3) in rohu. The average pH of rohu milt is equivalent to that of rainbow trout (7.2-7.9) (**Tekin *et al.,* 2003; Satybaldiyev *et al.,* 2023).** This result is similar to that of **Verma *et al.* (2009)**, who reported that IMC and exotic carp had continuous or linear motility greater than 60%. The aphrodisiac potential and health characteristics of a standardized extract of safed musli root (*C. borivilianum*) on the reproductive system were investigated by **Das *et al.* (2016).** They concluded that sperm motility was enhanced by the use of safed musli root (*C. borivilianum*). The period of spermatozoa motility described at the end of the experiment ranged from 105 to 119 seconds. Some fish species exhibit shorter periods of motility ranging from 30 to 120 sec (**Alavi and Cosson, 2005, 2006; Bakker *et al.,* 2006**); however, **Stoss (1983)** reported that fish spermatozoa have a motility period between 50 and 300 sec. Milt volume increases after induction, while the spermatocrit value decreases to 50-70 percent according to **Routray and Verma (2011)**. Spermatocrit values ranged from 69 to 81% in IMCs (**Verma *et al.,* 2009**) and from 35.93 to 98.07%, with a mean value of 63.19% in Tor *putitora* (**Charak *et al.,* 2018**). These results provide considerable support for our study, as the sperm concentration was 2.58×10^{10} ml^{-1}, and the average amount of milt generated was 2.18 ± 0.006 ml according to V**erma *et al.* (2009**) and **Bibi *et al.* (2021)**. The osmolality of IMCs for artificial propagation of fish seminal plasma commonly maintains the levels of Na+ and K+. According to Verma *et al*. (2009), the sperm latency of unmixed sperm generally ranges from 270-300 mOsm/kg. These results suggest that an SMRP-containing diet (CB2) for fish is extremely versatile for gonadal development and semen production and improves spermatocrit quantity due to the availability of altered kinds of phytochemicals, like saponins, alkaloids and glycosides.

In the existing study, the feeding of SMRP, particularly in the CB2 treatment (0.5 g/kg), resulted in increased Hb content, TEC, TLC, and haematocrit value in Jayanti rohu (*L. rohita*). A higher TLC enhanced the nonspecific immune response and immunostimulatory properties of Jayanti rohu. This finding is also supported by the findings of **Chelladurai *et al*. (2017), who studied** herbs such as *Allium cepa, Zingiber officinale, Allium sativum*, and *Urti cadioica* in *Mystus montanus*. Similar increases in TLC were observed in *Clarias gariepinus* and *Cyprinus carpio* when fed ethanolic extracts of *Garcinia kola* and *E. hirsutum*, respectively (**Dada & Ikuerowo, 2009; Pakravan *et al.,* 2012**). Similar results were observed in Nile tilapia fed *Roselle calyx* (**Mesallamy *et al*., 2016**). In contrast, **Nobahar *et al*. (2015)** reported no significant difference in the MCV of Beluga or *Huso huso* after the consumption of a 60-day diet containing *Allium sativum* and *Urtica dioica*. Our findings align with those of **Farahi *et al*. (2012)**, who reported a significant increase in the mean corpuscular haemoglobin concentration (MCHC) in rainbow trout, *Oncorhynchus mykiss*, fed diets containing garlic (*Allium sativum*) compared to the control group. Additionally, **Ojha *et al*. (2014)** noted that supplementing *L. rohita* with *Mucuna puriens* resulted in the highest mean corpuscular haemoglobin concentration (MCHC) in the treatment group and the lowest in the control group, and various types of phytochemicals were present in the SMRP. In the present study, the CB2 diet had a meaningful difference on the reproductive, haematological and growth parameters of Jayanti rohu.

Conclusion

The current study revealed that supplementation with SMRP enhances the physio-biochemical composition of Jayanti rohu (*L. rohita*), leading to heightened immunity. Additionally, findings from reproductive studies, specifically viability and fertility assessments, revealed that optimal reproductive performance is achieved with dietary supplementation of 0.5 g/kg SMRP. This study confirmed the potential of dietary SMRP as a pro-fertility agent in Jayanti rohu, but some field experiments are recommended for its inclusion in carp diets for intensified culture.

Declarations

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Competing Interests: The authors declare that there is no conflict of interest. Funding Information:

Ethical Statement:

The present study was conducted in accordance with institutional ethical guidelines for the use and care of experimental fishes. Ethical approval for the study was gotten from the Institutional Animal Ethics Committee

(IAEC). Ethical approval was approved under the approved CFSc/AC/583, certifying that all experimental procedures observed with the agreed standards and regulation.

Ethical Guidelines

All experimental procedures involving fishes adhered to institutional and national regulatory guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and complied with the ethical standards of the Basel Declaration and EU Directive 2010/63/EU.

Plant Ethics and Guidelines

Procurement of cultivated *C. borivilianum* adhered strictly to institutional, national, and international guidelines, including the National Biodiversity Authority (NBA) guidelines of India. Plant material was verified by (Dr. R. S. Chouhan), and specimen was deposited in the MPRDC.

Consent to participate Consent to participate declaration: Not applicable.

Consent to publish Consent to publish declaration: Not applicable.

Clinical trial number: Not applicable.

Data Availability Statements

All raw datasets and analyzed data generated during this study are included within the manuscript tables and figures, and additional underlying data are available from the corresponding author upon reasonable request.

Code availability: Not applicable

Author Contributions:

All the authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Mahendra Kumar Yadav, R.S. Chauhan, and Shri Ram Yadav. The first draft of the manuscript was written by Mahendra Kumar Yadav, and all the authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Competing Interests

The authors declare that there is no conflict of interest.

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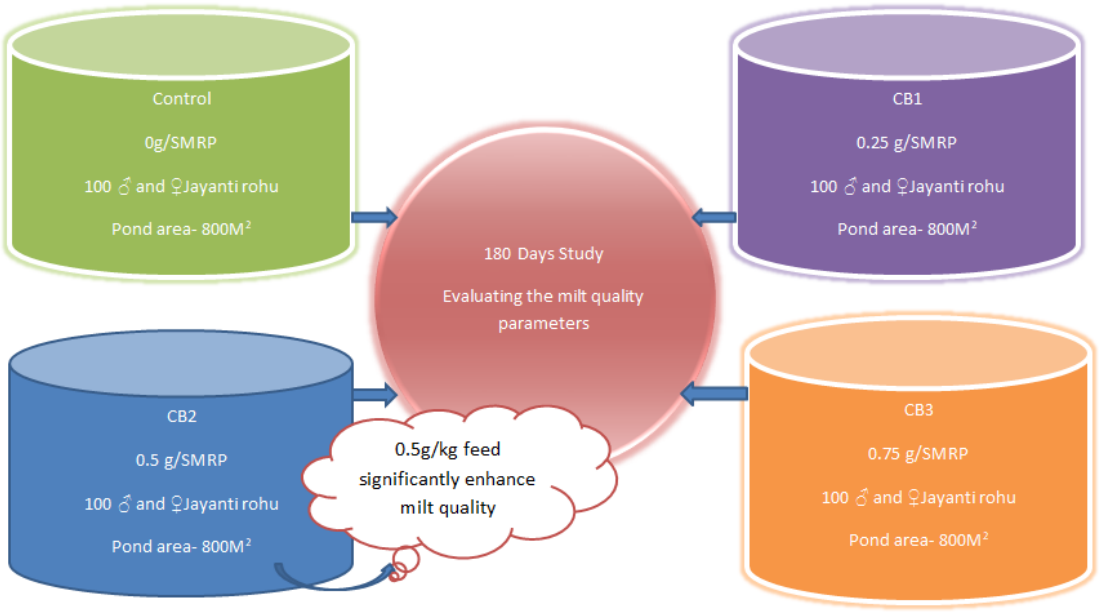
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Graphic Abstract



Highlights of Study

1. The results showed that inclusion of dietary safed musli (*Chlorophytum borivilianum*) in fish diet had direct relationship with various reproductive parameters under study up to the level of 0.5g CB/kg feed.
2. Inclusion of 0.5g/kg safed musli in fish diet would be helpful in maintaining good health of brood stock.
3. It is also helpful in making the strategy for mass seed production under the control condition.

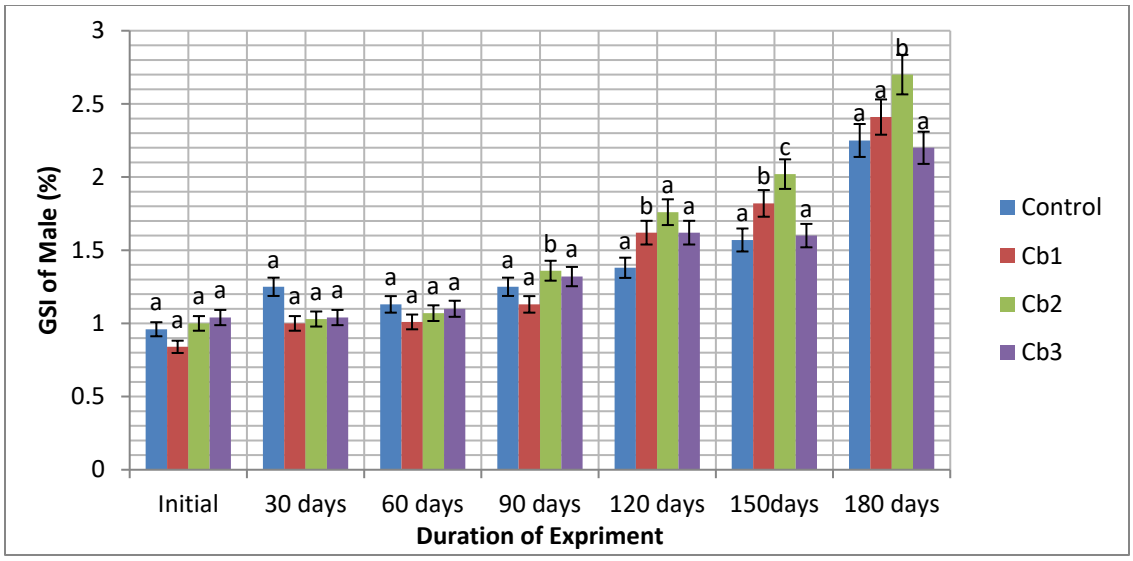


Fig. 1: Variations in male Jayanti rohu GSI during the experimental period

Table 1: Details of the experimental diet with proximate composition

S.No.	Particular	EXPRIMENTAL DIET			
A	**Diet Ingredients**	**C**	**CB1**	**CB2**	**CB3**
1	**Basal Diet*(g)**	100	100	100	100
2	**CB Root Powder(g/kg)**	00	0.25	0.50	0.75
*	**Basal Diet Ingredients ***	**% Composition**			
1	**Rice bran**	27.0	27.0	27.0	27.0
2	**Mustard oil cake**	35.0	35.0	35.0	35.0
3	**Soybean cake**	25.0	25.0	25.0	25.0
4	**Maize**	10.0	10.0	10.0	10.0
5	**Soybean oil**	1.0	1.0	1.0	1.0
6	**Lecithin**	1.0	1.0	1.0	1.0
7	**Agrimin Forte (Vitamin- mineral**	1.0	1.0	1.0	1.0

	mixture)				
8	**Total (%)**	100	100	100	100
B	**Proximate composition**	**(% dry matter basis)**			
1	**Dry matter**	09.40	09.28	09.23	09.10
2	**Crude protein**	28.26	28.52	28.34	28.29
3	**Fat**	07.58	07.68	07.60	07.55
4	**Ash**	06.50	06.44	06.75	06.54
5	**Crude Fibre**	06.34	06.20	06.54	06.44
6	**NFE (Nitrogen free Extract)**	41.92	41.88	41.54	42.08

Table 2: The profile of water quality metrics in various treatments during the study

Parameters	**Treatments (mean± SE)**			
	C	**CB1**	**CB2**	**CB3**
Temperature (°C)	27.30±0.65 (27.5-30.0)	27.33±0.63 (27.3-30.1)	27.43± 0.70 (27.4-30.6)	27.42±0.63 (27.2-30.2)
Dissolved oxygen (mg/l)	5.74±0.30 (5.5-6.1)	5.73±0.35 (5.3-6.2)	5.74±0.27 (5.6-6.1)	5.80±0.33 (5.5-6.4)
pH	7.74 ±0.06 (7.4-8.4)	7.79 ±0.06 (7.4-8.4)	7.78 ±0.01 (7.3-8.2)	7.78 ±0.04 (7.5-8.1)
Free CO_2 (mg/l)	0.13^{a}±0.07 (0-0.3)	0.15^{ab}±0.01 (0-0.3)	0.23^{ab}±0.01 (0-0.4)	0.28^{ab}±0.01 (0-0.4)
Total alkalinity (mg/l)	120.1±0.01 (76.5-200)	119.1±0.01 (76.0-205)	121.4±0.01 (81.5-210.5)	120.3±0.03 (78.8-207.8)
TDS(mg/l)	276.2±0.01	275.9±0.02	277.2±0.03	276.0±0.01

	(147.5-323.5)	(151.5-321.5)	(156.5-322.5)	(155.0-322.5)
Salinity (ppt)	0.13±0.78 (0.1-0.2)	0.11±0.04 (0.0-0.16)	0.15±0.05 (0.1-0.18)	0.14±0.03 (0.0-0.19)

The data are expressed as the means ± SEs (n=3).

Mean values in the same column sharing the same superscripts are not significantly different (P>0.05).

Table 3: The qualitative analysis of phytochemicals in Safed Musli (*C. borivilianum*)

S.No	Phytochemical Analysis	Observations	Present or Absent	Method
1	**Alkaloid**	Cream color Reddish brown solution/precipitate	+	Mayer's test
2	**Flavonoid**	Yellow orange Reddish brown/Orange color precipitate	+	Lead acetate test
3	**Terpenoid**	Reddish brown precipitate	+	Salkowski test
4	**Phenol**	Deep blue to Black color formation White precipitate	+	Lead acetate test
5	**Saponin**	Formation of persistent froth indicates the presence of saponins	+	Frothing test
6	**Tannin**	Brownish green/Blue black	-	Fecl3 test
7	**Glycoside**	A brown ring at the interface and green colour in the acetic layer were observed	+	Salkowski test

Present (+) or absent (-)

Table 4 Growth parameters

S.N	Treatments				
	Growth Parameters	**Control (C)**	**CB1**	**CB2**	**CB3**

1	**Initial weight of fish**		716^{a} ±2.3	718^{a} ±0.86	720^{a} ±1.15	715^{a} ±2.56
	Final weight		1878^{a} ±2.89	1997^{b} ±3.89	2489^{bc} ±3.96	1900^{a} ±3.88
	Net weight Gain(g)		1162^{a}±1.15	1279^{c}±2.30	1769^{d} ±0.57	1185^{b}±3.17
2	**Percent weight gain (%)**		162.30^{a}±0.68	178.01^{b}±0.53	245.69^{c}±0.47	165.92^{a}±0.15
3	**SGR**		0.53^{a}±0.05	0.57^{b}±0.003	0.69^{c}±0.01	0.54^{a}±0.001
4	**FCR**		4.86^{a}±0.0057	4.80^{b}±0.001	3.84^{c}±0.001	4.82^{a}±0.0057
5	**GCE**		0.21^{a}±0.001	0.22^{b}±0.01	0.25^{c}±0.003	0.21^{a}±0.006
6	**Initial Length(cm)**		25.19^{a}±2.89	22.58^{a}±0.66	23.21^{a}±1.73	23.63^{a}±1.15
	Final Length (cm)		48.0^{a}±0.60	48.01^{c}±0.57	49.15^{bc}±0.33	44.33^{a}±1.15
	Net length gain (cm)		22.81^{a}±1.49	25.43^{b}±2.49	25.94^{c}±2.08	20.7^{a}±1.89
7	**K Value**	**Initial**	1.8^{a}±0.05	2.3^{a}±0.06	1.8^{a}±0.03	1.7^{a}±0.01
		Final	1.7^{a}±0.01	1.5^{b}±0.01	1.4^{c}±0.01	1.7^{a}±0.01

The data are expressed as the means ± SEs (n=10).

The mean values in the same column with different superscripts are significantly different (P<0.05).

Table 5: Variations in male Jayanti rohu milt parameters in different treatments during the experimental period

S.No	Parameters	Months	Treatments (mean± SE)			
			Control	CB1	CB2	CB3
1	Mean milt volume (ml/kg body weight)	June	5.30^{a}±0.17	5.33^{a}±0.19	5.31^{a}±0.17	5.40^{a}±0.23
		July	7.22^{a}±0.12	8.0^{ab}±0.57	9.4^{c}±0.23	8.5^{bc}±0.28
		August	6.5^{a}±0.28	7.3^{ab}±0.19	7.5^{c}±0.28	7.2^{a}±0.11
2	Milt pH	June	6.9^{a}±0.18	7.03^{b}±0.03	8.0^{c}±0.03	7.5^{a}±0.05
		July	8.20^{a}±0.05	8.10^{a}±0.28	8.25^{a}±0.14	8.15^{a}±0.03
		August	7.2^{a}±0.10	8.1^{b}±0.20	8.26^{c}±0.26	8.13^{a}±0.13
3	Sperm motility (%)	June	81.0^{a}±0.14	82.6^{a}±0.69	88.0^{b}±1.15	81.6^{a}±0.40
		July	82.0^{a}±1.15	84.65^{b}±0.57	90.0^{c}±0.57	83.30^{a}±2.57

		August	81.33^{a}±0.38	83.33^{a}±0.57	89.0^{b}±0.57	85.66^{a}±0.63
4	**Osmolality (mOsm/kg)**	**June**	245.0^{a} ±2.31	253.7^{c} ±6.30	255.8^{c}±2.11	240.4^{a}±0.02
		July	263.0^{a} ±9.21	278.8^{b} ±4.38	288.6^{c}±4.81	260.0^{a}±2.06
		August	255.0^{a} ±4.45	258.8^{a} ±3.70	260.3^{c}±2.59	248.4^{a}±1.20
5	**Motility duration (sec)**	**June**	101.0^{a}±0.13	110.0^{ab}±1.15	116.0^{b}±0.48	110.0^{ab}±1.10
		July	104.0^{a}±0.48	114.0^{b}±1.34	118.0^{c}±1.15	108.0^{d}±1.15
		August	103.0^{a}±0.001	112.0^{a}±1.15	113.0^{a}±0.57	105.0^{a}±1.15
6	**Spermatocrit (%)**	**June**	57.5^{a}±1.44	60.5^{ab}±0.28	61.5^{c}±0.28	57.61^{a}±1.37
		July	60.0^{a}±0.57	68.0^{b}±1.15	73.0^{c}±0.57	67.0^{b}±0.57
		August	59.5^{a}±0.28	65.18^{c}±0.47	69.5^{d}±0.28	63.0^{b}±0.57
7	**Sperm Concentration (no.×10^{9}/ml)**	**June**	2.4^{a}±0.01	2.75^{c}±0.001	3.0^{d}±0.01	2.76^{b}±0.02
		July	2.63^{a}±0.005	2.98^{b}±0.01	3.10^{c}±0.02	2.69^{a}±0.005
		August	2.56^{a}±0.03	2.86^{c}±0.02	3.04^{d}±0.03	2.65^{b}±0.005

The data are expressed as the means ± SEs (n=10).

Mean values in the same column sharing different superscripts are significantly different (P<0.05).

Table 6: Variations in Jayanti rohu blood parameters in different treatment groups

S.No	**Parameters**	**Treatments**				
		Months	**Control**	**CB1**	**CB2**	**CB3**
1	**TEC (cell counts×10^{6} cells/mm^{3})**	**Initial**	1.24^{a}±0.4	1.35^{a}±0.15	1.39^{a}±0.15	1.47^{a}±0.16
		Final	1.68^{a}±0.01	1.75^{b}±0.02	2.05^{c}±0.02	1.80^{b}±0.005
2	**TLC (cell counts×10^{3} cells/mm^{3})**	**Initial**	20300^{a}±141	19400^{a}±145	19750^{a}±86	19900^{a}±106
		Final	21750^{a}±144	21800^{a}±57	21600^{a}±57	22300^{b}±99
3	**Hemoglobin (g %)**	**Initial**	7.5^{a}±0.05	7.6^{a}±0.05	7.8^{a}±0.05	7.8^{a}±0.05
		Final	7.8^{a}±0.11	8.3^{b}±0.05	10.5^{c}±0.11	8.2^{a}±0.03
4	**PCV (%)**	**Initial**	22.7^{a}±0.39	24.29^{a}±0.60	22.95^{a}±0.31	22.05^{a}±0.31
		Final	27.30^{a}±0.17	28.73^{a}±0.50	34.10^{b}±0.36	28.67^{a}±0.57
5	**MCV(µm3)**	**Initial**	194.01^{a}±2.11	223.5^{a}±1.34	202.79^{a}±0.90	125.28^{a}±2.34

		Final	163.05^{a}±2.84	164.30^{a}±4.41	166.77^{a}±4.02	161.59^{a}±3.87
6	**MCH(Pg)**	**Initial**	64.10^{a}±0.71	69.72^{b}±0.63	69.02^{b}±0.58	44.88^{a}±1.05
		Final	41.42^{a}±0.36	45.75^{ab}±1.08	51.74^{b}±1.29	44.72^{ab}±1.77
7	**MCHC(g/dl)**	**Initial**	33.03^{a}±1.36	31.2^{a}±1.23	33.98^{a}±1.06	35.82^{a}±1.36
		Final	28.52^{a}±0.28	26.94^{a}±0.57	31.36^{a}±0.57	28.50^{a}±1.08

37 The data are expressed as the means ± SEs (n=10).

38 Mean values in the same column sharing different superscripts are significantly different (P<0.05).

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