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Probiotic-supplemented Groundnut-oil Cake as a Sustainable Fish Meal Replacement in *Oncorhynchus mykiss* Fingerling Diet: Effect on Growth, Feed Utilization, Whole Body Composition and Dry Matter Digestibility

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Abstract

A sixty-day feeding trial was conducted to evaluate the effects of replacing dietary fish meal with probiotic-supplemented groundnut-oil cake (pGNOC) on the growth performance and survival of *Oncorhynchus mykiss* fingerlings (average initial weight 5±0.2 g). Four isonitrogenous (45%) and iso-lipidic (16%) diets were formulated by, replacing 0% (Control, T0), 10% (T1), 20% (T2), and 30% (T3) of fish meal with pGNOC at inclusion levels of 0% (Control, T0), 10% (T1), 20% (T2), and 30% (T3). The pGNOC added to different diets contained a constant dosage of *Lactobacillus acidophilus* at 3×10^7 CFU/kg diet. A total of 160 fingerlings were randomly randomly assigned to four treatments (4 replicates each) in 16 aquaria, with 10 fish per tankstocked in four treatments, each with four replicates (16 glass aquaria) with a stocking density of 10 individuals per aquarium. Fishes were fed twice daily at 5% of the body weight. No significant difference (P>0.05) was observed in terms of growth performance, whole-body composition, feed utilization and apparent dry matter digestibility between T1 and Control groups. However, increasing replacement levels beyond 10% adversely affected (P<0.05) these parameters. The survival rate, total erythrocyte count, and total leucocyte count indicated an insignificant variation (P>0.05) across all treatment groups. In conclusion, probiotic-supplemented GNOC, evaluated as a composite functional ingredient, can replace up to 10% of fish meal in the diet of rainbow trout fingerlings diets without any adverse effects on growth, feed efficiency and haematology.

Introduction

Aquaculture is frequently promoted for its pivotal role in contributing to global animal protein production and addressing global food security challenges (Boyd et al., 2022; Dewali et al., 2023). However, the rapid growth of the aquaculture sector poses sustainability challenges, mainly due to its dependence on traditional aquafeed formulations that use fish meal (FM) as a major protein ingredient (Gokulakrishnan et al., 2023).

Approximately 71.0% of FM is produced from wild-caught small pelagic fishes such as sardines, anchovies, and herrings, with the remaining portion derived from aquatic animal processing waste (Boyd et al., 2022). By 2033, 83% of global FM production is estimated to be utilized in aquaculture feeds (OECD/FAO, 2024). This heavy reliance, coupled with an all-time high surge in demand for FM as a protein source in aquafeeds, has placed an unprecedented pressure on wild fisheries, raising ecological and economic concerns about the

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sustainability of harvesting forage fish, which are integral to marine food webs (Naylor et al., 2021; Monteiro et al., 2024). In response, worldwide experts have mandated the approach of substituting the FM with other suitable sources of protein, with plant-based ingredients gaining prominence as one of the most commonly used alternatives (Tacon and Metian, 2008; Santigosa et al., 2008; Hardy, 2010; Nandakumar et al., 2013; Turchini et al., 2019; Jannathulla et al., 2019; Qian et al., 2024).

A suitable alternative to FM should possess specific attributes such as consistent availability, high nutrient quality, economic feasibility, good palatability and digestibility, low levels of anti-nutritional factors (ANFs) and non-starch polysaccharides (Jannathulla et al., 2019). Although various animal-derived protein sources have been tested as FM substitutes, their complete replacement potential remains limited at the commercial level. This is primarily due to their higher lipid and saturated fatty acid content, as well as high ash levels, which can impair digestibility in cultured fish (Bureau, 2006; Nandakumar et al., 2013). Moreover, certain regulations in many countries restrict the use of animal by-products in aquafeeds, further limiting their application (Shurson, 2020). Other similar evidences have supported the interest of researchers to explore plant-based protein sources as partial or total replacers of FM (Sharma et al., 1978; Jalili et al., 2013; Geurden et al., 2013; Mzengereza et al., 2016; Voorhees et al., 2019; Soltan et al., 2023; Li et al., 2023).

Oil cakes serve as rich sources of protein in aquafeeds and have demonstrated significant potential as economical and sustainable alternatives to fish meal (FM). Their abundant availability as by-products of the edible oil industry further supports their feasibility for large-scale inclusion in aquafeed formulations (Ramachandran et al., 2007; Jannathulla et al., 2019; Kumar et al., 2024). Among these, groundnut oil cake (GNOC) stands out for its excellent palatability and high crude protein content, approximately 41.73%. Several studies have reported its positive impacts on fish growth and performance, supporting its inclusion in aquafeed formulations (Lovell, 1989; Khan et al., 2003; Ghosh and Mandal, 2015).

Despite the potential of plant-based proteins as sustainable alternatives to FM, their inclusion in the diets of carnivorous fishes has produced variable results. Replacement levels have typically ranged from 20% to 75%, with only limited to moderate success (Burel et al., 2000; Pereira et al., 2003; Kaushik et al., 2004; Cabral et al., 2011; Acar and Turker, 2018; Hosseini et al., 2021). These limitations are primarily attributed to amino acid deficiencies and the presence of anti-nutritional factors (ANFs), which can negatively impact digestibility and nutrient absorption in fish (Øverland et al., 2009). Nonetheless, the inclusion of certain probiotics in aquafeeds may improve the digestibility of plant-protein by reducing the adverse effects of anti-nutritional factors and some non-starch polysaccharides (e.g., chitin and cellulose) (Bairagi et al., 2004; Wuertz et al., 2021). Primarily, the diets supplemented with *L. acidophilus* are known to improve feed utilization, overall growth performance and enhance the immune response in fishes (Lara-Flores et al., 2003; Villamil et al., 2014; Foysal et al., 2020).

Oncorhynchus mykiss (Rainbow trout) is a commercial fish species farmed mostly under semi-intensive or intensive aquaculture systems (Ghafarifarsani et al., 2021). *O. mykiss* has an annual global production (inland, marine and coastal aquaculture) of around 959,600 tons (FAO, 2022). Being a carnivore, the fish species has a high dietary requirement of digestible protein. Several studies have confirmed the positive effects of probiotics as additives in rainbow trout diets, thereby improving nutrient utilization, feed conversion ratio, growth performance, haematological indices and digestive enzyme activities (Enferadi et al., 2018; Hoseinifar et al., 2018; Mohammadian et al., 2019; Omidi et al., 2024).

With this background, the present study investigated the effect of dietary replacement of FM with probiotic-supplemented GNOC as a composite functional ingredient, on growth performance and welfare of *O. mykiss* fingerlings.

Material and Methods

Feed Formulation and Preparation

For diet preparation, all the dry ingredients were ground, sieved, weighed and blended uniformly as per the formulation, with the appropriate quantity of water to ensure good dough consistency. The wet dough was autoclaved for 15 minutes at 121°C. After cooking, the prepared dough was left to cool, followed by adding vitamin-mineral mixture and linseed oil (Malik et al., 2024). The dough was compressed through a manual hand pelletizer fitted with a die (2mm) to form wet pellets. The wet pellets were spread on a plastic sheet and subsequently semi-dried with forced air at room temperature for 13-15 h. Probiotic, *Lactobacillus acidophilus* was added to the diets by gradually spraying 250 mL of media containing 3.7×10^9 CFU/ml approximately of live *L. acidophilus* to the semi-dried pellets (Al-Dohail, 2009). The pellets were finally dried at room temperature, crumbled, sieved, and stored in air-tight jars at 4°C (Kahyani et al., 2021; Fan et al., 2021). Moreover, 0.1 g homogenized sample of dried pellets was added to 1 mL of deionized water to evaluate the viability of *L. acidophilus* in experimental diets. Serial dilutions were made up to 10^{-7} and spread unto MRS media (deMan, Rogosa, and Sharpe) agar plates in triplicate, followed by incubating the plates at 30°C for about 48 h. The colony-forming units (CFU) g^{-1} were determined using statistically viable plates (with 20–200 colonies), and the bacterial count was confirmed to be approximately 3.01×10^7 CFU/kg of diet.

A total of four iso-nitrogenous (45% crude protein), iso-lipidic (16% crude lipid) and isocaloric (475.34 kcal/100g) diets were prepared sequentially to replace FM with pGNOC (crude protein: 41%, crude lipid: 7.99%, ash: 3.53%) by weight at different inclusion levels (0%, 10%, 20%, 30%) (Table 1). Crude protein content of 45% was maintained in all the formulated diets based on earlier information on the protein requirement of *O. mykiss* fingerlings (Ahmed and Ahmad, 2020; Ahmad et al., 2021). The diets were designated as:

T0 (Control) – 0% FM replaced with pGNOC
T1 - 10% FM replaced with pGNOC
T2 - 20% FM replaced with pGNOC
T3 - 30% FM replaced with pGNOC

All experimental diets were analysed for proximate composition following the methods of the Association of Official Analytical Chemists (AOAC, 1990), including crude protein (CP), moisture content, crude fibre, lipid (fat), ash, and carbohydrates (calculated as nitrogen-free extract). The bomb calorimeter (Microprocessor Oxygen Bomb Calorimeter Model: CC01/M3, Toshniwal Technologies Pvt. Ltd., India) was used to calculate the gross energy of experimental diets.

Experimental Design

A healthy stock of rainbow trout (*Oncorhynchus mykiss*) fingerlings was procured from Rainbow Trout Hatchery, Mammar, Ganderbal. The 60-day feeding trial was conducted at the Experiential Learning Unit, Division of Aquaculture, Faculty of Fisheries, SKUAST-K. Following procurement, the fish were given prophylactic treatment for about 10-20 seconds with 0.05% potassium permanganate ($KMnO_4$) solution. From the procured stock, a total of 160 trout fingerlings with an average weight of 5±0.2 g were randomly allocated into sixteen glass aquaria (10 fingerlings per aquarium) supported by a flow-through system. Each aquarium had a total volume of approximately 60 L (50 cm × 30 cm × 40 cm), with an actual water volume maintained at around 50 L.

The experimental setup followed a completely randomised design consisting of four treatment groups viz, T_0, T_1, T_2 and T_3, each with four replicates. Before the start of the experimental trial, the fingerlings were acclimated for a period of 10 days and fed with control (T_0) diet at 5% body weight. Fish were fed to satiation twice (at 07:00 hours and 17:00 hours) a day and feed consumption was recorded for each tank during the entire study period.

Water Quality Maintenance

The water quality parameters were analysed at weekly intervals by following the standard procedures described by APHA (2012). Ideally, the samples were collected during early morning hours before the usual feeding schedule. Faeces and uneaten feed were removed by siphoning 10 minutes after feeding.

All the physico-chemical parameters were recorded to be within the optimal ranges for rainbow trout (Temperature: 14.1 to 15.9°C; pH: 7.53 to 8.16; Free-CO_2: 0.01 to 0.67 mg/L; D.O.: 7.6 to 8.41 mg/L; total alkalinity: 65.26 to 77 mg/L; ammonia-N: 0.01-0.02 mg/L; total hardness: 81.14 to 92.09 mg/L, Table 2). The water flow rate was maintained at 2.5 L/min $tank^{-1}$.

Table 1. Formulation and proximate composition (on dry matter basis) of different experimental diets fed to *O. mykiss* for a period of 60 days

Ingredients (g/100g)	Diets/Treatments[1]			
	Control (T_0)	(T_1)	(T_2)	(T_3)
Fish meal	45.00	40.50	36.00	31.50
Soybean flour	35.00	37.50	40.00	43.00
Groundnut oil cake	0.00	4.50	9.00	13.50
Wheat flour	6.00	4.50	3.00	1.00
Rice bran	4.00	3.00	2.00	1.00
Fish oil	4.00	4.00	4.00	4.00
Linseed oil	4.00	4.00	4.00	4.00
Vitamin-Mineral mix[2]	1.00	1.00	1.00	1.00
Carboxymethylcellulose	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Probiotic (*Lactobacillus acidophilus,* CFU/kg[3] of diet)	0	3.01×10^7	3.01×10^7	3.01×10^7
Proximate Composition				
Moisture %	8.95	8.04	8.10	8.00
Crude protein %	45.32	45.20	45.07	45.11
Lipid %	16.18	16.55	16.91	17.35
Crude Fibre %	5.03	3.71	3.67	2.69
Ash %	4.64	5.81	4.75	4.74
Nitrogen-Free Extract[4] %	19.88	20.69	21.50	22.11
Gross energy (kcal/100g)	471.65	478.32	485.00	492.22

[1]T_0 (Control) – 0% FM replaced with pGNOC, T_1 - 10% FM replaced with pGNOC, T_2 - 20% FM replaced with pGNOC, T_3 - 30% FM replaced with pGNOC, pGNOC-GNOC + *L. acidophilus*.
[2]Halver 2002 mineral ($AlCl_3·6H_2O$, 150; $ZnSO_4·7H_2O$, 3000; CuCl,100; $MnSO_4·4$-$6H_2O$, 800; KI,150; $CoCl_2·6H_2O$,1000 mg/kg; plus USP # 2 Ca $(H_2PO_4)_2$. H_2O, 135.8; $C_6H_{10}CaO_6$ 327.0; $C_6H_5O_7Fe.5H_2O$, 29.8; $MgSO_4.7H_2O$, 132.0; KH_2PO_4 (dibasic), 239.8; $NaH_2PO_4.2H_2O$, 87.2; NaCl, 43.5 (g/ kg) and Vitamin mix (choline chloride 5000: thiamin HCL 50; riboflavin 200; pyridoxine HCL 50; nicotinic acid 750; calcium pentothenate 500; inositol 2000; biotin 5.0; folic acid 15; ascorbic acid 1000; menadione 40; alpha-tocopheryl acetate 400; cyanocobalamine 0.1 (g/kg).
[3]CFU: Colony forming unit. [4]Nitrogen-free extract= 100 - (moisture% + crude protein% + lipid% + crude fibre% +ash%).

Sample Collection and Growth Performance

Before the experimental trial, 15 fingerlings of the same size were randomly selected from the procured stock, euthanized and a blood sample was collected from each fingerling. Then, the same fingerlings were frozen at -20 °C and later homogenized for estimating whole body composition.

The fish survival was recorded throughout the experimental trial. At the end of the trial, the fingerlings were starved for 24-hour and weighed individually for determining growth and feed utilization indices. Percentage weight gain (WG%), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival (%) were calculated using standard formulae as previously described by Kesbiç and Yigit (2019):

$$WG,\% = \frac{Ffw\ (g) - Ifw\ (g)}{Ifw\ (g)} \times 100$$

Where, Ffw: Final fish weight (g), Ifw: initial fish weight (g).

$$SGR,\%\ d^{-1} = \frac{Ln(Fww)\ (g) - Iww\ (g)}{Ed\ (g)} \times 100$$

Where, Fww: Final wet weight (g), Iww: Initial wet weight (g), Ed: Experimental days.

$$FCR = \frac{Tfc}{Twg}$$

Where, Tfc: Total feed consumed by fish (dry weight in g), Twg: Total weight gain by fish (wet weight in g)

$$PER = \frac{\text{Body weight gain (wet weight in g)}}{\text{Protein intake (dry weight in g)}}$$

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial of fish}} \times 100$$

Haematological Analysis

Three fish from each tank were randomly sampled and anaesthetized using clove oil (50 mg/l) prior to collection of blood (Javahery et al., 2012). Blood samples were drawn rapidly via venipuncture (i.e. from the caudal vein) using a medical syringe coated with EDTA solution (2.7%) and promptly transferred to heparinized Eppendorf tubes. For estimating total leukocyte count (TEC) and total erythrocyte count (TLC), a standard haemocytometer equipped with Neubauer chamber was used for manual counting of erythrocytes (RBCs) and leucocytes (WBCs) by employing the methods outlined by Gupta and Nigar, 2020.

Body Composition Analysis

After blood collection, the same fingerlings were euthanized, stored at -20°C and later homogenized to obtain a sample per replicate (Kasiga & Brown, 2019). These samples were then analysed for body composition parameters (AOAC, 1990).

Moisture content in the diets was estimated by drying samples at 105°C in a hot-air oven until constant weight was achieved (AOAC 1990, Method no.934.01), total crude protein content (Nitrogen content x 6.25) was estimated by Kjeldahl method (AOAC 1990; method no. 984.13), total lipid content was determined after diethyl ether extraction using Soxhlet method s (Soxtec System HT12, Foss Tecator AB; Hoganas, Sweden) and total ash content was determined by combusting the sample in an oven incineration muffle furnace (Labtech Muffle furnace, Model LEF-130S-2, India) at a temperature of 550°C for 6 h.

Apparent Digestibility of Dry Matter

Following the 8-week feeding trial, the four experimental diets were modified to include an inert marker (1% chromic oxide: Cr_2O_3 powder) for determination of apparent digestibility coefficients for dry matter. Prior to initiation of digestibility experiment, all the remaining fingerlings (18.5±2.1 g) present in each tank underwent a 3-day conditioning period for monitoring acceptability of the diets containing Cr_2O_3.

Table 2. Physico-chemical parameters of water recorded in different treatment groups during the experimental period of 60 days

Parameters	Treatments[1]			
	T_0 (Control)	T_1	T_2	T_3
Temperature (°C)	14.14–15.11	14.12–15.18	14.11–15.22	14.16–15.96
pH (mg/l)	7.70–8.05	7.53–7.98	7.74–8.16	7.64–8.11
Free-CO_2[2] (mg/l)	0.01–0.7	0.2–0.65	0.23–0.67	0.45–0.62
D.O.[3] (mg/l)	7.71–8.33	7.6–8.41	7.82–8.35	7.72–8.31
Total alkalinity (mg/l)	67.6–76.71	66.44–76.51	65.26–77.00	63.39–75.82
Total hardness (mg/l)	81.35–92.05	81.35–91.75	71.55–90.73	81.14–92.09

[1]T_0 (Control) – 0% FM replaced with pGNOC, T_1 - 10% FM replaced with pGNOC, T_2 - 20% FM replaced with pGNOC, T_3 - 30% FM replaced with pGNOC, pGNOC- GNOC + *L. acidophilus*. [2]Free-CO_2 – Free carbon dioxide, [3]D.O. – Dissolved oxygen.

As the fingerlings rapidly accepted these diets, no further conditioning period was required. The fingerlings in each tank were fed by hand to visual satiety twice daily for 10 days (Furukawa and Tsukahara, 1966). After every meal, the bottom of each tank was cleaned by siphoning to remove uneaten feed. Then, fish were left undisturbed for an hour and a half, and then faecal material was manually siphoned onto a fine-mesh net (Peña at al., 2020, Yigit and Keser, 2016). The faecal samples from each tank were filtered through a pre-weighed glass filter, pooled in accordance with the marked treatment group and stored at −20℃ until tested for digestibility.

To estimate Cr_2O_3, feed, faecal samples, and blanks were analysed in triplicate. Faecal samples were thawed, dried, ground, and homogenized. One gram from each was reserved for moisture and ash analysis. After adding 1 mL distilled water, samples were dried at 60–80°C and ashed in a muffle furnace at 550°C for 1 hour. The ash was digested with 5 mL of 6 M HCl, evaporated to dryness, and dissolved in 30 mL of warm 0.1 N N_2O_3. The solution was filtered and diluted to 50 mL with distilled water. Chromium concentration was measured using flame atomic absorption spectrophotometry, following Monteiro et al. (2002).

The apparent digestibility coefficient of dry matter (ADC_{DM}) was calculated as per Maynard et al. (1981) for all test diets:

$$ADC_{DM}(\%) = 100 - \{100[(Cr_{diet} / DM_{diet}) \times (DM_{faeces} / Cr_{faeces})]\}$$

Where, Cr_{diet}= % Cr_2O_3 in the diet, Cr_{faeces}=% Cr_2O_3 in the faeces, DM_{diet}= % Dry matter in diet, DM_{faeces}= % Dry matter in faeces

Statistical Analysis

The analysis was done using the statistical program, IBM SPSS Statistics V22.0. In order to compare mean values across different treatment groups, the recorded data were subjected to one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was then used to identify specific values between pairs of means. The differences were considered statistically significant at 5% level of probability (P<0.05). The results were reported as mean±standard error (SE).

Results

Growth, Feed Utilization Parameters and Survival

The dietary inclusion of probiotic-supplemented GNOC significantly (P<0.05) affected the growth performance of rainbow trout fingerlings over a 60-day experimental period (Table 3). The final weight was significantly (P<0.05) higher in the T_0 and T_1 groups across all the treatments. A significant (P<0.05) higher WG% and SGR was recorded in T_0 and T_1 while the T_3 group demonstrated significantly (P<0.05) lowest values. Moreover, a significant (P<0.05) lower FCR was observed in T_0 and T_1 groups. The PER was significantly (P<0.05) higher in the fingerlings fed with T_0 and T_1 diets. However, there was no significant effect (P>0.05) of any experimental diet on the survival (%) of *O. mykiss* fingerlings among different groups.

Haematology

The results of haematological parameters in different treatment groups are shown in Table 4. There was no significant (P>0.05) difference in haematological parameters such as TEC and TLC among the treatments.

Whole-Body Composition

At the termination of the experimental trial, the whole-body composition of fish showed a significant difference (P<0.05) in moisture, crude protein and lipid content. In contrast, no significant difference (P>0.05) was observed in total ash content among different treatment groups (Table 5). The T0 and T1 groups exhibited significantly (P<0.05) higher moisture and crude protein contents, along with significantly (P<0.05) lower lipid content compared to the other groups.

Apparent Dry Matter Digestibility

The apparent digestibility coefficient of dry matter (ADC_{DM}) was significantly (P<0.05) affected by various experimental diets (Figure 1). The T_0 and T_1 groups indicated significantly (P<0.05) highest ADC_{DM} among the various treatments. However, no significant variation (P>0.05) was observed between T_0 and T_1 groups.

Table 3. Growth performance, feed utilization and survival of *O. mykiss* fingerlings fed with different experimental diets for a period of 60 days

Treatments[1]	IW[2] (g)	FW[3] (g)	WG[4] (g)	WG (%)	SGR[5]	FCR[6]	PER[7]	Survival (%)
T_0	5.08±0.03	19.76±0.02^a	14.68±0.04^a	289.4±2.24^a	2.27±0.01^a	1.85±0.01^c	1.64±0.01^a	86.46±0.48
T_1	5.07±0.02	19.63±0.02^a	14.55±0.04^a	286.99±2.14^a	2.26±0.01^a	1.86±0.01^c	1.63±0.02^a	86.08±0.69
T_2	5.01±0.01	18.48±0.03^b	13.38±0.03^b	262.2±0.89^b	2.15±0^b	1.96±0^b	1.55±0.02^b	85.01±0.2
T_3	5.09±0.03	17.22±0.08^c	12.14±0.1^c	238.75±2.94^c	2.03±0.01^c	1.99±0.02^a	1.52±0.01^c	85.01±0.23
p-value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05	>0.05

[1]T_0 (Control) – 0% FM replaced with pGNOC, T_1 - 10% FM replaced with pGNOC, T_2 - 20% FM replaced with pGNOC, T_3 - 30% FM replaced with pGNOC, pGNOC- GNOC + *L. acidophilus*.
[2]IW= Initial weight, [3]FW= Final weight, [4]WG = Weight gain, [5]SGR= Specific Growth Rate, [6]FCR= Feed Conversion Ratio, [7]PER= Protein Efficiency Ratio. The data was expressed as Mean±Standard Error of Means (SEM), (n = 4). Different superscript letters in the same column denote significant differences (P<0.05) between the experimental groups.

Discussion

With the escalating costs of FM and its associated environmental impact, finding effective substitutes is essential for reducing production costs and ensuring the long-term sustainability of aquaculture. The results of this study provide important insights into the potential of *L. acidophilus*-supplemented groundnut oil cake (pGNOC) as a sustainable alternative to FM in aquafeeds. In this section, we discuss the observed effects on growth performance, feed utilization, haematology, and apparent dry matter digestibility (ADC_{DM}) in *O. mykiss* fingerlings, emphasizing the importance of reducing reliance on FM.

In the current study, similar growth performance and feed utilization were observed in the control (T0: 0% replacement of FM with pGNOC) and the T1 group (10% replacement of FM with pGNOC), as measured by percentage weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio. The *Lactobacillus* species used in this study contributes amino acids (e.g., methionine, cysteine), peptides, vitamins, enzymes (tannase and phytase), and organic acids (Odunfa et al., 2001; Osawa et al., 2006; Sharma et al., 2020; Sugahara et al., 2021), potentially mitigating the limitations of plant-based proteins. This likely explains the trend of insignificant variations in growth performance and feed utilization between the Control (T_0) and T_1 groups. However, an increase in FM replacement from 10% to 30% led to a gradual decline in growth performance and feed utilization. This decline is likely caused by higher proportions of anti-nutritional components (protease inhibitors (trypsin inhibitor), tannins, and phytic acid) present in GNOC, which are known to impair growth performance in fish (Maitra and Ray, 2003; Mandal and Ghosh, 2010; Nyina-wamwiza et al., 2010).

Previous research has shown that plant protein sources can only partially replace FM in *O. mykiss* diets, with reported dietary inclusion of up to 10% for peanut oil cake (Acar and Turker, 2018), 15% for hazelnut oil cake (Dogan, 2015), 20% for rice protein concentrate (Palmegiano et al., 2006), and 15% for red lentil meal (Özdemir and Yıldız, 2019). Beyond these levels, marked declines in growth performance, protein efficiency ratio, and increases in FCR have been documented. Consistent with these findings, our study also observed reductions in percent weight gain and specific growth rate, along with a marked increase in feed conversion ratio, as the level of FM replacement increased beyond 10% FM replacement. The survival rates for all experimental groups exceeded 95% with mortalities being attributed to fish that jumped out of the tanks. Moreover, there were no significant differences (P>0.05) in survival among the experimental groups aligning with the findings described by Montazeri Parchikolaei et al., 2021, who found no distinct effect on fish survival when FM was replaced with probiotic-supplemented soybean diets. The growth performance and feed utilization results of the present study depict that probiotic supplemented GNOC diets could replace 10% FM in the diet of rainbow trout fingerlings.

The haematological profile of a fish is a major indicator of its physiological state and general health status that ultimately impacts the production performance of a culture system. By combining standard diagnostic techniques, the haematological profile can be potentially used to monitor disease prevalence and/or

Table 4. Total erythrocyte count and total leucocyte count of *O. mykiss* fingerlings fed with different experimental diets for a period of 60 days

Treatments[1]	Total erythrocyte count ($\times10^6$/ mm^3) ±SEM	Total Leucocyte Count ($\times10^3$/ mm^3) ± SEM
Control (T_0)	1.29±0.1	8.31±0.23
T_1	1.42±0.03	8.41±0.25
T_2	1.43±0.03	8.57±0.14
T_3	1.44±0.02	8.58±0.21
p value	> 0.05	>0.05

[1]T_0 (Control) – 0% FM replaced with pGNOC, T_1 - 10% FM replaced with pGNOC, T_2 - 20% FM replaced with pGNOC, T_3 - 30% FM replaced with pGNOC, pGNOC- GNOC + *L. acidophilus*. The data was expressed as Mean±Standard Error of Means (SEM), (n=4). Different superscript letters in the same column denote significant differences (P<0.05) between the experimental groups.

Table 5. Whole body composition (% wet weight basis) of *O. mykiss* fingerlings fed with different experimental diets for a period of 60 days

Treatments[1]	Moisture	Crude Protein	Total Lipid	Total Ash
T_0	76.47±0.04^a	15.64±0.01^a	5.38±0.04^c	2.72±0.04
T_1	76.42±0.03^a	15.63±0.02^a	5.42±0.01^{bc}	2.68±0.06
T_2	76.41±0.02^a	15.56±0.03^b	5.47±0.01^{ab}	2.67±0.11
T_3	76.36±0.03^b	15.48±0.02^c	5.52±0.03^a	2.65±0.11
p value	<0.05	<0.05	<0.05	>0.05

[1]T_0 (Control) – 0% FM replaced with pGNOC, T_1 - 10% FM replaced with pGNOC, T_2 - 20% FM replaced with pGNOC, T_3 - 30% FM replaced with pGNOC, pGNOC- GNOC + *L. acidophilus*. The data was expressed as mean±standard Error of Means (SEM), (n=4). Different superscript letters in the same column denote significant differences (P<0.05) between the experimental groups.

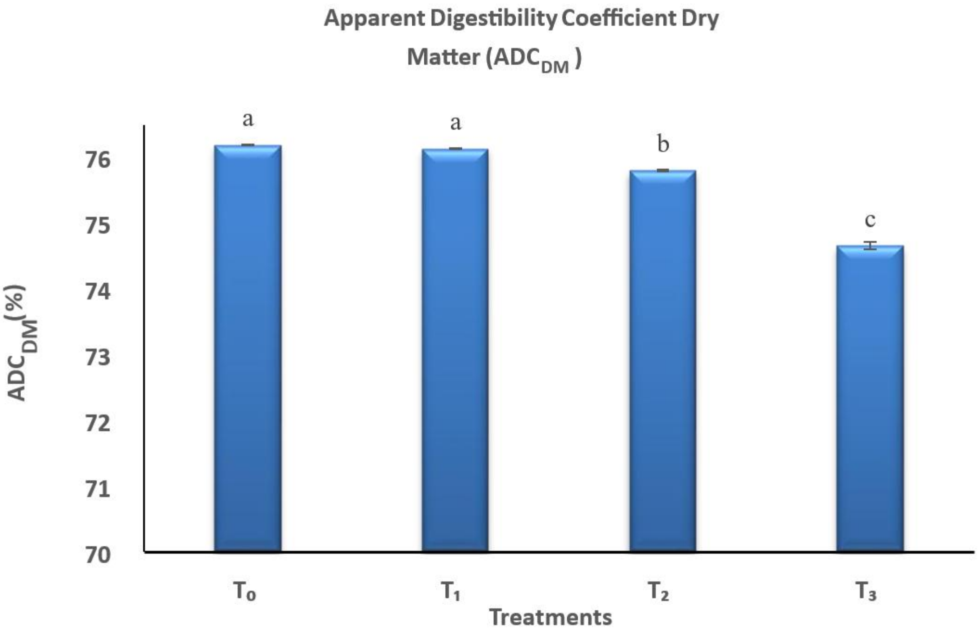

Figure 1. Apparent Digestibility Coefficients of Dry Matter (%) recorded for O. mykiss fingerlings subjected to different dietary treatments. Note: [1]T_0 (Control) – 0% FM replaced with pGNOC, T_1 - 10% FM replaced with pGNOC, T_2 - 20% FM replaced with pGNOC, T_3 - 30% FM replaced with pGNOC, pGNOC- GNOC + *L. acidophilus*.

assess stress-inducing conditions (Tavares-Dias and Moraes, 2006; Pavlidis et al., 2007). These blood components are important for monitoring feed toxicity, particularly concerning constituents that may influence blood formation during fish production (Oyawoye and Ogunkunle, 2004, Malik et al., 2024). In this study, TEC across different treatment groups did not indicate any adverse effects with increased inclusion levels of probiotic-supplemented GNOC in fingerling diets. These findings align with previous studies, which suggest that oil cakes used as FM replacements generally do not negatively impact TEC (Acar and Turker, 2018; Montazeri Parchikolaei et al., 2020). However, a slight, albeit insignificant increase in TEC was recorded in all treatment groups compared to the Control (T_0) group. This observation is consistent with the reports by Shahzad et al. (2023) and Irianto and Austin (2002), who also noted a minor, non-significant increase in TEC in *O. mykiss* fed with probiotic-supplemented diets.

In addition to known benefits for fish survival and growth, lactic acid bacteria, are reported to improve disease resistance in fish by their ability to stimulate both cellular and humoral immune responses (Wang et al., 2008; Qi et al., 2009). In this study, Total leukocyte count (TLC) did not differ significantly between treatment groups, which is consistent with findings by Montazeri Parchikolaei et al., 2020 that probiotic-supplemented plant-based protein sources did not significantly affect TLC when used to replace FM in fish diets.

The whole-body crude protein content exhibited a decreasing trend when dietary FM replacement levels increased. This is possibly due to the deficiency of essential amino acids such as methionine, cysteine, and lysine in GNOC (Green et al., 1988). Concurrently, total moisture content also decreased, while lipid content increased, likely due to the different sources of the dietary lipid content, as different lipid sources affect lipid deposition (Gong et al., 2024). This finding is consistent with the studies by Murray et al., 1977; Deng et al., 2006; Palmegiano et al., 2006; Shafaeipour et al., 2008; and Güroy et al., 2012. On the other hand, no significant variations were observed in the whole-body composition of fish fed with diets replacing 0% and 10% of FM with probiotic-supplemented GNOC (pGNOC). These results are consistent with Sealey et al., (2009) and Montazeri Parchikolaei et al., (2020), who reported insignificant variation between control and treatment groups fed probiotic-supplemented plant-based diets replacing FM. Additionally, total ash content remained consistent across all dietary treatments, corroborating the findings of Shafaeipour et al., (2008). Overall, the whole-body composition in this study indicates that replacing 10% of FM with probiotic-supplemented GNOC does not adversely affect *O. mykiss* fingerlings.

Apparent dry matter digestibility (ADC_{DM}) measures the efficiency with which feed or feed ingredients are digested, indicating the availability of nutrients for fish growth and maintenance. ADC_{DM} also provides a more accurate estimation of the indigestible material in animal feeds rather than focusing solely on some specific individual nutrients (Mzengereza et al., 2016). According to Li et al. (2013), a low ADC_{DM} typically suggests a higher percentage of indigestible materials in the feed. In the current feeding trial, the ADC_{DM} values decreased across treatments with increased inclusion levels of GNOC in the diets. As a carnivorous species, *O. mykiss* has a less specialized gut for digesting and absorbing plant-based materials. The reduced dry matter digestibility with higher incorporation of plant ingredients likely results from the increased fibre content and anti-nutritional factors (OrtizChura et al., 2018; Luo et al., 2008). Moreover, the general decreasing trend in ADC_{DM} observed with the dietary increase of probiotic-supplemented GNOC across treatments aligns with previous studies reporting reduced dry matter digestibility as plant proteins replacement for FM in *O. mykiss* diets (Pandey and Satoh, 2008; Nang Thu et al., 2011; OrtizChura et al., 2018). It has been reported that gram-positive bacteria, particularly *Lactobacillus* spp., can secrete exogenous enzymes that enhance digestive processes by increasing overall enzymatic activity in the fish intestine (Suzer et al., 2008; Essa et al., 2010; Mohapatra et al., 2012). These enzymes can tolerate a wider pH range, thereby extend the digestion period and maximize nutrient utilization (Essa et al., 2010). However, the ADC_{DM} for the group fed with pGNOC in replacement of 10% FM was similar to that of the control (T0) diet in trout, which can be attributed to the combinatorial effect of the presence of *L. acidophilus* and the lower fibre content and anti-nutritional factors present in the diet containing lower inclusion level of GNOC.

Conclusion

The study concludes that *L. acidophilus*-supplemented GNOC (pGNOC) can effectively replace up to 10% of fish meal in the diet of *O. mykiss* fingerlings without showing any detrimental effects on growth performance indices, feed utilization parameters, whole body composition, haematology and digestibility. In this study, pGNOC was evaluated as a composite functional ingredient, and the individual effects of GNOC and probiotic supplementation were not isolated. Further research is warranted to investigate the distinct roles of each component in fish meal replacement strategies. Future research should also explore optimizing probiotic formulations and reducing anti-nutritional factors in GNOC to enable higher FM replacement levels, contributing to more sustainable aquaculture practices.

Ethical Statement

The experimental design and methodology were approved by the members of the Institutional Animal Ethics Committee (IAEC), Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-Kashmir, India (Reg. No: 1809/GO/ReBiS/Rel/15/CPCSEA). The research methodology was in strict compliance with the protocols demonstrated by IAEC.

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Author Contribution

Sadiya Farooq: Conceptualization, Methodology, Investigation, Visualization, Data Curation, Formal analysis, Writing- Original draft preparation. Anayitullah Chesti: Conceptualization, Resources, Project administration, Validation, Supervision. Mansoor Ahmad Rather: Conceptualization, Methodology, Resources, Validation, Supervision. Mohd Ashraf Malik: Methodology, Data Curation, Formal analysis, Writing-review & editing, Supervision. Anam Aijaz: Methodology, Data curation, Writing-review & editing. Syed Shariq Nazir Qadiri: Supervision, Methodology, Data curation. Irshad Ahmad: Data curation, Formal Analysis, Validation. Bilal Ahmad Bhat: Formal analysis, Validation. Junaid Ahmad Khan: Methodology. Uzma Nazir: Reviewing. Sayima Majeed: Methodology. Farhat Zameer: Methodology.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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