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Effect of soybean meal (SBM) on growth and hematological parameters of rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*)

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Abstract
This study investigates the efficacy of soybean meal (SBM) as a potential protein source in aquafeed, focusing on its effects on the growth and hematological parameters of rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*). The research was conducted at the Microbiology and Immunology Laboratory, University of Agriculture, Faisalabad. With global demand for fishmeal exceeding supply, alternative protein sources, such as SBM, are increasingly crucial for sustainable aquaculture. The study, spanning 7 weeks, involved assigning rohu and grass carp into control (fish meal) and experimental soybean meal (SBM) groups. Growth parameters, including weight, length, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate, were monitored, alongside hematological analyses. The fish were fed on an experimental diet at a feeding level of 2% of body weight. The results indicated comparable growth performance between SBM-fed and fishmeal-fed groups, with SBM potentially enhancing survival rates. Body weight gain and SGR increased significantly compared to the control group fed with FM in rohu. However, for grass carp, there was no significant difference in weight gain and SGR values when fed SBM compared to FM. For rohu, the hematological studies showed significantly higher red blood cells, hemoglobin, hematocrit, white blood cells, and leucocytes. For grass carp RBCs, hemoglobin, and hematocrit values showed a declining tendency with experimental feed; besides that, there was a prominent rise in white blood cell (WBC) count and lymphocytes. These results suggested that SBM could be incorporated at varying percentages in carp feed, considering enhanced growth performance and hematological status as a cost-effective alternative protein source strategy to overcome the cost of fish meal protein in the aquaculture industry.

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Introduction

The global decline in ocean fisheries stocks has driven a significant growth in fish and shellfish farming, and it accounts for more than a quarter of all fish consumed directly by humans (Naylor et al., 2000). World hunger and severe food insecurity have increased after the COVID-19

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pandemic, highlighting the frailty of our society and agrifood systems. A significant way to raise the socioeconomic standing of a nation and combat hunger is through improved food security. Aquaculture significantly increases the amount of animal protein in diets worldwide, helping reduce poverty (Pradeepkiran, 2019; Boyd et al., 2022). It involves the organized raising, feeding, propagation, or protection of aquatic resources for various purposes, including commercial, recreational, or public use. Currently, aquaculture, encompassing both freshwater and marine environments, contributes over half (56% or 120 million tonnes) of the total global aquatic production. It stands as the fastest-growing form of food production worldwide (Mizuta et al., 2023).

The production of carp has seen a significant rise in Asia, particularly in China (Yue et al., 2024). In more modern and intensive systems, particularly for herbivorous and omnivorous finfish, there is a heavy reliance on added feeds because there are too many fish stocked at high densities, which cannot be sustained solely by natural food sources (Naylor et al., 2000). Freshwater fish production plays a crucial role in meeting global protein needs due to its high nutritional value with a significant presence of essential nutrients, including proteins, lipids, minerals, and vitamins (Abidi and Khan, 2004; Noreen et al., 2025).

The Cyprinidae family constitutes a prominent class of fish, essential for human food consumption. Within this family, the freshwater species *Labeo rohita*, commonly known as rohu, holds significant importance, particularly in Asian regions, notably the Indian subcontinent (Steffens and Wirth, 2005; Manam and Quraishi, 2024). Grass carp (*Ctenopharyngodon idella*), typically native to most rivers in China except the northwest, has experienced notable expansion in its habitats in recent years. Grass carp has been introduced to freshwater environments in numerous regions globally, spanning central Asia, Japan, America, and Europe (Ding, 2017).

In regions like Pakistan, aquaculture has become a rapidly growing sector as it deals with the increasing global demand for protein. Traditionally, fishmeal has been used as a primary protein source (Wu et al., 2004; Hussain et al., 2024). However, challenges like rising fishmeal costs have hindered the growth of the fish industry (Xie et al., 2001; Dhar et al., 2024). To tackle these challenges, researchers have explored many cost-effective alternatives as substitutes for fishmeal (Iqbal et al., 2016; Hussain et al., 2024). Soybean meal (SBM), a plant-based protein source, has been extensively researched and is regarded as a valuable ingredient for the production of aquatic feed (Shamna et al., 2017). It has attained significant interest as a potential substitute for fishmeal in fish feed because of its affordability, widespread availability, digestibility, and relatively stable amino acid profile (Pervin et al., 2020).

Nonetheless, soybean meal (SBM) contains antitrypsin, which can hinder fish growth. Therefore, the addition of soybean meal in fish diets must adhere to a specific threshold, not exceeding the tolerance limit (Yanti et al., 2019; Wang et al., 2022). However, the impact of substituting FM with soybean meal on the growth and physiological condition of fish varies depending on the species and their respective feeding mechanism. Most of the herbivorous and omnivorous fish species can incorporate SBM as a protein source, either partially or entirely, without experiencing adverse effects on their growth and well-being (Liu et al., 2021)

In biometrical research, it is crucial to assess the growth patterns of a fish that is influenced by various biological and environmental factors. Different studies have highlighted the significance of establishing the length-weight relationship of a fish, which offers valuable insight into various aspects such as growth patterns, overall health, conditions of a habitat, fish life history, and morphological characteristics of the species (Jisr et al., 2018; Oyebola et al., 2022; Faruque and Das, 2024). Hematological examination involves analysis of blood cell counts, as well as the measurement of various cell-related parameters and biochemical indices. It offers a complete understanding of various aspects of fish biology, including their ability to transport oxygen, immune system functionality, stress level, susceptibility to disease, nutritional status, and intoxication (Witeska et al., 2022; 2023).

The objective of the present study was to replace 100% fishmeal with soybean meal to assess the growth response and hematological parameters in rohu and grass carp, because it is a sustainable, low-cost protein source to replace the fishmeal.

Material and Methods

Study area and duration

The trial was conducted at the Fisheries Research Farm located at the Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. The duration of the trial was 7 weeks.

Experimental design

Fingerlings of both rohu and grass carp were collected and kept in glass aquaria to acclimatize at laboratory conditions, and dry pelleted feed was given for one week. The experiment was carried out in glass aquaria, and fingerlings of the same size of rohu and grass carp were kept as 20 fish per aquarium. The control group T_0 was composed of 100% fish meal. The other group consisted of 100% soybean meal. Regular water exchange was ensured to maintain water quality.

Feeding protocols

In both fish groups, feed was incorporated according to 2% of body weight. The feeding schedule was twice a day at 8:00 am and 4:00 pm. Modifications in the feed quantity were implemented according to the fish's weight gain. To streamline feed distribution, a weight scale was utilized to measure the daily food requirement.

Growth parameters

Throughout the trial period, growth parameters were measured to assess the development of the fish. These measurements include body weight, recorded in grams, and length in centimeters. The length and weight gain were determined by comparing the initial and final measurements of the fish over the trial period. The weight of the fish was monitored every week. Additionally, key metrics such as the specific growth rate (SGR), feed conversion ratio (FCR), and survival percentage were calculated using the following formulas:

Weight gain (g)

$$\text{Final weight (Wf) - Initial weight (Wi)}$$

Length gain (cm)

$$\text{Final length (cm) - Initial length (cm)}$$

Feed conversion ratio (FCR)

$$\text{FCR}=\frac{\text{Total feed given (grams)}}{\text{Wet weight gain (grams)}}$$

Specific growth rate (SGR)

$$\text{SGR}=\frac{\text{Final weight (g) - Initial weight (g)}}{\text{Trial duration (days)}}\times 100$$

Survival percentage (SP)

$$\text{SP (\%)} = \frac{\text{Number of harvested fish}}{\text{Number of stocked fish}}\times 100$$

Hematological parameters

The total red blood cell (RBC) and white blood cell (WBC) counts were assessed using a hematocytometer. Initially, the blood sample was divided into two portions. The first portion, containing 10% EDTA (ethylene diamine tetraacetate), was used to determine various hematological indices such as hemoglobin (Hb), hematocrit (HCT), lymphocytes, RBC count, and WBC count. To facilitate cell adhesion, 100 mL of the blood sample was incubated at 37 °C in microflat-bottomed plates for approximately 60 minutes. Following incubation, a saline buffer was used for washing, and the washing process was repeated three times to ensure accurate counts.

For differential staining of leukocytes, smears were prepared and stained. Meanwhile, the second portion of the blood sample was allowed to clot at 4 °C overnight. Subsequently, the clotted sample underwent centrifugation at 4000 rpm for 15 minutes and was then stored for future use.

Physicochemical parameters

Consistent monitoring of the water parameters was ensured by maintaining regulatory control. Electronic devices, such as a pH meter and HANNA HI-8424, measured pH levels, temperature, and dissolved oxygen (DO). Standard measurement techniques were employed to assess the total alkalinity and water hardness. The objective was to systematically track and analyze the various water quality indicators using appropriate instrumentation and recognized methods.

Statistical analyses

Data for each parameter were individually averaged. Quantitative results determined from the feed treatments were compared with each other using the *t*-test.

Results

Limnological parameters

Table 1 presents the average values of T_0 and T_1 for physicochemical parameters under two different feed treatments, T_0 and T_1, over 7 weeks. A slight fluctuation in temperature with time was observed, but it was not statistically significant. The same was the trend for dissolved oxygen (DO) values. Total alkalinity and water hardness values also remained almost uniform throughout the experimentation period (**Table 1**).

Table 1: Average physicochemical parameters of the two treatments, T_0 and T_1

Week	pH	Temperature (ºC)	DO (mg/L)	Total alkalinity (mg L^{-1})	Water hardness (mg L^{-1})
Initial	7.1	26.5	4.2	95	148
Ist week	7.3	28	4.1	101	150
2^{nd} week	7.4	28.8	4.3	96	147
3rd week	7.2	27	4.5	97	151
4^{th} week	7.3	26	4.2	95	149
5^{th} week	7.5	27.5	4.4	97	153
6^{th} week	7.1	29	4.2	96	147
7^{th} week	7.2	27	4.3	99	152
Mean ± SD	7.26 ± 0.14	27.47 ± 1.06	4.27 ± 0.12	97 ± 2.07	149.62 ± 2.26

Hematological parameters of rohu

After 7 weeks, significant differences were observed between the T_0 and T_1 groups across various hematological parameters (**Table 2**). In the T_0 group, red blood cell (RBC) counts ranged from 2.01 to 2.81 × 10^6/µL, while the T_1 group had higher RBC counts ranging from 2.86 to 3.12 × 10^6/µL. Hemoglobin (Hb) levels in the T_0 group ranged between 5.03 and 6.21 g/dL, whereas in the T_1 group, Hb levels ranged from 5.01 to 5.78 g/dL. Statistical analysis revealed a significant difference ($P \leq 0.05$) among the treatments and over time. Hematocrit (HCT) values in the T_0 group were between 21.32% and 29.79%, compared to 31.34% and 36.65% in the T_1 group. Significant differences ($P \leq 0.05$) were noted among the treatments and over time.

Mean cell volume (MCV) in the T_0 group ranged from 104 to 115 fL, while in the T_1 group, it ranged from 118 to 135 fL. Mean cell hemoglobin (MCH) levels were between 14.48 and 16.81 g/dL in the T_0 group, and significantly higher in the T_1 group, ranging from 18.48 to 23.02 g/dL. White blood cell (WBC) counts in the T_0 group were between 116 and 120 × 10^3/µL, whereas in the T_1 group, they were higher, ranging from 123 to 148 × 10^3/µL. Lastly, lymphocyte counts in the T_0 group ranged from 61.7% to 64.3%, while in the T_1 group, they were higher, ranging from 67.1% to 73.1%.

Table 2: Hematological parameters of control vs. treatment group in rohu

Parameters	T_0	T_1	*P* value	Sig/Non-sig
RBCs × 10^6/µL	2.27 ± 0.369	3.00 ± 0.13	0.0049	Significant
Hb (g/dL)	5.07 ± 0.081	5.52 ± 0.329	0.0364	Significant
HCT %	24.71 ± 3.812	34.42 ± 2.270	0.0023	Significant
MCV	109 ± 4.967	124.5 ± 7.593	0.0071	Significant
MCH (g/dL)	15.85 ± 1.036	21.36 ± 2.069	0.0015	Significant
WBCs × (10^3/µL)	118 ± 1.826	139.5 ± 11.56	0.0174	Significant
Lymphocytes %	62.9 ± 1.095	70.1 ± 2.703	0.0013	Significant

Hematological parameters of grass carp

After 7 weeks, significant differences were observed between the T_0 and T_1 groups across various hematological parameters (**Table 3**). In the T_0 group, red blood cell (RBC) counts ranged from 2.45 × 10^6/µL to 2.87 × 10^6/µL, while the T_1 group had lower RBC counts ranging from 2.06 × 10^6/µL to 2.53 × 10^6/µL. Statistical analysis revealed significant differences ($P \leq 0.05$) among the treatments. Hemoglobin (Hb) levels in the T_0 group varied between 5.49 g/dL and 6.41 g/dL, whereas in the T_1 group, Hb levels ranged from 5.23 g/dL to 6.28 g/dL. Hematocrit (HCT) values in the T_0 group were between 37.41% and 48.00%, compared to 21.32% and 29.79% in the T_1 group. Significant differences were noted between the treatments.

Mean cell volume (MCV) in the T_0 group ranged from 108 to 137 fL, while in the T_1 group, it ranged from 104 to 118 fL. Mean cell hemoglobin (MCH) levels were between 18.47 g/dL and 25.02 g/dL in the T_0 group, and lower in the T_1 group, ranging from 13.48 g/dL to 17.30 g/dL. White blood cell (WBC) counts in the T_0 group were between 116 × 10^3/µL and 120 × 10^3/µL, whereas in the T_1 group they ranged from 123 × 10^3/µL to 148 × 10^3/µL. Lastly, lymphocyte counts in the T_0 group ranged from 61.7% to 64.3%, while in the T_1 group, the counts were higher, ranging from 65.1% to 71.1%. Overall, the T_0 group exhibited higher values in RBC, HCT, MCV, and MCH, while the T_1 group had higher WBC and lymphocyte counts, indicating varying impacts of the treatments on these hematological parameters.

Table 3: Hematological parameters in grass carp under different feed treatments

Parameters	T_0	T_1	*P* value	Sig/Non-sig
RBCs × 10^6/µL	2.74 ± 0.200	2.36 ± 0.208	0.019	Significant
Hb (g/dL)	5.84 ± 0.415	5.53 ± 0.499	0.225	Non-significant
HCT %	42.78 ± 4.477	24.76 ± 3.826	0.0004	Significant
MCV	123.5 ± 12.069	109.75 ± 6.238	0.0447	Significant
MCH (g/dL)	22.66 ± 3.013	15.31 ± 1.631	0.0025	Significant
WBCs ×(10^3/µL)	118 ± 1.826	134.75 ± 9.105	0.0183	Significant
Lymphocyte %	62.9 ± 1.095	69.1±2.727	0.0028	Significant

Growth parameters

Growth in rohu

The research over a 7-week period investigated the growth performance of rohu (*Labeo rohita*) under two different dietary treatments: a control group (T_0) fed with fishmeal and an experimental group (T_1) fed with 100% soybean meal. At the start of the trial, the average weight of rohu in T_0 was 5.55 g, which increased to 6.78 g by the end of the experiment (**Figure 1**).

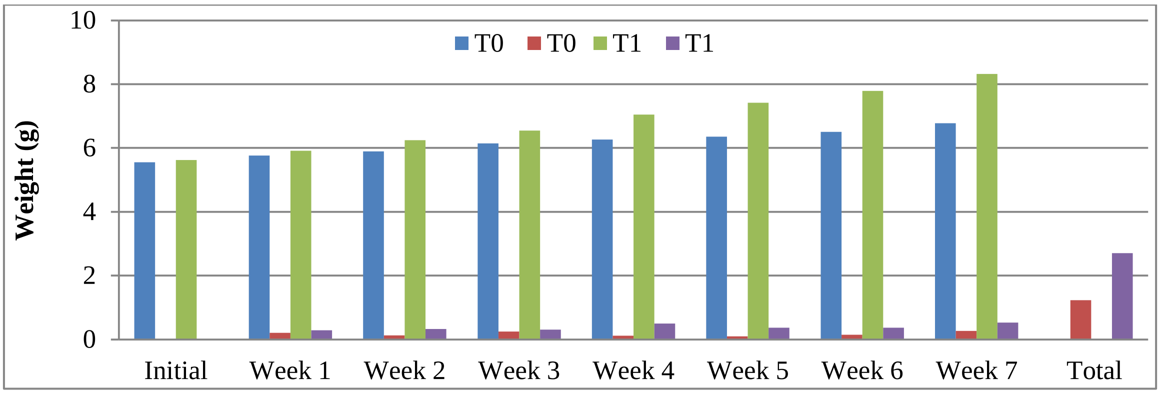

Figure 1: Average weight and weight gain in rohu each week under different feed treatments

In contrast, T_1 showed a greater increase, with average weights rising from 5.62 g to 8.32 g. The growth performance in T_1 was significantly higher than in T_0, as confirmed by a *t*-test indicating a significant difference in average weights between the treatments. The initial average length of rohu was 6.2 cm in T_0 and 5.9 cm in T_1. By the end of the study, the final average length was 8.2 cm in T_0 and 8.5 cm in T_1, respectively (**Figure 2**). Although T_1 showed a higher final length, the *t*-test revealed no significant difference in average length between the treatments (**Table 4**).

Table 4: Growth parameters of rohu under different feed treatments

Growth parameters	T_0	T_1	*P* value	Sig/Non-sig
Ave. weight	6.06±0.4074	6.86±0.9461	0.027*	Significant
Weight gain	0.17±0.0672	0.38±0.0934	0.0002*	Significant
Ave. length	7.13±0.7482	6.95±0.9165	0.3304	Non-significant
Length gain	0.28±0.1214	0.37±0.1603	0.1408	Non-significant
SGR (%)	2.47±0.9516	5.45±1.3252	0.0002*	Significant
FCR	1.91±0.2410	2.22±0.1112	0.0069*	Significant

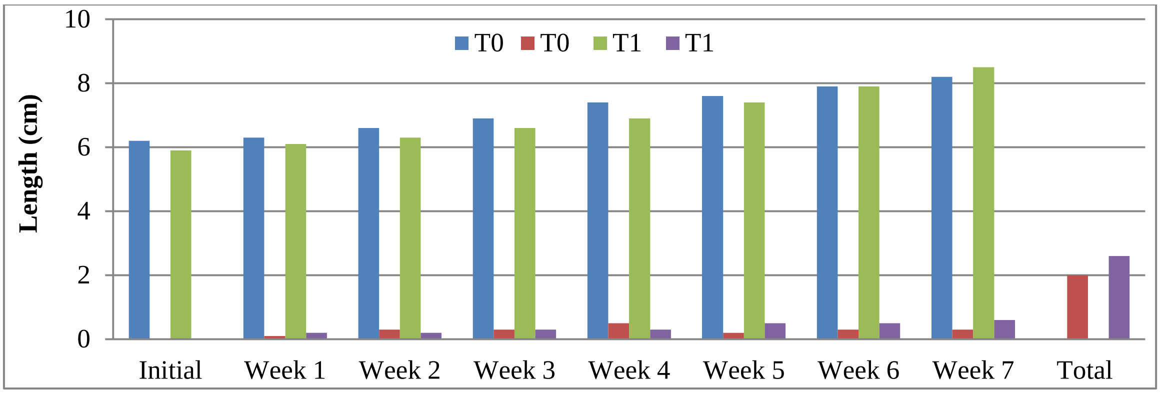

Figure 2: Average length and gain in length in rohu each week under different feed treatments

Specific growth rate (SGR) calculations indicated that T_1 had a higher SGR compared to T_0, reflecting more efficient growth in rohu. Feed conversion ratio (FCR) analysis showed a mean value of 1.91 for T_0 and 2.22 for T_1, indicating that T_0 had a better feed conversion efficiency. However, the survival rate was higher in T_1, with 90% survival compared to 80% in T_0.

Growth in grass carp

Initially, the average weight of grass carp in T_0 was 3.55 g, which increased to 6.07 g by the end of the trial (**Table 5**; **Figure 3**). In T_1, the average weight started at 3.62 g and increased to 6.32 g. T_1 exhibited greater weight gain; however, the *t*-tests indicated no significant difference in average weights between the treatments. The maximum weight gain occurred in both groups during the seven weeks, and the growth pattern observed was $T_0 < T_1$. Regarding length (**Table 5**; **Figure 4**), the initial average length of grass carp in T_0 was 6.1 cm, increasing to 8.1 cm at the end of the trial, while in T_1 it started at 5.9 cm and reached 8.4 cm by the end of the experiment. Although the mean final length in T_1 was higher, statistical analysis showed no significant difference in average lengths between the treatments.

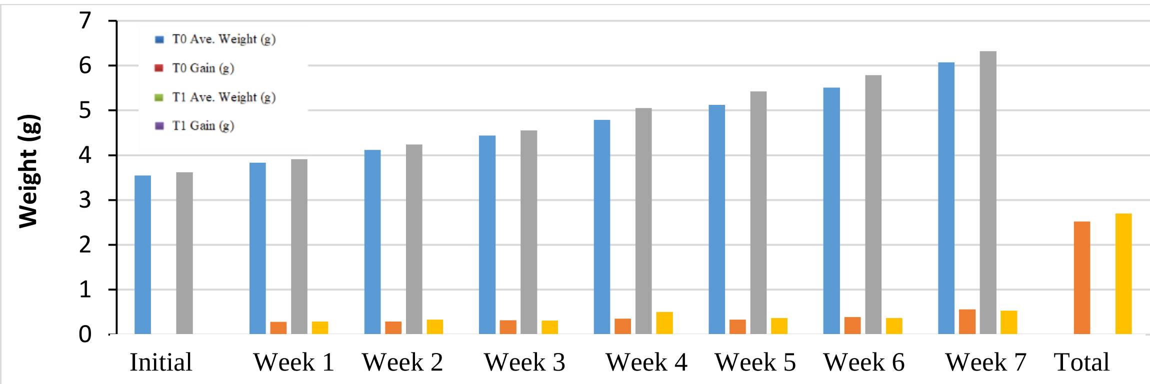

Figure 3: Average weight and weight gain in grass carp each week under different feed treatments

Specific growth rate (SGR) analysis of grass carp revealed that T_1 had a higher SGR compared to T_0, yet the *t*-test showed no significant difference in SGR between the treatments. Similarly, feed conversion ratio (FCR) values were recorded, with T_0 having a mean FCR of 1.70 and T_1 a slightly higher mean FCR of 1.79. The *t*-test indicated no significant difference in FCR between the treatments. Survival rates were also monitored weekly; T_0 showed a 90% survival rate, and T_1 achieved a 100% survival rate.

Table 5: Growth parameters of grass carp under different feed treatments

Growth parameters	T_0	T_1	*P* value	Sig/Non-sig
Ave. weight (g)	4.67 ± 0.8616	4.86 ± 0.9461	0.3454	Non-significant
Weight gain (g)	0.36 ± 0.0955	0.38 ± 0.0934	0.3099	Non-significant
Ave. length (cm)	6.95 ± 0.7030	6.9 ± 0.865	0.4504	Non-significant
Length gain (cm)	0.285 ± 0.1345	0.357 ± 0.1718	0.2017	Non-significant
SGR	5.11 ± 1.3728	5.45 ± 1.325	0.3215	Non-significant
FCR	1.70 ± 0.0945	1.79 ± 0.1106	0.0514*	Significant

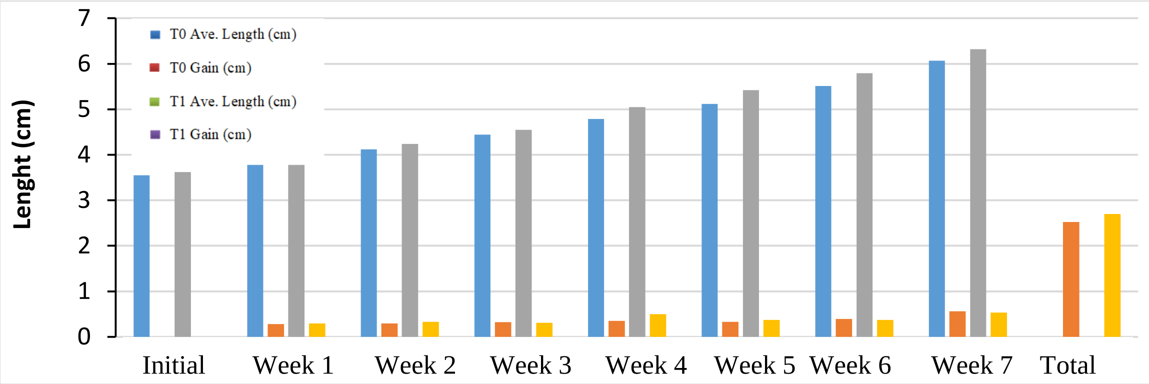

Figure 4: Average length and length gain in grass carp under different feed treatments

Discussion

The study observed significant improvements in hematological parameters of rohu in the T_1 group (soybean meal) compared to the T_0 group over 7 weeks. The T_1 group showed higher RBC counts, hematocrit, MCV, MCH, WBC counts, and lymphocyte percentages; these results resemble those of an earlier-published study (Howlader et al., 2023) in which the impact of replacing fish meal (FM) protein by soybean meal (SBM) on health condition and feed utilization of stinging catfish (*Heteropneustes fossilis*), hemoglobin, red blood cell (RBC) count, and white blood cell (WBC) count were recorded as higher with half substitution of FM by soybean meal. The findings indicate that substituting half of the FM protein in stinging catfish diets with SBM does not negatively impact the growth, feed efficiency, or the health of the catfish. Similar results were found for rohu in the present research, with higher mean values as compared to the control group, but with complete substitution of commercial feed with experimental soybean meal.

After 7 weeks, significant differences were observed between the T_0 and T_1 groups across various hematological parameters of grass carp. Overall, the T_0 group exhibited higher values in RBC, HCT, MCV, and MCH, while the T_1 group had higher WBC and lymphocyte counts, indicating varying impacts of the treatments on these hematological parameters. These results relate to the experiment conducted by Ye et al. (2019), wherein a lower red blood cell count was observed when fed with soybean meal to Obscure Puffer (*Takifugu obscurus*). Similar non-significant results were observed in the present research for grass carp when fed with soybean meal, with a decline of red blood cells; there was a significant rise in white blood cells and lymphocytes. Likewise, Yamamoto et al. (2021) observed a significant decline in red blood count in the red drum fish (*Sciaenops ocellatus* L.) fed with soybean meal. The present study observed the growth performance of grass carp under the two dietary treatments over a trial period. Although the average weight and length increased in both groups, with T_1 showing greater weight gain and slightly higher final length, statistical analyses did not reveal significant differences between the treatments. These results are relatable and contrasting to those of Liu et al. (2021), who investigated the impact of replacing fish meal (FM) with soybean meal (SBM) in diets for juvenile Redlip Mullet (*Liza haematocheilus*). They found that up to 75% substitution of FM with SBM had no significant negative effects on growth performance and feed utilization. However, beyond 75%, there was a declining trend in these parameters.

In conclusion, the study highlights the potential of soybean meal as a sustainable and cost-effective protein source for improving the growth performance and hematological parameters of freshwater fish species like rohu and grass carp. These findings contribute to the ongoing efforts in aquaculture to develop environmentally-friendly and nutritionally-balanced diets for sustainable fish production.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceptualization and design of the study: AF, SP, FR. Conduction of experiments: IH, SP, Arooj, Obra. Data collection, visualization, and interpretation: AF, SP, FR, A, ZFS, IM, TF. Formal statistical analysis: AF, IM, SP, Arooj, TF, BR. Writing of first draft: AF, SP, Arooj. Proof reading and approval of the final version: AF, SP, FR, Arooj, ZFS, IM, BR, Obra, TF, FB.

Permissions and ethical compliance
This work was approved by the Institutional Ethical Review Board/Committee (IERB/C) of the University of Agriculture, Faisalabad, Pakistan (Approval number 3245-48).

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination.

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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