



Blood Hematology and Health Impact Assessment of Fish *Labeo Rohita* with Potato Peel Incorporation

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ABSTRACT

Aquaculture is a rapidly expanding sector in global food production, now contributing more than half of the world's fish supply. Potato (*Solanum tuberosum* L.), the fourth most important food crop globally, generates substantial amounts of agro-industrial waste, particularly potato peels. This study aimed to evaluate the potential of potato peel as a sustainable and economical feed additive by assessing its impact on the blood hematology of *Labeo rohita*. An experimental trial was conducted using four dietary treatments: 0% (T₁), 5% (T₂), 10% (T₃), and 15% (T₄) potato peel inclusion, arranged in a completely randomized design with three replications. Hematological parameters including red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), hematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were analyzed to evaluate physiological responses and immune function. The results showed statistically significant differences ($p < 0.001$) among treatment groups. The 5% inclusion group (T₂) exhibited the most favorable hematological profile, with elevated RBC, WBC, Hb, and HCT values, indicating enhanced erythropoiesis, improved immune response, and better overall physiological health. In contrast, the 10% inclusion group (T₃) showed a pronounced decline in all hematological parameters, suggesting hematological suppression and signs of microcytic, hypochromic anemia. The 15% group (T₄) exhibited partial recovery compared to T₃ but still did not match the hematological performance observed in T₂. These findings suggest that moderate dietary inclusion of potato peel at 5% can improve blood health and immunity in *Labeo rohita*, whereas higher inclusion levels may exert negative effects due to the presence of anti-nutritional components. The study supports the selective use of potato peel as a functional, cost-effective, and eco-friendly feed ingredient in sustainable aquaculture systems.

INTRODUCTION

Aquaculture output has increased due to supply and demand-side variables, making it viable and enabling production to grow over time (Bjorndal *et al.*, 2024). Supply-side improvements, such as advancements in genetics, breeding programs, disease management, and feed efficiency, have contributed to higher yields. These innovations have enhanced growth rates, feed conversion ratios, and resistance to disease among aquaculture species. On the demand side, changing dietary preferences, urbanization, and increased awareness of the health benefits of fish consumption have fueled steady growth. With ongoing concerns such as climate change, feedstock shortages, and disease outbreaks, the sector faces critical challenges. These challenges are compounded by ecological constraints and rising operational costs. Finding substitute protein sources to feed aquatic creatures is currently the

aquaculture industry's biggest concern. The traditional reliance on fishmeal and fish oil has become economically and environmentally unsustainable. In order to meet customer demand for sustainable and eco-friendly food, the objective is to lessen the environmental impact of conventional feed production (Roccatello *et al.*, 2024). The adoption of circular economy principles, where waste materials are reused or repurposed into productive inputs, has gained traction as a strategy to promote sustainability in aquafeed production. Sustainable feeds are also important to reduce the carbon footprint of aquaculture and prevent further depletion of wild fish stocks used for fishmeal production.

The best protein sources for aquafeed are fish meals. There is dire need for an alternate source due to the world's extreme demand and limited supply (Hossain *et al.*, 2024). As wild fish populations decline, the availability of fishmeal continues to drop, making it a less reliable and



more expensive feed component. The production of fish feed

was significantly impacted by the rising prices of fish meal, which were predicted to be insufficient to meet the world's demand by 2050. Furthermore, the cost of fish meal has increased, its availability has decreased, its supply has been inconsistent, and its quality has deteriorated, which has expedited the partial or whole substitution of other protein sources (Kari *et al.*, 2023). These constraints emphasize the urgency of identifying plant- based or waste-derived protein sources that can provide similar nutritional value at lower cost and environmental impact. In this context, attention has turned toward the valorization of agricultural and food processing by-products as feed ingredients.

Potato peel waste is the most common by-product of potato processing, and depending on the peeling method, it can make up anywhere from 15% to 40% of the unprocessed weight of potatoes. The potato processing industry produces approximately 70 and 140 thousand tonnes of potato peels annually worldwide (Singh *et al.*, 2023). Disposing of such large quantities creates environmental and logistical challenges, but its conversion into animal feed could offer both ecological and economic advantages. Potato peel powder has a substantial quantity of nutritional components, especially higher amounts of carbohydrates, overall mineral content, adequate protein as well as fiber content, as well as a lower proportion of moisture and fats. 5.11–6.57% ash, 9.54–12.86% protein, 1.02–1.92% fat, 12.11–14.95% fiber, 59.64–68.71% carbs, and 4.78–6.82% moisture (Cozma *et al.*, 2024). These values suggest that potato peel can serve as a promising partial feed replacement in aquafeeds, improving nutritional profiles while cutting production costs.

Hematological analysis is a fundamental indicative tool used to estimate the bodily and pathological status of organisms, including fish. This form of analysis involves the quantitative and qualitative assessment of the cellular components of blood, such as hemoglobin concentration (Hb), red blood cell (RBC) and white blood cell (WBC) counts, hematocrit (Hct), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and erythrocyte sedimentation rate (ESR). These parameters provide vital insights into the oxygen-carrying capacity of blood, immune response, and overall metabolic condition. In the context of ichthyology, hematological profiles serve as reliable indicators of health status, stress response, nutritional deficiencies, and the presence of infectious or environmental stressors. Given their sensitivity to internal and external changes, these blood indices are widely recognized as primary diagnostic methods for assessing the wellbeing and physiological state of fish in both natural and experimental conditions (Ahmed *et al.*, 2022). The objective of this research is to assess *Labeo rohita* fingerlings hematological reaction to different dietary potato peel inclusion, with a focus on identifying any physiological changes or health-related effects resulting from this alternative feed ingredient and To determine the impact of potato peel incorporation on key blood parameters, specifically the hemoglobin concentration, white blood cell (WBC) count, and red blood cell (RBC)

count are used to evaluate the fish's hematological and immunological health.

MATERIALS AND METHODS

The purpose of this experiment was to determine the impact of potato peel incorporation within the diet of fish *Labeo rohita*. This research study was conducted at the Department of Zoology, Wildlife and Fisheries Laboratory, University of Agriculture Faisalabad, Constituent College Depalpur, Okara. Potato peels were collected from household kitchens. The peels were thoroughly washed, air-dried, and ground into powder before incorporation into the diet. A basal diet was formulated containing 30% crude protein, which served as the control diet. The experimental diets were prepared by replacing portions of the basal diet with potato peel powder at inclusion levels of 5%, 10%, and 15% by weight. A total of 360 healthy fingerlings were procured from a Fish Seed Hatchery Arifwala and acclimatized under laboratory conditions for two weeks prior to the experiment. The fish were divided into four treatment groups: Treatment1: Control diet, Treatment2: Diet with 5% potato peel incorporation, Treatment3: Diet with 10% potato peel incorporation, Treatment4: Diet with 15% potato peel incorporation

Experimental Setup

A 90-day feeding trial was conducted to evaluate the hematological and health impacts of dietary potato peel incorporation on *Labeo rohita* fingerlings. A total of 360 fish were randomly allocated into four treatment groups in triplicate, housed in 12 glass aquariums with 30 fish in each aquarium. The aquariums were equipped with continuous aeration using air stones connected to electric air pumps to ensure sufficient dissolved oxygen levels. The fish were fed twice daily (morning at 9:00 AM and evening at 5:00 PM) to apparent satiation. Water quality parameters such as temperature were monitored daily to maintain optimal rearing conditions.

Hematological Analysis

At the end of the trial fish were randomly selected from aquariums and carefully netted to reduce handling stress. The fish were anesthetized using a mild clove oil solution prior to blood collection (Tertoet *et al.*, 2024). Blood sample were collected by cardiac puncture using 3cc sterile disposable syringes, a method recognized for obtaining sufficient and uncontaminated blood volumes in fish (Witeska *et al.*, 2022).

Immediately after collection, the blood samples were transferred into K₂-EDTA-coated vials to prevent coagulation.

Red Blood Cell (Erythrocyte)

For red blood cell estimation, 20 µL of blood was diluted in 4.0 mL of modified Drabkin's fluid using a formal-citrate solution, yielding a 1:20,000 dilution (Eyiunmiet *et al.*, 2018). The diluted sample was loaded into a Neubauer hemocytometer. After allowing for sedimentation, red blood cells were counted in five small squares (each 0.2 mm²) under a microscope. The RBC count was calculated using the formula:

$$RBC \left(\frac{\text{cells}}{L} \right) = \frac{(\text{number of cell counted (N)} \times \text{Dilution factor (DF)} \times 106)}{(\text{Area counted (A)} \times \text{Depth of chamber (D)})}$$

White Blood Cells (Leucocytes)

For WBC estimation, 20 µL of blood was mixed with 0.38 mL of diluent in a test tube. The diluent lysed the red blood cells, enhancing the visibility of WBCs. A blood film was prepared and observed microscopically. WBC count was calculated as:

$$WBC \left(\frac{\text{cells}}{L} \right) = \frac{(\text{number of cell counted (N)} \times \text{Dilution factor (DF)} \times 106)}{(\text{Area counted (A)} \times \text{Depth of chamber (D)})}$$

Hemoglobin (Hb)

Hemoglobin concentration was estimated using the cyanmet hemoglobin method. A 20 µL blood sample was mixed with 4.0 mL of Drabkin's solution by gently inverting the tube 20 times. The mixture was analyzed using a spectrophotometer or colorimeter.

$$Hb = \frac{\text{Reading of test} \times \text{concentration of standard} \times \text{dilution}}{\text{Reading of standard}}$$

Packed Cell Volume (PCV)/Haematocrit

Heparinized capillary tubes (75 mm) were filled to the tenth mark, avoiding air bubbles. Samples were centrifuged at 3000 rpm for 30 minutes using the Wintrobe method. PCV was calculated as:

$$PCV = \frac{\text{Column reading}}{100 \text{ divisions}}$$

Mean Corpuscular Volume (MCV)

$$MCV = \frac{Hct \times 10}{RBC}$$

Mean Corpuscular Hemoglobin (MCH)

$$MCH = \frac{Hgb \times 10}{RBC}$$

Mean Corpuscular Hemoglobin Concentrations

$$MCHC (g/dL) = \frac{(Hgb/Hct)}{100}$$

All hematological parameters were cross-verified using an automatic hematology analyzer at Bio Tec Lab, Okara to ensure the reliability and accuracy of the results.

Statistical analysis

One-Way ANOVA was conducted using SPSS to evaluate statistical differences among treatment groups, followed by Tukey's HSD post hoc test for multiple comparisons to identify significant group variations (Field, 2013).

RESULTS AND DISCUSSION

The study was conducted to evaluate the impact of varying levels of potato peel incorporation on the hematological parameters of *Labeo rohita* under a controlled experimental setup. The treatments were arranged in a completely randomized design with three replications. The values presented in the table above show the mean hematological responses including RBCs, WBCs, hemoglobin, hematocrit, platelet count, and red cell indices (MCH, MCV, MCHC). Noticeable variations were observed among the treatment groups, indicating the physiological influence of different inclusion levels of potato peel in the diet. The detailed results are provided below.

Table 1

Hematological Parameters of Labeo Rohita Fed Diets with Varying Levels of Potato Peel Inclusion.

Treatment Group	RBCs (10 ⁶ /µL)	WBCs (10 ³ /µL)	Hb (g/dL)	HCT (%)	PLT (10 ³ /µL)	MCH (pg)	MCV (fL)	MCHC (g/dL)
T1R1	2	2.43	1.1	0.14	447	101	50.2	203
T1R2	1.85	2.17	1.21	0.05	402	110	55.3	223
T1R3	2.12	2.69	0.95	0.23	492	92	45.1	183
T2R1	2.55	3.7	3.5	1.76	5339	110	54.1	223
T2R2	2.43	3.36	3.2	1.6	4854	100	49.2	203
T2R3	2.31	3.02	2.9	1.44	4369	90	44.3	183
T3R1	1.5	0.21	0.3	0.05	1234	1.11	44.3	1.27
T3R2	1.6	0.26	0.1	0.06	2240	1.5	51.2	1.33
T3R3	1.7	0.3	0.3	0.07	3133	1.77	55.9	1.66
T4R1	1.95	1.85	0.8	0.09	3310	1.6	50.5	1.9
T4R2	2	2.1	0.95	0.12	3678	1.76	52	1.87
T4R3	2.05	2.2	1	0.11	4046	1.44	53	1.53

RBCs=Red Blood Cells WBCs=White Blood Cells Hb= Hemoglobin HCT=Hematocrit PLT=Platelet count MCH=Mean corpuscular hemoglobin MCV=Mean corpuscular volume MCHC=Mean corpuscular hemoglobin concentration.

In this study, *Labeo rohita* were fed diets containing varying levels of potato peel inclusion: T1 served as the control group (0% potato peel), T2 included 5% potato peel, T3 included 10%, and T4 included 15%. The effects of these dietary treatments on hematological parameters are expressed in Table 4.1. The results reveal a marked influence of potato peel levels on the blood health of the fish.

Red Blood Cells (RBCs): The RBC count was highest in T2 (2.31-2.55 × 10⁶/µL), suggesting enhanced erythrocyte production and improved oxygen-carrying capacity due to the beneficial effects of bioactive components present in potato peel at this level. T1 (control) and T4 (15%) had moderate RBC values, whereas T3 (10%) showed a significant decline (1.5-1.7 × 10⁶/µL), indicating potential hematological suppression or stress.

White Blood Cells (WBCs): WBC counts also peaked in T2 (3.02-3.7 × 10³/µL), indicating a strengthened immune response, likely due to phytochemicals or antioxidants in the potato peel. In contrast, the lowest WBCs were recorded in T3 (0.21-0.3 × 10³/µL), implying weakened immunity, possibly due to anti-nutritional factors at excessive inclusion levels.

Hemoglobin (Hb): The highest hemoglobin values were observed in T2, ranging from 2.9 to 3.5 g/dL, reflecting improved oxygen transport. As ever reduction in hemoglobin was found in T3, with Hb as low as 0.1 g/dL, indicating a possible onset of anemia or impaired hematopoiesis. T1 and T4 showed moderate values, with T4 slightly lower than the control.

Hematocrit (HCT): The highest hematocrit values were also observed in T2, ranging from 1.44 to 1.76%, indicating improved blood volume. T3 showed severe reductions, with HCT values below 0.07%, suggesting the onset of anemia or impaired hematopoiesis. T1 and T4 had moderate values, with T4 slightly lower than control.

Platelet Count (PLT): Platelet levels were significantly elevated in T2 (4369-5339 × 10³/µL), suggesting enhanced blood clotting function and overall physiological health. In T3, the lowest values (1234-3133 × 10³/µL) further

supported the signs of suppressed hematopoietic activity. T1 and T4 remained in the moderate range.

Red Cell Indices (MCV, MCH, MCHC): T2 displayed optimal values for MCV, MCH, and MCHC, indicative of healthy erythrocyte size, hemoglobin content, and concentration. T3 and T4, however, showed extremely low values, particularly T3 (MCH: 1.11-1.77 pg), (MCHC: 1.27-1.66 g/dL) suggesting microcytic, hypochromic anemia and compromised red blood cell integrity.

Table 2

Tukey HSD Post Hoc Comparison of Means for Treatments T1–T4

Comparison (IJ)	Mean Difference (I-J)	Std. Error	Sig. (p-value)	95% Confidence Interval
T1-T2	-0.4400	0.08679	0.004	[-0.7179, -0.1621]
T1-T3	0.3900	0.08679	0.009	[0.1121, 0.6679]
T1-T4	-0.0100	0.08679	0.999	[-0.2879, 0.2679]
T2-T3	0.8300	0.08679	0.000	[0.5521, 1.1079]
T2-T4	0.4300	0.08679	0.005	[0.1521, 0.7079]
T3-T4	-0.4000	0.08679	0.008	[-0.6779, -0.1221]

Interpretation

Tukey's HSD post hoc analysis revealed statistically significant differences in the measured parameter among several treatment groups. A significant decrease ($p=0.004$) was observed in the mean value of T1 (control) compared to T2 (5% PP), indicating that 5% potato peel incorporation significantly elevated the parameter. Conversely, T1 differed significantly from T3 (10% PP) ($p=0.009$), with T3 showing a higher mean, suggesting further elevation at 10% PP inclusion. There was no significant difference between T1 and T4 (15% PP) ($p=0.999$), implying that at higher levels, the effect plateaued or regressed toward control levels. The most pronounced difference was between T2 (5% PP) and T3 (10% PP), with a highly significant increase ($p < 0.001$), reinforcing a dose-responsive trend up to 10%. Significant differences were also noted between T2 and T4 ($p = 0.005$) and between T3 and T4 ($p = 0.008$), suggesting that the 15% PP group (T4) experienced a decline compared to both T2 and T3, potentially due to the adverse effects of higher fiber or anti-nutritional factors in potato peels at excessive inclusion rates.

Figure 1

Comparison of Mean Red Blood Cell (RBC) Counts among Labeo rohita Subjected to Four Dietary Treatments.

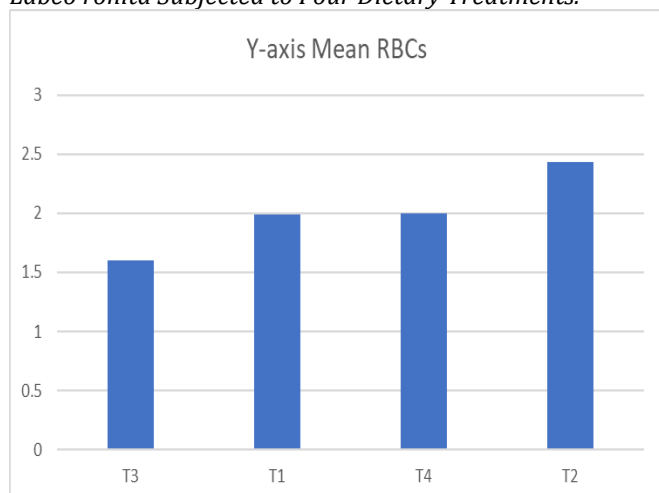


Figure 2

Effect of Dietary Treatments on White Blood Cell (WBC) Counts in Labeo rohita

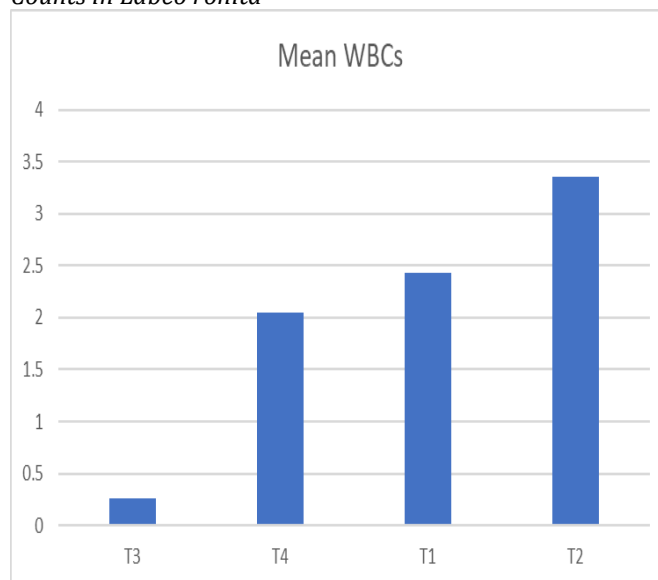


Figure 3

Variation in Mean Hemoglobin (Hb) Concentration Across Different Dietary Groups in Labeo rohita.

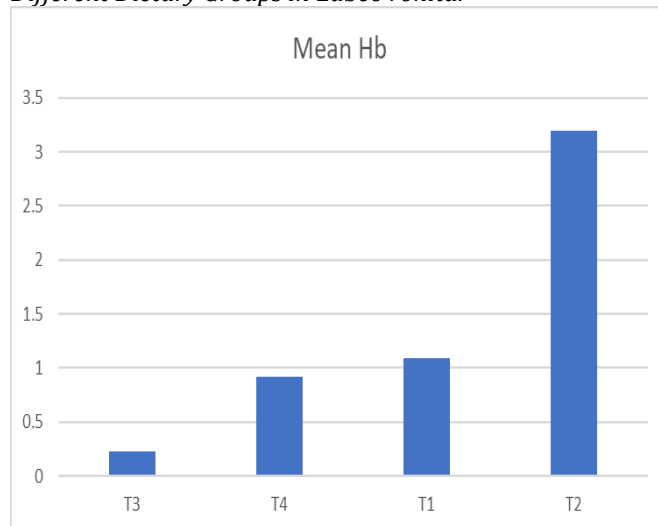


Figure 4

Mean Hematocrit (Hct) Percentages in Labeo rohita Across Dietary Treatments.

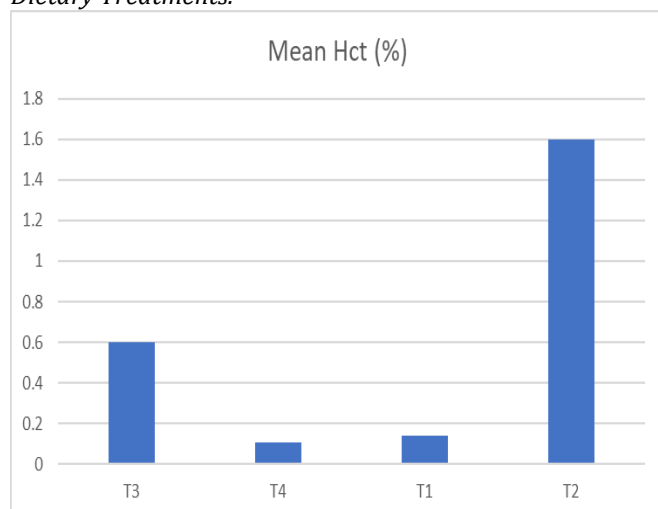
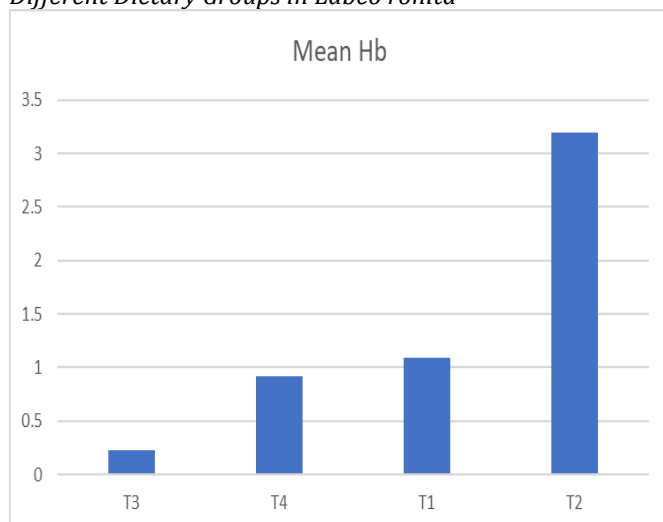
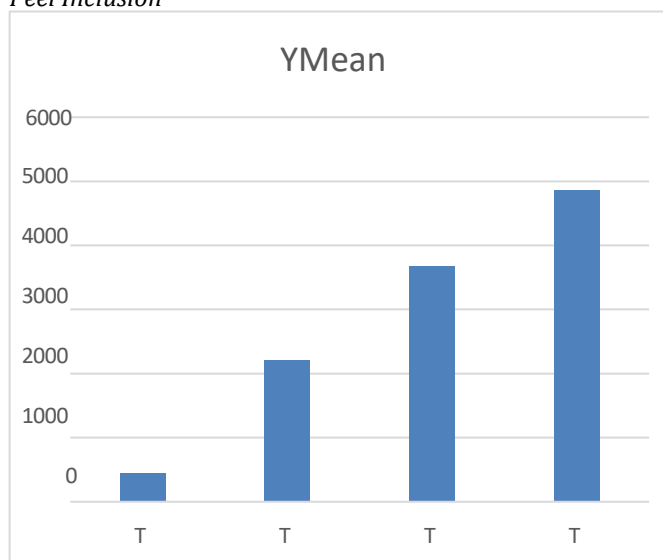


Figure 5

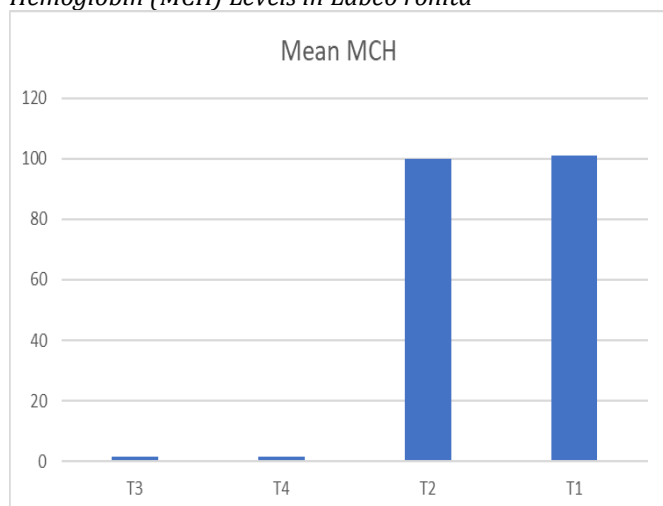
Variation in Mean Hemoglobin (Hb) Concentration Across Different Dietary Groups in *Labeo rohita*

**Figure 6**

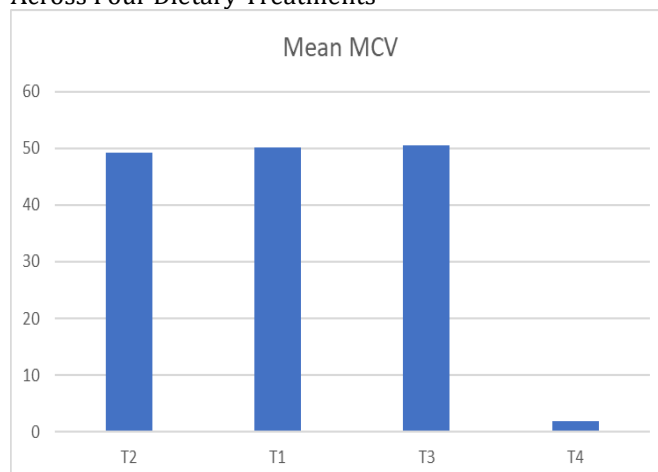
Mean Platelet (PLT) Counts in *Labeo rohita* Across Different Treatment Groups (T1–T4) with Varying Levels of Potato Peel Inclusion

**Figure 7**

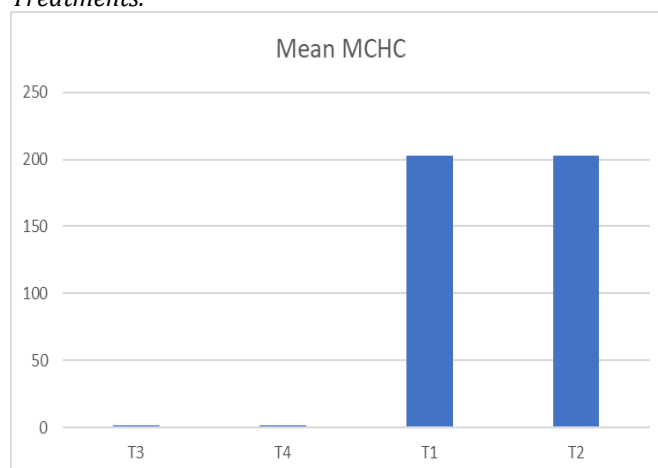
Effect of Dietary Treatments on Mean Corpuscular Hemoglobin (MCH) Levels in *Labeo rohita*

**Figure 8**

Mean Corpuscular Volume (MCV; fL) in *Labeo rohita* Across Four Dietary Treatments

**Figure 9**

Mean Corpuscular Hemoglobin Concentration (MCHC; g/dL) in *Labeo rohita* Fed with Different Dietary Treatments.



The findings of the present study demonstrate that moderate dietary inclusion of potato peel (PP) at 5% (T2) significantly enhances hematological parameters in *Labeo rohita*, while higher inclusion levels of 10% (T3) and 15% (T4) result in pronounced hematological suppression. These outcomes point toward a dosage threshold, beyond which the fibrous and anti-nutritional characteristics of potato peel outweigh its physiological benefits. The dietary modulation of blood indices clearly indicates that potato peel, when included judiciously, can serve as a functional feed ingredient with hematological advantages. Among all the experimental groups, T2 (5% PP) exhibited the most favorable hematological profile. The RBC counts in T2 ranged from 2.31 to $2.55 \times 10^6/\mu\text{L}$, indicating enhanced erythropoiesis and better oxygen-carrying capacity, possibly due to the presence of beneficial phytochemicals and micronutrients in the potato peel at this concentration. Furthermore, WBC count peaked in T2 (3.02 – $3.7 \times 10^3/\mu\text{L}$), highlighting a pronounced immunostimulatory effect. This suggests that moderate potato peel inclusion may contribute to improved immune readiness and systemic defense mechanisms. The group also recorded the highest hemoglobin values (2.9–3.5 g/dL) and hematocrit levels

(1.44–1.76%), reflecting enhanced blood oxygen transport and volume. Platelet levels were also elevated in T2 ($4369\text{--}5339 \times 10^3/\mu\text{L}$), reinforcing the idea that overall hematopoietic and physiological health was optimized at this inclusion level. These results underscore the functional potential of potato peel as a low-cost, nutritionally valuable supplement when incorporated at optimal levels.

This positive response in T2 is in strong agreement with the findings of (Wei *et al.*, 2024), who reported that dietary supplementation with potato starch in *Clarias gariepinus* improved digestive efficiency and overall health without negatively impacting hematological indices. The similarity in response supports the hypothesis that moderate incorporation of tuber-derived ingredients especially in partially processed or digestible forms can favorably impact fish physiology. Additionally, (Baleta *et al.*, 2022) found that *Oreochromis niloticus* (Nile tilapia) fed diets enriched with sweet potato shoot extracts exhibited significant enhancements in RBCs, WBCs, hemoglobin, hematocrit, and platelet values. These findings further corroborate the role of bioactive compounds in tuber crops in promoting hematological and immunological health.

In contrast, higher levels of potato peel inclusion (10% and 15%) were associated with substantial hematological impairments, particularly in T3, where all parameters dropped drastically. In T3, RBC values declined to $1.5\text{--}1.7 \times 10^6/\mu\text{L}$, WBCs dropped as low as $0.21\text{--}0.3 \times 10^3/\mu\text{L}$, and hemoglobin plummeted to 0.1 g/dL. The hematocrit values were also critically low, registering below 0.07%. Such severe reductions point to a state of compromised erythropoiesis, possible anemia, and immune suppression. Red blood cell indices like MCH ($1.11\text{--}1.77 \text{ pg}$) and MCHC ($1.27\text{--}1.66 \text{ g/dL}$) were also significantly lowered, indicating microcytic, hypochromic anemia. This hematological profile suggests not just nutritional inadequacy but also metabolic distress, possibly induced by the accumulation of anti-nutritional factors such as phenolics, oxalates, tannins, and enzyme inhibitors present in unprocessed or poorly digested potato peel.

The moderate responses observed in T1 (control group) and T4 (15% inclusion) further confirm this trend. While these groups did not exhibit the severe suppression seen in T3, they also failed to show the enhanced hematological profiles found in T2. Notably, the T4 group consistently performed slightly below the control group in nearly all hematological measures. This suggests that while some physiological adaptation may occur at higher PP levels, it is insufficient to counteract the adverse effects of excessive fiber and anti-nutrients.

The results of the post hoc Tukey's HSD analysis reinforce this interpretation. Significant differences were found between T2 and all other groups, particularly T2–T3 ($p = 0.000$, MD = 0.8300) and T2–T4 ($p = 0.005$, MD = 0.4300), reflecting T2 distinct hematological advantages. T1 also differed significantly from both T2 and T3, while the

comparison between T1 and T4 was statistically insignificant ($p = 0.999$), emphasizing the suppressive nature of higher PP inclusion levels.

To mitigate such negative effects, the inclusion of other plant-based functional ingredients with known antioxidant and hematological support properties may be beneficial. Helmiati *et al.* (2021) demonstrated that fermented *Moringa oleifera* leaf meal

significantly elevated hematocrit, leukocrit, and phagocytic activity in red tilapia, indicating a potential role in reversing immunosuppressive effects. Similarly, Khattab *et al.* (2024) showed that *Moringa oleifera* based diets improved growth, feed utilization, and gut health, reinforcing the potential synergy of multiple plant-based supplements in aquafeed formulations.

It is also worth noting the importance of dietary protein levels in supporting hematological function. According to (Ahmed and Maqbool, 2017), increasing protein content from 25% to 50% in *Labeo* species led to significant improvements in RBC, Hb, and HCT values. This indicates that the benefits observed in T2 may partly be due to enhanced protein utilization facilitated by the bioavailability of nutrients at this inclusion level. In contrast, the diminished performance at higher PP levels may result from interference with protein digestion and absorption due to excessive fiber and antinutrients. In conclusion, the results of this study confirm that the hematological and physiological benefits of potato peel inclusion in aquafeed are dose-dependent. A 5% inclusion level (T2) offers significant enhancements in blood parameters, immune function, and hematopoietic health, while higher levels (10–15%) exert a suppressive effect, likely due to compromised nutrient bioavailability and increased metabolic burden. These outcomes highlight the necessity of careful inclusion level optimization when using agricultural products like potato peel in aquaculture diets, ensuring nutritional gain without compromising fish health.

CONCLUSION

These feeds are very nutritive and help in the growth of fish. The integration of potato peel as a dietary supplement in *Labeo rohita* aquaculture shows promise for improving blood health and immune resilience, provided it is used at appropriate inclusion levels. This research contributes to the growing body of knowledge aimed at developing nutritionally balanced, health-promoting, and environmentally sustainable aquafeeds. The findings have practical implications for feed formulation strategies, resource recycling, and cost reduction, ultimately supporting the long-term sustainability and productivity of the aquaculture industry.

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