

COMPARATIVE ASSESSMENT OF HAEMATOLOGICAL PARAMETERS AND PROXIMATE COMPOSITION CULTURED AND WILD GOLDEN MAHSEER (TOR PUTITORA)

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Abstract

The present study compared the haematological characteristics and proximate composition of cultured and wild *Tor putitora* and examined the effect of captive rearing on the physiological and nutritional status of this threatened species. Five cultured fish were collected from Garyala Mahseer Hatchery, Attock, whereas four wild fish were collected from the Indus River near Attock, Pakistan. Standard fish haematological procedures were employed for the determination of the haematological parameters and standard AOAC official methods were used for the analysis of the proximate composition. The cultured fish had significantly higher ($P < 0.05$) red blood cell count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration than the wild fish. Although haemoglobin concentration and packed cell volume were higher in culture fish, these differences were not statistically significant ($p > 0.05$), supporting the increased erythropoietic activity and oxygen-carrying capacity of fish under captive conditions. However, the platelet count was significantly higher in the wild fish while the total white blood cells count and the differential leucocyte count did not differ significantly between the two groups. The crude protein content was significantly higher in cultured fish whereas crude fat was significantly higher in wild fish ($P < 0.05$). There was no significant difference in the moisture and ash content. The observed differences were probably associated with variations in feeding regime, environmental conditions and energy allocation between cultured and wild populations. The results provide baseline information on haematological and nutritional parameters for *Tor putitora*, and could be useful for conservation planning, management of hatcheries, stock enhancement and sustainable aquaculture of this threatened species.

INTRODUCTION

The golden mahseer (*Tor putitora*) ranks among the largest freshwater cyprinid species and is characteristically distributed across the river system

of Himalayan region of South Asia, with major population concentrations occurring at altitudes ranging from 400 to 1600 meters above sea level

(Abass *et al.*, 2024). It has been found in Pakistan, India, Nepal, Bhutan, Afghanistan and Myanmar (IUCN, 2015). *Tor putitora* is considered as the “king of freshwater fishes” and “the tiger of the water” due to its beautiful golden colour, large body size, good proximate composition and outstanding sporting qualities (Bhatt and Pandit, 2016; Gupta *et al.*, 2016; Mushtaq *et al.*, 2024). It is a potamodromous species that migrates upstream during the breeding season. Its successful reproduction depends heavily on free-flowing river systems (Bhatt and Pandit, 2016).

In addition to the contribution of golden mahseer to inland fisheries, it plays a significant role in the recreational fishing industry as well as in ecotourism and livelihoods to the local communities of the Himalayas. It is also very cold tolerant and has seen a substantial demand in the market for aquaculture, captive breeding and stock enhancement programmes. It is a widespread species in Pakistan in the Indus River system and its associated tributaries and was earlier reported as *Tor macrolepis* but later studies classified the species as *Tor putitora* (Yousaf *et al.*, 2021). The species is found in free-flowing, well oxygenated, clean streams in warm waters of 10 to 20 °C which promote its normal growth, metabolism and reproduction (Shahi *et al.*, 2014). Changes in conditions in the environment can affect various pieces of physiology, such as oxygen consumption, immune response and energy consumption (Brijs *et al.*, 2015).

The population of *Tor putitora* has significantly dropped in recent decades making it an Endangered species on the IUCN Red List (Jha *et al.*, 2018). The major reasons for this decline have been identified as overexploitation, illegal fishing, habitat degradation, pollution and blocking of the river through dams (Bhatt and Pandit 2016). The slow growth rate, delayed sexual maturity, and low reproductive potential further reduce the species' ability to withstand environmental disturbance. As a result, considerable efforts have been made towards the captive breeding, hatchery production and stock enhancement to support conservation programmes and sustainable aquaculture (Akhtar and Ciji, 2024; Sarma *et al.*, 2024).

While some progress has been made in the understanding of the ecology, culture and reproduction of golden mahseer, there is comparatively little interest in determining the physiological status of the fish in various rearing conditions. Hatchery fish are fed diets that are different than those found in wild fish, and experience significantly different stocking densities and environmental conditions. These differences can have a physiological impact on the performance of the fish and consequently on the quality and viability of the management and restocking programmes. Therefore, reliable physiological indicators are required to assess the similarity between cultured fish and naturally adapted populations.

Haematological evaluation of fish health is one of the most informative methods to evaluate the fish physiological status, immune competence and responses to environmental changes, among the various methods used. Blood parameters are sensitive to changes in environmental parameters, nutrition, disease and management practices, and thus a good indicator of fish health in natural ecosystems and aquaculture systems (Cooke *et al.*, 2013). Routine haematological parameters such as haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and haematocrit (Ht), in addition to the haematological indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), offer important insights into oxygen transport, immune function, and fish physiology (von Tempelhoff *et al.*, 2016; Witeska *et al.*, 2023). Similarly, blood slides stained for various purposes allow for the evaluation of erythrocyte morphology as well as differential counts of leucocytes and other disorders of the blood cells that may be associated with stress, disease or environmental changes (Witeska *et al.*, 2022).

However, a number of biological and environmental factors must be taken into account for the interpretation of haematological variables. Blood parameters can be affected by age, sex, reproductive status, nutritional status, water

temperature, dissolved oxygen, season, feeding regime and stress during handling (Filiciotto *et al.*, 2012; Ahmed *et al.*, 2020). Thus, it is important to establish baseline values and differences between species to ensure accurate health assessment and to identify normal physiological variation from pathological changes (Fazio, 2019). Although there is an increasing interest in conservation of *Tor putitora*, there is a limited amount of comprehensive haematological data available for fish populations in the Indus River system, including comparisons between wild and cultured fish.

Besides the physiological assessment, proximate composition has been extensively employed to assess the nutritional quality and metabolic state of fishes. The relative proportions of moisture, crude protein, crude lipid and ash are indicative of differences in feeding ecology, habitat and growth and energy allocation (Ahmed *et al.*, 2022; Francis *et al.*, 2024). These components are also responsible for the nutritional and commercial values of fish for human consumption. Several factors such as diet, age, environment and rearing practices have been shown to affect the muscle composition (Begum *et al.*, 2016). While cultured fish are fed formulated feeds in relatively stable environments, wild fish tend to feed on a wide range of natural feed resources, and under different conditions of high and low fish mobility may differ in terms of the composition of tissue and proximate composition (Glencross, 2020; Raymond *et al.*, 2020). Hence, the comparison of proximate composition is helpful to understand the nutritional impact of captive reared fish and the overall quality of the reared fish.

While much progress has been made in the study of the biology and ecology of *Tor putitora*, there is limited information available on the physiological and nutritional status of wild and cultured populations. Comparative studies combining haematological parameters with the proximate composition of the muscles are rather lacking in particular, and haematological reference values for the populations of the Indus River system are still poorly documented. This is hindering the

application of blood markers as a regular health assessment tool or for monitoring and evaluation of stock enhancement programmes using blood markers. Moreover, there may be physiological and biochemical variations caused by the differences in environmental conditions, feeding regime and activity between natural habitats and culture systems, which can affect the health of fish, the quality of their flesh and post-release performance. Thus, it is necessary to compare and evaluate the wild versus cultured populations to assess if the cultured populations have similar physiological traits.

In the present study, a comparative study of proximate composition and haematological profile of wild and cultured *Tor putitora* was done. The results yield basic haematological data for this vulnerable species, gain insight into the haematological and nutritional effects of captive production, and create scientific knowledge that can be applied in conservation planning, stock enhancement, hatchery management and sustainable aquaculture development.

MATERIALS AND METHODS

Sample collection and blood sampling

In the present study, a total of nine healthy *Tor putitora* (five cultured and four wild) were used. The total length of fish was about 10 to 14 inches and their weight was about 300 to 500 g. In August, wild fish were caught from the Indus River at the local village of Attock, Punjab, Pakistan, with the help of local fishermen who were using gill nets and mesh nets. The cultured fish were collected from the Garyala Mahseer Hatchery, Attock. Immediately after capture, blood samples were obtained by taking blood from the caudal vein in sterile disposable syringes and placed into EDTA coated vacutainer tubes that prevent coagulation. All the samples were transported in insulated ice boxes at approximately 4°C. Soon after transportation Haematological analyses were carried out at the Laboratory. For each fish, samples of dorsal muscle were taken for proximate composition analysis.

Haematological analysis

Standard methods described by Blaxhall and Daisley (1973) and Wedemeyer and Yasutake (1977) were used for haematological analyses. All the blood parameters (total RBC count and total WBC count) were measured after proper dilution in a standard diluting solution containing sodium citrate, formalin, brilliant cresyl blue and distilled water by using a Neubauer haemocytometer. The estimation of haemoglobin (Hb) concentration was done by the cyanmethaemoglobin method using Drabkin's reagent while the determination of packed cell volume (PCV) was made by the microhaematocrit capillary technique after centrifugation. Thin blood smears were immediately prepared after the collection, air-dried, fixed in methanol and stained with Giemsa stain for the differential leucocyte count. The formulae used by Houston (1990) were used to calculate mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Proximate composition analysis

Proximate composition was analysed by using the Official Methods of AOAC (2019). Moisture content was determined using the oven-drying method at 105°C until a constant weight was achieved (AOAC Method 950.46). The crude protein content was estimated by multiplying the total nitrogen content by a conversion factor of 6.25 using the Kjeldahl method (AOAC Method 981.10) which included acid digestion, steam distillation and titration. The crude fat content was determined by Soxhlet extraction with diethyl ether (920.39) for six hours while the ash content was determined by dry ashing in a muffle furnace at 550°C for five hours (938.08). The proximate composition values were all on a wet-weight basis and reported as percentages.

Statistical Analysis

Independent samples t-test was used in SPSS software to compare the data of haematological

and proximate parameters of wild and cultured *Tor putitora*. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION**Haematological parameters**

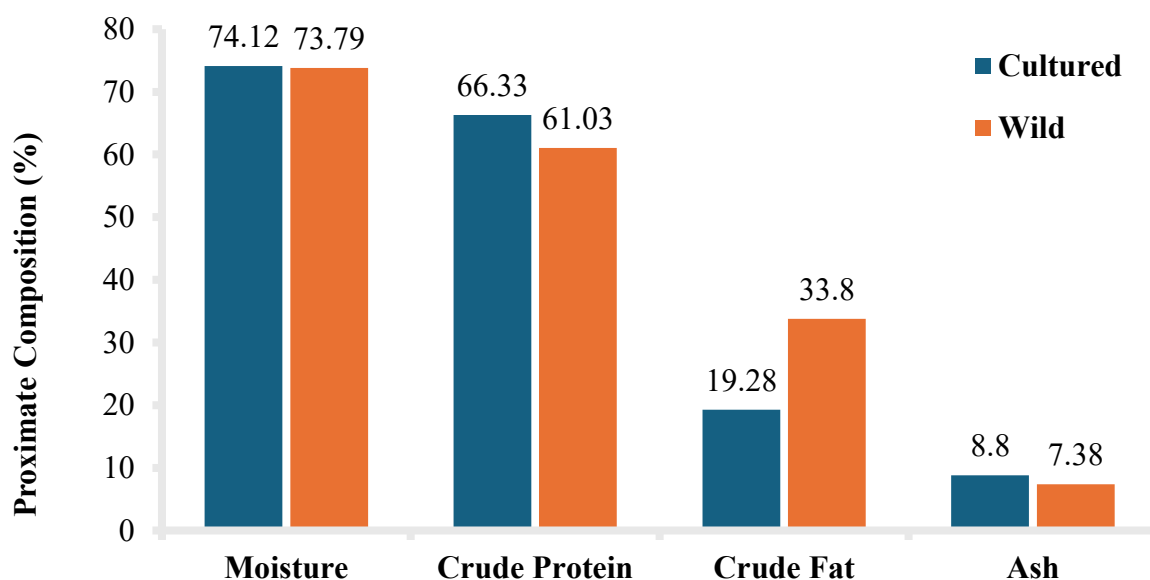
The haematological profile of cultured and wild *Tor putitora* is shown in Table 1. Significant difference between cultured and wild fish were observed mainly in erythrocytic parameters. Cultured fish showed higher mean haemoglobin concentration ($16.92 \pm 3.06 \text{ g dL}^{-1}$), packed cell volume ($34.78 \pm 18.68\%$) than wild fish; however, these differences were not statistically significant ($P > 0.05$). In contrast, red blood cell count, mean corpuscular volume ($191.92 \pm 28.82 \text{ fL}$), mean corpuscular haemoglobin ($44.70 \pm 3.38 \text{ pg}$) and mean corpuscular haemoglobin concentration ($53.38 \pm 3.75 \text{ g dL}^{-1}$) were significantly higher in cultured fish than wild fish ($P < 0.05$). However, platelet count was significantly higher in wild fish than cultured fish ($P < 0.05$). There was no significant difference in total WBC, neutrophils, lymphocytes, monocytes and eosinophils between the cultured and the wild fish ($P > 0.05$), however, slightly higher values of WBC and lymphocytes were recorded in cultured fish and slightly higher percentage of neutrophils in wild fish.

Proximate composition

The proximate composition of the muscle tissue of cultured and wild *Tor putitora* is shown in Figure 1. Moisture content was comparable between cultured ($74.12 \pm 1.94\%$) and wild fish ($73.79 \pm 1.21\%$) and did not differ significantly ($P > 0.05$). Cultured fish contained significantly higher crude protein than wild fish (66.33 ± 0.73 vs. $61.03 \pm 1.10\%$; $P < 0.05$), whereas crude fat was significantly higher in wild fish ($33.80 \pm 4.05\%$) than in cultured fish ($19.28 \pm 3.84\%$; $P < 0.05$). The ash content was numerically higher for cultured fish but the difference was not statistically significant ($P > 0.05$).

Table 1: Haematological comparison between cultured and wild golden mahseer (*Tor putitora*)

Group	Cultured	Wild
Parameters	Mean±SD	Mean±SD
Hb (g dL ⁻¹)	16.92±3.06	13.20±0.35
WBC (×10 ³ cells μL ⁻¹)	86.92±14.13	84.35±9445.45
Platelets (×10 ³ cells μL ⁻¹)	114.00±1.52	255.00±27.39
Neutrophils (%)	27.20±32.73	31.00±12.90
Lymphocytes (%)	68.20±32.95	65.00±12.90
Monocytes (%)	2.20±0.45	2.00±0.00
Eosinophils (%)	2.40±0.55	2.00±0.00
RBC (10 ⁶ cells μL ⁻¹)	1.42±0.35	0.36±0.13
PCV/HCT (%)	34.78±18.68	29.11±0.46
MCV (fL)	191.92±28.82	168.05±9.22
MCH (pg)	44.70±3.38	37.37±1.76
MCHC (g dL ⁻¹)	53.38±3.75	41.97±1.31

Figure 1. Proximate composition of cultured and wild golden mahseer (*Tor putitora*)

In the present study the comparative study of the haematological parameters and proximate composition of muscle of cultured and wild *Tor putitora* was done to assess the effect of rearing environment on the physiological and nutritional status of this endangered species. The results showed significant variations in some haematological parameters and in muscle characteristics, thus indicating that the rearing conditions of a captive environment affect both

the physiological performance and the composition of the tissues. Red blood cell count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were significantly higher in cultured fish than in wild fish. Although haemoglobin concentration and packed cell volume were higher in cultured fish, these differences were not significantly significant, but the platelet count was significantly higher in wild fish. Contrary to this, there were no

significant differences in total leucocyte counts and differential leucocyte percentages between the two groups indicating similar immune status in the two groups under the conditions of the experiment.

The higher haemoglobin concentration, erythrocyte count and packed cell volume observed in cultured fish may indicate improved oxygen transport and erythropoietic activity under hatchery conditions. These parameters are known to be markers of metabolic efficiency and overall physiology due to the fact that they reflect the oxygen carrying capacity of blood to actively respiring tissues. The improved erythrocytic parameters observed in the cultured fish may be attributed to balanced nutrition, regular feeding and more stable environmental conditions, provided in the hatchery. In cultured fish, a similar increase in erythrocytic parameters has been observed under favorable rearing conditions with the provision of sufficient dietary protein and optimum water quality that stimulates the production of erythrocytes and improves the physiological performance (Fazio, 2019; Ahmed *et al.*, 2020; Habib *et al.*, 2021). The comparatively lower values found in wild fishes, however, might reflect higher energetic requirements related to the increased swimming activity, food search and the environmental variability of wild fish.

The enhanced oxygen carrying capacity of hatchery reared fish is further confirmed with significantly high MCV, MCH and MCHC values of cultured *Tor putitora*. These RBC indices give details about the size of the RBCs and the amount of haemoglobin in every cell. Erythrocyte maturation values are generally higher when there is efficient maturation of erythrocytes, and lower when nutritional status is poor and/or there is chronic stress or environmental disturbance. Similar associations between improved haematological parameters and favourable culture conditions have been reported in several freshwater fish species, in which the improvement in culture conditions stimulated the growth of their erythrocytes and haematological stability (Witeska *et al.*, 2023). Based on these observations, it may be inferred that the controlled

hatchery environment promoted normal haematological function.

There were no significant differences between cultured and wild fish in total white blood cell counts or percentages of differential leucocytes. Changes in numbers of white blood cells are often correlated with infection, inflammation or physiological stress and are an important part of the innate immune system. While cultured fish showed a slight increase in lymphocytes, and wild fish showed a slight increase in neutrophils, neither difference was statistically significant. The absence of significant differences suggests that neither population was experiencing severe immunological disturbance at the time of sampling. In fish maintained under favourable environment conditions, the leucocytes profile showed a relative stability, despite the changes in habitat and/or production system (Ahmed *et al.*, 2020; Witeska *et al.*, 2023). However, the comparatively high neutrophil numbers in wild caught fish could reflect greater exposure to natural environmental stressors such as changing water quality and pathogen exposure and handling stress during catch.

The present study revealed interesting results, that is, the number of platelets was significantly higher in wild fish. Fish thrombocytes are involved in homeostasis, and in innate immune defence by phagocytosis and inflammatory reactions. An increase in the number of thrombocytes has often been linked to tissue damage and to the activation of protection mechanisms in response to environmental stress. Thus, the higher number of platelets seen in wild *Tor putitora* could be considered a physiological response to greater physical stress, parasites or fluctuations in environmental conditions found in natural habitats. Thrombocyte abundance has also been shown to increase in fish exposed to environmental stressors and habitat disturbance, which suggests that a role in immune surveillance and tissue repair may also apply to thrombocytes (Fazio, 2019; Witeska *et al.*, 2022). These observations highlight the potential for haematological studies to contribute to understanding the physiological status of

endangered fish stocks and to be a useful monitoring tool for conservation.

The proximate composition of fish muscle serves as an indicator of the nutritional state, feeding history and energy allocation, and hence can be used as an indicator of the nutritional quality of the fish produced under different environmental conditions. The crude protein content was significantly higher in cultured *Tor putitora* while the content of crude fat was significantly higher in the wild fish in the present study. The moisture and ash contents of both groups were not significantly different, which meant that the main nutritional differences were related to protein and lipid metabolism and not to the water or mineral content.

The higher value of crude protein found in cultured fish is probably related to the feeding of nutritionally balanced formulated diets for cultured fish and the favorable culture conditions. The quality of the feed given to the hatchery-reared fish is high and sufficient dietary protein, essential amino acids and energy for tissue growth and muscle development are constantly provided. In such circumstances, nutrients are optimally used to build proteins and not wasted on finding food or adapting to the environment. This has also been observed in the cultured freshwater fishes fed nutritionally balanced feeds, which showed a significant increase in the muscle protein content with the better-quality feeds that led to better feed utilization resulting into increased growth performance (Desta *et al.*, 2019; Francis *et al.*, 2024). The greater protein level in cultured *Tor putitora* is thus indicative of the nutritional conditions available for muscle growth in the current hatchery management.

The crude lipid content, however, was found to be significantly higher in the wild fish than in cultured ones. The lipid reserves are the main source of metabolic energy in fish and are variable, depending on food availability, swimming activity, seasonal factors, and reproductive investment. Wild fish have to deal with fluctuating feeding opportunities and higher energetic requirements of migration, predator avoidance, and foraging behaviour compared to cultured fish. Lipids are

crucial for energy balance and physiological adaptation under these conditions. This is also consistent with previous studies that have noted higher levels of lipid deposition in wild vs cultured fish, which is believed to be due to natural feeding habits, availability of food and adaptable energy storage strategies (Raymond *et al.*, 2020; Glencross, 2020). Hence, the high level of crude fat in wild *Tor putitora* could be a natural adaptation to changing environmental conditions in riverine habitats.

The moisture content in both of the groups was fairly uniform and not significantly different. There is limited variation in muscle moisture unless the fish is nutritionally stressed, dehydrated or under extreme physiological stress. It can be inferred that the muscle tissue hydration level was also stable across production systems, given the similar moisture levels. Similar findings were observed in a few cultured and wild freshwater fishes with no significant changes in moisture content due to feeding regime or habitat conditions (Raymond *et al.*, 2020). Also, no statistically significant difference existed between cultured and wild fish in ash content, indicating that mineral deposition in muscle tissue was relatively similar in both fish groups. Cultured fish showed slightly higher ash content but there were no significant differences in mineral content.

As a whole, it can be concluded from the proximate composition results that the effect of a captive rearing does not have a negative impact on the quality of the flesh. In cultured fish protein content levels are correlated with good nutritional status whereas in wild fish lipid levels are correlated with adaptation to natural environmental factors. The results indicate that *Tor putitora* from hatcheries have desirable nutritional traits and similar tissue composition to those of the wild populations and may be utilized in hatchery and conservation programmes.

CONCLUSION

The present study revealed that the rearing environment plays an important role in influencing the physiological status and proximate composition of *Tor putitora*. Cultured golden

mahseer exhibited higher RBC count, MCH and MCHC than wild fish whereas haemoglobin concentration and PCV showed no significant differences. Wild fish showed significantly higher platelet count and lipid content, whereas the difference in neutrophil percentage was not significantly significant. These facts imply that the fish population which are found in the wild develop a physiological response to cope with the environment and food availability. The proximate analysis revealed significantly higher protein content in cultured fish, whereas moisture and ash content did not differ significantly between two groups.

DECLARATIONS

Authors contribution

S.R. and M.K. conducted the research, performed laboratory work and prepared the manuscript. M.S.N. and M.Z.A. supervised the research and guided the statistical analysis. N.A. conducted the sampling.

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Ethical Statement

Ethical approval was not required for this study.

Conflict of interest

Authors declare there is no conflict of interest.

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