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Research article

Haematological profile, blood cell characteristic and serum biochemical composition of cultured brown trout, *Salmo trutta fario* with respect to sex

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ABSTRACT

The purpose of this research is to see how sex affects several haematological, biochemical and blood cell morphology in cultured brown trout, *Salmo trutta fario*. Different haematological parameters, for instance haemoglobin (Hb) concentration, haematocrit (Hct), red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and serum biochemical parameters such as glucose, cholesterol, total protein, albumin, globulin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), sodium, potassium and phosphorus were analysed. Throughout the study period, male fish had considerably greater Hb content, RBC count and Hct content than female fish, but insignificant ($P > 0.05$) difference in WBC count, differential leucocyte count, MCV, MCH and MCHC was seen between the two sexes of *Salmo trutta fario*. The majority of biochemical markers showed no significant ($P > 0.05$) difference, with the exception of AST, ALP, sodium, potassium, and phosphorus, which showed a significant ($P < 0.05$) difference. Under light microscopy, RBC and differential leukocyte count (neutrophils, lymphocytes, and monocytes) as well as thrombocytes were characterised and discriminated, revealing that the majority of the cells were normal and intact in shape. As a result of the foregoing findings, it is suggested that the data generated be used to identify the health state of fish and to determine the occurrence of various clinical and subclinical disorders, which might assist in enhancing the overall fish population production.

1. Introduction

Global aquaculture is currently one of the fastest expanding sectors of food production, accounting for 50% of total food supply (FAO, 2018; Okocha et al., 2018; Ibrahim et al., 2020). Fish aquaculture, also known as aquafarming, is quickly growing in popularity and has a significant economic influence around the world. Because of its high nutritional quality and biologically active chemicals with good effects on human health, fish is consumed by a wide range of individuals, regardless of their income, age, or health (Gormley, 2006; Lund, 2013). The haematological and biochemical profile of fish is a useful tool for determining the well-being position of various aquatic organisms, together with both farmed and wild fish (Adel et al., 2016; da Silva Correa et al., 2017; Zhao et al., 2018; Sheikh and Ahmed, 2019; Fazio, 2019; Sidiq and Ahmed, 2020; Jan and Ahmed, 2021; Jan et al., 2021), because these indices provide valuable information for learning about fish reactions to stress, contaminants, hypoxia, nutrition, and habitat, as well as ecological and physiological circumstances (Cnaani et al., 2004; Caruso et al., 2005; Walencik and Witeska, 2007; Faggio et al., 2014; Ahmed and Sheikh, 2019, 2020; Ahmed et al., 2019; Fazio, 2019; Suljevic and Mitrasinovic-Brulic, 2020). Age, diet, sex, fish species and strains, sexual maturity cycle, stocking density and feeding regime, seasonal variations, photoperiod, nutritional state, geographical location, disease, physico-chemical variations, temperature and salinity, sampling conditions, anaesthesia type and laboratory techniques, handling and transport, blood collection, handling, storage time of blood samples, anticoagulants used can strongly influence the results obtained from a haematological and biochemical analysis (Adeyemo et al., 2009; Ferri et al., 2011; Gul et al., 2011; Jeronimo et al., 2011; Witeska and Wargocka, 2011; Faggio et al., 2013; Fazio et al., 2013, 2014; Witeska et al., 2015; Cieplinski et al., 2019; Ahmed et al., 2020; Jan et al., 2021). Erythrocytes, one of the major blood constituent, could be utilised to make a diagnosis of anaemia and identify various strategies in fish populations in terms of metabolic oxygen demand (Wilhelm Filho et al.,

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1992). Furthermore, erythrocytes are considered as the primary signal for many adaptive physiological methods in response to different environmental changes (Val et al., 1992). Blood cell characteristics are thought to be a good tool for evaluating the physiological state of fish and the environment in which they reside (Li and Wang, 1995; Zhou et al., 2003; Zhang et al., 2011; da Silva Correa et al., 2017). This blood cell count method has been used in fish farms for a variety of purposes, including medicine administration, parasite infection, pollution, and toxicity (Dias et al., 2011; Ventura et al., 2015; da Silva Correa et al., 2017).

Brown trout, *Salmo trutta fario,* a member of the Salmonidae family, can be found in inland waters in North America, New Zealand, Southern Europe, and Western Asia (Rawat et al., 2011). In the year 1900, trout was introduced to Kashmir (Mitcheli, 1918). Brown trout is currently thriving in the snow-fed streams of Kashmir, and it is the most well-known freshwater fish found in the Lidder, Sindh, Vishaw, Bringi, Ferozpora, and gushing streams. In Jammu and Kashmir, salmonid fish is the most important aquaculture product. Fish housed in hatcheries are primarily used to restock natural populations that are targeted by recreational anglers (Frank-Gopolos et al., 2015). Hatcheries must ensure that the animals employed in the hatching practice are well cared for. As a result, managing the physiological situation of brood stock fish is extremely important (Wedemeyer, 2002; Cieplinski et al., 2019). Blood tests are the simplest and most comprehensive approach to determine animal's health. During the study period, we decided to examine the impact of sex on fundamental haematological, serum biochemical including blood cell characteristics of farmed mature *S. trutta fario*.

2. Materials and methods

2.1. Collection of fish samples

A total of 38 adult brown trout, *Salmo trutta fraio* were furnished by the Dachigam, Laribal, Srinagar: State Government of Fishery Department Hatchery (J&K) for carrying out the current experimental work. Fish were kept in a concrete rectangular raceway and were fed with commercial pellets before the experiment began. Both male and female samples were deemed healthy i.e. bearing no symptoms of deformities or infestations externally. Preceding blood sampling, the fish were moved to a tank and anaesthetized (MS-222 @ 0.3 g/L of water) (Iaria et al., 2019). With 26 gauge plastic syringes having no coagulant, the blood was drawn from the caudal vein and transferred to collecting vials containing lithium heparin which were available commercially. The samples of blood were promptly examined for various haematological parameters after being deposited in lithium heparin anticoagulant vials. The remaining blood samples were transferred to anticoagulant-free eppendorf tubes for the process of serum collection and thereafter biochemical parameter analysis.

2.2. Gender identification and biometric parameters

As indicated in Figure 1, both genders mean length and body weight were measured. *Salmo truttra fario* sexes were determined by looking at the mouth. Male trout have a longer elongated snout and lower jaw with kype, whereas female trout have a small round upper jaw (hooked lower jaw).

2.3. Physico-chemical parameters

Water chemistry parameters of the Laribal hatchery, for instance, pH, water temperature, dissolved oxygen and dissolved free carbon dioxide were calculated by making the use of standardized methods (APHA, 1998) Figure 2.

2.4. Examination of haematological profile

The cyanmethemoglobin technique was used to determine haemoglobin concentration (Lavanya et al., 2011). By making the use of an upgraded Neubauer haemocytometer and Natt-Herrick's diluent, total erythrocyte and leukocyte count was performed (Natt and Herrick, 1952). The amount of corpuscular count was measured according to Pal et al. (2008) and Parida et al. (2012). The total RBC count per mm^3 was calculated as $200 \times 50 \times N = 10{,}000 \times N$ (N = total number of calculated RBC, dilution factor = 200), and the total WBC count per mm^3 was calculated as $20 \times 1 \times L/0.4$ cells $= 50 \times L$ (L = total number of calculated WBC, dilution factor = 50). The amount of haematocrit was determined as per Adebayo et al. (2007). Micro-haematocrit capillaries

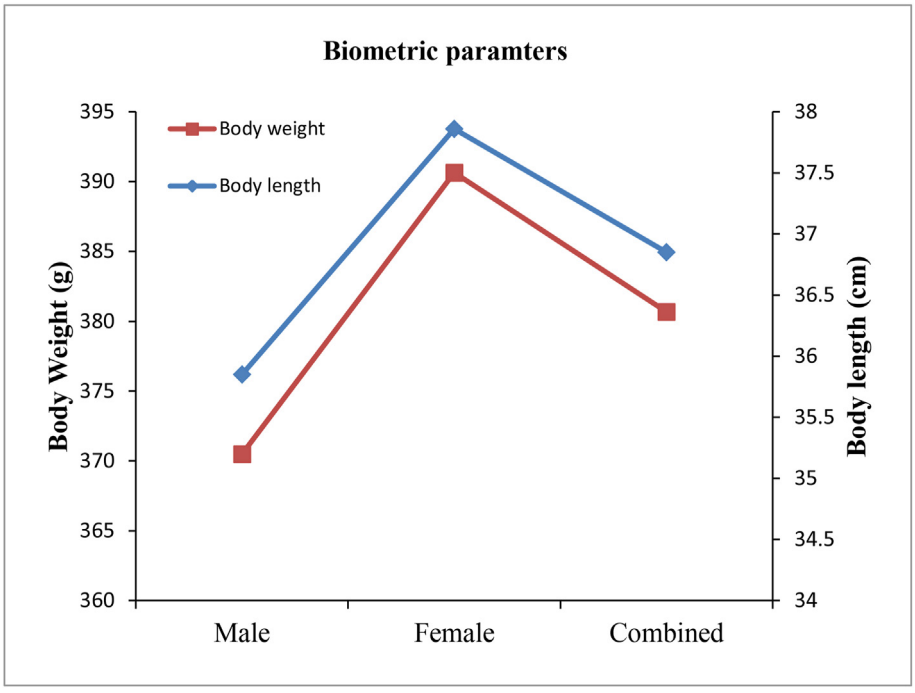


Figure 1. Biometric parameters (Length × weight) of male and female *Salmo trutta fario*.

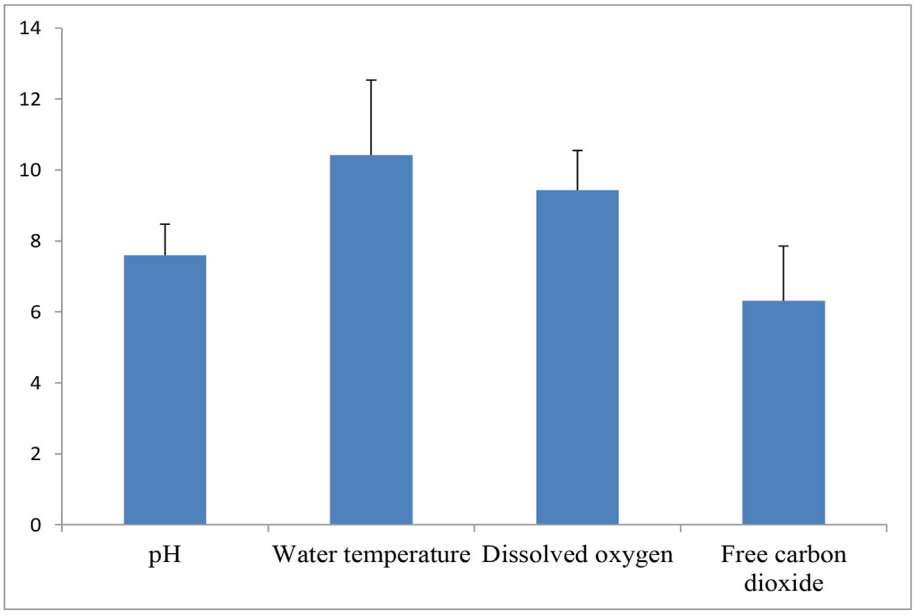


Figure 2. Physico-chemical characteristics of Laribal hatchery.

were used for the computation of Hct content, spun at 12,000 rpm for 5 min in a micro-haematocrit centrifuge (REMI RM-12C BL, India) with the results represented as percentage. Dacie and Lewis (1991) equations were used to calculate erythrocyte indices.

2.5. Serum biochemical parameter estimation

Aside from haematological testing, serum was collected through centrifugation for 5 min at 5000 g for biochemical testing. Using the Vet scan biochemistry analyzer (VS2 USA), the serum parameters such as glucose (mg dL^{-1}), cholesterol (mg dL^{-1}), total protein (g dL^{-1}), albumin (g dL^{-1}), globulin (g dL^{-1}), alanine aminotransferase (IUL^{-1}), aspartate aminotransferase (IUL^{-1}), alkaline phosphate (IUL^{-1}), sodium, potassium and phosphorus were evaluated.

2.6. Differential leukocyte count and blood cell size measurement

Smears of blood of four for each specimen of fish were made instantly utilising a precise volume of blood from every vial of blood sample for the morphological investigation of blood cells. The blood smears were air dried before being preserved for 1 min in absolute methanol and stained properly with Wright-Giemsa and Toluidine blue. The stained smears were examined on a Leica DM 7 microscope with Las 4.12 software for the evaluation of blood cell characteristics at 1000 x magnification, and then accordingly the photos were obtained.

2.7. Statistical analysis

Microsoft Excel was used to sort the experimental data, which was then processed and analysed using SPSS 20 statistical software. To investigate the difference in haematological, biochemical parameters and blood cell characteristics between genders, the data was summarised as mean, standard deviation, and difference between sexes were statistically assessed using the students t-test. Results with a level of significance ($P < 0.05$) were judged as significant.

3. Results

Haematological parameters, for instance, haemoglobin, total erythrocyte and leukocyte counts, haematocrit, and erythrocyte indices; mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration including differential leucocyte count; lymphocytes, thrombocytes, neutrophils, and monocytes were investigated. Table 1 shows significant ($P < 0.05$) differences in major haematological parameters of *Salmo trutta fario*. All through the study period, male fish had significantly ($P < 0.05$) greater values of haematological parameters such as haemoglobin concentration (Hb), total erythrocyte count (RBC), and haematocrit (Hct), but insignificant ($P > 0.05$) difference in total white blood cell count, differential leucocyte count, mean corpuscular haemoglobin (MCH), and haematocrit (Hct) was noted.

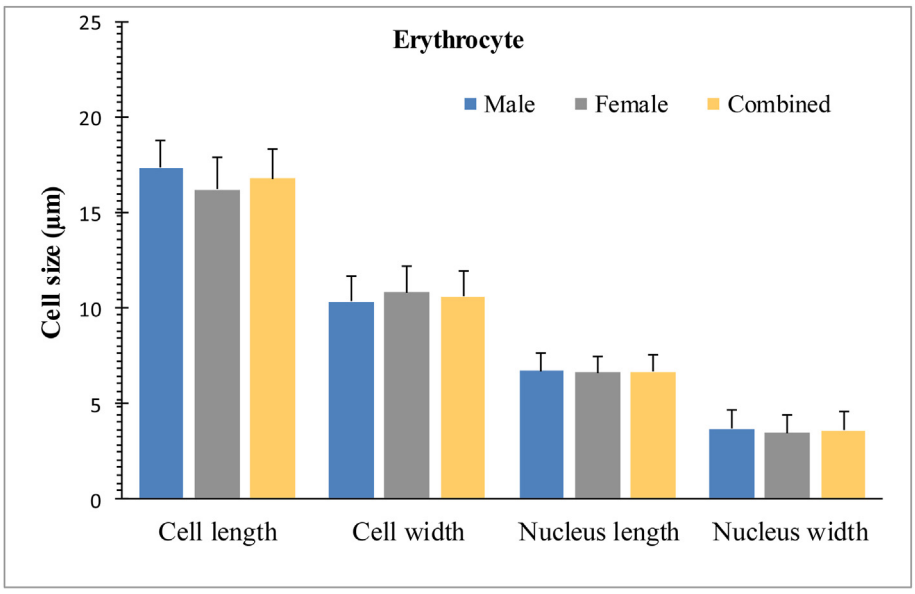
3.1. Morphology and differential blood cell count

The erythrocyte size and blood cell dimensions of both sexes of *Salmo trutta fario* is presented in Figures 3, 4, 5, 6, and 7. The study revealed that erythrocytes (RBC), leucocytes and thrombocytes including neutrophils, lymphocytes and monocytes were the most common blood corpuscles recorded in the blood of *S. trutta fario* as depicted in Figure 8. The differences in blood cell sizes between the two sexes of *S. trutta fario* showed

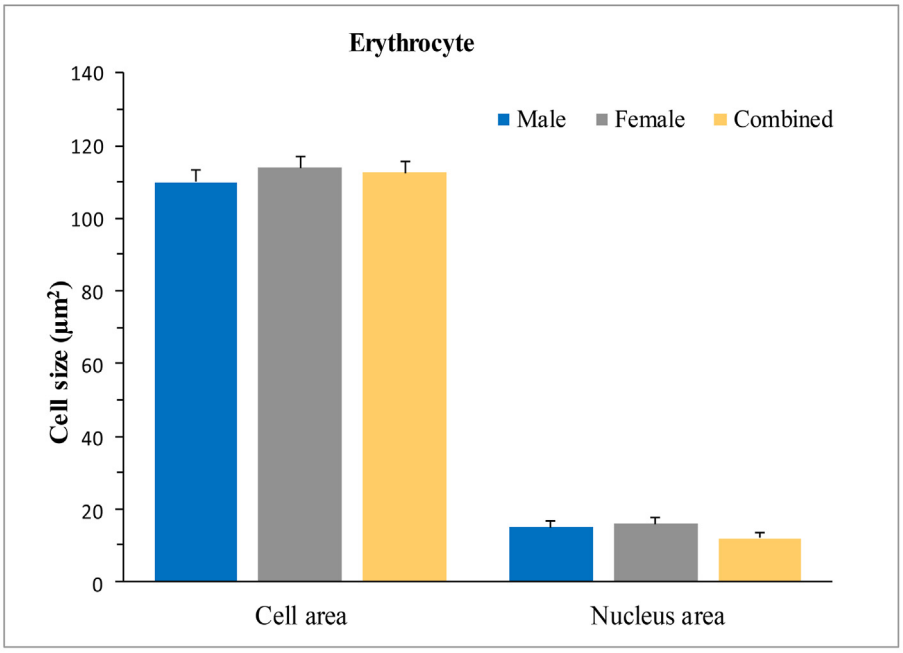
Table 1. Hematological parameters of cultured brown trout, *Salmo trutta fario*.

Parameters	Male	Female	Combined
Hb (g/dL)[1]	10.68 ± 1.53^{a}	9.18 ± 0.94^{b}	9.93 ± 1.47
RBC ($\times 10^{6}$ mm^{-3})[2]	1.89 ± 0.14^{a}	1.58 ± 0.36^{b}	1.74 ± 0.32
WBC ($\times 10^{3}$ mm^{-3})[3]	3.06 ± 0.64a	$2.\ 83 \pm 0.71^{a}$	2.95 ± 0.68
Lymphocytes (%)	45.31 ± 2.11^{a}	43.69 ± 1.88^{a}	44.50 ± 2.0
Thrombocytes (%)	32.16 ± 1.82^{a}	33.76 ± 1.13^{a}	33.00 ± 1.48
Neutrophils (%)	14.68 ± 1.62^{a}	15.87 ± 1.43^{a}	15.29 ± 1.52
Monocytes (%)	7.84 ± 0.82^{a}	6.86 ± 0.72^{a}	7.40 ± 0.78
Hct (%)[4]	38.47 ± 3.09^{a}	32.02 ± 2.31^{b}	35.25 ± 4.23
MCH (pg)[5]	56.28 ± 4.82^{a}	54.62 ± 5.91^{a}	55.46 ± 5.04
MCHC (%)[6]	29.10 ± 3.19^{a}	27.19 ± 2.74^{a}	28.15 ± 3.09
MCV (fL)[7]	169.67 ± 12.93^{a}	167.09 ± 10.83^{a}	168.38 ± 11.85

[1] Haemoglobin (Hb),
[2] Red blood cell (RBC) count, [3]White blood cell (WBC) count,
[4] Haematocrit, (Hct),
[5] Mean corpuscular haemoglobin (MCH),
[6] Mean corpuscular haemoglobin concentration (MCHC).
[7] Mean corpuscular volume (MCV), *Mean values in a rows sharing the same superscript are not significantly different ($P > 0.05$).



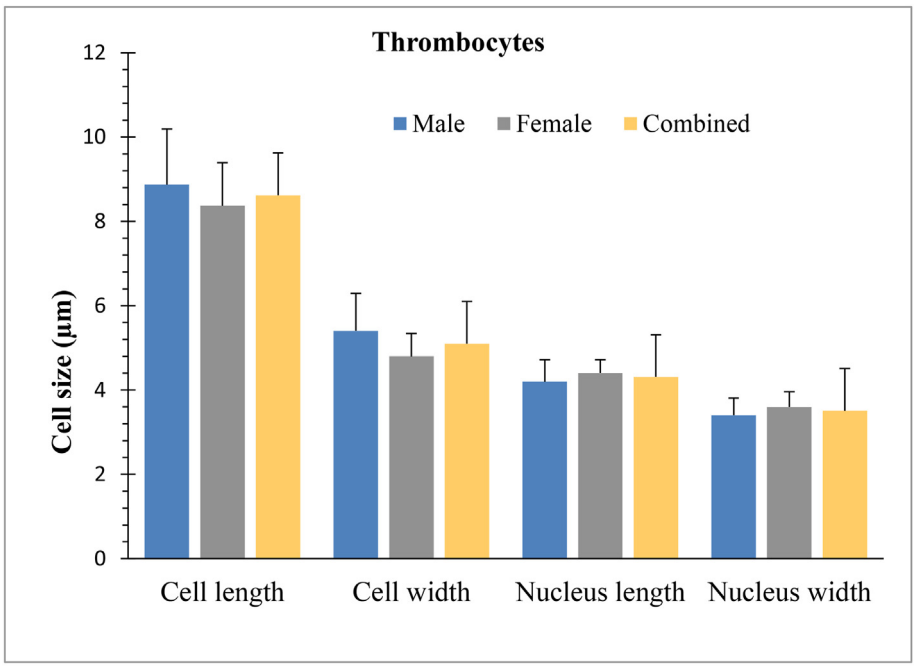
A



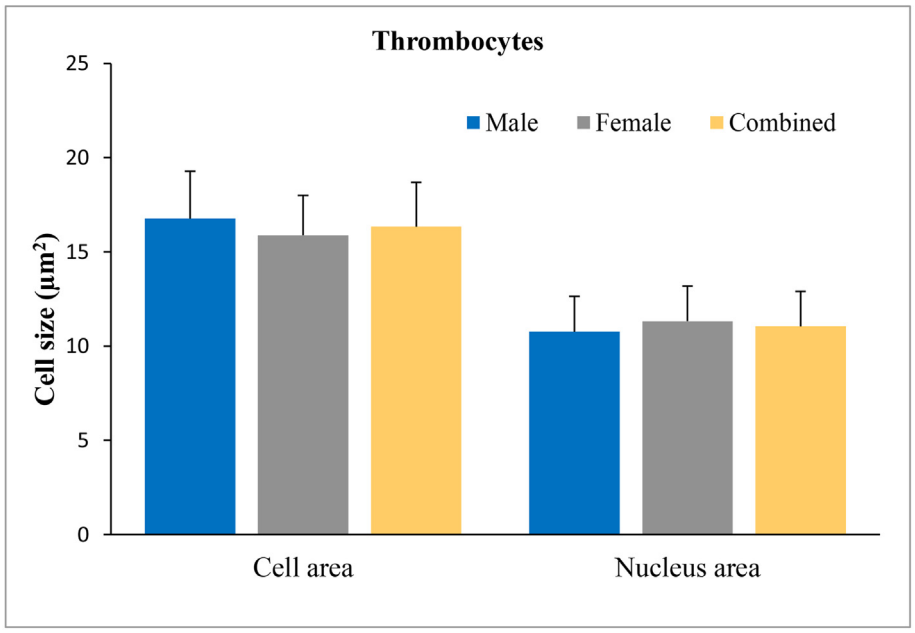
B

Figure 3. A, B cell size and area of erythrocyte from different genders.

no significant ($P > 0.05$) difference except erythrocyte during the current investigation. The adult erythrocytes of both male and female *S. trutta fario* were almost elliptical in shape, with a blue stained centrally positioned nucleus including purple stained homogeneous cytoplasm. The immature erythrocytes, on the other hand, were found to be spherical in shape, smaller in size and lower in number compared to mature erythrocytes, with an oval to round nucleus and mildly purple stained coloured cytoplasm. Similarly, no morphological changes in thrombocytes were seen in both sexes of *S. trutta fario*. Thrombocytes were fusiform, circular, and elliptical in shape, with nuclei that took on the shape of the cells, however kidney-shaped nuclei were detected on rare occasions. In both sexes of *S. trutta fario*, such cells existed alone or in groups and had a bigger nucleus with chromatin clusters and pink stained cytoplasm using Wright-Giemsa stain. Neutrophils, on the other hand, are generally spherical or irregular in shape, with a purple stained nucleus that is placed centrally or eccentrically, and a band or segment shaped nucleus (bilobed, trilobed, tetralobed). Extremely little pale pink or purple granules were seen in the cytoplasm. Lymphocytes were the most prevalent cell type after erythrocytes, with a centrally located nucleus that is dark purple coloured and oval in shape, with the cytoplasm taking up the majority of the cell's area. On the basis of relative amounts of cytoplasm and diameters, two types of lymphocytes were identified: small and giant lymphocytes. The small as well as the larger lymphocytes had a nucleus which took up the majority of the cell area with cytoplasm being reduced to periphery of the cell. On its overall body surface, lymphocytes occasionally expanded into pseudopodia. The monocytes were oval to round in form, with kidney-shaped eccentrically positioned nuclei. These cells have a bluish green cytoplasm with neither acidophilic nor basophilic



C



D

Figure 4. C, D cell size and area of Thrombocytes from different genders.

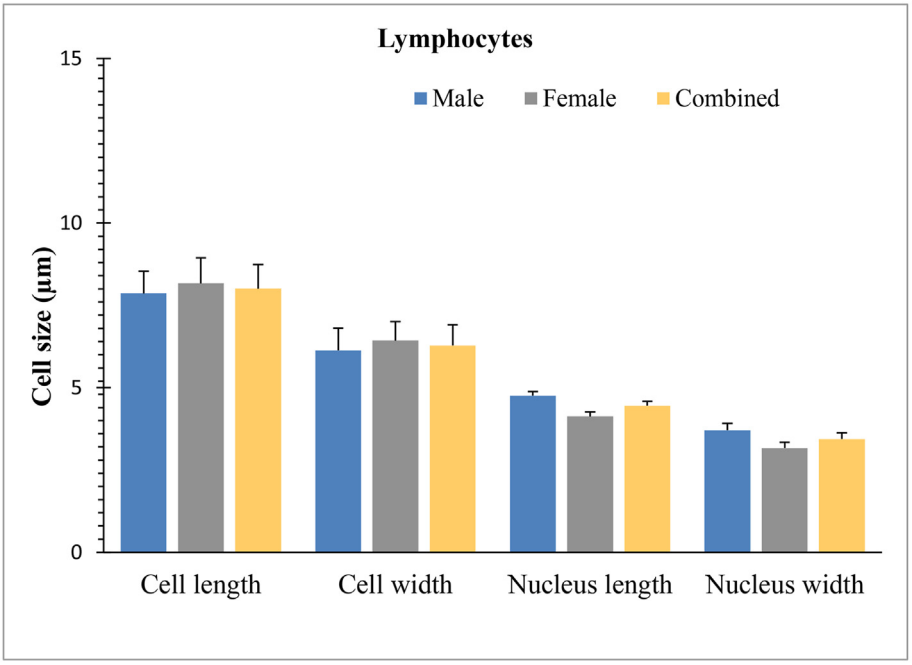
granules. Vacuolated monocytes were uncommonly found. Due to the absence of characteristic granules, monocytes were clearly separated from neutrophils in the current investigation under a light microscope.

3.2. Size and area of peripheral blood cells

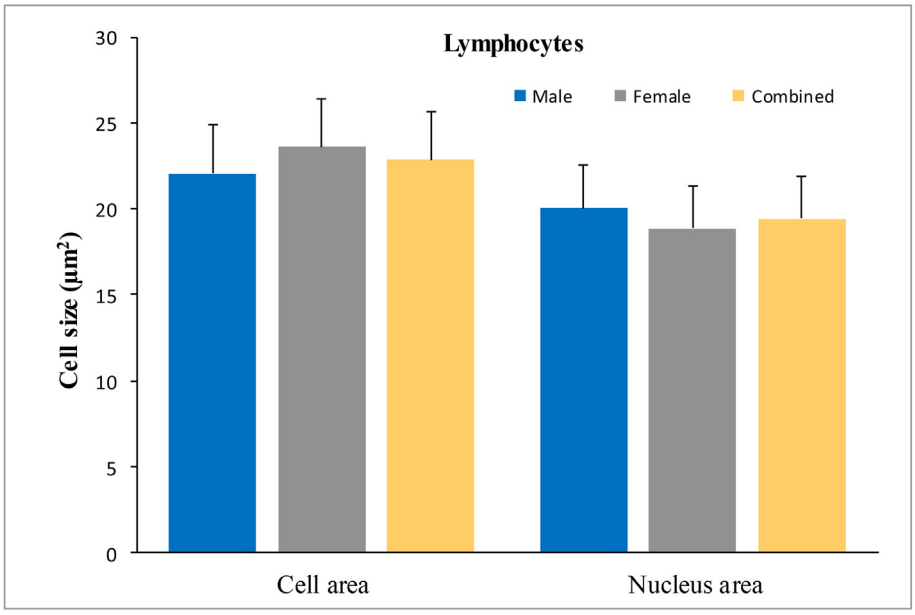
We measured and compared the size of each cell type in male and female *Salmo trutta fario*, including erythrocytes, lymphocytes, neutrophils, monocytes and thrombocytes. With the exception of erythrocytes, no significant ($P > 0.05$) differences in size across genders have been observed.

3.3. Serum biochemical parameters

Table 2 shows the sex-specific blood biochemical characteristics of *Salmo trutta fario*. The majority of biochemical markers showed no significant ($P > 0.05$) change, except for aspartate aminotransferase (AST), alkaline phosphatase (ALP), sodium, potassium and phosphorus, which showed a significant ($P < 0.05$) difference.



E

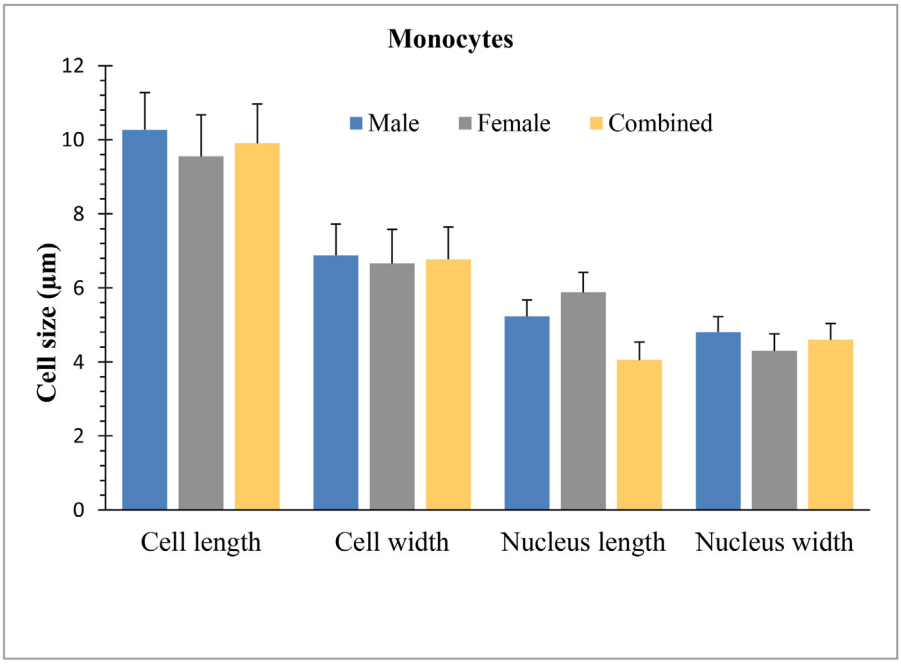


F

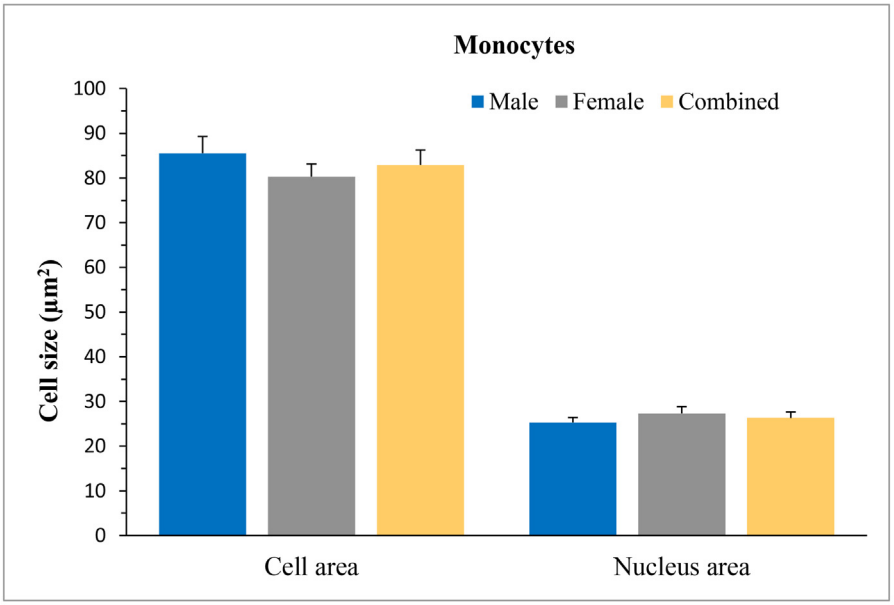
Figure 5. E, F cell size and area of lymphocytes from different genders.

4. Discussion

Fish are the largest and most diversified species of aquatic organisms because they live in close proximity to their aquatic environment, and any changes in the ecology are promptly reflected in the blood of fish (Jan et al., 2021). Various investigators and researchers have discovered an increasing trend related to the study of haematological and serum biochemical characteristics of fish blood to evaluate the overall health position of fish in recent years, since such blood indices offer dependable knowledge on chronic symptoms, metabolic disorders and deficiencies, before they manifest in the environment (Sheikh and Ahmed, 2019). *Salmo trutta fario*, a very active fish, has sufficient values for all major haematological markers, according to the current study (Hb, Hct, RBC, WBC, MCV, MCH and MCHC). Several researchers discovered that active fish have greater levels for almost all haematological markers (Ahmed et al., 2020). Aside from fish activity, it has been discovered that sex plays a significant effect on the haematological parameter values of fish (Gabriel et al., 2004; Akinrotimi et al., 2007; Khadjeh et al., 2010; Ahmed et al., 2019; Jan and Ahmed, 2021; Jan et al., 2021). Significantly ($P < 0.05$) all main haematological indices such as Hb, Hct, and RBC were greater in case of males of *S. trutta fario* than females, with the exception of leucocyte (WBC) count that was recorded to be higher in case of females. Our findings are consistent with those of various other researchers that studied fish species such as *Tor putitora* (Kapila et al., 2000), *Onchorhynchus mykiss* (Rehulka et al., 2004), *Rutilus kutum* (Nikoo et al., 2010), *Clarias batrachus* (Acharya and Mohanty, 2014), *Cirrhinus mrigala* (Pradhan et al., 2014), *Sander lucioperca* (Zakes et al., 2016), *Barilius bendelisis* (Sharma et al., 2017), *Hypophthalmichthys molitrix* (Ahmed



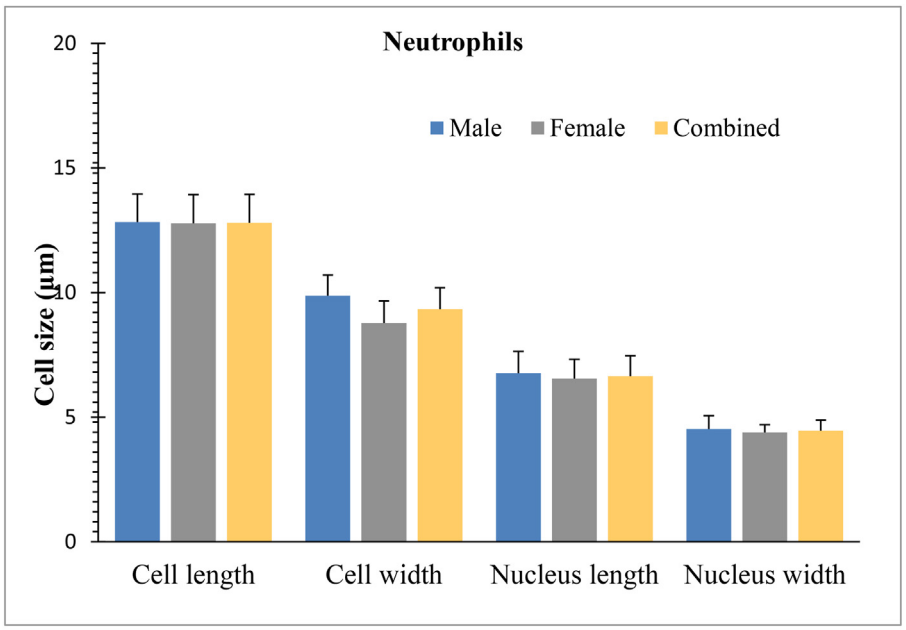
G



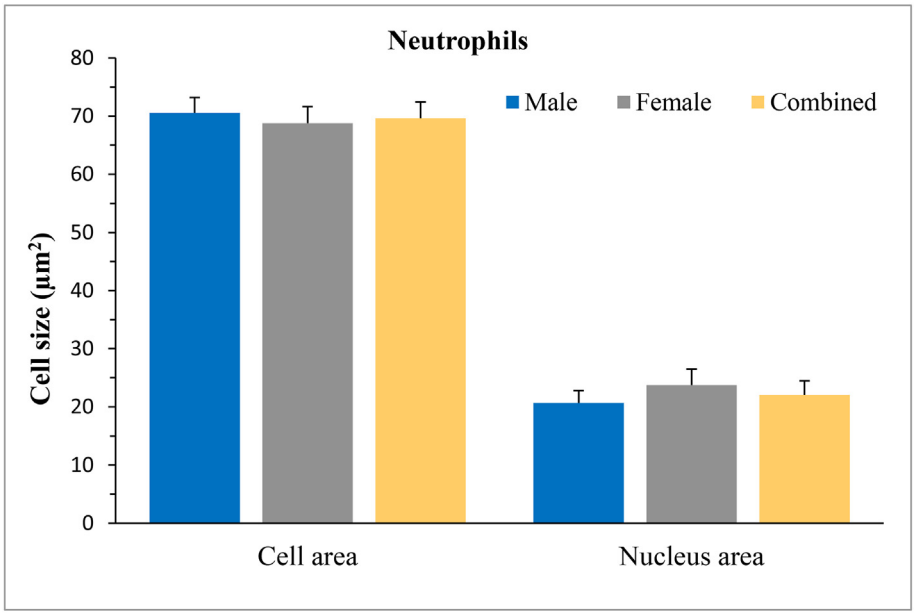
H

Figure 6. G, H cell size and area of monocytes from different genders.

et al., 2019), *Schizothorax plagiostomus* (Sheikh and Ahmed, 2019), *S. labiatus* (Jan and Ahmed, 2021) and *S. labiatus* (Jan et al., 2021). According to Zakes et al. (2016), the hormone erythropoietin is accountable for enhanced haematological parameters in case of males. The differences in numerous blood cell components associated to sex can also be attributable to the male fish's higher metabolic rate (Sharma et al., 2017). In contrast, the females of *S. trutta fario* exhibited higher levels of WBC count compared to males that agrees with the studies on other different fish species, for instance, *Labeo rohita* and *T. putitora* (Kapila et al., 2000), *C. batrachus* (Acharya and Mohanty, 2014), *Heterobranchus longifilis* (Suleiman et al., 2016), *B. bendelisis* (Sharma et al., 2017), *Rhinogobio ventralis* (Zhao et al., 2018), *H. molitrix* (Ahmed et al., 2019),



I



J

Figure 7. I, J cell size and area of lymphocytes from different genders.

S. plagiostomus (Sheikh and Ahmed, 2019), *S. labiatus* (Jan and Ahmed, 2021) and *S. labiatus* (Jan et al., 2021). Female fish with a greater WBC count have the capability to react rapidly to the multiple changes occurring in the medium as a result of xenobiotic transformation, implying that female fish possess a higher tolerance to deal with harmful toxic stress compared to male fish (George and Akinrotimi, 2017). Other researchers have observed similar findings (Satheeshkumar et al., 2012; Sharma et al., 2017; Ahmed et al., 2019; Jan et al., 2021). Erythrocyte indices such as MCV, MCH and MCHC are based on the amount of Hb occurring in the blood periphery and can aid in the measurement of erythrocyte size. The current study found insignificant ($P > 0.05$) difference in case of erythrocyte indices within two sexes of *S. trutta fario*, which is consistent with previous findings (Zhao et al., 2018; Sheikh and Ahmed, 2019; Jan and Ahmed, 2021; Jan et al., 2021).

In addition to haematological parameters, information on biochemical parameters is critical for evaluating the well-being of fish, as majority of pathological indicators arise in the plasma prior to occurrence of clinical diseases (Sheikh and Ahmed, 2019). As a result, biochemical markers play an important role in assessing and detecting disease-related issues in fish (Ahmed et al., 2019). There were insignificant ($P > 0.05$) differences in glucose, cholesterol, total protein, albumin, globulin and ALT values between the sexes in this investigation. Several researchers have already experimented in numerous fish species and found that the various metabolic parameters do not differ considerably between sexes (Khajevand et al., 2007; Ahmed et al., 2019). Although the plasma glucose level in males of *S. trutta fario* was found to be greater than in females in our study, many other investigators have also reported similar findings (Charoo et al., 2013; Zakes et al., 2016; Kulkarni, 2017; Zhao et al., 2018;

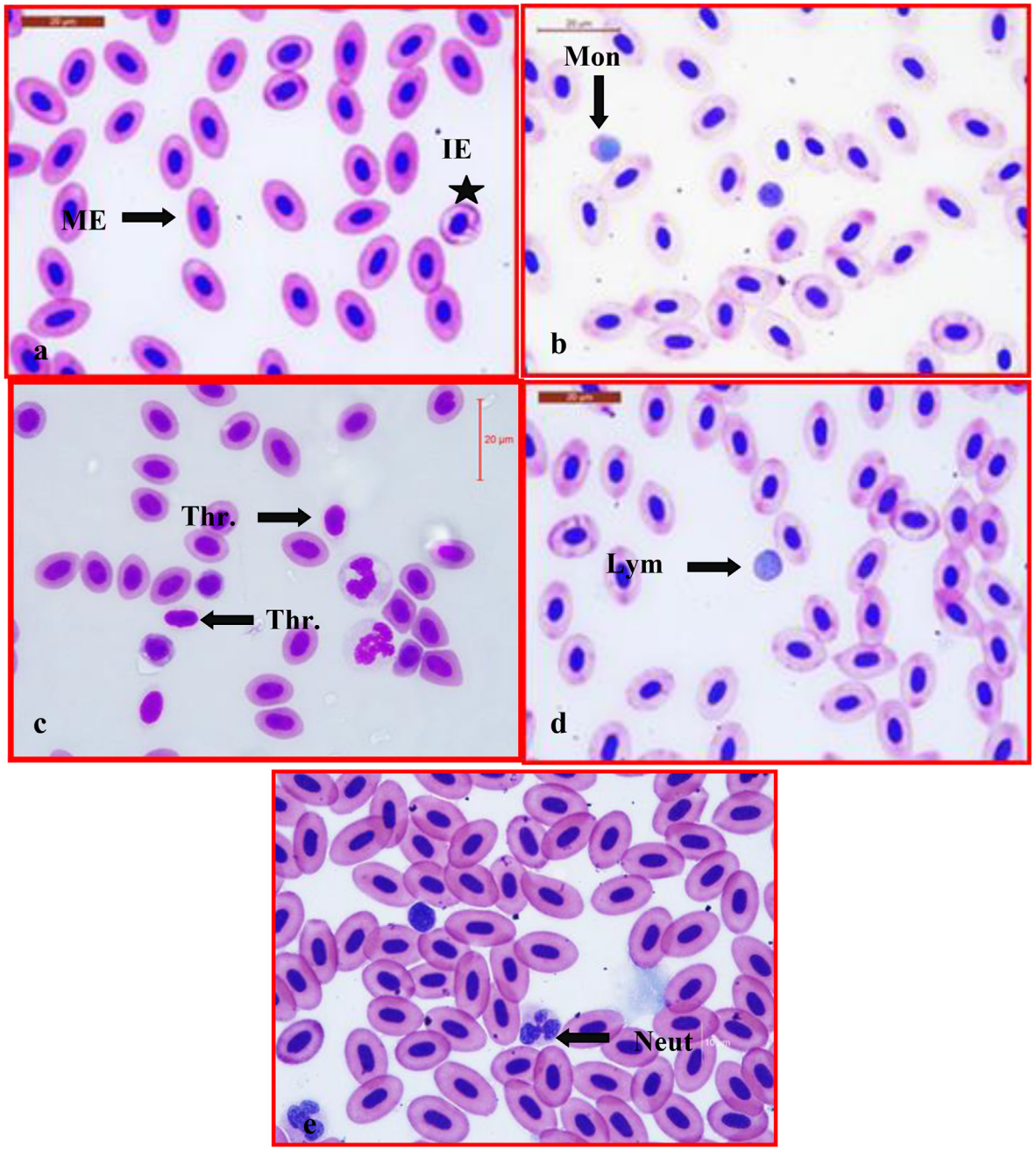


Figure 8. Pictorial images of different blood cells of *Samo trutta fario* a. (ME) Mature Erythrocyte, (IE) Immature Erythrocyte, b. Monocyte c. Thrombocytes d. Lymphocyte e. Neutrophil.

Ahmed et al., 2019; Jan et al., 2021). Serum glucose is regarded as a critical indicator of a fish's nutritional state and stress level. According to reports, blood glucose levels in fish vary depending on their age, reproductive stage, size and nutritional state (Percin and Konyalioglu, 2008; Prasad and Charles, 2010). Furthermore, the higher glucose concentration in males could be linked to their faster growth rate and higher efficiency of food conversion (Giberson and Litvak, 2003; Baker et al., 2005). Females of *S. trutta fario* have higher cholesterol levels than males, contrary to popular belief. It's possible that female fish have a greater cholesterol level since it's thought to be necessary for gonad development, basal steroid production, and adrenal steroidogenesis (Sharma et al., 2015, 2017; Sheikh and Ahmed, 2019; Jan and Ahmed, 2021; Jan et al., 2021). Our findings are consistent with those of various other researchers (Acharya and Mohanty, 2014; Zakes et al., 2016; Zhao et al., 2018; Ahmed et al., 2019; Jan et al., 2021). Total protein serum level is thought to be the main stable component of blood, and as such, it could be utilised to evaluate the nutritional state, physiological status, stress and fish well-being (Friedrich and Stepanowska, 2001; Riche, 2007). Throughout the investigation, we observed that the values of total protein, albumin and globulin were somewhat more in case of males compared to females of *S. trutta fario*. Other researchers have reported similar findings for different species of fish in the past (Owolabi, 2011; Charoo et al., 2013; Zakes et al., 2016; Kulkarni, 2017; Zhao et al., 2018; Ahmed et al., 2019; Jan et al., 2021).

Enzymes like ALT, AST, and ALP have been found in the blood of all species, including fish, and they perform various physiological activities inside the blood (Kulkarni, 2017). Such enzymes are produced mostly in the liver, and when they are found in blood plasma or serum, they provide specific information on organ disease. It has been discovered that an increase in the level of ALT indicates liver illness more specifically than

Table 2. Biochemical parameters of cultured brown trout, *Salmo trutta fario*.

Parameters	Male	Female	Combined
Glucose (mg dL^{-1})	114.60 ± 9.57[a]	110.54 ± 8.76[a]	112.57 ± 9.17
Cholesterol (mg dL^{-1})	178.76 ± 10.56[a]	182.0 ± 14.42[a]	180.86 ± 12.50
Total protein (g dL^{-1})	6.2 ± 0.87[a]	5.56 ± 1.27[a]	6.1 ± 1.08
Albumin (g dL^{-1})	3.23 ± 0.60[a]	2.89 ± 0.68[a]	3.06 ± 0.64
Globulin (g dL^{-1})	2.17 ± 0.78[a]	2.16 ± 0.88[a]	2.16 ± 0.83
Alanine aminotransferase (UL^{-1})	230.6 ± 13.21[a]	220.20 ± 11.20[a]	225.43 ± 12.20
Aspartate aminotransferase (UL^{-1})	162.65 ± 8.65[b]	184.60 ± 8.46[a]	173.67 ± 8.56
Alkaline phosphatase (UL^{-1})	40.21 ± 3.21[a]	41.10 ± 5.42[a]	40.65 ± 4.31
Sodium (mmol L^{-1})	122.4 ± 6.65[b]	135.58 ± 7.87[a]	129.01 ± 7.26
Potassium (mmol L^{-1})	1.34 ± 0.23[b]	2.51 ± 0.43[a]	1.92 ± 0.33
Phosphorus (mmol L^{-1})	4.20 ± 0.87[b]	6.32 ± 1.32[a]	5.25 ± 1.09

*Mean values in a rows sharing the same superscript are not significantly different ($P > 0.05$).

the level of AST, despite the fact that the latter is similarly high during acute liver necrosis. Similarly, alkaline phosphatase (ALP) is the most clinically relevant enzyme generated by the liver, and it has been observed that the enzyme ALP is produced in higher numbers during some physiological situations, such as liver and skeletal problems (Kulkarni, 2017). Significant ($P < 0.05$) differences in the levels of AST and ALP were detected during the present study, though insignificant ($P > 0.05$) changes in the readings of ALT were found. When comparing males and females of *S. trutta fario*, AST values were found to be higher in females. Similar conclusions were drawn in the cases of several fish species such as *Acipenser stellatus* (Shahsavani et al., 2010) and *H. molitrix* (Ahmed et al., 2019). Significant ($P < 0.05$) variations in sodium (Na^+), potassium (K^+), and phosphorus (P) between the sexes were also observed. Kulkarni (2017) found similar results in the case of *Notopterus notopterus*. The usage of these blood electrolytes is commonly used to identify the physiological properties, health state and toxicity of fish (Greenwell et al., 2003). Furthermore, these electrolytes are involved in several homeostatic mechanisms in the body of fish (Clarke, 1998). The values of all these electrolytes were found to be higher in males than females of *S. trutta fario* in this investigation. Contrarily, lower values of serum electrolytes were recorded by various workers who carried out their work in case of different female fish such as *Thunnus thynnus* (Percin et al., 2010), *N. Notopterus* (Kulkarni, 2017). Such inferences indicated that female fish are more vulnerable and feeble than males (Kavya et al., 2016).

The ability to recognise the properties of blood cells aids in determining the health position of fish and this has been well documented (Sheikh and Ahmed, 2020). Erythrocytes were found to be the most prevalent cell type in the peripheral blood of both sexes of *S. trutta fario* during the current investigation. Both sexes of *S. trutta fario* had large erythrocytes, which implies physiological adaptation to hypoxia tolerance and mimics the normal converse connection between erythrocyte size and aerobic swimming performance of fish (Tang et al., 2015; Sheikh and Ahmed, 2020). After erythrocytes, thrombocytes are the most common cell type, accounting for nearly half of all circulating leucocytes. They have a dark purple nucleus and light purple cytoplasm. In *S. trutta fario*, such cells seemed to be oval, fusiform, round, or spike shaped, which matches the findings in *A. sinensis* (Gao et al., 2007), *Glyptosternum maculatum* (Zhang et al., 2011), *S. prenanti* (Fang et al., 2014), *S. niger* (Sheikh and Ahmed, 2020). The staining of the peripheral blood during the present study endorsed the identification of five categories of leukocytes, which were divided into two primary cell types, agranulocytes and granulocytes in both sexes of *S. trutta fario*. The presence of two types of granulocytes, neutrophils and eosinophils were seen in *Oreochromis* hybrid (Hrubec et al., 2000), *C. dimerus* (Vazquez and Guerrero, 2007), *Betta splendens* (Motlagh et al., 2012), *S. niger* (Sheikh and Ahmed, 2020). The polymorphic, segmented, band or horse-shaped and centrally or ecentrally placed nucleus was typically seen in neutrophils. On the other hand, eosinophil might include bar-shaped crystalloids analogous to those noted in human eosinophil. Although there is insufficient data about the function of fish eosinophils, Ellis (2001) noted that they may be involved in performing functions comparable to mammalian mucosal mast cells. The most frequent type of leucocytes found in fish blood was lymphocytes, which were spherical or horseshoe shaped with a heavily pigmented nucleus. Different fish species, such as *A. sinensis, C. dimerus, B. splendens, G. eckloni* and *S. niger* had similar types of cells (Gao et al., 2007; Vazquez and Guerrero, 2007; Motlagh et al., 2012; Tang et al., 2015; Sheikh and Ahmed, 2020). Monocytes were also shown to exist as peripheral blood cells in the current study, which is comparable to findings made in many fish species such as *C. carpio, O.* hybrid*, C. dimerus, G. eckloni, S. niger* and others (Groff and Zinkl, 1999; Hrubec et al., 2000; Vazquez and Guerrero, 2007; Tang et al., 2015; Sheikh and Ahmed, 2020). Several fish species, including Atlantic herring (Boyar, 1962), *Carassius auratus* (Weinreb and Weinreb, 1969), *Sarpa sarpa* (Weinreb and Weinreb, 1969) and *S. trutta* (Blaxhall and Daisley, 1973) have been shown to lack such cells (Pavlidis et al., 2007).

5. Conclusion

The current findings provide a baseline of sex-based data on the haematological, serum biochemistry and characterization of blood cells of *Salmo trutta fario* under normal conditions. Such data gathered in this study could be helpful in monitoring the sex-specific physiological health condition of fish populations. Our study on haemato-biochemical characteristics and differential blood cell counts represent a significant scientific contribution to future blood cell characterization and data comparison with other salmonid species. Such fish-related data has also offered an imperative role in promoting the significance of pisciculture and raising environmental consciousness. Furthermore, the findings of this research will assist environmental and aquaculture officials in making informed judgments on the proper management and breeding of fish for human consumption in the future.

Declarations

Author contribution statement

Zubair Ahmad Sheikh, Kousar Jan, Naveed Nabi: Performed the experiment; Wrote the paper.

Imtiaz Ahmed: Conceived and designed the experiments.

Francesco Fazio: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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