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RESEARCH ARTICLE

The haematology of clinically healthy, farmed juvenile Asian seabass (*Lates calcarifer* Bloch)—reference intervals, and indicators of subclinical disease

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Abstract

This study establishes the blood reference intervals (RIs) for clinically healthy and farmed juvenile Asian seabass (*Lates calcarifer*), within 4–6 weeks after stocking into flow-through, marine aquaculture systems. The 90% percentile RIs ($n = 156$, mean bodyweight 41.8 g) are as follows: glucose (GLU) 2.4–11.3 mmol/L, haematocrit (Hct) 18.9%–39.2%, haemoglobin concentration (Hb) 56.0–85.0 g/L, total plasma protein (TPP) 56.0–77.0 g/L, total red blood cell (RBC) count $4.1–11.2 \times 10^{12}$/L, total white blood cell (WBC) count $5.3–69.9 \times 10^{9}$/L, total lymphocytes $4.7–51.4 \times 10^{9}$/L, monocytes $0.3–16.2 \times 10^{9}$/L and heterophils count $0.6–8.4 \times 10^{9}$/L. Pearson's method analysis showed weak but significantly positive correlations between fish bodyweight and Hct, Hb, TPP and total RBC count ($p < 0.05$). Histopathology of 42 of the 156 clinically healthy fish used to derive the RIs, with blood values within the 90% percentile range, did not exhibit any abnormal pathology. In contrast, histopathology from a different group of clinically healthy *L. calcarifer* ($n = 72$, mean bodyweight 31.3 g) with blood values falling outside of these established 90% percentile RIs showed that 25% of these fish had severe, chronic granulomatous enteritis, and 13% had severely depleted lipid stores in their liver. Point biserial correlation analysis of blood values from this second group of 72 fish showed that elevated total WBC, monocyte and heterophil counts and reduced Hct levels are significantly associated ($p < 0.05$) with the occurrence of severe, chronic granulomatous enteritis and depleted lipid stores in their liver. Reduced blood GLU and TPP levels in the second group of fish were significantly associated with fish that had depleted lipid stores in liver ($p < 0.05$), corroborating a period of malnutrition. This study is among the first to establish blood RIs for clinically healthy, farmed juvenile *L. calcarifer* and detection of subclinical diseases in fish to support early intervention.

KEYWORDS
aquaculture, blood reference intervals, health management, histopathology, subclinical disease

1 | INTRODUCTION

Barramundi or Asian seabass (*Lates calcarifer*) is an increasingly important aquaculture species in Southeast Asia, Australia and the United States. This is due to its versatility in both freshwater and marine aquaculture (Jerry, 2014). The global production of *L. calcarifer* has grown by almost twofold, from 63,000 tonnes in 2016 to 117,000 tonnes in 2020 (valued at 540 million $USD) (FAO, 2022). However, disease is estimated to cause greater than 40%–70% of production losses in aquaculture, costing the industry millions of dollars annually (Bromage et al., 1999; de Groof et al., 2015; Loch et al., 2017). Chemical overuse to combat bacterial or parasitic diseases threatens the sustainability of aquaculture, contributing to environmental degradation and loss of biodiversity. There is a need for cost-effective and reliable methods to detect subclinical disease and allow farms to institute early intervention to reduce production losses.

Haematology, or the analysis of blood parameters, is a quick and potentially non-lethal means of monitoring health in commercial fish farms (Fazio, 2019; Witeska et al., 2022; Yildiz, 2009). Blood analyses reflect the immune status and ability of cultured fish to respond to husbandry stressors, infection by pathogens and disease outbreaks (Thrall et al., 2012). Broadly, haematological values may vary due to several factors: (a) intrinsic—sex, age, species, (b) extrinsic—photoperiod, temperature, seasons and (c) pre-analytical sampling methods (Braceland et al., 2017; Fazio, 2019; Manna et al., 2021). There have been previous studies on differences in haematological values in fish based on age (Okorie-Kanu & Unakalamba, 2015), culture conditions (Liu et al., 2017; Montero et al., 1999; Tort et al., 1996), dietary additives (Chiu et al., 2015; De et al., 2019; Siddik et al., 2018), exposure to heavy metals (Javed et al., 2016), handling (Alexander et al., 2011; Fazio et al., 2015), high stocking density or poor water quality (Ardiansyah & Fotedar, 2016), sex (Akinrotimi et al., 2011; Gabriel et al., 2004), species (Parrino et al., 2018; Sayed et al., 2020), temperature (Kim et al., 2019; Stewart et al., 2019), transportation (Paterson et al., 2003) and infectious disease (Pomposini et al., 2019; Qiang et al., 2013). However, interpretation requires baseline blood values or reference intervals (RIs) to be first established for fish under different culture conditions and age groups (Friedrichs et al., 2011).

RIs have been established in several important cultured catfish (Akinrotimi et al., 2011; Bianchi et al., 2014; Manna et al., 2021), tilapia (Chen et al., 2003; Hrubec et al., 2000; Mauel et al., 2007), salmonids (Casanovas et al., 2021; Nabi et al., 2022; Rozas-Serri et al., 2022) and other teleost species such as the shortnose sturgeon (*Acipenser brevirostrum*) (Knowles et al., 2006), spotted rose snapper (*Lutjanus guttatus*) (Del Rio-Zaragoza et al., 2011) and Senegalese sole (*Solea senegalensis*) (Peres et al., 2015). However, RIs for clinically healthy, farmed *L. calcarifer* are lacking. The requirements for sufficient sample size make it challenging to establish statistically reliable and robust RIs (Katayev et al., 2010; Schubiger et al., 2021). In addition, teleost possesses nucleated red blood cells (RBC), which present a challenge for automated analysis commonly used in other domesticated animal species (Fazio et al., 2012). RBC, white blood cell (WBC) and differential WBC counts require laborious manual-counting techniques (Witeska et al., 2022). Nevertheless, studies have investigated the mean blood values of *L. calcarifer* in response to dietary additives or supplementation (Ali et al., 2017; Longbaf Dezfouli et al., 2019; Talpur & Ikhwanuddin, 2012), high stocking density (Ardiansyah & Fotedar, 2016; Sadhu et al., 2014), harvest stress (Wilkinson et al., 2008), probiotics (Adorian et al., 2018) and transportation stress (Paterson et al., 2003), but RIs have not been established.

This study aims to establish baseline blood RIs for clinically healthy, farmed juvenile *L. calcarifer*. Juvenile fish in intensive aquaculture are more susceptible to disease, due to developing immune systems (Al Khaziri et al., 2019; Azad et al., 2004; Bastos Gomes et al., 2017; de Groof et al., 2015; Dong et al., 2017; Fenner et al., 2006; Gibson-Kueh et al., 2011). The blood parameters analysed and methods used are selected based on their practical applications in commercial farms. This study will allow fish farms to identify farmed stocks with blood values outside established RIs, to facilitate early detection of subclinical disease and manage production losses.

2 | MATERIALS AND METHODS

2.1 | Background of fish in this study

Fish were subsampled from 8 batches of juvenile *L. calcarifer* shortly after stocking into 80-tonne flow-through nursery tanks at a commercial marine fish farm in Singapore, over a 15-month period (September 2020 to November 2021). Clinically healthy (i.e. with no disease signs) *L. calcarifer*, displaying good feeding response and active swimming behaviour were sampled for haematology and histopathology ($n = 156$, mean bodyweight S.D. = 41.8 ± 26.6 g). Fish were sampled from each batch over several time points, in the first 4–6 weeks post-stocking. These flow-through systems run on sand-filtered sea water, with approximately one full-cycle water exchange per hour. Fish were fed commercial slow-sinking pellets two to three times a day, at feed rates of approximately 3% bodyweight. Water quality recorded included temperature at 30°C ± 0.5, dissolved oxygen above 6 ppm, pH of 7.8–8.2, salinity at 29–31 ppt, ammonia levels at 0–0.25 mg/L, nitrite at 0 mg/L and nitrate at 0–5 mg/L, throughout the study period.

2.2 | Blood sample collection and analyses

Twelve fish were taken at each sampling point before the first feed of the day, and all blood samples were kept chilled until processed within 1–2 h, to minimize post-collection artefacts. Fish were anaesthetised using a 40 ppm AquiS™ immersion bath immediately post-capture for blood sampling, and promptly killed for

bodyweight-length measurements and tissue sampling. Blood was drawn from the caudal vein using a 1 mL syringe (Nipro), and a 21G or 23G needle (BD PrecisionGlide™), and immediately placed in 1.5 mL lithium heparin tubes (BD Microtainer®).

Blood glucose (GLU) (mmol/L) was measured using a handheld Instant ACCU-CHEK™ glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Haemoglobin (Hb) concentration (g/L) was determined using a handheld haemoglobin analyser DiaSpect™ (DiaSpect Medical GmbH, Barleben, Germany) according to manufacturer instructions. Heparinised blood in microcapillary tubes was spun at 12,000 rpm at ambient temperature for 3 min to determine haematocrit (Hct) (%), using a portable micro-haematocrit ZipCombo™ centrifuge (LW Scientific, USA). Approximately three microliters of heparinised whole blood from each fish was used to prepare blood smears on clean glass slides, air dried and stained with Diff Quik™ (Thermo Shandon Limited, USA) to determine differential white blood cell (WBC) count, as percentages of lymphocyte, monocyte and heterophils counts per 100 leukocytes. Differential WBC counts were conducted under bright-field microscopy using an Olympus BX53 transmission light microscope, by differentiating 100 WBC under oil immersion (100× objective) (Rozas-Serri et al., 2022). Heparinised blood was spun down in a centrifuge (CF-5, DAIHAN Scientific, Korea) at 1210 *g* (6000 rpm) for 5 min, and approximately 30 µL of plasma was placed on a MT-200ATC clinical handheld protein refractometer (QA Supplies LLC, USA) to measure total plasma protein (TPP) (g/L). Blood samples that clotted or haemolysed were excluded from analyses. Blood GLU with readings beyond the detection limits of ACCU-CHEK™ Instant glucometer (Roche Diagnostics GmbH, Mannheim, Germany) were excluded.

Total red blood cell (RBC) counts (10^{12}/L) and WBC counts (10^{9}/L) were carried out on 1:200 dilution or 5 µL heparinised blood diluted in 1 mL of Natt and Herrick's solution (Vetlab supply, Palmetto Bay, FL, USA). Blood samples were mixed well before approximately 15 µL of blood diluted in Natt & Herrick's solution were fed into a KOVA® Glasstic® counting chamber (Kova International Inc., USA). WBC was counted in all 81 small grids, and RBC counts in 9 grids (four corners, one in the centre and four centre grids of the outermost rows and columns) under 400× magnification. Total WBC and RBC counts were derived using the formulae below. Total WBC count was subsequently multiplied with differential counts (%) of monocytes, lymphocytes and heterophils to obtain their respective counts. Samples with lysed or swollen RBCs were excluded from total RBC counts.

$$\text{White blood cell count (per } \mu\text{L)} = \left(\frac{\text{Total No. of WBC counted in all 81 grids}}{0.9}\right) \times 200$$

$$\text{Red blood cell count (per } \mu\text{L)} = \left(\frac{\text{Total No. of RBC counted in 9 grids}}{9} \times 90\right) \times 200$$

Mean corpuscular volume (MCV) (fl), mean corpuscular haemoglobin (MCH) (pg) and mean cell haemoglobin concentration (MCHC) (g/L) values were derived from Hct, Hb concentration and total RBC counts according to the formulae below (Thrall et al., 2012).

$$\text{MCV (fL)} = \left(\frac{\text{Hct (\%)}}{\text{RBC count (per } \mu\text{L)}/10^{6}}\right) \times 10$$

$$\text{MCH (pg)} = \text{Hb (g/L)} \times \left(\frac{10}{\text{RBC count (per } \mu\text{L)} / 10^{6}}\right)$$

$$\text{MCHC (g/L)} = \left(\frac{\text{Haemoglobin (g/L)}}{\text{Hematocrit (\%)}}\right) \times 100$$

2.3 | Histopathology

Tissues (Brain, gills, gut, heart, kidney, liver, muscle, spleen and stomach) from freshly euthanized *L. calcarifer* were fixed in 10% phosphate-buffered formalin, for routine histoprocessing at Institute of Molecular & Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR) Singapore, or Diagnostic Veterinary Pathology Laboratory, Murdoch University, Australia, into haematoxylin and eosin (H&E) stained tissue sections. Of the 156 clinically healthy *L. calcarifer* used to establish the RIs, H&E stained tissue sections from 42 fish (i.e. with GLU, TPP, Hct, Hb, total RBC count and total WBC count within the 90% percentile RIs) were examined on the Olympus BX53 transmission light microscope, and images captured using Digital Camera DP74 and CellSens™ Standard Imaging System (Olympus Corporation). Additionally, a separate group of 72 clinically healthy *L. calcarifer* (mean bodyweight S.D. = 31.3 ± 26.7 g) from the same farm, with at least one blood value (GLU, TPP, Hct, total RBC count or total WBC count) falling outside the 90% percentile RIs were examined for histopathology. Out of these 72 fish, 45 (63%) fish have at least one blood value outside the 90% percentile RIs, while the rest had more than one blood value outside the 90% percentile RIs. In this study, the presence or absence of severe chronic enteritis is as described in previous studies, or in brief, multifocal to coalescing, chronic granulomatous enteritis (Gibson-Kueh et al., 2004, 2021). Researchers may refer to Domingos et al.'s (2021) for images of how livers with good lipid and glycogen reserves or depleted fat stores look like histologically.

2.4 | Statistical analysis and development of reference intervals

This study follows the protocol proposed by the ASVCP Quality and Laboratory Standards Committee (QALS) Guidelines, for the determination of RIs in veterinary species (Friedrichs et al., 2011). Data was analysed using IBM SPSS ver. 27. As sample size exceeded the recommended minimum of $n = 39$, RIs were generated using non-parametric methods. Blood values within the central 90% percentile of reference values were adopted in this study for increased robustness and stringency (Friedrichs et al., 2011). Normality of data were determined using Shapiro–Wilk test and equal variances were verified with Levene's test. The correlation between bodyweight of clinically healthy fish ($n = 156$) used to establish the RIs for this study and their blood values were analysed using Pearson's correlation

coefficient. In a different group of clinically healthy fish ($n=72$), point-biserial correlation test was used to determine if there is an association between blood parameters of fish with values outside the 90% percentile RIs and the occurrence of severe chronic enteritis in the gut or depletion of liver lipid stores. The blood data of these 72 fish were log-transformed to meet the assumptions for point-biserial correlation tests.

3 | RESULTS

The RIs of blood values from clinically healthy, farmed juvenile *L. calcarifer* (this study) are presented in Table 1. RIs (90% percentile) values are as follows: GLU 2.4–11.3 mmol/L, Hb 56.0–85.0 g/L, Hct 18.9–39.2%, TPP 56.0–77.0 g/L, total RBC counts 4.1–11.2 $\times 10^{12}$/L, MCV 25.8–78.1 fL, MCH 6.5–17.4 pg, MCHC 197.4–317.6 g/L, total WBC counts 5.3–69.9 $\times 10^{9}$/L and lymphocytes 4.7–51.4 $\times 10^{9}$/L, monocytes 0.3–16.2 $\times 10^{9}$/L, heterophils counts 0.6–8.4 $\times 10^{9}$/L. Lymphocytes are the dominant WBCs in clinically healthy, farmed juvenile *L. calcarifer*. Eosinophil and basophils were not observed in *L. calcarifer* examined in this study. These blood values did not show a normal distribution, except for Hct and total RBC counts. Pearson correlation analysis showed a weak, but significantly positive correlation between bodyweight and Hct ($r=0.32$; $p<.01$), Hb ($r=0.19$; $p<.05$), TPP ($r=0.25$; $p<.01$) and total RBC counts ($r=0.28$) (Figure 1 & Table 2) ($n=156$). In contrast, a significantly weak, negative correlation was observed between fish bodyweight and MCHC ($r=-0.31$) ($p<.01$) (Figure 1 & Table 2) ($n=156$).

Histopathological examination of 42 out of 156 clinically healthy fish used to develop blood RIs for this study did not reveal any

TABLE 1 Blood reference intervals (RIs) in clinically healthy, farmed juvenile *L. calcarifer* in this study.

Blood parameters	*n*	Min	Max	Median	Mean (S.E.)	90% percentile reference intervals	90% confidence values of mean
Glucose (mmol/L)	151	1.4	15.4	6.4	6.7 ± 0.2	2.4–11.3	6.3–7.0
Haemoglobin (g/L)	156	38.0	91.0	74.0	72.2 ± 0.08	56.0–85.0	71.0–73.5
Haematocrit (%)	156	15.0	43.0	29.0	29.3 ± 0.4	18.9–39.2	28.5–30.0
Total plasma protein (g/L)	156	51.0	89.0	66.0	66.2 ± 0.05	56.0–77.0	65.4–67.0
Total red blood cells (10^{12}/L)	60	3.2	13.1	7.3	7.2 ± 0.2	4.1–11.2	6.8–7.6
MCV (Mean corpuscular volume) (fL)	60	20.6	92.9	44.8	46.6 ± 1.8	25.8–78.1	43.5–49.6
MCH (Mean cell haemoglobin) (pg)	60	5.5	23.2	10.1	11.0 ± 0.4	6.5–17.4	10.3–11.7
MCHC (Mean cell haemoglobin concentration) (g/L)	156	183.0	383.0	250.0	251.7 ± 2.7	197.4–317.6	247.1–256.2
Total white blood cells (10^{9}/L)	91	4.2	85.3	26.2	29.2 ± 2.1	5.3–69.9	25.7–32.7
Lymphocyte (10^{9}/L)	91	3.4	70.0	18.3	21.2 ± 1.4	4.7–51.4	18.8–23.6
Monocyte (10^{9}/L)	91	0.1	27.2	2.5	4.6 ± 0.5	0.3–16.2	3.7–5.5
Heterophil (10^{9}/L)	91	0.5	10.2	3.1	3.5 ± 0.3	0.6–8.4	3.1–3.9

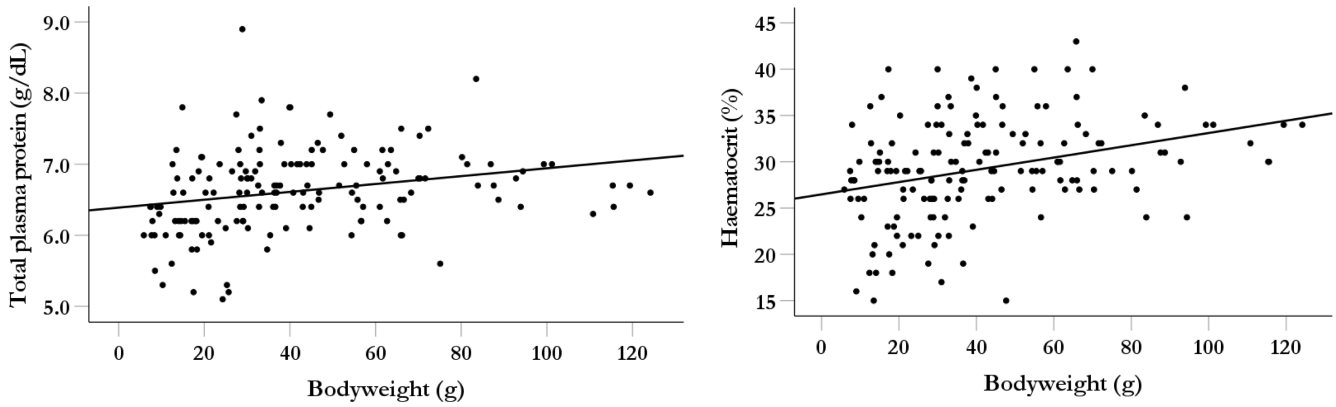

FIGURE 1 Scatter plot displaying weak but significantly positive correlation of haematocrit ($n=156$, $r<0.32$) and total plasma protein ($n=156$, $r<0.25$) to bodyweight in clinically healthy *L. calcarifer* used to establish RIs in this study ($n=156$, $p<.01$). It is possible that analyses of blood values against a wider bodyweight range may show a stronger positive correlation.

significant pathology (Table 3). In contrast, histopathological examination of a different group of clinically healthy fish ($n=72$) with blood parameters outside the established RIs revealed that 25% ($n=18/72$) suffered from severe, chronic granulomatous enteritis, while 13% ($n=9/72$) had severely depleted lipid and glycogen stores in the liver (Figure 2 & Table 3). Moreover, 11.1% ($n=8/72$) of examined fish suffered from both severe, chronic granulomatous enteritis and depleted lipid and glycogen stores in the liver. Some fish with chronic enteritis displayed the occasional intralesional, scattered clusters of large coccobacilli, resembling 'big belly' disease (Gibson-Kueh et al., 2004, 2021) (Figure 2).

Point biserial correlation analysis of blood parameters from this second group of 72 clinically healthy *L. calcarifer* showed that elevated total WBC counts ($r=0.31/0.28$; $p<.05$), monocyte ($r=0.28/0.27$; $p<.05$) and heterophil counts ($r=0.28/0.28$; $p<.05$) and reduced Hct levels ($r=-0.38/-0.34$; $p<0.01$) are significantly associated with the occurrence of severe, chronic granulomatous enteritis and depleted lipid stores in the liver (Table 4). Reduced blood GLU ($r=-0.39$; $p<.01$) and TPP levels ($r=-0.24$; $p<.05$) were also significantly associated with *L. calcarifer* that had depleted liver lipid stores (Table 4).

4 | DISCUSSION

This is the first study to establish blood reference intervals (RIs) for clinically healthy, farmed juvenile *L. calcarifer*, as a quick and simple health monitoring tool (Table 1). RIs may vary across fish species, size and husbandry practices, such that specific baseline values are needed for interpretation (Casanovas et al., 2021; Mauel et al., 2007; Nabi et al., 2022; Rozas-Serri et al., 2022; Schubiger et al., 2021). Twenty-five percent of clinically healthy, juvenile *L. calcarifer* with at least one blood value (GLU, TPP, Hct, total RBC count or total WBC count) outside the 90% percentile RIs had severe chronic granulomatous enteritis due to 'big belly' disease, which can cause high mortality rates of up to 80%–100% (Chew et al., 2023; Gibson-Kueh, 2012; Gibson-Kueh et al., 2004). This study demonstrated the usefulness of RIs in the detection of subclinical diseases. Chronic gastrointestinal bacterial diseases are common in many aquaculture species: yellowtail kingfish (*Seriola lalandi*) (Legrand et al., 2020), grouper (*Epinephelus coioides*) (Lee et al., 2002), marine cobia (*Rachycentron canadum* L.) (Liu et al., 2004) and grass carp (*Ctenopharyngodon idella*) (Song et al., 2014).

Severe chronic bacterial enteritis and depleted liver lipid stores in clinically healthy juvenile *L. calcarifer* are significantly associated with elevated total WBC, monocyte and heterophil counts and low Hct levels. Leucocytosis, or increased total WBC counts in fish, is an inflammatory response (Clauss et al., 2008), that has been reported in bacterial disease in Nile tilapia (*O. niloticus* L.) (Martins et al., 2008), African catfish (*Clarias gariepinus*) (Adeyemi et al., 2014), Senegalese sole (*S. senegalensis*) (Costas et al., 2013) and Koi (*Cyprinus carpio*) infected with *Flavobacterium columnare* (Tripathi et al., 2005). Low Hct levels or non-regenerative anaemia are common in chronic diseases (Collet et al., 2015; Gallaugher & Farrell, 1998; Smith et al., 2006). Anaemia has been reported in salmonids with gill disease (Currie et al., 2022), winter flounder (*Pseudopleuronectes americanus*) with fin rot (Ziskowski et al., 2008) and striped bass (*Morone saxatiles*) and Korean catfish (*Silurus asotus*) experimentally infected with *Edwardsiella tarda* (Lee Herman & Bullock, 1986; Yu et al., 2010).

The blood glucose RI of 2.4–11.3 mmol/L (n = 151) observed in clinically healthy juvenile *L. calcarifer* in this study is comparable to other cultured teleost species such as Nile tilapia (2.9–8.7 mmol/L), striped catfish (4.9–7.7 mmol/L), Atlantic salmon (2.9–9.6 mmol/L) and rainbow trout (3.1–6.7 mmol/L) (Table 5). However, the mean GLU (6.7 ± 0.2 mmol/L) of clinically healthy, juvenile *L. calcarifer* in this study is higher than that reported in previous studies on the same species (Table 7). This study was conducted on *L. calcarifer* less than 4–6 weeks after stocking at a commercial fish farm, while previous studies were on experimental fish in laboratory settings. Farmed fish often experience high levels of stress due to factors such as high stocking density and regular handling (Barcellos et al., 2004; Sadhu et al., 2014). Stress-induced spikes in blood cortisol levels can lead to gluconeogenesis, insulin resistance and hyperglycaemia, as a response to meet increased metabolic energy demands (Bonga, 1997; Geer et al., 2014; Small, 2004; Stewart et al., 2019). Hyperglycaemia is transient and will abate

TABLE 2 Correlation analyses of blood parameters to bodyweight (g) in clinically healthy *L. calcarifer*.

Blood parameters	n	Pearson correlation score (*r*-value)	*p*-Value
Haemoglobin (g/L)	156	0.185	<.05
RBC count (10^{12}/L)	60	0.279	<.05
MCHC (g/L)	156	−0.308	<.01

TABLE 3 Histopathological findings in clinically healthy *L. calcarifer* with blood values within and outside the 90% percentile RIs.

Histopathology examination	Number and percentage of *L. calcarifer* with severe chronic enteritis in gut	Number and percentage of *L. calcarifer* with depleted lipid stores in liver
Clinically healthy fish with blood values within 90% percentile RIs ($n=42$)	0 (0%)	0 (0%)
Clinically healthy fish with blood values outside 90% percentile RIs ($n=72$)[a]	18 (25%)	9 (13%)

[a]These 72 *L. calcarifer* are a separate group of fish that were distinct from the fish used to derive the RIs.

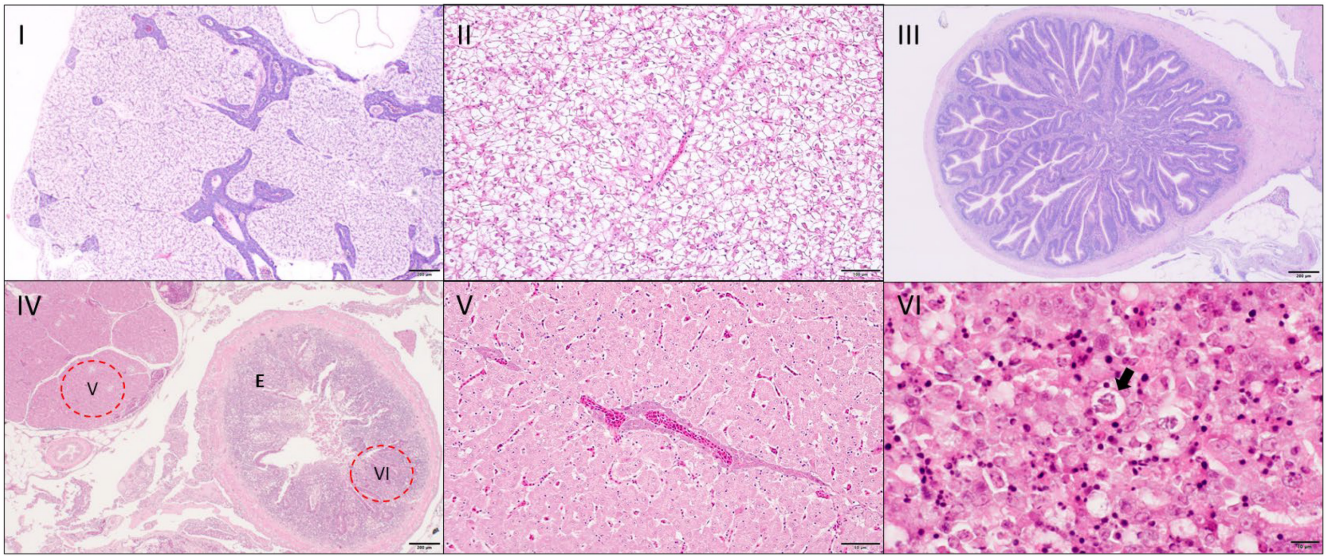

FIGURE 2 H&E tissue sections from clinically healthy, juvenile *L. calcarifer* with blood parameters within (I, II & III) and outside (IV, V & VI) the 90% percentile RIs established in this study. (I & II) Liver with light staining, wispy appearance, indicate good lipid and glycogen storage, shown at higher magnification in II. (III) Intestine with no abnormal histopathology. (IV) Clinically healthy *L. calcarifer* with blood parameters outside the 90% percentile RIs, suffering from severe, extensive chronic granulomatous enteritis (E). (V) Liver with severely depleted lipid and glycogen storage, stain more intensely eosinophilic. (VI) Intralesional, clusters of large coccobacilli (arrow), in intestine with chronic granulomatous enteritis due to 'big belly' disease.

TABLE 4 Point biserial correlation analysis of blood values from clinically healthy *L. calcarifer* with blood values outside RIs ($n = 72$) showed a significant association between some blood values and presence of severe chronic enteritis and depleted lipid stores in liver.

Blood parameters	*n*	Mean	Std. deviation	Association with severe chronic enteritis in gut (*r*-value)	Association with depleted lipid stores in liver (*r*-value)
Glucose (mmol/L)	57	5.5	3.7	−0.118	**−0.392****
Haemoglobin (g/L)	72	52.4	34.9	0.203	0.069
Haematocrit (%)	72	28.9	8.4	**−0.375****	**−0.342****
Total plasma protein (g/L)	72	6.7	0.9	−0.178	**−0.235***
Total red blood cells (10^{12}/L)	30	7.8	2.9	−0.241	−0.100
MCV (Mean corpuscular volume) (fL)	30	44.3	17.4	−0.079	−0.229
MCH (Mean cell haemoglobin) (pg)	30	10.8	3.9	−0.040	−0.114
MCHC (Mean cell haemoglobin concentration) (g/L)	72	25.8	4.3	0.021	0.083
Total white blood cells (10^9/L)	59	29.2	31.4	**0.308***	**0.278***
Lymphocyte (10^9/L)	59	65.9	20.9	0.213	0.182
Monocyte (10^9/L)	59	17.9	10.7	**0.279***	**0.267***
Heterophil (10^9/L)	59	16.2	14.7	**0.279***	**0.281***

Note: The *r*-value represents the strength of the association and ranges between −1 and 1. Positive *r*-value represents a positive correlation, while a negative *r*-value represents a negative correlation.
*Blood parameters with a significant *p*-value of <.05; **Blood parameters with a significant *p*-value of <.01.

when stressors are removed, but poor husbandry practices that persist may result in chronic stress and sustained hyperglycaemia (Manuel et al., 2014). Decreased blood GLU levels were observed in *L. calcarifer* with chronic enteritis and low liver lipid stores in this study. Hypoglycaemia had been reported in *L. calcarifer* that were not eating post-challenge with *Vibrio harveyi* (Talpur, 2014),

TABLE 5 A summary of blood reference intervals (RIs) in clinically healthy juvenile *L. calcarifer* (this study) and other teleost fish species.

Fish groups	Species	Salinity	Body wt (g)	GLU (mmol/L)	Total/plasma protein (TP/PP) (g/L)	RBC (10^{12}/L)	Hct (%)	Hb (g/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	References
Tilapia	Tilapia (*O. hybrids*)	FW	240.0	1.7–3.8	29.0–66.0 (TP)/48.0–78.0 (PP)[a]	1.9–2.8	27.0–37.0	70.0–98.0	115.0–183.0	28.3–42.3	220.0–290.0	Hrubec et al. (2000)
	Hybrid Tilapia (*O. aureus* × *O. niloticus*)	FW	72.0–556.0	1.8–7.6	27.0–50.0 (TP)/30.0–77.0 (PP)[a]	–	22.0–45.0	–	–	–	–	Mauel et al. (2007)
	Nile Tilapia (*O. niloticus*)	FW	393.2	2.9–8.7	33.0–50.0 (TP)[a]	–	33.3–48.5	–	–	–	–	Chen et al. (2003)
Catfish	Striped catfish (*P. hypophthalmus*)	FW	23.6–39.4	4.9–7.7	42.5–67.0 (TP)[a]	6.8–8.5	38.8–43.3	67.0–83.0	63.9–83.9	11.5–15.3	163.8–202.0	Manna et al. (2021)
	Duckbill catfish (*S. lima*)	FW	54.1 ± 15.1	–	42.0–50.0 (PP)	2.1–2.5	26.8–30.0	49.6–72.1	116.9–134.5	–	173.7–254.6	Bianchi et al. (2014)
	Yellow catfish (*H. brachysoma*)	FW	36.0–161.0	70.4–141.3 (g/L)	20.6–86.3 (PP)[b]	1.7–2.4	21.4–55.6	72.0–99.0	–	–	–	Prasad and Charles (2010)
	Striped catfish (*P. hypophthalmus*)	FW	141.7 ± 41.2	4.6–7.6	30.0–42.0 (TP)/126.0–173.0 (PP)[a]	1.8–2.8	23.8–35.9	–	106.3–156.6	–	–	Galagarza et al. (2017)
	Channel catfish (*I. punctatus*)	FW	98.5–834.5	0.9–4.8	26.0–66.0 (TP)	1500.0–4100.0	27.0–54.0	44.0–109.0	88.6–186.7	–	205.0–226.0	Tavares-Dias and Moraes (2007a)
	African catfish (*C. gariepinus*)	FW	1800.0	–	–	3.1–8.6	32.6–45.7	100.2–186.4	72.1–91.3	30.2–46.7	382.1–467.4	Akinrotimi et al. (2011)
Salmonid	Coho Salmon (*O. kisutch*)	FW	50.0–150.0	1.3–7.5	20.0–59.6 (TP)[a]	22258.9–16,0179.8	38.3–68.5	299.2–749.9	40.1–177.8	–	0.7–1.4	Rozas-Serri et al. (2022)
	Atlantic salmon (*S. salar*)	FW	50.0–150.0	2.9–9.6	25.7–64.9 (TP)[a]	21312.0–13,0669.2	24.0–56.6	154.3–636.3	110.7–206.0	–	0.7–1.4	Rozas-Serri et al. (2022)
	Rainbow trout (*O. mykiss*)	FW	50.0–150.0	1.1–7.5	25.1–55.4 (TP)[a]	10716.9–86834.6	33.0–60.7	–	86.0–192.7	–	0.7–1.1	Rozas-Serri et al. (2022)
	Rainbow trout (*O. mykiss*)	FW	–	3.1–6.7	35.0–58.0 (TP)[a]	–	33.0–51.0	–	–	–	–	Marancik et al. (2014)
	Rainbow trout (*O. mykiss*)	FW	131.0–385.3	1.9–2.7	30.0–39.0 (TP)	1.0–2.0	29.0–40.0	83.2–122.8	188.8–288.5	56.8–82.7	281.4–327.6	Nabi et al. (2022)
	Chinook salmon (*O. tshawytscha*)	FW & SW	44.0–3164.0	2.7–6.1	27.0–46.6 (TP)	–	27.4–44.3	81.7–128.5	106.3–156.6	–	254.3–332.8	Casanovas et al. (2021)
Cichlid	South American cichlid (*C. dimerus*)	FW	27.1–50.2	–	–	1.7–4.3	22.5–39.1	52.3–83.3	70.1–198.0	14.5–40.6	174.3–303.1	Rey Vázquez and Guerrero (2007)
	Red top ice blue cichlid (*M. greshakei*)	FW	99.7 ± 4.1	2.1–2.7	34.6–46.2 (TP)[a]	1.7–2.7	21.0–29.5	63.0–91.3	95.3–132.4	26.9–40.3	270.0–320.0	Snellgrove and Alexander (2011)

(Continues)

TABLE 5 (Continued)

Fish groups	Species	Salinity	Body wt (g)	GLU (mmol/L)	Total/plasma protein (TP/PP) (g/L)	RBC (10^{12}/L)	Hct (%)	Hb (g/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	References
Others	Common carp (*C. carpio*)	FW	20.6–60.7	–	–	1.4–1.5	24.0–25.5	62.4–69.6	178.6–200.1	47.3–53.1	256.6–284.9	Witeska et al. (2016)
	Sobaity sea bream (*S. hasta*)	SW	35.1 ± 0.9	6.2–7.9	32.0–39.0 (TP)[a]	1.7–2.2	24.5–33.8	28.0–60.0	126.3–191.7	14.4–26.5	92.0–180.0	Torfi Mozanzadeh et al. (2015)
	Asian seabass (*L. calcarifer*)	**SW**	**41.8 ± 2.1**	**2.4–11.3**	**56.0–77.0 (PP)**	**4.1–11.2**	**18.9–39.2**	**56.0–85.0**	**25.8–78.1**	**6.5–17.4**	**197.4–317.6**	**Present study**
	Spotted rose snapper (*L. guttatus*)	SW	59.0 ± 23.7	–	44.0–117.5 (TP)	0.8–3.7	33.5–71.1	72.9–170.3	135.7–369.8	20.0–91.5	111.6–310.9	Del Rio-Zaragoza et al. (2011)
	Yellow Perch (*P. flavescens*)	FW	125.0 ± 14.0	3.4–10.1	37.0–50.0 (TP)/60.0–82.0 (PP)[a]	0.002–0.003	29.0–47.0	–	–	–	–	Hrubec and Smith (2004)
	Koi (*C. carpio*)	FW	200	–	–	1.7–1.9	29.7–33.9	63.2–75.5	166.3–190.0	37.7–42.7	204.0–229.0	Tripathi et al. (2004)
	Senegalese sole (*S. senegalensis*)	SW	288.0 ± 56.0	1.1–4.8	26.0–63.3 (TP)	1.1–2.3	9.4–26.7	26.1–60.2	82.5–165.9	17.9–41.2	138.9–273.4	Peres et al. (2015)
	Sablefish (*A. fimbria*)	SW	186.0–440.0	1.0–4.9	25.2–44.9 (TP)/43.0–79.2 (PP)[a]	–	36.0–62.0	–	–	–	–	Schubiger et al. (2021)
	Striped bass (*M. saxatilis*)	FW	1800.0 ± 150.0	6.4–10.8	35.0–48.0 (TP)	2.3–3.9	25.3–46.3	87.0–125.0	89.0–151.0	31.9–47.2	25.9–37.8	Fazio et al. (2020)
	Shortnose sturgeon (*A. brevirostrum*)	FW	2590.0 ± 860.0	2.1–4.1	27.0–53.0 (TP)/28.0–60.0 (PP)[a]	0.7–1.1	26.0–46.0	57.0–87.0	307.0–520.0	65.9–107.1	150.0–300.0	Knowles et al. (2006)

[a]Studies that reported plasma protein (PP) as total protein (TP) despite using anticoagulants.
[b]One study that did not state the use of anticoagulants although PP was presented.

exposed to heavy metals (Javed & Usmani, 2015), or suffered short-term food deprivation (Norouzi et al., 2021).

TPP show smaller fish-to-fish variations with a range of 56.0–77.0 g/L and a mean of 66.2 ± 0.05 g/L ($n = 156$), which makes TPP a more reliable biomarker compared to other blood parameters (Table 1). The RI for TPP in clinically healthy *L. calcarifer* in this study aligns with several other fish species such as tilapia (48.0–78.0 g/L), yellow perch (60.0–82.0 g/L) and sablefish (43.0–79.2 g/L) (Schubiger et al., 2021), except for significantly higher values in striped catfish (126.0–173.0 g/L) (Table 5). Variations in blood protein levels between species may be species-related or due to different methodologies used to obtain the measurements (Tables 5 and 7). Elevated blood serum protein levels have been observed in fish given immunity-enhancing feed additives such as essential oil (citrus EO) (Acar et al., 2015; Ranjan et al., 2014) and dietary chitosan (Ranjan et al., 2014). In Asian seabass (*L. calcarifer*), total protein levels were significantly increased 12 h post-challenge with *Aeromonas hydrophilia*, thought to be due to elevated globulins levels (Kathirkaman et al., 2018). There is a weak but significantly positive correlation between TPP and *L. calcarifer* bodyweight (Figure 1). Higher blood protein levels have been reported in adult catfish (*Heteroclarias hybrid*) (Okorie-Kanu & Unakalamba, 2015) and rainbow trout (*O. mykiss*) compared to younger fish (Pastorino et al., 2020). Other studies did not find a significant positive correlation between total serum protein and bodyweight in striped bass (*M. saxatilis*) (Fazio et al., 2020). *L. calcarifer* with severely depleted liver lipid stores in this study exhibited significantly reduced blood TPP (hypoproteinaemia), which may be due to impaired nutrient absorption or increased protein loss in fish associated with chronic enteritis (Gibson-Kueh et al., 2021; Řehulka, 2002; Řehulka & Minarik, 2007). Hypoproteinaemia has been reported in bacterial, viral and parasitic infections across multiple fish species, including snapper, salmonids and trout (Del Rio-Zaragoza et al., 2011; Řehulka & Minarik, 2007; Yildiz & Aydin, 2006). Similarly, hypoproteinaemia was reported in carp (*Cyprinus carpio* L.) and Asian seabass (*L. calcarifer*) kept at high stocking densities (Sadhu et al., 2014; Yin et al., 1995), in channel catfish (*I. punctatus*) subjected to acute temperature spikes (Stewart et al., 2019) and in koi (*Cyprinus carpio*) with severe skin ulcerations (Tripathi et al., 2005).

The RI for total RBC count in clinically healthy *L. calcarifer*, 4.1–11.2 × 10^{12}/L ($n = 60$), is relatively higher as compared to tilapia (1.9–2.8 × 10^{12}/L), duckbill catfish (2.1–2.5 × 10^{12}/L), South American cichlid (1.7–4.3 × 10^{12}/L) and Koi (1.7–1.9 × 10^{12}/L) and lower than Coho Salmon, Atlantic salmon and rainbow trout (10716.9–160179.8 × 10^{12}/L) (Rozas-Serri et al., 2022) and channel catfish (1500.0–4100.0 × 10^{12}/L) (Tavares-Dias & Moraes, 2007b) (Table 5). Erythrocyte indices are useful indicators of fish health, providing insights into their overall well-being (Witeska, 2015). The variations in total RBC counts in different fish species reflect physiological differences in meeting oxygen demands between sedentary versus active swimmers (Franklin et al., 1993; Putnam & Freel, 1978; Satheeshkumar, 2012; Witeska, 2013). The

TABLE 6 A summary of total and differential leucocyte counts or percentages in clinically healthy juvenile *L. calcarifer* (this study) and other teleost fish species.

Fish groups	Species	Salinity	Mean wt (g)	WBC (10^9/L)	Lymphocytes (10^9/L)	Small lymphocytes (10^9/L)	Large lymphocytes (10^9/L)	Monocytes (10^9/L)	Neutrophils (N)/ heterophils (H) (10^9/L)	References
Tilapia	Tilapia (*O. hybrids*)	FW	240.0	21.6–154.7	–	6.8–136.4	2.9–30.8	0.4–4.3	0.6–9.9 (N)	Hrubec et al., 2000
	Hybrid Tilapia (*O. aureus* × *O. niloticus*)	FW	72.0–556.0	18.8–151.8	8.2–139.6	–	–	0.2–6.1	0.5–16.0 (N)	Mauel et al., 2007
Catfish	Striped catfish (*P. hypophthalmus*)	FW	23.6–39.4	10.0–11.6	–	–	–	–	–	Manna et al., 2021
	Duckbill catfish (*S. lima*)	FW	54.1 ± 15.1	30.2–52.5	24.1–42.1	–	–	0.0–1.0	1.0–5.2 (N)	Bianchi et al., 2014
	Yellow catfish (*H. brachysoma*)	FW	36.0–161.0	20.4–36.7	–	–	–	–	–	Prasad & Charles, 2010
	Striped catfish (*P. hypophthalmus*)	FW	141.7 ± 41.2	36.3–94.3	19.0–60.0	13.8–51.5	0.7–21.2	0–7.6	–	Galagarza et al., 2017
	Channel catfish (*I. punctatus*)	FW	98.5–834.5	27.5–41.5	5.4–11.6	–	–	2.9–7.5	12.5–22.7 (N)	Tavares-Dias & Moraes, 2007b
	African catfish (*C. gariepinus*)	FW	1800.0	18.7–25.6	51.1–70.2 (%)	–	–	1.9–4.0 (%)	27.6–40.1 (%) (N)	Akinrotimi et al. (2011)
Salmonids	Coho Salmon (*O. kisutch*)	FW	50.0–150.0	3.7–24.2	2.4–19.3	–	–	0.06–1.0	0.5–5.3 (N)	Rozas-Serri et al. (2022)
	Atlantic salmon (*S. salar*)	FW	50.0–150.0	4.5–16.5	3.8–13.0	–	–	0.05–0.4	0.7–3.5 (N)	Rozas-Serri et al. (2022)
	Rainbow trout (*O. mykiss*)	FW	50.0–150.0	7.2–24.5	9.1–15.7	–	–	–	0.3–5.9 (N)	Rozas-Serri et al. (2022)
	Rainbow trout (*O. mykiss*)	FW	131.0–385.3	34.1–85.0	28.1–64.0	14.9–42.0	7.5–37.7	1.2–7.0	4.5–18.8 (N/H)	Nabi et al. (2022)
	Chinook salmon (*O. tshawytscha*)	FW/SW	44.0–3164.0	11.3–44.1	86.3–99.0 (%)	–	–	–	–	Casanovas et al. (2021)
Cichlid	South American cichlid (*C. dimerus*)	FW	27.1–50.2	6.6–18.6	2.6–7.1	–	–	1.2–3.3	1.9–5.2 (H)	Rey Vázquez and Guerrero (2007)
	Red top ice blue cichlid (*M. greshakei*)	FW	99.7 ± 4.1	22.9–55.2	21.2–52.4	–	–	0.1–1.7	0.3–2.4 (N/H)	Snellgrove and Alexander (2011)
Others	Common carp (*C. carpio*)	FW	20.6–60.7	51.3–60.8	87.3–91.7 (%)	–	–	0.7–2.0	5.6–8.9 (N)	Witeska et al. (2016)
	Sobaity sea bream (*S. hasta*)	SW	35.1 ± 0.9	8.8–14.5	85.0–96.0 (%)	–	–	–	2.0–6.0 (%) (N)	Torfi Mozanzadeh et al. (2015)
	Asian seabass (*L. calcarifer*)	**SW**	**41.8 ± 2.1**	**5.3–69.9**	**4.7–51.4**	**–**	**–**	**0.3–16.2**	**0.6–8.4 (H)**	**Present study**
	Spotted rose snapper (*L. guttatus*)	SW	59.0 ± 23.7	25.2–111.2	82.6–100.0 (%)	–	–	0.6–5.4 (%)	0.2–8.4 (%) (N)	Del Rio-Zaragoza et al. (2011)
	Yellow Perch (*P. flavescens*)	FW	125.0 ± 14.0	52.6–186.5	–	36.8–153.4	3.5–23.1	0.7–12.6	1.9–36.0 (N)	Hrubec and Smith (2004)
	Koi (*C. carpio*)	FW	200	19.8–28.1	74.5–83.7 (%)	–	–	2.3–3.4 (%)	8.0–13.9 (%) (N)	Tripathi et al. (2004)
	Senegalese sole (*S. senegalensis*)	SW	288.0 ± 56.0	66.1–200.4	31.2–63.1 (%)	–	–	0.7–7.1 (%)	3.0–8.4 (%) (N)	Peres et al. (2015)
	Sablefish (*A. fimbria*)	SW	186.0–440.0	4.3–33.1	66.0–96.0 (%)	–	–	0.0–12.1 (%)	1.8–23.4 (%) (N)	Schubiger et al. (2021)
	Striped bass (*M. saxatilis*)	FW	1800.0 ± 150.0	14.7–88.3	–	–	–	–	–	Fazio et al. (2020)
	Shortnose sturgeon (*A. brevirostrum*)	FW	2590.0 ± 860.0	28.4–90.8	–	9.1–56.7	2.1–10.4	0–7.1	3.8–33.6 (N)	Knowles et al. (2006)

TABLE 7 A summary of mean blood parameters in clinically healthy juvenile *L. calcarifer* as reported in this and other studies on the blood of clinically healthy *L. calcarifer*.

Fish size (g)	*n*	Salinity	GLU (mmol/L)	Hb (g/L)	Hct (%)	Total/plasma protein (TP/PP) (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Lymphocyte (10^9/L)	Monocyte (10^9/L)	Neutrophils (N)/ heterophils (H) (10^9/L)	References
1.5±0.2	9	SW	–	62.0±0.03	20.3±0.1	–	0.1±0.01	–	–	–	1.6±0.1	–	–	–	Adorian et al. (2018)
2.7±0.7	3	SW	–	60.0±1.0	–	–	2.1±0.01	–	–	–	24.1±0.1	–	–	–	Devakumar and Chinnasamy (2017)
5.2±0.2	6	SW	–	73.0 (%)	31.6	–	–	–	–	–	–	–	–	–	Ilham et al. (2016)
7.1±0.1	9	SW	2.6±0.3	72.0±1.0	34.7±0.2	38.8±8.3 (TP)	3.5±0.03	101.5±0.4	19.5±0.3	184.0±3.0	7.8±0.1	–	–	–	Syed Raffic Ali et al. (2018)
7.8±0.02	6	SW	2.7±0.2	82.0±3.0	34.3±1.7	–	3.3±0.3	92.0±1.0	20.9±0.7	215.0±10.4	7.3±0.1	–	–	–	Syed Raffic Ali et al. (2017)
8.1±0.1	9	SW	5.6±0.3	72.5±0.3	33.2±2.7	13.2±5.9 (TP)	3.3±0.5	79.7±1.4	24.8±3.9	301.2±22.7	8.3±0.6	–	–	–	Ali et al., 2017
12.2±0.4	9	SW	–	74.3±1.5	38.5±0.4	–	3.1±0.03	96.3±0.2	21.4±0.2	213.0±5.0	7.3±0.03	–	–	–	Syed Raffic Ali et al. (2016)
18.0±1.0	5	SW	6.5±0.1	70.0±2.0	25.3±1.3	0.001±0.0004 (TP)	0.1±0.01	–	–	–	0.2±0.001	28.2±1.4 (%)	21.3±1.4 (%)	38.7±1.4 (%) (N)	Talpur et al. (2013)
18.0±2.0	5	SW	6.3±0.1	–	–	0.002±0.001 (TP)	–	93.8±1.4	21.2±1.1	26.4±0.2 (%)	–	22.8±1.2 (%)	16.1±1.2 (%)	43.5±1.4 (%) (N)	Talpur and Ikhwanuddin (2013)
20.0±1.0	6	SW	6.3±0.1	–	–	0.001±0.001 (TP)	–	–	–	–	–	–	–	–	Talpur (2014)
20.0±2.0	5	SW	5.7±0.2	0.6±0.1 (g %)	18.2±1.4	0.002±0.0002 (TP)	9.2±0.1	–	–	–	0.2±0.001	21.4±2.5 (%)	13.2±1.7 (%)	53.3±2.4 (%) (N)	Talpur and Ikhwanuddin (2012)
28.2±0.1	3	SW	2.5±0.2	105.0±2.7	32.7±0.9	36.0±1.1 (TP)[a]	3.0±0.1	–	–	–	0.5±0.01	–	–	–	Lim et al. (2019)
32.8±2.2	3	FW	2.3±0.2	–	–	37.5±2.0 (TP)	–	–	–	–	–	–	–	–	Longbaf Dezfouli et al. (2019)
35.0±5.0	3	SW	5.3±0.2	–	–	31.3±2.0 (TP)[a]	–	–	–	–	–	–	–	–	Norouzi et al. (2021)
41.8±2.1	**60–156**	**SW**	**6.7±0.2**	**72.2±0.08**	**29.3±0.4**	**66.2±0.05 (PP)**	**7.2±0.2**	**46.6±1.8**	**11.0±0.4**	**251.7±2.7**	**29.2±2.1**	**21.2±1.4**	**4.6±0.5**	**3.5±0.3 (H)**	**Present study**
46.6±0.2	20	–	–	61.1±0.2	28.8±0.1	36.7±0.03 (TP)[a]	–	94.4±0.1	15.2±0.01	2.9±0.2	–	–	–	–	Kathirkaman et al. (2018)
235.0–410.0	25	SW	3.6±0.1	53.2±2.4	44.3±1.3	0.05±0.01 (TP)[a]	3.0±0.3	13.4±2.3	156.6±5.5	120.1±9.5 (%)	21.6±1.5	–	–	–	Satheeshkumar (2012)
350.0–500.0	5	SW	3.6±0.2	53.2±0.03	44.3±5.9	0.05±0.01 (TP)[a]	3.0±0.2	13.4±9.9	156.6±2.1	120.1±1.2 (%)	21.6±0.2	–	–	–	Satheeshkumar et al. (2011)
350.0–600.0	5	SW	3.6±0.2	53.2±0.03	44.3±5.9	0.05±0.01 (TP)[a]	3.0±0.2	13.4±9.9	156.6±2.1	120.1±1.2 (%)	21.1±3.6	–	–	–	Satheeshkumar et al. (2012)
468.0±12.9	2	SW	4.2±0.1	–	–	–	–	–	–	–	–	–	–	–	Wilkinson et al. (2008)
500.0	16	SW	–	10.1±1.1	31.1±3.0	–	–	–	–	32.4±2.1 (g 100 mL^{-1})	–	–	–	–	Paterson et al., 2003
40.0–100.0 cm	4	Brackish	–	–	–	–	–	–	–	–	–	64.0–92.0 (%)	4.0–16.0 (%)	6.0–36.0 (%) (N)	Masterman and Barnes (2016)

[a]Represents studies that reported plasma protein (PP) as total protein (TP) despite using anticoagulants.

RIs of haematocrit (Hct) and haemoglobin (Hb) in *L. calcarifer* established in this study, ranging from 18.9% to 39.2% and 56.0 to 85.0 (g/L), respectively, are comparable to those reported in most fish species (Table 5) and other studies on *L. calcarifer* (Table 7). Typically, Hct levels in fish range between 20% and 45% (Clauss et al., 2008; Torfi Mozanzadeh et al., 2015). Lower Hct ranges 9.4%–26.7% reported in Senegalese sole (*S. senegalensis*) may be attributed to the lower oxygen demand in this bottom-dwelling species (Peres et al., 2015). Higher Hct levels (>45%) have been reported in teleost species such as salmonids (38.3%–68.5%) (Rozas-Serri et al., 2022), spotted rose snapper (33.5%–71.1%) (Del Rio-Zaragoza et al., 2011) and sablefish (36%–62%) (Schubiger et al., 2021) (Table 5). Occasionally, transient spikes in Hct levels may occur as a result of various factors such as splenic contraction in response to stressors, such as fish capture or handling (Frisch & Anderson, 2000; Gallaugher & Farrell, 1998), hypoxia (Silkin & Silkina, 2005) and dehydration (Clauss et al., 2008).

The blood cell indices of *L. calcarifer* in this study revealed that their RBCs are smaller but exhibit similar Hb concentration compared to other fish species (Table 5). In this study, clinically healthy *L. calcarifer* demonstrated an MCV range of 25.8–78.1 fL and MCH of 6.5–17.4 pg, differing from 150 to 350 fL for MCV and 30–100 pg for MCH typical in teleost species (Hrubec & Smith, 2010). However, the MCHC RI of *L. calcarifer* (197.4–317.6 g/L) in this study compares well with channel catfish (205.0–226.0 g/L), South American cichlid (174.3–303.1 g/L) and spotted rose snapper (111.6–310.9 g/L) (Table 5). The mean MCV (46.6 ± 1.8 fL), MCH (11.0 ± 0.4 pg) and MCHC (251.7 ± 2.7 g/L) in this study are also consistent with the findings from other studies on *L. calcarifer* (Table 7). Natural variations in the size of erythrocytes exist between fish species and tend to be smaller in more active swimming fish (Hrubec & Smith, 2010; Lahnsteiner, 2021).

The RI for the total WBC count in this study is $5.3–69.9 \times 10^9$/L compares well with studies on *L. calcarifer* and other teleost species (Tables 6 and 7). Variations in total WBC count in fish may be attributed to species differences (Fazio et al., 2013), sex (Motlagh et al., 2012), or the feeding habits and environment in which they reside (Parrino et al., 2018). Notably, the mean WBC count of *L. calcarifer* ($29.2 \pm 2.1 \times 10^9$/L) in this study is higher than reported in other studies on this species (Table 7). This discrepancy may be due to the sampled *L. calcarifer* originating from a commercial farm setting, where husbandry stressors such as handling, high stocking density and poor water quality could trigger immune responses, leading to elevated WBC counts (Thrall et al., 2012). Differential WBC count is a valuable indicator of fish health (Modra et al., 1998). Dominantly high lymphocytes, low heterophils neutrophils and low monocytes differential counts were reported in healthy *L. calcarifer* in this and one other study (Masterman & Barnes, 2016). B lymphocytes are the predominant WBC type in the peripheral blood circulation of normal, healthy fish (Haugland et al., 2012; Sunyer et al., 2006). Studies, where neutrophils were elevated in clinically healthy *L. calcarifer* (Talpur et al., 2013; Talpur & Ikhwanuddin, 2012, 2013), would suggest challenges from infectious agents (Stakauskas et al., 2007). Migration of neutrophils into the blood circulation to inflammatory sites occurs within minutes and peaks within 1–2 days in zebrafish and Korean catfish (Martin & Feng, 2009; Yu et al., 2010). Neutrophilia precedes the arrival of monocytes from the circulatory system to the inflammatory site (Roberts, 2012). Few studies have reported the presence of eosinophil and basophils in *L. calcarifer*, and this concurs with our observations (Talpur & Ikhwanuddin, 2012, 2013). Similarly, eosinophils and basophils are either scarce or absent in sablefish (Schubiger et al., 2021), yellow perch (Hrubec & Smith, 2004), African catfish (Akinrotimi et al., 2011), wild Australian catfish (Kelly & Gibson-Kueh, 2015) and Koi fish (Tripathi et al., 2004).

This study is among the first to establish blood RIs for clinically healthy, farmed juvenile *L. calcarifer* and detection of subclinical diseases in fish to support early intervention. Blood RIs can vary with fish size, species and husbandry practices. Therefore, it is important to establish farm-specific baseline values or RIs for different age groups under varying culture conditions, for evaluating the health status of farmed *L. calcarifer*.

AUTHOR CONTRIBUTIONS

Xian Zhe Chew and **Susan Gibson-Kueh** conceived and designed the study. **Xian Zhe Chew** conducted the field sampling and blood analysis. **Xian Zhe Chew** and **Susan Gibson-Kueh** performed data analysis of histopathology. **Xian Zhe Chew** and **Susan Gibson-Kueh** wrote, reviewed and edited the manuscript. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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