Received: 14 April 2024 | Revised: 12 June 2024 | Accepted: 17 June 2024

DOI: 10.1111/jfd.13994

Journal of Fish Diseases WILEY

RESEARCH ARTICLE

Changes in blood serum parameters in farmed rainbow trout (*Oncorhynchus mykiss*) during a piscine lactococcosis outbreak

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Abstract

The aquaculture sector plays a vital role in global food security, yet it grapples with significant challenges posed by infectious diseases. Piscine lactococcosis is one of the significant threats in rainbow trout aquaculture due to its potential to cause severe economic losses through mortalities, reduced growth rates, and increased susceptibility to other pathogens. It poses challenges in disease management strategies, impacting the sustainability and profitability of rainbow trout farming. The current study focuses on the variations in serum blood parameters of farmed rainbow trout *Oncorhynchus mykiss* during a lactococcosis outbreak caused by *Lactococcus garvieae*. Blood samples were collected for biochemical analysis, fish were examined for parasites and bacteria, and DNA from bacterial colonies was PCR-amplified and sequenced for identification. Overall, 13 biochemical parameters, including proteins, enzymes, lipids, chemicals, and minerals, were measured in serum blood samples from both diseased and healthy fish. The results indicate significant alterations in the levels of these parameters during the outbreak, highlighting the impact of infections on the blood profile of farmed rainbow trout. Urea levels were significantly higher in diseased fish compared to controls, and creatinine, phosphorus, and magnesium also showed similar trends. Alanine aminotransferase and total protein levels were higher in control fish. Chloride levels differed significantly between groups. Iron levels were higher in controls and lower in diseased fish. No significant differences were found in other parameters. This study reveals significant changes in serum blood parameters of rainbow trout during a lactococcosis outbreak caused by *L. garvieae*. These changes highlight the potential of these parameters as tools for monitoring health status, stress, and aquaculture management. Continuous monitoring can provide valuable insights into disease severity and overall fish health, aiding in the development of improved management practices. The presented data contribute to understanding

the pathophysiology of piscine lactococcosis and developing effective mitigation strategies for farmed rainbow trout.

KEYWORDS
aquaculture, blood parameters, fish health, *Lactococcus garvieae*

1 | INTRODUCTION

Aquaculture emerges as a rapidly advancing sector within global food production, serving as a pivotal contributor to both the worldwide food supply and economic growth (FAO, 2022; Subasinghe et al., 2009). Over the years, aquaculture practices have evolved significantly, leveraging technological advancements to increase efficiency and production. However, this growth in the aquaculture industry encounters various challenges, with infectious diseases posing a substantial threat to farmed species health and production (Behringer et al., 2020; Lafferty et al., 2015; Murray & Peeler, 2005; Toranzo et al., 2005).

Piscine lactococcosis is one of the emerging bacterial diseases that affects both freshwater and marine fish species in various geographical locations including Africa, Europe, South America, North America, and Asia (Bwalya et al., 2021; Colussi et al., 2023; Fukushima et al., 2016; Gibello et al., 2016; Meyburgh et al., 2017; Ortega et al., 2020; Shahin et al., 2021; Vendrell et al., 2006). Multiple species of the genus *Lactococcus* have been recovered from different wild and farmed fish including *L. garvieae*, *L. plantarum*, *L. piscium*, *L. petauri* and *L. formosensis* (Abraham et al., 2023; Shahin et al., 2021; Soltani et al., 2021; Vela et al., 2024; Vendrell et al., 2006). The clinical signs of piscine lactococcosis include acute hemorrhagic septicemia, erratic swimming, lethargy, exophthalmia, anorexia, skin lesions, alterations in blood parameters and moderate to high mortality (Pastorino et al., 2019, 2020; Shahin et al., 2021; Vendrell et al., 2006). *L. garvieae* is one of the etiological agents of piscine lactococcosis causing significant economic losses globally. *L. garvieae* is a gram-positive bacterium that infects a wide range of fish in warm, temperate and cold-water environments including tilapia *Oreochromis* spp. (Bwalya et al., 2021; Egger et al., 2023; Evans et al., 2009), rainbow trout *Oncorhynchus mykiss* (Shahin et al., 2021), largemouth black bass *Micropterus salmoides* (Abraham et al., 2023), wels catfish *Silurus glanis* (Ravelo et al., 2003), bastard halibut *Paralichthys olivaceus* (Lee et al., 2001), cage-cultured so-iuy mullet *Planiliza haematocheilus* (Han et al., 2015), flathead grey mullet *Mugil cephalus* (Chen et al., 2002; Tsai et al., 2012), sea bass *Dicentrarchus labrax* (Salogni et al., 2024), yellowtail amberjack *Seriola lalandi* and Japanese amberjack *S. quinqueradiata* (Kawanishi et al., 2005), rockfish *Sebastes schlegelii* (Kang et al., 2004), snubnose pompano *Trachinotus blochii* (Neupane et al., 2023) and cobia *Rachycentron canadum* (Rao et al., 2022), clown fish *Coris aygula* (Colorni et al., 2003) as well as lemon damsel *Pomacentrus moluccensis* and striped catfish *Pangasianodon hypophthalmus* (Choi et al., 2019). In addition, the bacterium has been also detected in crustaceans and molluscs including farmed giant river prawn *Macrobrachium rosenbergii* (Chen et al., 2001), wild and cultured octopus *Octopus vulgaris* (Fichi et al., 2015) and recently white leg shrimp *Penaeus vannamei* (Ballantyne et al., 2023).

Among mammals, cases of *L. garvieae* have been investigated in bottlenose dolphins *Tursiops truncates* (Evans et al., 2006). The pathogen's adaptability and resilience across diverse environmental conditions contribute to its extensive global distribution. Its impact extends beyond aquatic organisms, with its presence observed in polygastric and monogastric mammals, including humans (Colussi et al., 2023; Francés-Cuesta et al., 2022).

Rainbow trout *O. mykiss*, holds a notable position as a species of particular importance in aquaculture. The total European rainbow trout production is estimated at 364,930 tonnes of live weight, and Italy stands as one of the leading producers, contributing with ~11% of total production equal to 40,441 tonnes (FAO, 2023). In addition, it is the most sensitive cultured fish species to *L. garvieae* are presenting acute disease associated with elevated mortalities and significant economic losses (Khalil, Bulfon, et al., 2023; Khalil, Orioles, et al., 2023; Khalil, Saccà, et al., 2023; Pastorino et al., 2019; Shahin et al., 2021). Thus, more research investigating the effect of piscine lactococcosis on health and sustainability of rainbow trout aquaculture are warranted. Recent studies have emphasized the importance of establishing haematological and biochemical reference intervals for different fish species to monitor health and disease states. For instance, reference intervals for haematological and serum biochemical parameters were established for chirruh snowtrout *Schizothorax esocinus* (Reshi & Ahmed, 2022), sea trout *Salmo trutta* (Sheikh et al., 2022), and *O. mykiss* (Nabi et al., 2022). These studies have shown significant variations in blood parameters based on sex and other physiological factors, highlighting their utility in health assessments.

A comprehensive understanding of changes in serum blood parameters is of great importance as it can assist in the development of indicators of the health status of fish in response to changes related to diet, water quality and various diseases. In addition, the blood profile of a cultured fish can provide insights into its physiological condition and overall health (Fazio et al., 2013; Fazio, 2019; Forouhar Vajargah et al., 2019). Integrating haematology with other standard diagnostic techniques allows to detect and evaluate stress-inducing factors and diseases that may impact production efficiency. Fish haematological studies began in 1943 (Field et al., 1943), with subsequent significant advancements in literature and techniques, enhancing understanding of fish blood cell analysis (Fazio, 2019).

While haematology is a reliable and non-lethal method extensively used in humans to assess health status affordable, comprehensive and standardized parameters are still lacking in farmed fish (Chen & Luo, 2023).

Moreover, blood analyses offer valuable insights into animal welfare by assessing neuroendocrine and immune system activation, impacts of adverse breeding conditions, xenobiotics, disease and genetic predispositions (Azadikhah, Varcheh, et al., 2023; Azadikhah, Yalsuyi, et al., 2023; da Silva Liebl et al., 2022; Pastaki et al., 2023; Rashidian et al., 2022; Saha et al., 2023; Seibel et al., 2021).

Unlike mammals, fish have nucleated erythrocytes, rendering traditional automated analysis methods unsuitable: automated blood cell count analysis holds promise as a future diagnostic tool in aquaculture, potentially replacing manual methods while addressing this challenge (Fazio, 2019).

Physiological parameters in blood, plasma, or serum respond to stress conditions across various fish species. For example, handling stress in Atlantic salmon *Salmo salar*, can trigger changes in O-phosphocholine, lactate, carbohydrates, alanine, valine, trimethylamine-N-oxide, and Di-O-acetylated sialic acids (Karakach et al., 2009; Liu et al., 2008). Similarly, in rainbow trout, stress from "hook and line" methods affect cell counts, haematocrit, glucose, and clotting time (Casillas & Smith, 1977), while manual stripping influences plasma cortisol levels (Stone et al., 2008).

Stocking density also impacts the haematological parameters of fish. In Atlantic salmon, a density of 30 kg/m^3 alters alkaline phosphatase, immunoglobulin M, cortisol, and maleic dialdehyde (Liu et al., 2015). In rainbow trout (40–70 kg/m^3), stocking density affects cortisol, cholesterol, glucose, triglyceride, and lactate (Conde-Sieira et al., 2010; North et al., 2006; Yarahmadi et al., 2014), while in brook trout *Salvelinus fontinalis*, at 120 kg/m^3, glucose levels change (Vijayan et al., 1990).

The diet also plays a significant role, with factors such as food deprivation or a plant-based diet with yeast fraction affecting very-low-density lipoproteins, high-density lipoprotein, choline, β-glucose, lactate, and histidine in *O. mykiss* (Kullgren et al., 2010; Yonar et al., 2011). Additionally, pollution, like oxytetracycline, can lead to changes in sodium dismutase, erythrocyte, and leukocyte numbers (Roques et al., 2020). Recent studies describe variations in blood parameters during fish infections, especially cyprinids (Harikrishnan et al., 2003; Reda et al., 2024) and salmonids (Isla et al., 2022). Specifically, they highlight significant changes in levels of white blood cells (WBCs), red blood cells (RBCs), haemoglobin (Hb) and haematocrit/packed cell volume (PCV), and other blood parameters in response to infection by pathogenic bacteria such as *Aeromonas hydrophila*, *Shewanella* spp., and *Piscirickettsia salmonis*.

Despite the extensive research on piscine lactococcosis, encompassing clinical signs, histopathological changes, conventional and advanced diagnostics (Abraham et al., 2023; Shahin et al., 2021, 2022; Vendrell et al., 2006), changes in serum blood parameters during lactococcosis in rainbow trout are yet to be addressed.

This study aims to fill this existing gap by conducting a detailed analysis of changes in serum blood parameters during a lactococcosis outbreak in farmed rainbow trout, with a specific focus on exploring potential biomarkers to advance knowledge and enhance disease prevention and control strategies in aquaculture.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling activities

The study was conducted in a trout facility located in northern Italy, where cases of piscine lactococcosis were reported in November 2023.

Four tanks within the farm were selected including three naturally infected tanks (R1-3) and a single control tank (i.e., uninfected fish). The main physicochemical parameters [temperature (°C), dissolved oxygen (mg/L) and pH] of the fish rearing water were measured at the time of sampling. Both the control group and infected trouts were reared at a density of 25 kg/m^3 in raceway systems supplied by well water (13–14°C). The rainbow trout of the control group were reared in raceways measuring 100 × 6 × 1 m, while trout affected by lactococcosis were raised in raceways of 43 × 6 × 1 m. Fish were fed twice daily, with an amount equivalent to 1% of the tank biomass.

Fifty-two unvaccinated adults of rainbow trout *Oncorhynchus mykiss* (280.40 ± 25.31 g), including both symptomatic fish with lactococcosis and a control group ($n = 13$ from each tank), were randomly sampled. Euthanasia of the fish was done using an overdose (170 mg/kg) of tricaine methanesulfonate (Sigma-Aldrich, Milan, Italy) before sampling.

2.2 | Blood sampling

Blood samples were immediately collected via caudal vein puncture using a 2.5 mL sterile syringe and placed into Vacuette® tubes containing serum clot activator (Greiner Bio-One GmbH, Kremsmünster, Austria). Collected blood samples were kept at 4°C till processing.

2.3 | Diagnostic investigations

After blood sampling, each fish was weighed (g) and measured for total length (cm).

Following the necropsy of the sampled fish, the disease status of the fish was assessed via external and internal examination. A comprehensive parasitological examination was conducted to detect both ecto- and endoparasites. Macroscopic and microscopic analyses were performed on the gills, skin, abdominal cavity, and digestive tract using a NIKON ECLIPSE Ci-L microscope (Nikon, NY, USA).

Bacteriological analysis was carried out using kidney, eyes and brain swabs inoculated on Columbia Blood Agar (CBA) and Tryptic Soy Agar (TSA), incubated at 22 ± 1°C for 72 h, and checked daily. Dominant pure colonies were subcultured on CBA and sent for

identification by MALDI-TOF (Bruker Daltonics Inc., Billerica, MA, USA) and subsequent molecular analyses.

2.4 | Blood sample preparation and analysis

The blood serum was collected through centrifugation for 15 min, at 2000 rpm at 10°C using an ALC multi-speed refrigerated centrifuge (PK131R, USA). A visual inspection of the centrifuged samples was done to eliminate any potential haemolysis that might impact the accuracy of results followed by storing at −80°C until further analysis.

The following blood parameters were examined in the retrieved serum samples using an automated system photometer (I-Lab Aries Chemical Analyser-Instrumentation Laboratory, Bedford, MA, USA), proteins such as total proteins and albumin; enzymes like alanine aminotransferase and aspartate aminotransferase; lipids, including cholesterol and triglycerides; and metabolites such as urea, creatinine, and chloride, as well as minerals like magnesium, phosphorus, calcium, and iron, following the method previously described by Pastorino et al. (2019, 2020).

2.5 | Molecular identification of bacterial colonies

DNA was extracted from selected bacterial colonies on CBA using a combination of boiling and freeze-thawing procedures. In brief, the bacterial colonies were suspended in RNase and DNase-free water. The suspension was then heated at 95°C for 15 min, followed by freezing at −20°C. DNA was collected by centrifugation at 7000 rpm for 5 min, and the resulting supernatant was utilized for further analysis.

The 16S-23S rRNA Internal Transcribed Spacer (ITS) region was amplified in GeneAmp PCR System 9700 (Applied Biosystems) using polymerase chain reaction (PCR) following the method described by Stoppani et al. (2023). Amplicons were analysed on a 2% agarose gel. PCR products were purified using the Qiaquick purification kit (Qiagen®) and then sequenced with the Brilliant Dye Terminator chemistry (v1.1) from NimaGen using the genetic analyser (SeqStudio genetic analyser, Thermo Fisher®). Alignment was performed using DNASTAR Lasergene Software, and the consensus sequence was compared to sequences in the GenBank database utilizing the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi; Accessed on 10/01/2024).

2.6 | Statistical analysis

All statistical procedures and analyses were performed using R software (v. 4.3.2). Normality of the data was assessed using the Shapiro–Wilk test, and homoscedasticity was evaluated using the Levene test. The analysis of significant differences among the groups for each considered biochemical parameter involved the application of the Kruskal-Wallis test. A nonparametric pairwise multiple comparison procedure was performed using Dunn's post-hoc test.

Principal Component Analysis (PCA) was also performed to simplify complex data by identifying principal components, reducing dimensionality, and preserving significant variance ("Factoextra" R package).

A level of $p < .05$ was set for statistical significance.

2.7 | Ethics statement

All samples were analysed for diagnostic purposes as a part of routine activities conducted at the laboratory where the authors are employed (http://www.izsplv.it/; Accessed on January 11, 2024). Therefore, the inclusion of an Ethics Committee was not deemend necessary. Animal handling was performed following the European/Italian guidelines on animal welfare norms (L.D. No. 26/2014, implementation of the European Directive 2010/63/EU).

3 | RESULTS

3.1 | Morphometrics of fish, water quality characters and clinical investigation

The average body length and weight of the 52 specimens were 30.2 ± 2.86 cm, and 280.40 ± 25.31 g, respectively. In the trout raceways facilities, the reported water quality values were as follows: temperature at 15.9 ± 0.3°C, dissolved oxygen at 7.4 ± 0.5 mg/L, and pH at 7.1 ± 0.2.

Up on necropsy of the fish, no clinical signs of diseases were recorded in the control group. However, the diseased fish (groups R1-3) showed general anorexia, melanosis, lethargy, loss of orientation, and erratic swimming. Internally, fish showed exophthalmia, ascites, and anal prolapse.

In addition, different levels of haemorrhages and petechiae were observed externally (peri-orbital and intraocular, cutaneous) and in internal organs including swim bladder, intestine, liver, peritoneum, spleen and kidney, and accumulation of ascitic fluid in the peritoneal cavity (Figure 1). Hepatic steatosis and splenomegaly were observed in several specimens. Moreover, no parasites were detected following parasitological examination.

3.2 | Bacteriological analysis and molecular confirmation

Presence of *Lactococcus garvieae* was confirmed following bacteriological analysis, which was detected in 34 out of 39 of the clinically diseased fish (87.17%) while was not detected in any of the control fish. Comparing the bacterial prevalence between the affected tanks, showed that tank R1 recorded the lowest rate of 69.23% (9/13

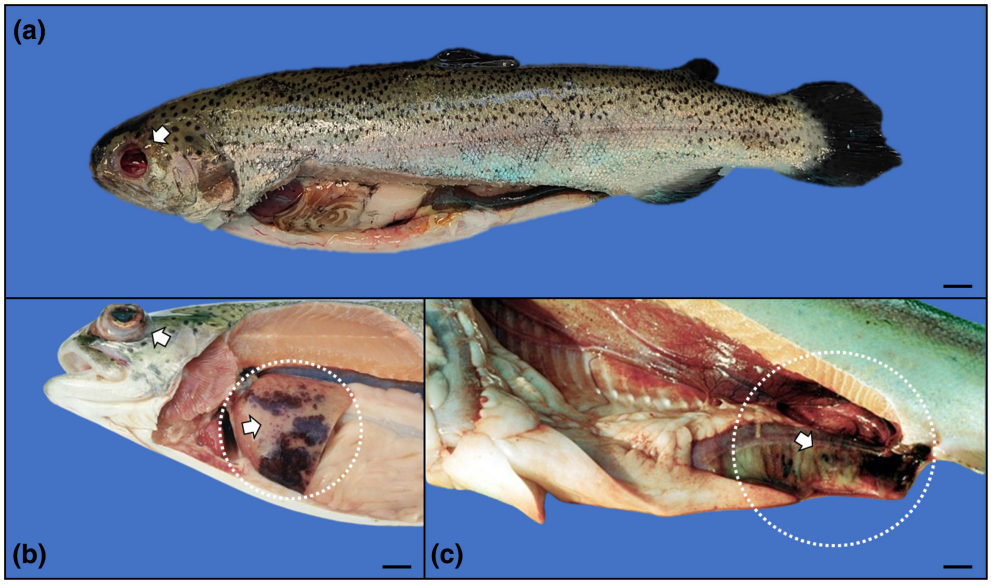

FIGURE 1 Diseased farmed rainbow trout *Oncorhynchus mykiss*, showing typical external and internal clinical signs of piscine lactococcosis (white arrows). (a) Eye enucleation. (b) Unilateral exophthalmia with the presence of haemorrhages in the periorbital and intraocular area; vascular congestion with petechial haemorrhages in the liver. (c) The final segment of the intestine is heavily haemorrhagic. Scale bar: 1 cm.

positive), followed by R3 at a rate of 92.31% (12/13 positive), and the last tank R2 exhibited the highest positive detection rate at 100.00% (13/13 positive). Genetic analysis also confirmed the absence of *L. petauri*, in all examined raceways. The BLAST analysis demonstrated, 100% similarity to *L. garvieae* ITS sequences obtained from rainbow trout in Italy previously deposited into the GenBank database (*L. garvieae* strain 1IT; 1998 Accession number OQ108343).

3.3 | Serum analysis

The urea levels in the screened serum showed that tank R3 presented a statistically significant ($p < .05$) higher value of 1.8 ± 0.42 mg/dL (mean ± SD) in comparison to the other tanks. Notably, there were no significant differences between the control group (1.82 ± 0.09 mg/dL), and tanks R1 and R2, which recorded values of 1.78 ± 0.18 and 1.73 ± 0.10 mg/dL, respectively. Statistical significances were also recorded in the R3 group for creatinine, phosphorus, and magnesium. For creatinine, the control values stand at 0.40 ± 0.06 mg/dL, while R1, R2, and R3 showed values of 0.29 ± 0.10, 0.35 ± 0.09, and 0.30 ± 0.12 mg/dL, respectively ($p < .001$). For phosphorus, the control value was 16.61 ± 3.11 mg/dL, while R1, R2, and R3 showed values of 17.07 ± 3.93, 16.83 ± 2.43, and 19.48 ± 2.16 mg/dL, respectively ($p < .05$). The values of magnesium were 3.70 ± 0.36, 3.53 ± 0.67, 3.64 ± 0.39, and 4.47 ± 0.55 mg/dL for controls, R1, R2 and R3, respectively ($p < .01$).

The control group exhibited statistically significant ($p < .001$) higher alanine aminotransferase value of 56.30 ± 46.45 U/l (in comparison to the other groups), which showed no significant differences between each other and presented values of 16.15 ± 14.68, 11.23 ± 8.76, and 12.60 ± 9.68 U/l, at R1, R2, and R3 groups, respectively. A similar pattern was observed for total proteins, where the control group reported a value of 3.00 ± 0.26 g/dL, which was significantly lower than the other diseased fish tanks ($p < .05$).

Also, statistically significant differences ($p < .05$) were observed for chloride in R1 (109.23 ± 4.66 mmol/L), while no significant differences were detected between the control (115.30 ± 0.85 mmol/L) and tanks R2 and R3 (113.31 ± 2.93 and 109.80 ± 26.31 mmol/L, respectively).

For iron, the highest value was observed in the control group (166.85 ± 119.20 mg/dL), while the lowest value was found in group R3 (40.54 ± 13.11 mg/dL). In addition, values of 72.31 ± 37.91 and 89.46 ± 34.02 mg/dL were found in tanks R2 and R2, respectively. Thus, statistically significant differences were observed for the control ($p < .05$) and for group R3 ($p < .01$).

No statistically significant differences ($p > .05$) were detected among the considered groups for cholesterol, triglycerides, aspartate aminotransferase, calcium, and albumin. The values for all the analysed blood parameters in the 4 tanks are visualized in Figure 2.

The PCA yielded a representative Biplot (Figure 3; Data S1). This enabled the clear categorization of individuals into two distinct groups, corresponding to healthy and diseased fish. The primary axis (Dim1) contributed to 34.7% of the total variance, while the second axis (Dim2) contributed to 20.3%. The substantial cumulative contribution of these two dimensions, amounting to 55.0%, suggests that they capture a significant percentage of the overall variation in the data. Figure 3 shows a clear separation between the two groups (i.e., healthy vs. diseased), indicating that variations in the data distinctly reflect the health status of the specimens.

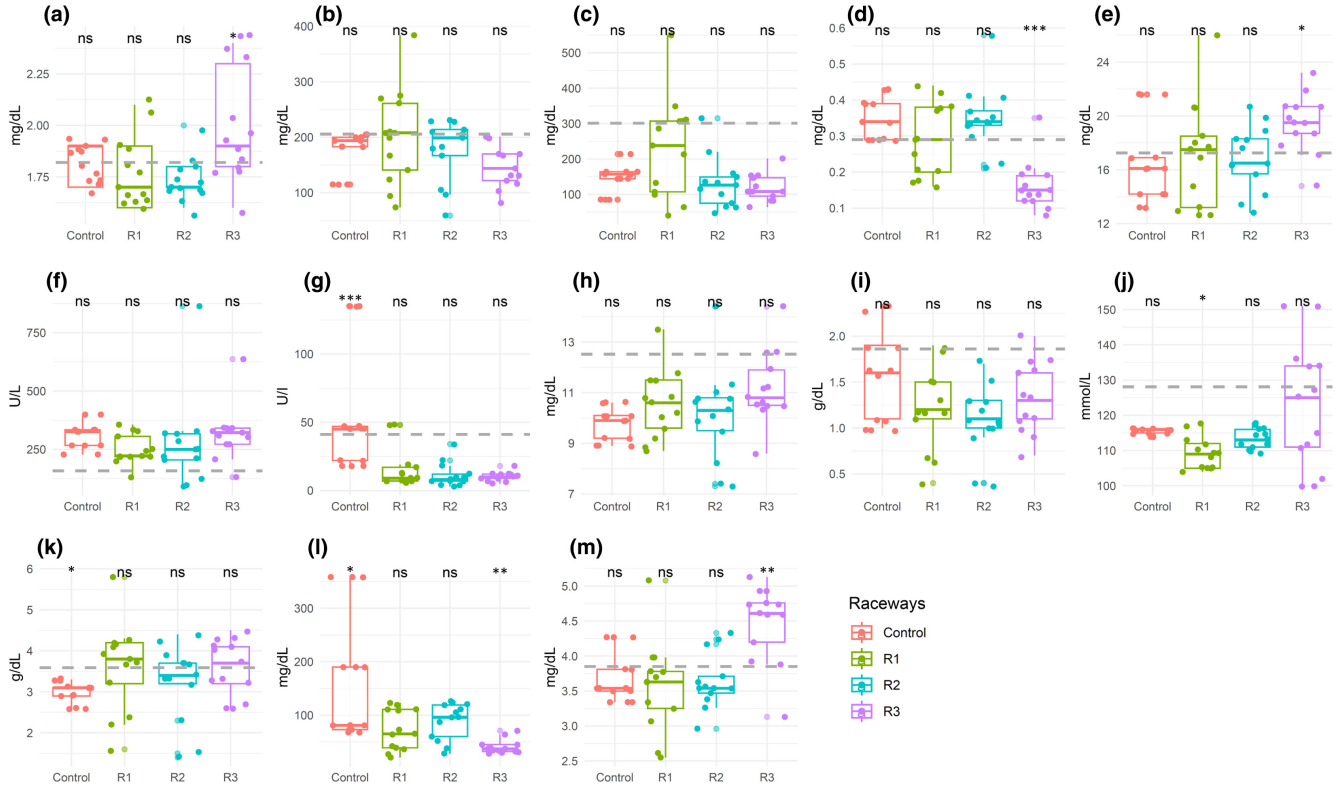

FIGURE 2 Boxplots of serum blood biochemical parameters in farmed rainbow trout *Oncorhynchus mykiss* during a lactococcosis outbreak: a = Urea; b = Cholesterol; c = Triglycerides; d = Creatinine; e = Phosphorus; f = Aspartate aminotransferase; g = Alanine aminotransferase; h = Calcium; i = Albumin; j = Chloride; k = Total protein; l = Iron; m = Magnesium. Asterisks denote significant differences based on Dunn's post-hoc test (ns = not significant; *$p < .05$; **$p < .01$; ***$p < .001$). Grey dashed lines denote baseline values according to Pastorino et al. (2020) and Manera and Britti (2006) (Data S1).

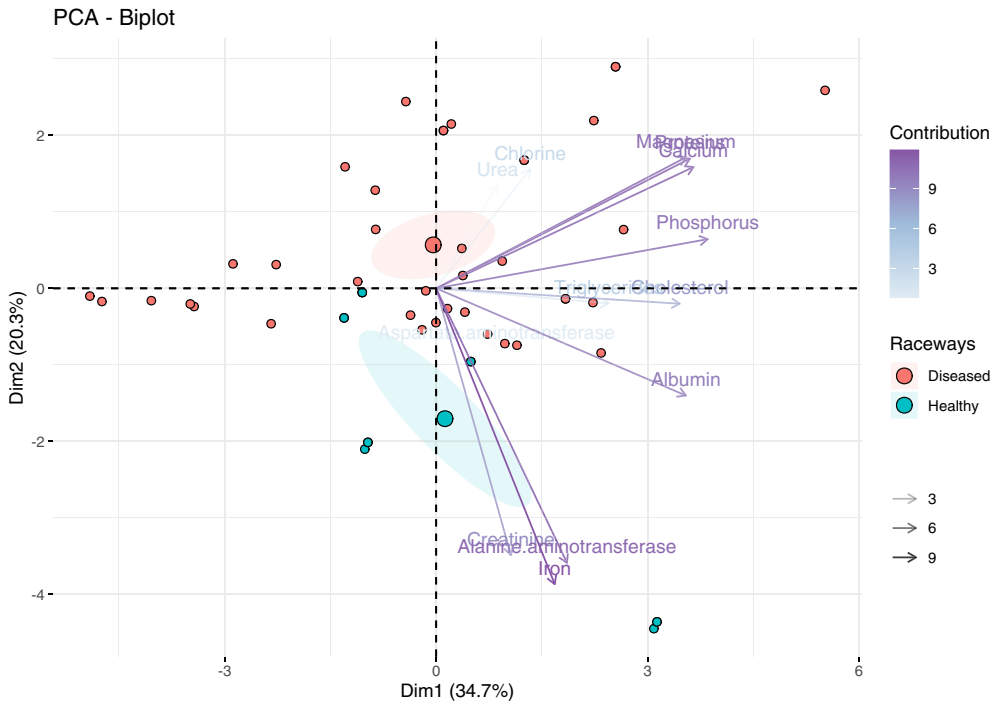

FIGURE 3 PCA-biplot illustrating serum blood biochemical parameters in farmed rainbow trout *Oncorhynchus mykiss* during a lactococcosis outbreak. Two distinct groups were established: diseased and healthy fish. The biplot provides a visualization of relationships among the measured variables, highlighting differences and trends between the two groups concerning the analysed serum biochemical parameters.

4 | DISCUSSION

The assessment of blood biochemical parameters plays a crucial role in assessing the health status of fish (Řehulka & Minařík, 2001). These parameters provide valuable insights into various physiological processes, such as metabolism, immune function, and organ function.

In this study, we collected serum biochemical data for the first time from three distinct diseased groups and a control group of rainbow trout from an aquaculture facility affected by a lactococcosis outbreak caused by *Lactococcus garvieae*. The baseline serum blood biochemical values for rainbow trout were established using the findings of Pastorino et al. (2020) and Manera and Britti (2006) for which the control group showed similar values to them with exception for some individual variability. This variability may be due to the effect of biometric indices like body weight and length which was previously reported to influence certain haematological and biochemical parameters (Fazio et al., 2020).

The results of the Principal Component Analysis demonstrate a clear separation between the group of diseased fish and the group of healthy fish. This separation distinctly highlights significant differences in the measured variables between the two groups. Variables contributing most to this separation could be associated with health factors, immune response, or metabolism, suggesting potential biomarkers for early diagnosis or health monitoring of fish in aquatic environments.

Triglycerides and cholesterol are vital energy substrates demanded in higher quantities during growth phases which orchestrated by reserve mobilization and consumption (Fazio et al., 2020). During lactococcosis outbreaks in trout, changes in triglyceride and cholesterol levels may be attributed to various factors. Significant factors are the clinical signs associated with lactococcosis which often results in anorexia, melanosis, lethargy, disorientation, and erratic swimming (Vendrell et al., 2006). These variable signs may alter lipid metabolism and results in the exhaustion of fish's immune system. Lipids, including fatty acids and their derivatives like eicosanoids, are vital for the well-being of finfish species, influencing growth, health, reproduction and immunity (Balfry & Higgs, 2001).

The inflammatory response may lead to increased lipid production, including triglycerides, to support the energy needs of the immune system and fight the infection. However, it was previously reported that alterations in cholesterol synthesis and metabolism may occur due to physiological stress induced by the disease (Barton & Iwama, 1991).

In this study, while rainbow trout was infected by lactococcosis, lower values were observed compared to basal ones (Figure 2). This suggests a negative impact of the disease on trout health and negatively influences nutrient absorption or metabolism. Previous studies have shown that diseases can compromise metabolic homeostasis in fish (Mateus et al., 2017), but further study is needed to fully understand this phenomenon in presence of lactococcosis and to develop more effective management strategies. Additionally, changes in feeding and feeding behaviour of fish affected by lactococcosis may influence blood triglyceride and cholesterol levels. In contrast to the previous observation, a recent study examined the effect of *Aeromonas hydrophila* infection and high-fat diets on grass carp *Ctenopharyngodon idella* where an increase in fat deposition was reported in the liver, especially with high-fat diets, also impairing the antioxidant capacity of the liver and causing liver damage (Zhao et al., 2019). Elevated levels of Hydroxy lipids (L-OH) and 4-hydroxy-2-hexenal (HHE) were found in the liver of diseased Japanese pufferfish *Takifugu rubripes*, in response to HHE and propanal. These results were also found in other fish species [e.g., *Paralichthys olivaceus*, *Seriola quinqueradiata* and greater amberjack *S. dumerili*] in association with different diseases (Tanaka et al., 2002). Therefore, this could explain the hepatic steatosis found in this study. It is likely that this fat deposition in the liver occurs in the early stages of the disease while more advanced stages cause inappetence resulting in weight loss (anorexia), lethargy, etc.

A slight variation in protein values, including total proteins and albumin, in trout investigated in this study was observed (Figure 2) specifically a decrease from baseline values was found for albumin. This alteration may be attributed to the effects of the disease on liver function and the circulatory system. Reduced levels of proteins may indicate an inflammatory state or liver dysfunction. Previous research has demonstrated that diseases can alter protein profiles in fish (Barton & Iwama, 1991; Ellis, 2001; Piazzon et al., 2016), suggesting that such changes may serve as indicators of health status and the degree of physiological stress in fish affected by lactococcosis. The decrease in albumin concentration may result from increased catabolism during inflammation and reduced food intake in diseased fish (Řehulka & Minařík, 2007). Our results disagree with Aydin et al. (2001) who observed elevated albumin levels in diseased rainbow trout infected with *Serratia liquefaciens*. On the other hand, our findings matched the results of Yildiz and Aydin (2006) and Aydin et al. (2000) who noted lower albumin and total serum protein levels in rainbow trout infected with *Arcobacter cryaerophilus*, and in fish naturally infected with *Campylobacter cryaerophila*, respectively. Moreover, a similar observation was reported in rainbow trout naturally infected by *Aeromonas* spp. (Rehulka, 2002) or co-infected with *Streptococcus* spp. (Barham et al., 1980) where significant decrease of albumin was highlighted. Other studies have also reported a decrease in albumin in diseased salmonids (Řehulka & Minařík, 2007). However, our results for total protein reported a significant increase compared to the control group with values almost double those reported by Řehulka and Minařík (2007) in brook trout *Salvelinus fontinalis* affected by *Flavobacterium columnare*. Our results are yet in agreement to what is reported in cultured striped bass *Morone saxatilis*, although some values, such as cholesterol, protein and triglycerides may be affected by fish size (Fazio et al., 2020).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are valuable biomarkers that are commonly used to assess liver health and respond to environmental and physiological stresses in fish. In addition, it is known that parameters of hepatic function including ALT and AST can vary depending on stage of evolution of the pathology at the time of sampling. In our study, a higher value of ALT

was reported in the control group, while AST levels were stable for all groups (Figure 2). Several studies explored variations in the levels of AST and ALT in fish affected by diseases, including lactococcosis (Gatlin, 2007). These studies demonstrated that fish exposed to disease conditions such as lactococcosis are commonly expressing significant increase in AST and ALT levels serum or liver tissues. These elevations indicate liver damage and/or intense activation of the immune system in response to the disease. Such changes can serve as indicators of disease severity and overall fish health, providing valuable insights for diagnosing and treating diseases in farmed fish.

Metabolites such as urea, creatinine and chloride are crucial for assessing renal function and electrolyte balance and are frequently utilized to evaluate the health status of fish and their response to environmental stressors (Azadikhah, Varcheh, et al., 2023; Azadikhah, Yalsuyi, et al., 2023; Currie & Evans, 2020; Gatlin, 2007; Grosell et al., 2010; Wendelaar Bonga, 1997). In coho salmon *Oncorhynchus kisutch* affected by proliferative kidney disease (PKD), an increase in urea levels indicates a loss of renal function (Wedemeyer & Ross, 1973). In our study, a significant increase was recorded in tank R3 which may be attributed to an advanced stage of lactococcosis associated pathology (Figure 2). These results are in agreement with the findings of Řehulka and Minařík (2007) for natural infection caused by *F. columnare*. Anaemia was characterized by decreased erythrocyte count and haemoglobin levels as well as increased mean corpuscular volume and reduced mean corpuscular haemoglobin concentration. Clinical chemistry analyses showed lower total protein, glucose, and calcium levels, and higher urea levels. Enzyme analyses revealed elevated activity in alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase, with decreased alkaline phosphatase activity. Electrophoretic analysis indicated reduced albumin levels (Řehulka & Minařík, 2007). In addition, high levels of urea were found in diseased fish, potentially related to kidney and skin pathologies. Similar findings were also observed in rainbow trout infected with viral haemorrhagic septicaemia (VHS) infection, *Aeromonas* spp., and PKD (Hoffmann & Lommel, 1984; Rehulka, 2002).

In this study, creatinine showed significantly higher values than baseline values; however, a significant decrease was recorded in group R3 (Figure 2). Since no renal changes were observed on necropsy, the increase in creatinine concentration can related to increased muscle activity (Campbell, 2012).

Essential macro- and microminerals are vital for fish health and growth (Lall & Kaushik, 2021). Microminerals serve various functions, including catalytic, structural, and regulatory roles in enzyme systems (Andreini et al., 2009; Maret, 2010; Mertz, 1998). Deficiencies in these minerals can often impair enzyme activities (Lall, 2010). Fish have diverse iron requirements based on variable factors such as weight gain and body iron levels (Lall & Kaushik, 2021). However, weight gain alone may not accurately reflect iron needs, as iron primarily resides in haemoglobin. As fish grow, their iron requirements may change due to alterations in blood volume and tissue iron deposition rates (Antony Jesu Prabhu et al., 2016; Suttle, 2010). It is of not that basal values for iron are not reported in the literature; however, significantly lower values were found in our study compared to the control group (Figure 2). This may be due to a variety of factors, such as diet, surrounding environment, or possible diseases such as lactococcosis, which may affect iron absorption or metabolism in fishes. Iron is vital for many organisms, including pathogenic bacteria, which must contend with its limited availability in the host environment (Zughaier & Cornelis, 2018). They employ various mechanisms to acquire it, such as siderophore production and uptake of heme or ferrous iron. Some fish-infecting pathogens, like *Vibrio anguillarum* and *Photobacterium damselae*, exhibit specific adaptations for iron acquisition as described in the review article by Li and Ma (2017). Additionally, the *Burkholderia* bacterial genus, encompassing species such as the *B. cepacia* complex and *B. pseudomallei*, causes infections requiring highly adaptive iron acquisition strategies (Butt & Thomas, 2018). Iron uptake is a critical factor in bacterial pathogenesis, affecting both the pathogen and host response (Zughaier & Cornelis, 2018).

Calcium and phosphorus are essential for skeletal development and maintenance, as well as various physiological functions such as maintaining acid–base balance (Zimmer et al., 2019). Low calcium levels disrupted the calcium-phosphorus balance, likely due to hypoproteinaemia, especially hypo-albuminemia, as calcium primarily binds with albumin. Similar values were recorded in our study which is in agreement with Řehulka & Minařík (2007) who reported a correlation between low total protein and low calcium levels.

Magnesium plays an essential role in numerous physiological functions in fish. Its deficiency in fish can lead to various symptoms including anorexia, reduced growth, lethargy, increased mortality, etc. (Lall, 2010; Lall & Kaushik, 2021). In rainbow trout, it can also cause kidney calcinosis, vertebral deformities, and organ degeneration (Cowey et al., 1977; Dabrowska et al., 1989). Our values are in agreement with Manera and Britti (2006), except for group R3 which showed a significant increase of magnesium in response to *L. garvieae* infection (Figure 2). This could be a physiological response to disease, in which magnesium levels increase as part of an immune or defence reaction.

5 | CONCLUSION

The present study highlights significant changes in serum blood parameters of rainbow trout *Oncorhynchus mykiss* during a lactococcosis outbreak caused by *Lactococcus garvieae*. These findings indicate that the assessment of these parameters may serve as a useful tool for monitoring health status, stress, and poor aquaculture management. Additionally, ongoing monitoring of these parameters can offer valuable insights into disease severity and the overall health status of fish populations in aquaculture environments. Understanding these changes can help improve management practices and promote the health and well-being of farmed fish in aquatic environments against piscine lactococcosis caused by *L. garvieae*.

While the standard deviation of the morphometric data indicates a relatively narrow range, implying limited variability around

the mean, it is critical to acknowledge the possibility of subtle differences among the studied fish. Despite their less pronounced nature, these differences could still exert an influence on the serum blood profile and disease outcomes. Even minor variations in morphometric parameters might bear implications for disease severity or progression. Hence, it remains essential to carefully consider these nuances and their potential impact on the interpretation of findings.

AUTHOR CONTRIBUTIONS

Giuseppe Esposito: Conceptualization; writing – review and editing; writing – original draft; methodology; investigation; software; formal analysis; data curation. **Stefania Bergagna:** Methodology; investigation; validation; visualization. **Silvia Colussi:** Writing – review and editing; writing – original draft; conceptualization; methodology; investigation; supervision. **Khalid Shahin:** Writing – original draft; writing – review and editing; methodology; investigation; conceptualization; supervision. **Roberta Rosa:** Methodology; investigation; validation; visualization. **Donatella Volpatti:** Writing – review and editing; methodology; investigation. **Caterina Faggio:** Writing – review and editing; methodology; investigation. **Camilla Mossotto:** Methodology; investigation. **Alice Gabetti:** Methodology; investigation. **Alessandra Maganza:** Methodology; investigation. **Elena Bozzetta:** Writing – review and editing; supervision. **Marino Prearo:** Methodology; investigation; writing – review and editing; conceptualization; supervision. **Paolo Pastorino:** Writing – review and editing; methodology; investigation; conceptualization; supervision; writing – original draft.

ACKNOWLEDGEMENTS

We thank the fish farmers for their availability and assistance during all phases of sampling.

FUNDING INFORMATION

None.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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How to cite this article: Esposito, G., Bergagna, S., Colussi, S., Shahin, K., Rosa, R., Volpatti, D., Faggio, C., Mossotto, C., Gabetti, A., Maganza, A., Bozzetta, E., Prearo, M., & Pastorino, P. (2024). Changes in blood serum parameters in farmed rainbow trout (*Oncorhynchus mykiss*) during a piscine lactococcosis outbreak. *Journal of Fish Diseases*, *00*, e13994. https://doi.org/10.1111/jfd.13994