



Functional effects of quercetin via the haematology–gut axis: Resistance to *Lactococcus petauri* in *Oncorhynchus mykiss*

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ABSTRACT

This study evaluated the effects of low-fat (LFD), high-fat (HFD), and quercetin-supplemented high-fat (HFD+Q; 0.2 g/kg) diets on the haematological responses, intestinal histomorphology/histopathology, and resistance to *Lactococcus petauri* infection in rainbow trout (*Oncorhynchus mykiss*). A total of 270 fish (initial mean weight: 38.37 g) were randomly distributed into three dietary groups with three replicates per treatment (30 fish per tank) and fed the experimental diets for eight weeks. At the end of the trial, haematological parameters were determined using an automated analyser; histomorphometric measurements (muscle layers, villus parameters, and goblet cell counts) and histopathological evaluations were performed on intestinal samples. Furthermore, an intraperitoneal challenge with *L. petauri* was conducted to evaluate disease resistance. The HFD group exhibited significant suppression of leukocyte profiles (*WBC*, *LYM*, *MID*, *GRAN*) and a downward trend in HGB/HCT and erythrocyte indices. This group also displayed intestinal alterations consistent with muscular layer irregularities, submucosal oedema, and loss of goblet cells. In contrast, the HFD+Q group maintained more balanced haematological parameters and intestinal barrier indicators, contributing to the recovery of mucosal defence by increasing goblet cell counts compared to the HFD group. In the challenge trial, the survival probability was significantly higher in the HFD+Q group. In conclusion, quercetin is a functional feed additive with the potential to enhance resistance to *L. petauri* infection by supporting haematological homeostasis and intestinal integrity under high-fat feeding conditions. Further immunological and molecular studies are recommended to elucidate the mechanism of action and optimal dosage.

Keywords: *Oncorhynchus mykiss*, *Lactococcus petauri*, Quercetin, High-fat diet, Haematology, Intestinal histology



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Introduction

Ensuring sustainable production in aquaculture largely depends on maintaining the physiological health of fish (Oliveira et al., 2024; Awad, 2025). In this context, enriching the nutritional content of commercial fish feeds with functional ingredients has become a focal point of increasing interest in recent years (Onomu & Okuthe, 2024). Bioactive compounds of natural origin, in particular, have come to the fore in aquaculture nutrition due to their potential effects, such as reducing oxidative stress and supporting the immune system (Awad, 2025; X. Hu et al., 2025). The evaluation of physiological parameters is considered a fundamental approach to reliably determining the effects of such nutritional interventions on fish health (Oliveira et al., 2024; Onomu & Okuthe, 2024).

Physiological indicators are widely used to determine health status, stress response, and adaptation to environmental conditions in fish. Among these, haematological parameters are evaluated as reliable biomarkers reflecting the effects of nutritional practices, feed additives, and environmental stressors on the organism (Fazio et al., 2013; Seibel et al., 2021). It has been reported that various nutritional applications, such as plant-derived additives, prebiotics, and mineral supplements, can modulate physiological processes related to the immune system, and these effects are frequently observed through haematological changes (Czech et al., 2009; Jalali et al., 2009; Esmacili, 2021). Therefore, assessing physiological changes is of great importance for a holistic understanding of the impact of nutritional interventions on immune functions and overall fish health (Esmacili, 2021; Oliveira et al., 2024).

Intestinal histology represents a fundamental indicator of fish health. Histological investigations of the digestive system are widely regarded as reliable approaches for assessing metabolic status, overall health, nutritional condition, and the effects of environmental factors on fish (Matulić et al., 2020; Vatsos, 2021). The examination and monitoring of the histological architecture of the intestine and liver, which are the primary organs responsible for digestion and nutrient absorption, are commonly employed to evaluate the effects of feed formulations enriched with plant-derived ingredients (Raskovic et al., 2011). Given that intestinal tissue can undergo rapid and pronounced structural alterations in response to dietary components or stress-related factors, histomorphometric analyses enable the quantitative or semi-quantitative assessment of these changes, thereby providing valuable information on the impact of feed ingredients on intestinal function (Vatsos, 2021). Based on these considerations, it can be concluded that histomorphometric evaluations effectively

reveal diet-induced intestinal alterations and, when interpreted alongside other physiological parameters (e.g., haematological indices), allow for a more comprehensive understanding of the physiological responses to nutritional interventions.

Quercetin (3,3',4',5,7-pentahydroxyflavone), a member of the flavonoid family, is a common polyphenol naturally found in many fruits and vegetables (Bischoff, 2008). Studies have revealed that quercetin possesses antiviral, anticancer, antibacterial, anti-inflammatory, and immunomodulatory properties; it suppresses oxidative stress, supports the innate immune response, and exhibits hepatoprotective effects (Gasmi et al., 2022; Muderrisoglu et al., 2022; Duan et al., 2025; Köse, 2025). Due to these characteristics, quercetin is gaining increasing attention as a functional feed additive in aquaculture nutrition, particularly regarding its effects on fish physiology and immune-related parameters (Armobin et al., 2023; Y. Hu et al., 2025; Ming et al., 2025).

The regulation of immune responses and oxidative stress in fish plays a critical role not only in nutritional physiology but also in resistance against bacterial diseases. Lactococcosis, which causes significant economic losses in rainbow trout (*Oncorhynchus mykiss*) farming, is a globally widespread disease that progresses more severely under stress, immunosuppression, and inadequate farming conditions (de Ruyter et al., 2023; Egger et al., 2023). Although the disease was long associated with *Lactococcus garvieae*, recent studies have revealed that closely related species such as *Lactococcus petauri* can also be causative agents, a finding confirmed in *O. mykiss* isolates in Türkiye (Altinok et al., 2022; Vela et al., 2024). Considering previous studies that reported that plant-based additives such as *Capsicum annuum* (Yilmaz et al., 2024) and blends of organic acids and plant essential oils (Huyben et al., 2021; Balta et al., 2025) strengthen immune and antioxidant defence systems and increase survival rates against *L. garvieae* infections, it is clear that nutrition-based strategies are indispensable for disease management in *O. mykiss* aquaculture.

Although the physiological, immunological, and antioxidant effects of quercetin have been extensively investigated in various species, to the best of our knowledge, no studies have yet addressed the effects of this bioactive compound against *L. petauri*-induced infections, nor its role in haematological and intestinal histomorphometric responses in *O. mykiss*. In this study, the effects of low-fat (LFD), high-fat (HFD), and quercetin-supplemented HFD diets on physiological parameters related to the haematology and intestinal histology of *O.*

mykiss were evaluated, independent of growth performance. Furthermore, an experimental infection (challenge) trial was conducted against *L. petauri*, one of the causative agents of lactococcosis, to demonstrate the effects of quercetin-supplemented feeding on disease resistance. The findings provide holistic and up-to-date information for the literature regarding the role of quercetin as a functional feed additive in fish physiology and disease management.

Materials and Methods

Ethical Approval and Study Permissions

This study was conducted within the scope of project number FBA-2023-15, supported by the Scientific Research Projects Coordination Unit of Recep Tayyip Erdoğan University. Ethical approval was obtained from the Local Ethics Committee for Animal Experiments of Recep Tayyip Erdoğan University on June 13, 2023 (Decision No: 2023/30). All procedures involving the animals used in the study were carried out in accordance with the Experimental Animal Use Guide and standard operating procedures of Recep Tayyip Erdoğan University.

Fish Material, Feeding Conditions, Experimental Diets, and Experimental Design

Details regarding the fish materials, feeding regimen, formulation of the experimental diets, preparation methods, and experimental design have been presented in a previous publication (Köse, 2025). Briefly, fish were obtained from a private farm and transported to the Recep Tayyip Erdoğan University Iyidere R&D Unit, where they were acclimated to a commercial feed for 14 days. Following acclimation, fish were randomly assigned to three experimental groups (LFD, HFD, and HFD+Q). Each group consisted of three replicates with 30 fish per tank (initial mean weight: 38.37 g). The experiment was conducted in 100-L fibreglass tanks equipped with a flow-through system (0.2 L/sec) and aeration under natural daylight conditions. During the 8-week trial, fish were fed their respective experimental diets daily at a rate of 2% of their body weight, divided into three meals. Over the course of the experiment, the mean water temperature, pH, and dissolved oxygen were determined to be 14.2 °C, 7.74, and 7.72 mg/L, respectively.

The nutritional compositions of the experimental diets were as follows: Crude protein levels for LFD, HFD, and HFD+Q were 45.91%, 45.96%, and 45.94%, respectively; crude fat levels were 11.38%, 22.53%, and 22.33%; moisture levels were 9.89%, 9.54%, and 9.27%; ash levels were 7.69%, 7.87%, and 7.94%; and crude fiber levels were 1.79%, 1.82%, and 1.83%. Nitrogen-free extract (NFE) values were 23.34,

12.28, and 12.69, while gross energy (MJ/kg diet) levels were 19.69, 22.21, and 22.19, respectively. Distinct from the LFD and HFD groups, the HFD+Q group was supplemented with 0.20 g/kg of quercetin (3,3',4',5,7-pentahydroxyflavone; >95% purity, Sigma Chemical Co., USA). The quercetin dosage was determined based on effective dose ranges (150–800 mg/kg) reported for fish species such as *Channa argus* and *Cyprinus carpio* (Ghafarifarsani et al., 2022; Kong et al., 2022; Armobin et al., 2023), and levels reported to pose no toxicity risk in mice (250 mg/kg) (Cunningham et al., 2022). In the diets, fish meal, soy protein, and corn gluten were used as protein sources; fish oil and soybean oil (1:1 ratio) were used as lipid sources; and potato starch was used as a carbohydrate source. Diets were prepared at the Recep Tayyip Erdoğan University Iyidere R&D Unit Feed Laboratory according to protocols established in our previous publications (Köse et al., 2021, 2024; Kose & Karabulut, 2022).

Blood Sampling and Haematological Analyses

At the end of the trial, fish were fasted for 24 hours prior to sampling. Five fish were randomly selected from each tank and anaesthetized with clove oil at a dose of 2–5 mg/L. Blood samples were collected from the caudal vein using a sterile 2.5 mL syringe with a 22G needle. The collected blood samples were transferred to tubes containing EDTA-K3 and stored at +4 °C for approximately 4 hours until analysis.

For each blood sample, the following parameters were measured: leukocyte (*WBC*), lymphocyte (*LYM*), monocyte (*MID*), granulocyte (*GRAN*), erythrocyte (*RBC*), haemoglobin (*HGB*), hematocrit (*HCT*), mean corpuscular volume (*MCV*), mean corpuscular haemoglobin (*MCH*), mean corpuscular haemoglobin concentration (*MCHC*), platelet (*PLT*), and mean platelet volume (*MPV*). Analyses were performed using an automated haematological analyser, Prokan 6800VET (Prokan, China). Prior to the study, the instrument was calibrated specifically for fish blood, in accordance with the protocols outlined by Er et al. (2024). The operational principle of the analyser was detailed in a study by Minaz et al. (2022).

Histological Method and Sample Collection

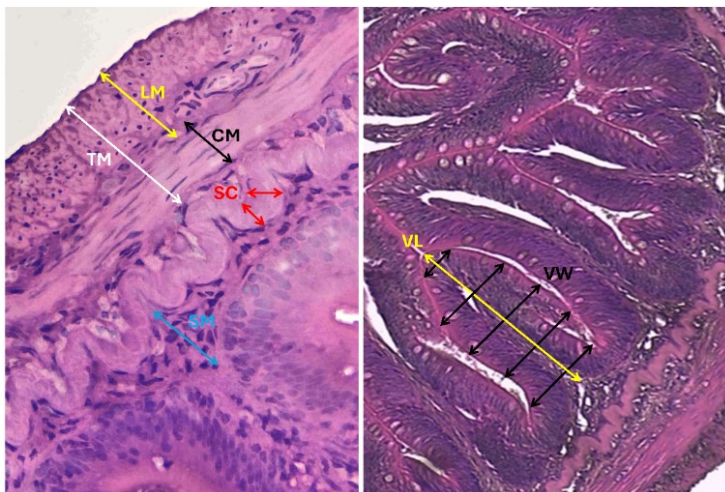
Intestinal tissues from the five fish previously selected for haematological analysis were used for histological examination. For fixation, samples were taken 0.5–1 cm distal to the portion identified as the second segment of the intestine. (Lokka et al., 2013).

Collected intestinal samples were fixed in Davidson's fixative for approximately 36 hours, followed by immersion in 70% ethanol for 24 hours. Tissue processing was carried out using

a LEICA TP1020 tissue processor, blocking with LEICA HistoCore Arcadia H, and tissue sectioning with a LEICA RM2125RT microtome (Leica Microsystems Limited, Switzerland). Hematoxylin-Eosin (H&E) staining was performed automatically using an MLT FS-16 Combo Automated Slide Stainer (MLT LLC, Dubna, Moscow, Russia).

The staining procedure involved the following steps: tissues were cleared of ethanol residues and passed through successive series of alcohol and xylene. Samples were incubated in a 65 °C paraffin bath, embedded in paraffin blocks, sectioned at 5 µm thickness, and mounted on slides. Slides were kept in an oven at 65 °C for paraffin removal, then stained with H&E, and finally coverslipped with Entellan™ (Luna, 1968). Histological images were acquired using a Leica DM500 light microscope integrated with a high-resolution camera (Taup-Cam YW3609EH, CMOS 4K UHD). Images were recorded via ToupTek ToupView software (version 4.11, ToupTek, Hangzhou, Zhejiang, China).

Intestinal tissues were evaluated in accordance with procedures previously described in the literature. Goblet cell count was calculated as the average number of goblet cells per villus. Villus absorption surface (*VA*) was obtained by multiplying villus length (*VL*) by villus width (*VW*). For this calculation, at least five measurement points were used for each villus (Baeza-Ariño et al., 2016; Köse et al., 2024) (Figure 1). Histopathological evaluation was confirmed by a blinded observer, with group information concealed for the samples.



In the histological micrograph, *TM* represents tunica muscularis; *LM*, longitudinal muscularis; *CM*, circular muscularis; *SC*, stratum compactum; *SM*, submucosa; *VL*, villus length; and *VW*, villus width (H&E, 40X)

Figure 1. Measurement points for intestinal wall and villus lengths in *O. mykiss*

Challenge Test with *Lactococcus petauri*

After sampling for other experimental parameters was completed, the tanks for each dietary group were pooled. Subsequently, fish randomly selected from each pool were used to establish challenge groups in triplicate (LFD, HFD, and HFD+Q; n=10 per replicate). One replicate from each group served as a control and was injected with sterile physiological saline solution (PSS). The mean body weights of the fish in the LFD, HFD, and HFD+Q groups were determined to be 100.11 ±2.14 g, 123.21 ±1.03 g, and 128.32 ±0.95, respectively.

Throughout the challenge trial, the mean water temperature, pH, dissolved oxygen, conductivity, and TDS were 15.95 ±0.35 °C, 7.90 ±0.08, 7.05 ±0.18 mg/L, 144.0 ±2.0 µS/cm, and 74.9 ±1.13 mg/L, respectively. The trial was conducted under natural daylight conditions, and the fish were hand-fed three times a day at a rate of 2% of their body weight. However, with the onset of disease symptoms and loss of appetite, feeding was carried out until the fish were satiated to prevent feed wastage.

The *L. petauri* strain (NCBI Accession number: JAQIFV000000000.1) used in the challenge study was obtained from the Fish Diseases Laboratory at the Faculty of Fisheries, Recep Tayyip Erdoğan University. The bacterial culture was incubated in Tryptic Soy Broth (TSB) medium for 24 hours, then centrifuged at 10000×g at 4°C for 5 minutes. The resulting bacterial pellets were diluted with physiological saline solution (PSS) to reach the McFarland 0.5 standard. Before infection, the bacterial concentration was determined to be 3.4×10⁷ CFU mL⁻¹ using the Plate Count Agar (PCA) method. The bacteria were injected intraperitoneally (0.2 mL per fish) into the fish using a sterile insulin syringe. Sterile PSS was administered to the control groups using the same method (Er et al., 2021; Köse et al., 2021, 2024).

Re-isolation was performed on the dead fish to confirm the relationship between the cause of mortality and the pathogen. Spleen, liver, and kidney samples were aseptically collected from dead individuals and inoculated onto tryptic soy agar (TSA) medium. After 48 hours of incubation at 22 °C, the obtained colonies were evaluated morphologically and biochemically. The phenotypic characteristics of the isolates, identified as Gram-positive, chained cocci, were found to be consistent with the *L. petauri* isolate used.

Cumulative survival rates were calculated using Kaplan-Meier analysis.

The following formula was used to calculate the survival rate:

$$S(t) = \prod_{t_i \leq t} \left(1 - \frac{d_i}{n_i}\right)$$

Where $S(t)$ represents the probability of survival at time t , t_i represents the time at which death events occurred, d_i represents the number of individuals who died at time t_i , and n_i represents the number of individuals at risk at time t_i .

Statistical Analysis

Data analysis and graphical representations were performed using the SigmaPlot 15.0 software package (AlfaSoft, Umeå, Sweden) and OriginLab Pro 2025 (OriginLab Corporation, Massachusetts, USA). All data are presented as mean \pm standard deviation (SD). The normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using the Brown–Forsythe test. To determine the effects of dietary treatments, a one-way ANOVA was applied to all parameters. The tank was considered the experimental unit ($n = 3$), and values for each tank were analysed based on the means calculated from five randomly sampled fish per tank. Tukey’s honestly significant difference (HSD) post-hoc test was used to identify differences between groups. Statistical significance was set at $p < 0.05$; however, relevant ANOVA results are reported with their actual p -values.

Results and Discussion

Haematological Analysis Findings

A significant portion of the haematological parameters in *O. mykiss* was influenced by the dietary treatments, resulting in statistically significant differences between groups ($p < 0.05$). These parameters were categorised into three groups based on their physiological functions: immune-related parameters (*WBC*, *LYM*, *MID*, and *GRAN*), oxygen transport-related parameters (*RBC*, *HGB*, *HCT*, *MCV*, *MCH*, and *MCHC*) and coagulation-related parameters (*PLT* and *MPV*).

All immune-related haematological parameters, including *WBC*, *LYM*, *GRAN*, and *MID*, exhibited statistically significant differences among the groups ($p < 0.001$). Significant decreases were observed in all of these parameters within the HFD group, suggesting a physiological state consistent with leukopenia, lymphopenia, and granulocytopenia ($p < 0.05$). In contrast, the mean values obtained in the HFD+Q group were found to be similar to those of the LFD group and were significantly higher compared to the HFD group ($p < 0.05$) (Figure 2).

Regarding haematological parameters related to oxygen transport, statistically significant differences were determined among the groups in terms of haemoglobin concentration (*HGB*), hematocrit (*HCT*), mean corpuscular volume (*MCV*) and mean corpuscular haemoglobin (*MCH*) ($p < 0.001$) (Figure 3). In contrast, no statistically significant difference was detected between the groups regarding erythrocyte count (*RBC*) and mean corpuscular haemoglobin concentration (*MCHC*) values ($p > 0.05$). Significant decreases in *HGB* and *HCT* values were observed in the HFD group compared to the LFD group ($p < 0.05$), and a significant reduction in *MCH* values was determined in parallel. These findings suggest a possible decrease in the oxygen-carrying capacity of erythrocytes in parallel with the decrease in *HGB*, *HCT*, and *MCH* without a distinct change in erythrocyte count (*RBC*) and show consistency with a hypochromic haematological profile. The downward trend observed in *MCV* values in the HFD group points toward microcytic changes. Conversely, *HGB*, *HCT*, *MCV*, and *MCH* values in the HFD+Q group were found to be significantly higher than those in the HFD group ($p < 0.05$) and showed statistically similar values to the LFD group regarding these parameters ($p > 0.05$). *RBC* and *MCHC* values remained similar among the group ($p > 0.05$).

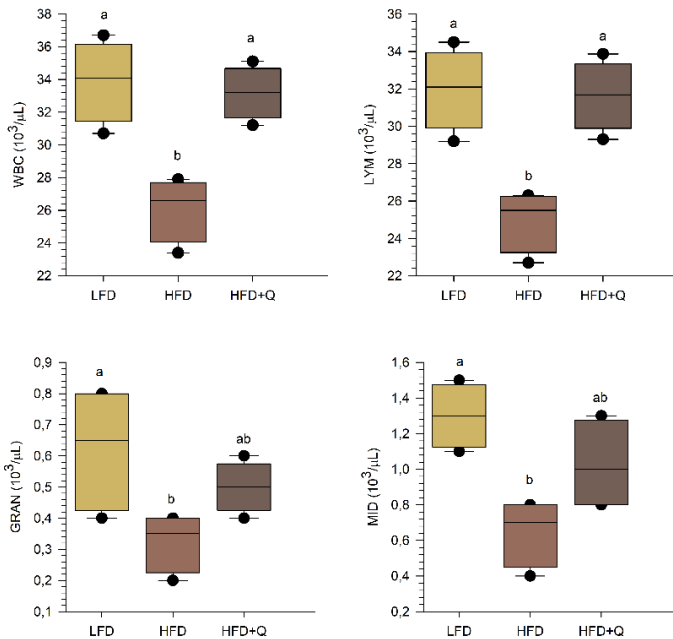
Regarding haematological parameters related to coagulation, no statistically significant difference was detected among the groups in terms of *PLT* and *MPV* levels ($p > 0.05$) (Figure 4). However, it was observed that *PLT* values in the HFD group showed a lower trend compared to the LFD group, while *PLT* values in the HFD+Q group followed higher levels compared to the HFD group. Similar distributions were observed in *MPV* values, and a wide range of variation was noted in the HFD+Q group.

Intestinal Histology

Morphometric findings

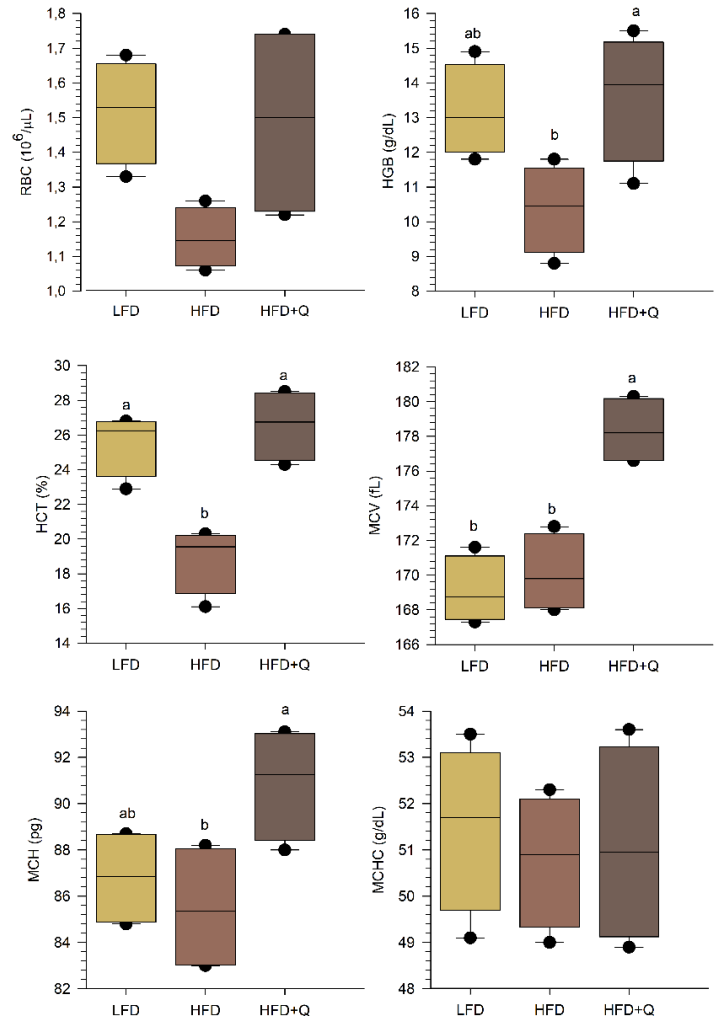
The intestinal histomorphometry evaluation revealed significant differences among the experimental groups for various structural parameters (Figure 5). Quercetin exhibited a trophic effect, significantly increasing the thickness of the longitudinal muscularis (*LM*) and tunica muscularis (*TM*) ($p < 0.05$). Accordingly, *LM* and *TM* thickness showed significant differences between groups, highest in the intestines of *O. mykiss* fed the HFD+Q diet and lowest in those fed the LFD diet ($p < 0.001$). In contrast, there was no significant difference in the circular muscularis (*CM*) thickness among the groups ($p = 0.288$), suggesting that this layer was not significantly affected by either dietary fat content or quercetin supplementation. The thickness of the stratum compactum (*SC*) was significantly increased in the HFD and HFD+Q groups

compared to the LFD group ($p < 0.001$). However, no significant difference was found between the HFD and HFD+Q groups for this parameter ($p > 0.05$). Similarly, the thickness of the submucosa (*SM*) was significantly higher in the HFD group compared to the other groups ($p < 0.001$). However, there was no significant difference between the LFD and HFD+Q groups for this parameter ($p > 0.05$).



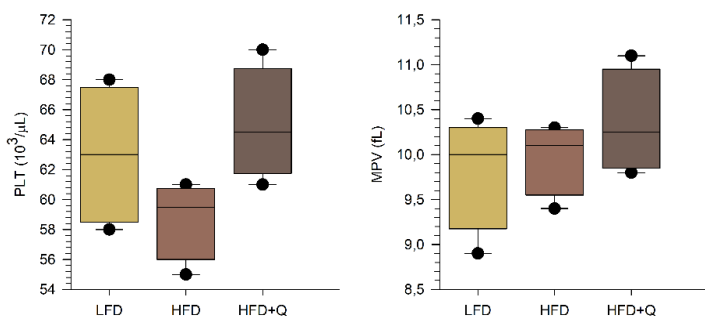
This figure illustrates the effects of different dietary treatments on immune-related haematological parameters, including white blood cell count (*WBC*), lymphocytes (*LYM*), granulocytes (*GRAN*), and mid-range cells (*MID*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD ($n = 3$ tanks per diet), with each value representing the tank mean calculated from five fish sampled per tank. Different lowercase letters indicate statistically significant differences among dietary groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$)

Figure 2. Effects of experimental diets on immune-related haematological parameters in *O. mykiss*



This figure illustrates the effects of different dietary treatments on oxygen transport-related haematological parameters, including red blood cell count (*RBC*), haemoglobin concentration (*HGB*), hematocrit (*HCT*), mean corpuscular volume (*MCV*), mean corpuscular hemoglobin (*MCH*), and mean corpuscular hemoglobin concentration (*MCHC*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD ($n = 3$ tanks per diet), with each value representing the tank mean calculated from five fish sampled per tank. Different lowercase letters indicate statistically significant differences among dietary groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$)

Figure 3. Effects of experimental diets on oxygen transport-related haematological parameters in *O. mykiss*



This figure illustrates the effects of different dietary treatments on coagulation-related haematological parameters, including platelet count (*PLT*) and mean platelet volume (*MPV*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD ($n = 3$ tanks per diet), with each value representing the tank mean calculated from five fish sampled per tank. Different lowercase letters indicate statistically significant differences among dietary groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$).

Figure 4. Effects of experimental diets on coagulation-related haematological parameters in *O. mykiss*

Measurements performed to evaluate the effects of experimental diets on villus morphology in *O. mykiss* revealed significant differences between the groups (Figure 6). Lamina propria (*LP*) thickness was significantly increased in both the HFD and HFD+Q groups compared to the LFD group, with the highest value observed in the HFD+Q group ($p < 0.001$). Similarly, a significant increase in villus length (*VL*) was detected in the HFD and HFD+Q groups compared to the LFD group, with the highest value observed in the HFD+Q group, which was significantly different from the LFD group ($p < 0.001$). Conversely, no significant difference was found among the groups regarding villus width (*VW*) ($p = 0.206$). The Villus absorption area (*VA*) was significantly increased in the HFD group compared to the LFD group. This increase was even more pronounced with quercetin supplementation in the HFD+Q group, which recorded the highest value. A significant difference was observed among all groups for this parameter ($p < 0.001$).

The high-fat diet (HFD) caused a decrease in goblet cell count (9.39 ± 0.93) in the HFD group, showing a significant difference compared to other groups ($p < 0.001$). Quercetin supplementation significantly increased the goblet cell count (11.73 ± 0.87) in the HFD+Q group compared to the HFD group ($p < 0.05$), while the LFD group showed a similar goblet cell count (11.07 ± 0.76) ($p > 0.05$) (Figure 7). These results indicate that HFD reduced goblet cell abundance, whereas quercetin supplementation restored it to levels comparable to LFD.

Histopathological findings

As shown in Figure 8, histological examination revealed distinct morphological differences between the experimental groups in both the intestinal muscle layers and the mucosal architecture of *O. mykiss*. In Figure 8A, corresponding to the LFD group, the circular (*CM*) and longitudinal (*LM*) muscle layers were clearly demarcated, and the tunica muscularis (*TM*) maintained a smooth and compact structure. No oedema or cellular infiltration was observed in the submucosal (*SM*) region, and the stratum compactum (*SC*) appeared thin and well-organised, preserving the muscular integrity. In contrast, Figure 8B shows that the HFD group exhibited pronounced disorganisation in the *CM* region, thickening of the *SC*, and eosinophilic staining within the *SM* layer, indicating expansion and oedema. These changes suggest hypertrophic remodelling of the muscle layers and submucosal inflammation induced by high-fat feeding. However, Figure 8C demonstrates that the HFD+Q group largely retained normal structural integrity. The *TM*, *CM*, and *LM* layers displayed compact organisation similar to the LFD group, and no signs of oedema were observed in the *SM*, confirming the protective effect of quercetin supplementation on intestinal wall morphology. The mucosal morphology also exhibited diet-dependent alterations. As shown in Figure 8D, the LFD group presented short and thick villi (*VL*) with irregular contours and localised oedema in the lamina propria (*LP*). Although slight epithelial irregularities were noted, goblet cells (*Gb*) were uniformly distributed and morphologically distinct.

In Figure 8E, the HFD group exhibited long and cylindrical villi with preserved epithelial continuity; however, goblet cells were sparse and less distinguishable, suggesting reduced mucus secretion and weakened mucosal defence. Finally, Figure 8F illustrates that the HFD+Q group exhibited partially regular villi with well-preserved epithelial integrity. The *LP* was compact with minimal oedema, and goblet cells were more numerous and evenly distributed compared to both other groups. These observations clearly indicate that quercetin supplementation mitigated the adverse effects of a high-fat diet, maintaining both muscular structure and mucosal defence integrity.

Lactococcus petauri challenge

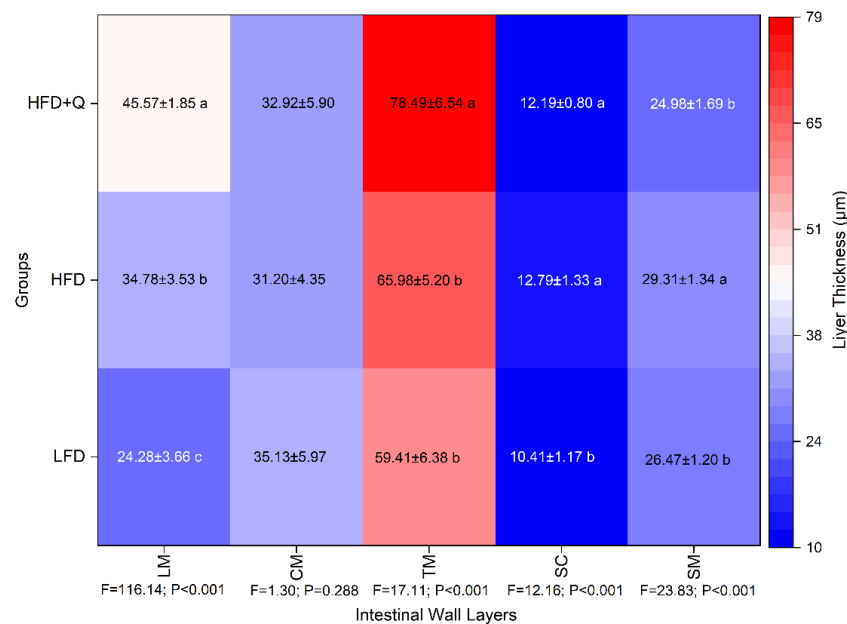
Environmental parameters, feeding conditions, and feeding regimen during the challenge trial were described in previous sections. The first death in the LFD group occurred on day 6 post-infection. The majority of deaths across all groups occurred between days 7 and 12. By day 15, the LFD group recorded the lowest survival probability at 20%, while the HFD group's rate was 45%. In contrast, the HFD+Q group had a

75% survival probability, with no further deaths occurring after day 10. Given the absence of additional mortality after day 15, this day was designated as the end of the experiment (Figure 9). The survival probability of the HFD+Q group was found to be significantly higher than both the LFD and HFD groups ($p < 0.05$). No mortality occurred in the control groups that received PSS throughout the trial. These results indicate that quercetin supplementation confers resistance against *L. petauri* infection.

Hematological Parameters

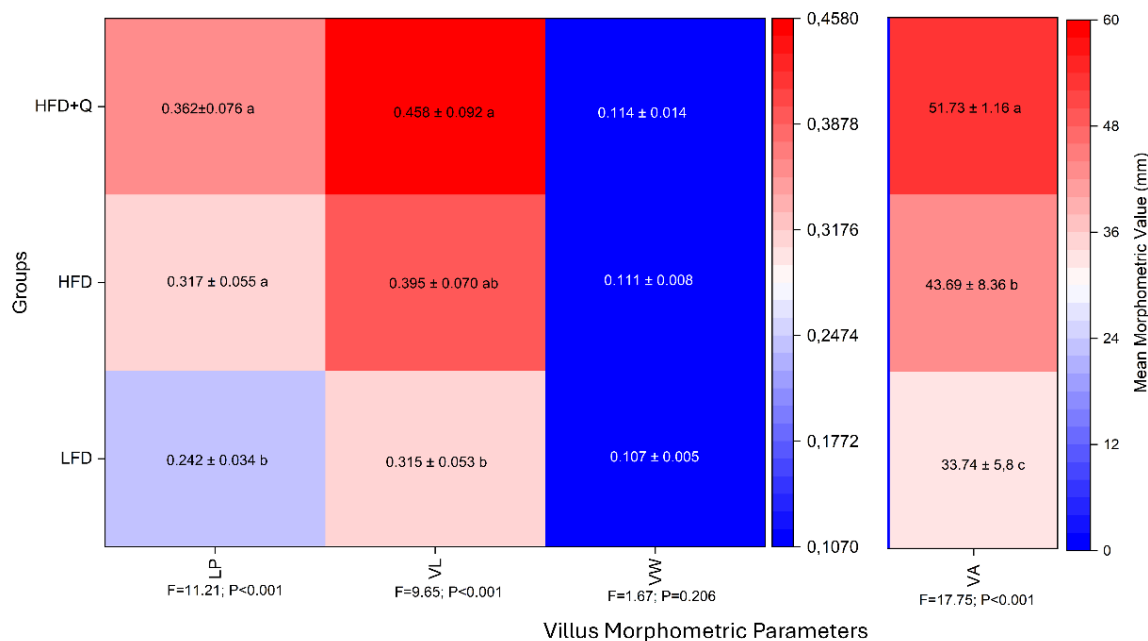
Haematological parameters are recognised as reliable biomarkers widely used to evaluate the physiological reflections of nutritional practices and environmental stressors in fish (Tavares-Dias et al., 2011; Fazio et al., 2013; Seibel et al., 2021). In particular, total leukocyte count (*WBC*), leukocyte subgroups (*LYM*, *MID*, and *GRAN*), erythrocyte indices, and platelet parameters can provide holistic information regarding the immune and physiological homeostasis of the organism. In the literature, it has been reported that diets with

high lipid content can adversely affect immune cell homeostasis in fish, potentially leading to decreases in total leukocyte count (*WBC*) and leukocyte subgroups. Specifically, long-term high-fat feeding regimens have been reported to disrupt immune homeostasis and negatively affect immune cell dynamics (Bujamma & Padmavathi, 2018; Zahran et al., 2018). Conversely, it is reported that biologically active compounds such as plant-derived additives, prebiotics, and flavonoids can exhibit supportive effects on the immune response in fish, and this effect is frequently reflected through haematological parameters (Czech et al., 2009; Jalali et al., 2009; Esmacili, 2021). Consistent with this literature, the present study observed a decrease in *WBC* and leukocyte subgroups levels in *O. mykiss* individuals fed a high-fat diet; however, these parameters were maintained at levels similar to those in the LFD group in the HFD+Q group. These findings suggest that quercetin may play a balancing role in the immune cell profile under high-fat diet conditions and may contribute to the maintenance of immune homeostasis.



This figure illustrates the effects of different dietary treatments on the thickness of intestinal wall layers, including longitudinal muscularis (*LM*), circular muscularis (*CM*), tunica muscularis (*TM*), stratum compactum (*SC*), and submucosa (*SM*) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean ±SD (n = 3 tanks per diet). Different letters in the columns indicate statistically significant differences among groups (one-way ANOVA with Tukey’s HSD post hoc test, $p < 0.05$)

Figure 5. Effects of experimental diets on intestinal wall histomorphometry in *O. mykiss*



This figure illustrates the effects of different dietary treatments on villus morphometric parameters, including villus length (VL), villus width (VW), lamina propria thickness (LP), and villus area (VA) in *O. mykiss*. Fish were fed a low-fat diet (LFD; 11.38% crude fat), a high-fat diet (HFD; 22.53% crude fat), and a high-fat diet supplemented with quercetin (HFD+Q; 22.33% crude fat + 0.2 g/kg quercetin). Values are presented as mean \pm SD (n = 3 tanks per diet). Different letters in the columns indicate statistically significant differences among groups (one-way ANOVA with Tukey’s HSD post hoc test, $p < 0.05$)

Figure 6. Effects of experimental diets on intestinal villus histomorphometry in *O. mykiss*

Dietary lipid levels and alterations in lipid metabolism have previously been reported to influence erythrocyte function and oxygen-carrying capacity in various fish species. Several studies have shown that high-fat diets may lead to reductions in *HGB* concentration, *HCT*, and erythrocyte indices such as *MCV* and *MCH*, while *RBC* count does not necessarily change in parallel with these alterations (Shiogiri et al., 2017; Witeska et al., 2023). Such discrepancies have been attributed to factors including feeding duration, lipid source, species-specific responses, and experimental conditions (Alami et al., 2024;).

Consistent with this body of literature, the present study demonstrated that exposure to a high-fat diet exerted suppressive effects on erythrocyte-related functional parameters represented by *HGB*, *HCT*, *MCV*, and *MCH*, without inducing a marked change in *RBC* levels. This pattern suggests that erythrocyte functional performance, rather than numerical integrity, was adversely affected. In contrast, the recovery observed in these parameters in the HFD+Q group indicates preservation of erythrocyte oxygen-carrying capacity. It has been previously reported that flavonoids, owing to their antioxidant properties, can mitigate oxidative stress-induced cellular damage and support haemoglobin synthesis, thereby

contributing to the maintenance of erythrocyte integrity (Pasdar et al., 2020). Moreover, a recent study conducted in *O. mykiss* reported that quercetin supplementation enhanced oxygen-carrying capacity and supported resistance against environmental stressors (Hoseini et al., 2025). In line with these findings, the recovery of erythrocyte functions observed following quercetin supplementation in the present study is consistent with the proposed regulatory role of flavonoids in maintaining haematological balance.

Parameters associated with coagulation, including platelet count (*PLT*) and mean platelet volume (*MPV*), have been reported to exhibit lower sensitivity to nutritional interventions compared with erythrocyte and leukocyte parameters (Witeska et al., 2023). Nevertheless, pronounced alterations in *PLT* and *MPV* values have been documented under conditions of prolonged stress, toxic exposure, or severe environmental disturbances (Zahran et al., 2018). Within this context, the absence of statistically significant differences in *PLT* and *MPV* among the experimental groups in the present study suggests that platelet dynamics may be relatively resilient to high-fat dietary interventions. However, the distributional trends observed in the quercetin-supplemented group imply that quercetin may assume a supportive, regulatory role in

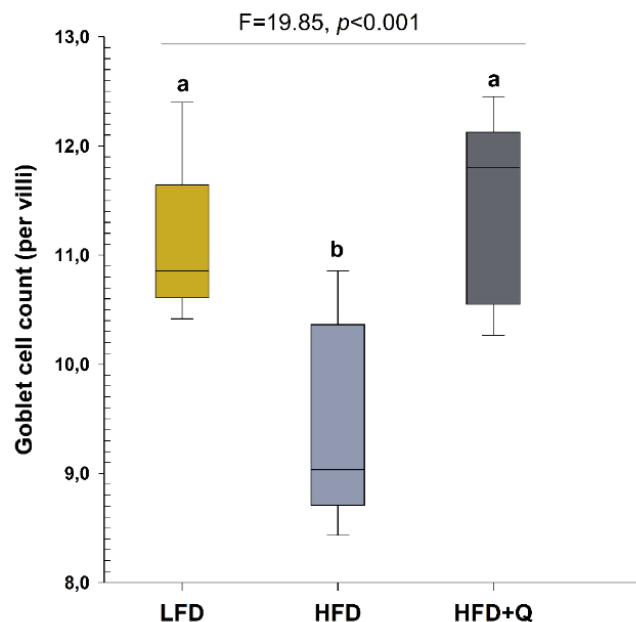
haematological homeostasis rather than exerting a direct effect on platelet-specific parameters.

Intestinal Histomorphology and Histopathology

Intestinal health plays a critical role in maintaining physiological homeostasis in aquatic organisms (Uyanga et al., 2021). It is well established that dietary composition directly affects intestinal structure and cellular organisation, thereby shaping the physiological capacity related to digestion and nutrient absorption (Hamedi et al., 2011). In this context, alterations in intestinal histomorphology provide important indicators for evaluating diet-induced physiological responses. Villus height (*VL*) and regularity are among the primary structural determinants of intestinal surface area (Awad et al., 2009), while mucins secreted by goblet cells play a central role in maintaining epithelial integrity and sustaining mucosal barrier function (Zahran et al., 2020; Feng et al., 2023). These structural and cellular characteristics are therefore considered to be closely associated with the maintenance of normal and healthy growth in fish.

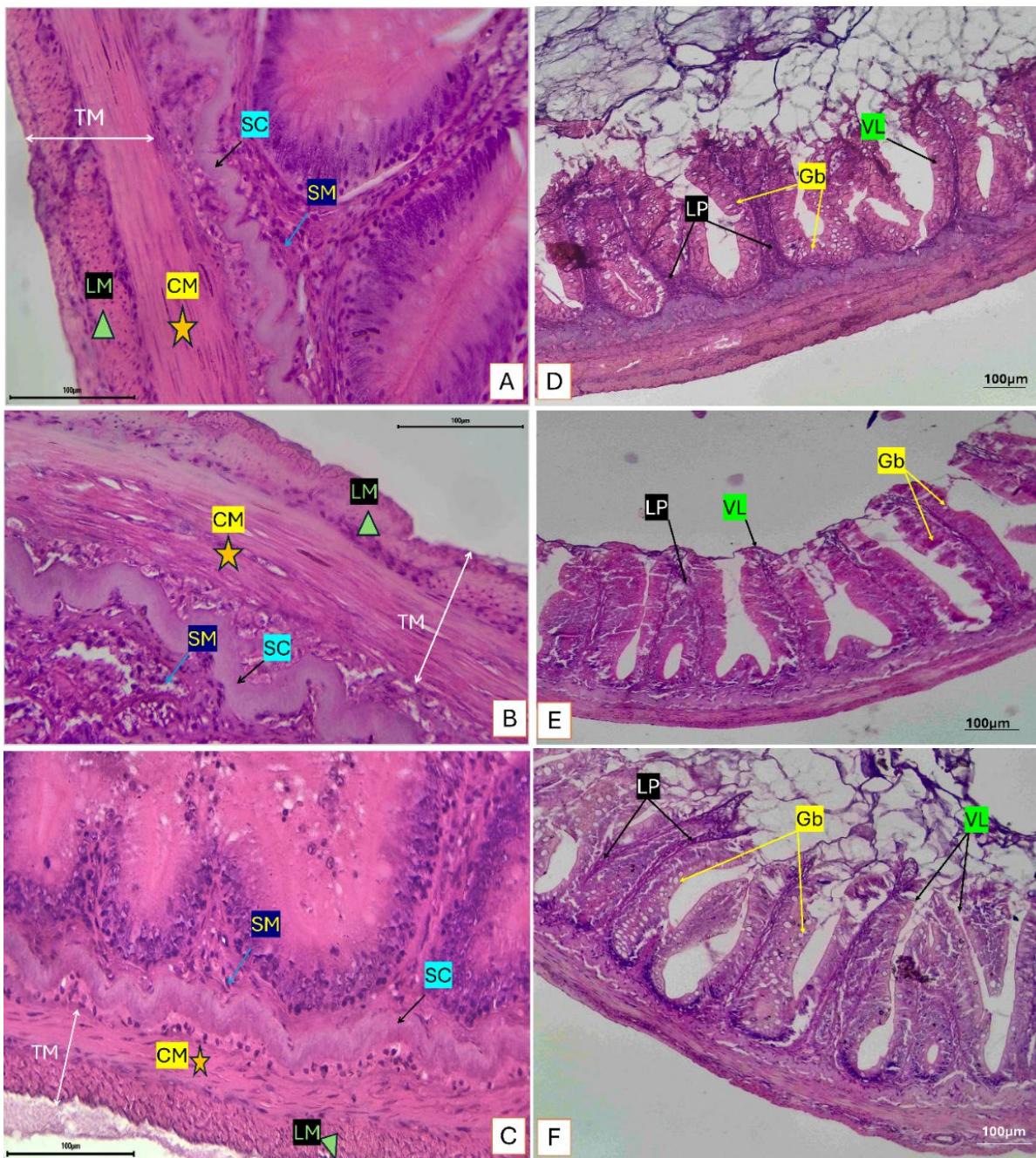
It has been reported that marked differences exist among fish species with respect to intestinal morphology and function. Herbivorous species, due to their adaptation to fibre-rich diets, are suggested to require less intensive peristaltic activity

(Nasruddin et al., 2014). In contrast, carnivorous species fed low-fibre diets may require more pronounced structural development of the muscular layers responsible for peristaltic movements to ensure efficient transport of intestinal contents. Accordingly, the thickness and organisation of intestinal muscle layers have been described as reflecting the structural basis of peristaltic activity (Nasruddin et al., 2014; Witeska et al., 2023). Within this framework, the increases observed in the thickness of the longitudinal muscularis (*LM*) and tunica muscularis (*TM*), particularly in the HFD and HFD+Q groups, suggest that the muscle layers responsible for peristalsis in *O. mykiss* may be structurally modulated in response to physiological demands. These structural alterations may represent a potential adaptive response aimed at supporting intestinal motility-related physiological capacity. When the studies demonstrate that dietary lipid levels can influence intestinal muscular layer thickness in *O. mykiss* (Liu et al., 2021) The findings indicate that remodelling of longitudinal and circular smooth muscle layers is closely associated with intestinal transport and motility capacity (Niessen et al., 2005; Khasanov et al., 2023), and the studies reporting the motility-regulatory effects of quercetin on the gastrointestinal tract (Kim et al., 2018; Modzelewska et al., 2021) When considered together, the present findings are strongly supported by the existing literature.



Values are presented as the mean \pm SD (n = 3 tanks per diet). Different letters above the bars indicate statistically significant differences among the groups (one-way ANOVA with Tukey's HSD post hoc test, $p < 0.05$)

Figure 7. Effects of experimental diets on goblet cell counts in *O. mykiss*



Panels A–C show the tunica muscularis (TM), including the circular (CM) and longitudinal (LM) muscle layers, stratum compactum (SC), and submucosa (SM). Panels D–F display the villus (VL) structure, lamina propria (LP), and goblet cells (Gb). Representative micrographs illustrate: (A, D) normal muscular and epithelial organisation in the LFD group; (B, E) disorganisation of CM, thickened SC, submucosal oedema, and reduced goblet cells in the HFD group; (C, F) preserved structural integrity and restoration of goblet cell abundance in the HFD+Q group.

(A-C panels = 40X, D-F panels = 10X; scale bar = 100 µm, H&E)

Figure 8. Histopathological alterations in the intestinal structure of *O. mykiss* fed different experimental diets

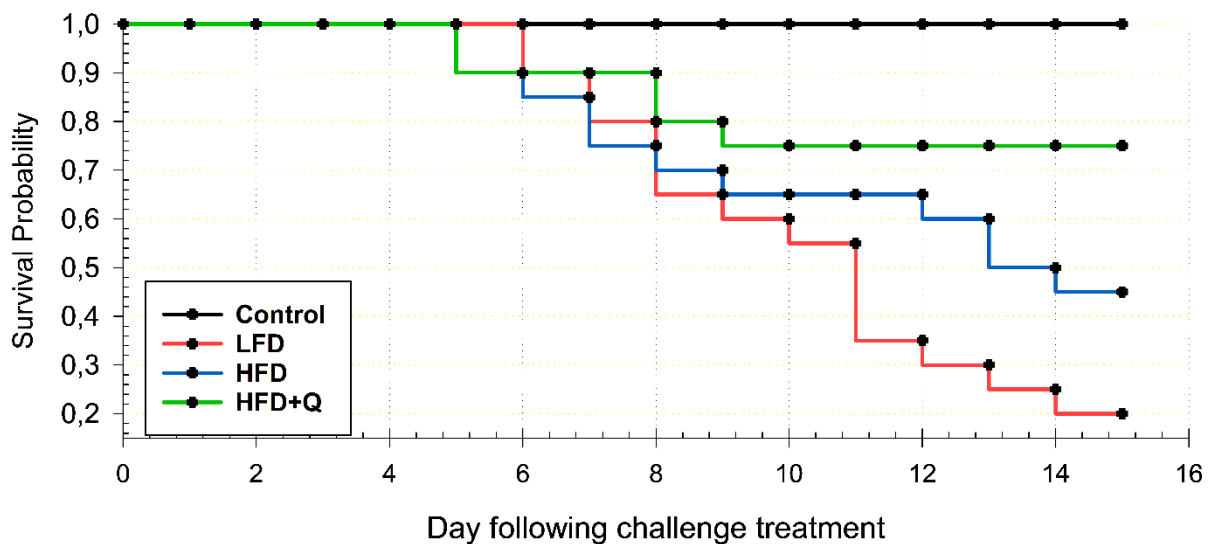


Figure 9. Kaplan–Meier survival curves of *O. mykiss* following *L. Petauri* infection after feeding with experimental diets

However, the effects of a high-fat diet on intestinal structure were not unidirectional. In the present study, histological impairments such as disorganised muscle layers, submucosal oedema, and a reduction in goblet cell number were observed in the HFD group. Goblet cell–derived mucins are known to play a critical role in maintaining mucosal barrier function, and reductions in these cells have been reported to compromise intestinal defence capacity (Zahran et al., 2020; Feng et al., 2023). Therefore, the loss of goblet cells observed in the HFD group may represent a risk condition associated with weakened mucosal integrity.

In the low-fat diet (LFD) group, although the muscle layers appeared regular, villi were notably shorter and thicker, and edematous areas were present within the lamina propria. This morphological profile suggests that intestinal surface area and tissue organisation may be physiologically constrained. Similarly, previous studies in different fish species have reported that dietary composition can be a determining factor influencing villus architecture and lamina propria integrity (Awad et al., 2009; Hamed et al., 2011; Hasan et al., 2024; Lee et al., 2024; Milián-Sorribes et al., 2024; Yan et al., 2024; Zhou et al., 2024).

In contrast, intestinal architecture was largely preserved in the HFD+Q group, as evidenced by more regularly organised muscle layers, longer and more homogeneous villi, and an increased number of goblet cells. These findings suggest that quercetin supplementation exerts a protective and regulatory effect on intestinal mucosal integrity and tissue organization. Although some studies have reported that high-fat diets do not always induce pronounced negative effects on intestinal

morphology in *O. mykiss* (Liu et al., 2021) The goblet cell loss observed in the HFD group in the present study indicates that mucosal defence mechanisms may be sensitive to dietary conditions. The ability of quercetin supplementation to compensate for this loss by increasing goblet cell density suggests a strengthening of the mucus layer and enhanced preservation of epithelial integrity. Similarly, the supportive effects of quercetin on intestinal mucosal integrity have also been reported in *O. niloticus* (Abdo et al., 2024).

Resistance Against *Lactococcus petauri* Infection

The challenge trial conducted with *Lactococcus petauri* provides a functional model for evaluating how dietary composition and the inclusion of alternative feed additives can shape physiological responses to infection in fish. In this context, experimental disease challenge trials are widely used to assess the effects of dietary interventions and feed additives on host resistance and host–pathogen interactions in fish (Austin & Zhang, 2006).

Haematological parameters in fish are influenced by multiple factors, including age, sex, life stage, seasonal variations, and nutritional status (Alcorn et al., 2003; Morgan et al., 2008; Seibel et al., 2021; Nabi et al., 2022). This multifactorial structure makes it difficult to establish reliable haematological reference intervals for fish species. Notably, the only study reporting reference intervals for *O. mykiss* in accordance with the standards of the American Society for Veterinary Clinical Pathology (ASVCP) was presented by Nabi et al. (2022). In the present study, although the total leukocyte (*WBC*) counts and leukocyte subgroups in the HFD group were lower than those in the LFD and HFD+Q groups, they

remained within the reported reference ranges. On the other hand, high-energy diets and polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA), have been previously reported to support faster and more effective immune responses (Calder, 2015; Cornet et al., 2018; Mendivil, 2021; Magalhães et al., 2023). In addition, adequate energy availability has been shown to be critical for sustaining host defence during infection, whereas insufficient energy intake may indirectly suppress immune responses (Austin & Zhang, 2006). In the present study, the gross energy content of the diets was higher in the HFD group than in the LFD group (22.21 MJ kg⁻¹ vs 19.69 MJ kg⁻¹). In this context, although statistically significant reductions in WBC and leukocyte subgroups were observed in the HFD group, these findings appear to be consistent with previously described pathophysiological mechanisms and host responses during lactococcosis in salmonids (Vendrell et al., 2006). Nevertheless, the higher survival rate observed in the HFD group compared with the LFD group may seem contradictory to the haematological findings at first glance. Evidence from fish challenge studies indicates that improvements in nutritional and energetic status, including lipid-related components, can enhance post-challenge outcomes such as survival. This highlights that infection outcome is shaped not only by single haematological indices but also by the host's metabolic capacity (Deng et al., 2013; Seibel et al., 2021; Nabi et al., 2022). This apparent discrepancy suggests that short-term survival during acute bacterial infections is determined not only by WBC counts or leukocyte abundance, but also by the host's capacity to meet the high energy demands associated with inflammation and tissue repair. Although the underlying biochemical mechanisms were not directly evaluated in the present study, the findings indicate that reduced lipid intake in the LFD group may have limited the ability of fish to develop an effective physiological response against *L. petauri* infection. Consequently, the higher survival rate observed in the HFD group, despite leukocyte suppression, may be attributed to improved infection tolerance supported by sufficient energy and lipid availability. This finding emphasises that host defence during infection is shaped not only by immune cell dynamics but also by metabolic and energy-related factors.

The survival advantage observed in the quercetin-supplemented HFD+Q group may be attributed to the combined effects of metabolic support provided by high energy and lipid availability and the regulatory role of this compound in maintaining haematological balance. The preservation of WBC and leukocyte subgroups in this group may have contributed to sustaining immune responsiveness during infection. Plant-

derived flavonoids have been reported to support host defence by modulating immune cell functions (Awad et al., 2015; Zhang et al., 2021). Furthermore, quercetin has been shown to exert antimicrobial activity by disrupting bacterial cell wall and membrane integrity (Wang et al., 2018). In this context, the effects of quercetin are considered to play a regulatory and supportive role in enhancing the physiological capacity provided by high-energy diets.

Given the increasing recognition of *L. petauri* as an emerging pathogen in salmonids, the survival advantage associated with quercetin supplementation observed in this study is particularly noteworthy. The reclassification of some outbreaks previously attributed to *L. garvieae* as being caused by *L. petauri* has necessitated a reassessment of the role of this pathogen in aquaculture (de Ruyter et al., 2023). In this context, evidence suggesting that plant-derived compounds such as quercetin can support physiological resistance to infection under high-fat dietary conditions offers an important perspective for the development of non-antibiotic strategies.

Overall, the findings of the present study indicate that dietary lipid level and quercetin supplementation can modulate physiological responses to *L. petauri* infection in fish. The positive effects of a quercetin-supplemented high-fat diet on survival may be associated with the regulatory role of this compound in maintaining physiological homeostasis. Nevertheless, further detailed immunological and molecular investigations are required to elucidate the underlying mechanisms responsible for the observed effects.

Conclusion

This study demonstrated that dietary lipid levels in *O. mykiss* exert significant effects on haematological homeostasis, intestinal structural integrity, and resilience against *L. petauri* infection. The high-fat diet (HFD) negatively impacted immune cell dynamics through a suppressive effect on the leukocyte profile (*WBC*, *LYM*, *MID*, and *GRAN*) and pointed toward a potential functional weakening of oxygen-carrying capacity by creating a downward trend in *HGB*, *HCT*, and erythrocyte indices (*MCV*, *MCH*) without distinct changes in erythrocyte count (*RBC*). Additionally, the HFD was associated with histopathological changes such as irregularities in intestinal muscle layers, submucosal oedema, and a reduction in goblet cells, which may weaken mucosal defence.

The quercetin-supplemented high-fat diet (HFD+Q) significantly limited these adverse effects of the HFD, presenting a more balanced physiological profile characterised by the preservation of leukocyte subgroups, more stable intestinal wall/mucosa organisation, and recovery of goblet cell density. These structural and haematological improvements

found a functional counterpart to the significant survival advantage observed in the HFD+Q group during the *L. petauri* challenge trial. In particular, the earlier cessation of mortality and the higher survival probability suggest that quercetin can support disease resistance.

In conclusion, quercetin supplementation can be considered a functional feed additive that supports haematological balance and intestinal barrier integrity under high-fat feeding conditions, and accordingly, carries the potential to increase resistance capacity against *L. petauri*, one of the causative agents of lactococcosis. However, due to the use of a single dose level and the fact that mechanistic endpoints (antioxidant/immune gene expression, mucosal immunity markers, etc.) were not directly measured, it is recommended to further detail the mechanism of action through dose-response designs and molecular/immunological validations.

Although the present findings support the functional potential of quercetin in aquaculture nutrition, further immunological and molecular studies are recommended to elucidate its mechanisms of action and to determine optimal dietary inclusion levels under different culture conditions.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The animal study was approved by Recep Tayyip Erdoğan University Experimental Animals Ethics Committee (decision number: 2023/30, date: June 13, 2023). The study was conducted in accordance with the local legislation and institutional requirements.

Data availability: The data will be made available upon request from the author.

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Disclosure: -

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