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Evaluation of the Effect of Three Florfenicol Doses Against Salmonid Rickettsial Septicemia (SRS) in Atlantic Salmon (*Salmo salar* Linnaeus) Challenged by Intraperitoneal Injection

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Abstract

The emergence and spread of pathogens pose significant challenges to the sustainability and productivity of aquaculture globally. For the Chilean salmon farming industry, salmonid rickettsial septicemia (SRS), caused by the facultative intracellular bacterium *Piscirickettsia salmonis*, constitutes one of the main disease challenges. In this study, the efficacy of various oral doses of florfenicol (FFC) (5, 7.5, and 10 mg/kg BW/day) against SRS was assessed in Atlantic salmon, when treatment was initiated at an early stage of infection. Since salmonids infected with *P. salmonis* typically lose appetite as the disease progresses, and the therapeutic FFC dose is dependent on a normal specific feeding rate (SFR), the treatments were administered 5 days post-challenge (DPC5). On the day of challenge, experimental fish were intraperitoneally (IP) injected with 0.2 mL of *P. salmonis* genogroup LF-89 inoculum (9.07×10^7 CFU mL^{-1}). Fish mortality, behavior, clinical signs of disease, feed intake and SFR were monitored throughout the study. Conclusions: An important finding in this study was that all tested antibiotic doses halted disease progression and prevented mortality in fish challenged with *P. salmonis* when administered DPC5. In the control group, mortality reached 32.2% with fish displaying clinical signs of SRS.

Keywords: antibiotic dose; Atlantic salmon; aquaculture; florfenicol; SRS

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1. Introduction

Globally, Chile is the world's second-largest producer of salmon, following Norway, with an estimated production of approximately one million tons of farmed salmonids in 2023 [1]. Despite continuous growth and technological advancements, the Chilean salmon farming industry still faces significant fish health challenges, with salmonid rickettsial septicemia (SRS) being one of the most prevalent and difficult diseases to manage [2]. Although vaccines against SRS are routinely used in the industry, the disease continues to pose significant challenges, necessitating the ongoing use of antibiotics for effective management. In 2024, the Chilean salmon farming industry consumed a total of 351.1 tons of antibacterial agents (AB), active ingredient. The majority of this AB use (97.90%) occurred during seawater production, with 96.15% specifically used to treat SRS [3]. This extensive use of AB raises several concerns, such as antimicrobial resistance (AMR), environmental impact and the industry's ecological footprint [4]. These concerns highlight the urgent need to reduce AB usage, which can be partly achieved by optimizing the treatment doses

and refining the application procedures currently employed to manage SRS in Chilean salmon farming.

SRS, also known as piscirickettsiosis, is caused by *Piscirickettsia salmonis*, a Gram-negative facultative intracellular bacterium [5,6]. The emergence of *P. salmonis* infection in farmed Coho salmon (*Oncorhynchus kisutch*) was first reported in Chile in 1989 by Bravo & Campos [5,7,8]. Following this initial report, the bacterium has been detected in various salmonid species around the world [9–12]. Salmonids infected with *P. salmonis* demonstrate clinical signs such as reduced appetite, erratic swimming, skin lesions, pale gills, anemia, intestinal necrosis, enlarged spleen and bacterial aggregates in multiple organs including the ovaries, brain, gills and heart [10,11,13,14]. These internal and external clinical signs collectively contribute to the diagnostic profile of SRS.

Several genomic and phylogenetic studies have shown that *P. salmonis* can be divided into two distinct genogroups: LF-89 and EM-90 [12,15–17]. This classification has been further supported by the analysis of 19 published *P. salmonis* genomes [18]. Recently, an additional genogroup was designated as NC, which comprised isolates from the Norwegian and Canadian farming industries, as well as four new subgroups of the *P. salmonis* EM-90 group (EM1-EM4) [11]. Data from ADL Diagnostics Chile (a diagnostic and biotechnology laboratory in Chile) also suggest that the LF-89 genogroup has five genovariants (B-I to B-V) that show varied susceptibility to antibiotics (florfenicol) and differentiated virulence [19,20]. Florfenicol is a broad-spectrum bacteriostatic antibiotic extensively used in aquaculture, cattle, swine, and poultry. It is especially critical in the salmon industry for controlling bacterial infections, such as SRS, when vaccination efficacy is limited or during acute disease outbreaks [3]. The florfenicol minimum inhibitory concentration (MIC) values for the predominant genovariants BI and BIII generally range from 0.5 to 2 and 0.25 to 1, respectively [21].

For Atlantic salmon, infectious diseases are the primary cause of mortality, accounting for 22.1% of total deaths in 2024. Of these infectious cases, 43.1% were specifically attributed to Piscirickettsiosis (SRS) [2]. In Chile, there are currently 17 commercially authorized vaccines against SRS approved by the Agricultural and Livestock Service (SAG) [22]. However, the effectiveness of these vaccines varies considerably, resulting in continued dependence on antibiotic treatments [23]. This further highlights the severity of the disease, emphasizing the substantial impact on fish welfare and the considerable economic consequences it imposes on the industry and the urgent need for additional and alternative mitigation tools.

Currently, Chilean government authorities and the farming industry are implementing several initiatives to improve SRS control while also focusing on reducing antibiotic consumption in salmonid farming. Examples of these initiatives include the Pincoy Project [24], the Yelcho Project [25], and the Chilean Salmon Antibiotic Reduction Program (CSARP) [26], the latter aiming to reduce antibiotic use in Chile's farmed salmon industry by 50% by 2025 [26].

To optimize AB use and enhance animal welfare in salmon farming, efficient and effective therapies are essential. This study aims to evaluate the appropriate dosage and therapeutic effects of early-stage administration of florfenicol (FFC) in Atlantic salmon experimentally challenged with *Piscirickettsia salmonis* (genogroup LF-89).

2. Materials and Methods

2.1. Bacteria and Culture Conditions

Isolates from the LF-89 genogroup currently predominate in clinical cases of SRS in Chile [27]. Accordingly, an isolate from this genogroup was selected for use in the present study. The challenge material was prepared using an in-house *P. salmonis* isolate (Strain

code: PM-120832) obtained from ADL Diagnostic Chile SpA (ADL), and affiliated with the LF-89 genogroup, following standard procedures at the ADL laboratory. The *P. salmonis* isolate was cultured on ADL-PSA plates (prepared by ADL; marine agar supplemented with NaCl, sodium citrate, peptone, L-cysteine, glucose, FBS and MEM) for 9 days at 19 °C, as described previously by Henriquez et al. [28]. Bacterial colonies were collected from ADL-PSA plates, suspended in PBS medium and adjusted to the required colony-forming units (CFU) per milliliter. The cultured challenge material for the intraperitoneal (IP) challenge was transported to the challenge facility in containers with gel packs to maintain a stable temperature. Temperature was measured prior to and at the end of the IP challenge and ranged between 7.0 and 7.9 °C in both the pre-study for inoculum dose determination and the treatment study.

2.2. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of florfenicol for the challenge isolate was previously determined by ADL in accordance with CLSI guidelines, incorporating the modifications described by Henríquez et al. [28].

2.3. Production of the Medicated Feed

Experimental medicated feeds for the study were prepared in the medicated feed line in Skretting Chile using the standard manufacturing protocol of commercial medicated feeds. The FFC was top-coated onto a commercial base feed (Optimax Pro, Skretting, Puerto Montt, Chile) using the FFC premix Duflosan (Veterquimica S.A., Sanitago, Chile). The active ingredient is florfenicol.

Duflosan feed doses are shown in Table 1. To achieve the target FFC therapeutic dose (5, 7.5 and 10 mg/kg BW/day), a restricted feeding rate of 1.5% was determined (SFR (%) = (Feed consumed per day (g) ÷ Fish biomass (g)) × 100).

Table 1. FFC (florfenicol) doses and specific feeding rate (SFR, %) for each treatment group (T1–T3) and control group.

	Florfenicol			**Florfenicol**
Treatment Groups	**Duflosan Feed Dose (g/kg)**	**Florfenicol Feed Dose (g/kg)**	**SFR (%)**	**Target Therapeutic Dose mg/kg BW/day**
Control	0.000	0.000	1.5	0
T1	0.667	0.333	1.5	5
T2	1.000	0.500	1.5	7.5
T3	1.333	0.667	1.5	10

Before using the feeds in the treatment study, recovery analysis of FFC was performed in the Skretting Chile Laboratory by using high-performance liquid chromatography (HPLC) to ensure targeted medicine levels were met.

2.4. Fish and Fish Husbandry

For this study, unvaccinated Atlantic salmon post-smolts (Aquagen stock) were used. Prior to admission to the TEKBios Fish Trial Center, the fish were screened for common pathogens using RT-qPCR and confirmed to be free of *P. salmonis*, *Renibacterium salmoninarum*, infectious pancreatic necrosis virus (IPNv), infectious salmon anemia virus (ISAv), *Aeromonas salmonicida*, *Flavobacterium psychrophilum*, and *Flavobacterium columnare*. TEKBios operates a flow-through production system, where the fish were initially housed in circular 5000 L holding tanks supplied with seawater (30–33 parts per thousand (ppt)) at 12 ± 1 °C. In these holding tanks, the fish were fed Optimax Pro (Skretting Chile) according to appetite

and monitored for normal behavior before being used in the pre-study for inoculum dose determination and the treatment study, as described below.

2.5. Pre-Study for Inoculum Dose Determination

To determine the appropriate bacterial inoculum dose for the treatment study, a pre-study was conducted following the standard *P. salmonis* IP challenge protocol of TEKBios, as outlined in the section "IP Challenge with *P. salmonis*." On the day of distribution, the fish were transferred from the holding tank to the study tanks (1 m^3) at a salinity of 30–33 ppt and a temperature of 14.2 ± 1 °C. Fish were weighed individually and randomly assigned to one of the five study tanks (30 fish per tank). A total of four challenge doses of *P. salmonis* (genogroup LF-89) were tested (Table 2). The fish were on average 159 g ± 13.7 g at IP challenge. Fish were IP injected with 0.2 mL of the respective bacterial inoculum on day 0 post-challenge (DPC0). The pre-study lasted a total of 42 days and included an acclimation period (12 days), the IP challenge with *P. salmonis* (1 day), and a 29-day monitoring period for the challenged fish.

Table 2. Challenge inoculum (*P. salmonis*) used in the dose determination pre-study.

Challenge Inoculum	Bacterial Count (CFU mL^{-1})
1	8.30×10^7
2	2.90×10^7
3	1.13×10^7
4	4.86×10^6

Daily feeding rates and mortality were recorded throughout the study, serving as a guide for when to initiate the 10-day medicated feed treatment in the main study. Based on the results of this pre-study, the inoculum dose to be used in the treatment study was determined.

2.6. Treatment Study

A total of 400 Atlantic salmon post-smolts, with an average weight of 255 g, were transferred from the holding tank to eight study tanks, each with a volume of 1 m^3 (50 fish per tank), in a separate challenge room. Each of the three treatment groups was kept in duplicate tanks supplied with seawater (30–33 ppt). The temperature was slightly increased from 12.2 ± 0.9 °C during the acclimatation period to 14.3 ± 0.3 °C during the challenge study. Temperature was measured continuously throughout the study. The initial fish density was 12.7 kg/m^3 per tank and the oxygen levels were 10.5 ± 1.1 mg/L. Two tanks served as control groups.

In the first part of the treatment study, the fish underwent a 13-day acclimation period before the challenge. During this period, the fish were fed Optimax Pro (Skretting Chile). Prior to the IP challenge, the fish were subjected to a 24 h starvation period. On the day of challenge (DPC0), the fish were transferred from the study tank and placed in a container with an anesthetic solution (Benzocaine 20%) for deep sedation. IP administration of the challenge material was performed on anesthetized fish by inserting the needle perpendicularly (90°) to the ventral midline, towards the abdominal wall, and injecting 0.2 mL of the challenge material (9.07×10^7 CFU mL^{-1}) per fish. After inoculation, the fish were transferred back to their original study tanks, and their recovery progress was continuously monitored. During the 35-day challenge period, the temperature was 14.3 ± 0.3 °C. The salinity, pH, temperature and oxygen levels in the tanks were measured daily to ensure optimal fish welfare. Following the IP challenge, the fish were monitored

daily, and data on feed intake, specific feeding rate (SFR), fish behavior, and mortality were recorded.

The FFC treatment started five days post-challenge (DPC5). The medicated feed was distributed in the tanks by using an automatic feeder (brand: IMENCO Aqua Chile S.A., Puerto Montt, Chile; model: Beltfeeder Inox 120 mm) between 9:00 a.m. and 15:00 p.m. for 10 consecutive days, with a calculated SFR of 1.5% for groups T2 (7.5 mg/kg BW/day) and T3 (10 mg/kg BW/day). For group T1 (5 mg/kg BW/day), the SFR was adjusted to 1.6%, due to the 5% lower FFC level in the feed (Table 3).

Table 3. Laboratory results of the FFC recovery concentration in the feeds, the adjusted SFR% during treatment, and the actual FFC therapeutic dose for each treatment group in the study. The recovery concentration in the produced feed for T1 was slightly below the target level. Therefore, the SFR was adjusted from 1.5% to 1.6% to compensate.

	Florfenicol			Florfenicol
Treatments	**Target Level in Feeds (g/kg)**	**Recovery Level in Feeds (g/kg)**	**Adjusted SFR (%)**	**Actual Therapeutic Dose mg/kg BW/day**
Control	0.000	0	1.5	0.00
T1	0.340	0.317	1.6	5.07
T2	0.510	0.505	1.5	7.58
T3	0.680	0.668	1.5	10.02

During the treatment period (10 days), the control group was fed standard commercial feed with an SFR of 1.5%. After the treatment period, all groups were fed standard commercial feed until the end of the challenge study (DPC35) (Figure 1). Daily records were kept of feed intake, SFR (%), uneaten feed (%), fish behavior, clinical external signs, and mortality across all study tanks.

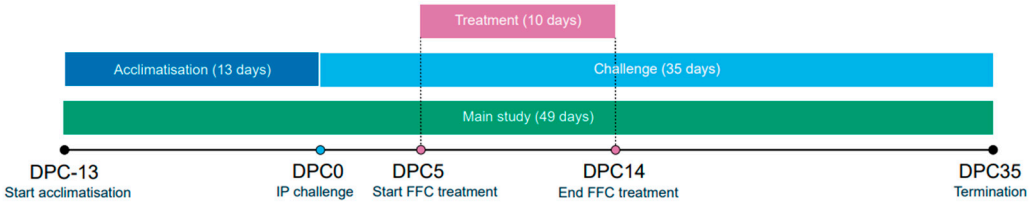


Figure 1. Timeline for the treatment study. The experiment lasted 49 days (in green) in total. The study started with a thirteen-day acclimation period (dark blue). On day 14 (DPC0), the fish were IP challenged with *P. salmonis* (blue dot). Five days post-challenge (DPC5), the FFC feed treatment was initiated and continued for 10 days (in pink). After the treatment period, the challenge ran until the termination day (DPC35).

The protocol used for this study was approved by all parties and aligned with the protocol of the TEKBios scientific committee. This study was conducted at the TEKBios Fish Trial Center, ensuring ethical compliance in animal experimentation, in accordance with the recommendations provided by the local National Agency for Research and Development (ANID) to uphold ethical standards in animal research [29].

2.7. Sampling

Mortality was recorded daily. All fish in the main study were assessed for clinical signs of SRS using an SRS necropsy score to evaluate disease progression and overall health status. At the end of the study, surviving fish were sampled and analyzed for clinical signs of SRS.

2.8. Statistical Analysis

Data was analyzed using two-way repeated measures ANOVA to assess differences in SFR over time and among treatment groups. When significant effects were detected, post hoc comparisons were performed using Tukey's HSD. Statistical significance was set at $p < 0.05$, and trends were noted for p-values between 0.05 and 0.1. All analyses were conducted using GraphPad Prism version 10.5.0 (GraphPad Software, Boston, MA, USA).

3. Results

3.1. Production of the Medicated Feed

Skretting Chile Laboratory analyzed the recovery of FFC in the feed by using HPLC to ensure targeted medicine levels were met. The results confirmed that the medication levels (FFC) in the feed fall within the required range for this study. For group T1 (5 mg/kg BW/day), the SFR was adjusted to 1.6%, due to the 5% lower FFC level in feed (Table 3).

3.2. Minimum Inhibitory Concentration (MIC)

ADL Diagnostics determined the MIC for the challenge isolate in this study to be 1 µg/mL for FFC.

3.3. Pre-Study for Inoculum Determination

Fish mortality was first observed on day 12 post-challenge and persisted throughout the study period. The cumulative mortality data for all tanks are presented in Figure 2. The fish groups inoculated with IP doses ranging from 1.13×10^7 to 8.30×10^7 CFU mL^{-1} exhibited cumulative mortality rates ranging from 10% to 93.3%. No mortality was recorded in the group challenged with the lowest dose (4.86×10^6 CFU mL^{-1}). A significant decrease in feed intake and specific feed ratio (SFR) was observed between 3 and 8 days prior to the first mortality. The highest dose tested in the LD50 experiment was selected for use in the main study (8.30×10^7 mL^{-1}).

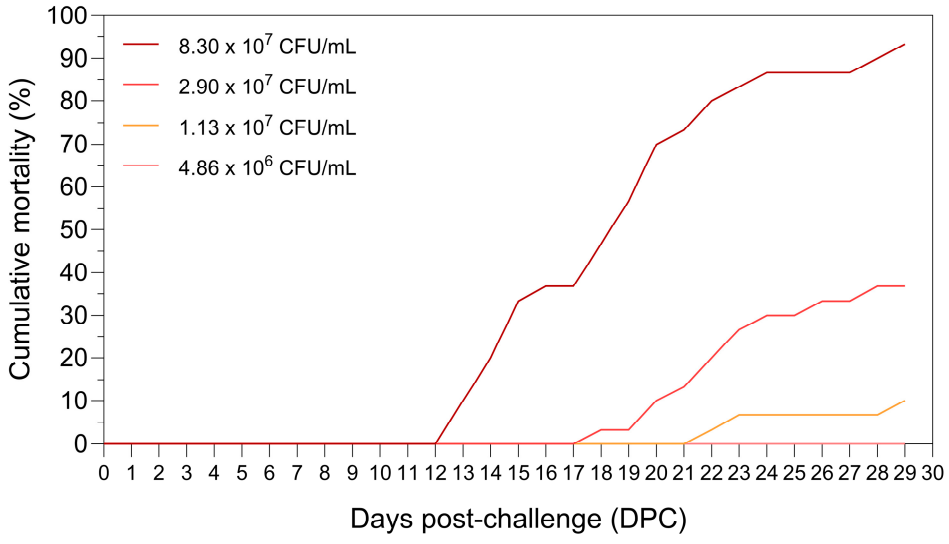


Figure 2. Cumulative mortality in the LD50 pre-challenge, testing five different challenge doses of *P. salmonis*. The intraperitoneal (IP) doses ranging from 1.13×10^7 to 8.30×10^7 CFU mL^{-1} resulted in cumulative mortality rates between 10% and 93.3%.

3.4. Treatment Study

In the treatment study, the target mortality in the control group was 50%. To achieve this, fish were intraperitoneally injected with a challenge dose of *P. salmonis* at

9.07×10^7 CFU mL^{-1} per fish. This dose was selected based on the results from the pre-study and was adjusted to account for the higher average body weight of the fish used in the treatment study. The bacterial concentration was calculated to achieve the intended infection pressure, given the increased biomass of the experimental animals. Mortality was first observed on days 19 and 20 post-challenge in the control group, with the average cumulative mortality reaching 32.2% (Figure 3). No mortality was recorded in the test groups fed medicated feeds (T1, T2, and T3). The cumulative mortality rates for each group are presented in Figure 3. Survival analysis indicated no significant difference between replicate tanks fed the control feed (log-rank (Mantel–Cox) test, *chi − squared* 0.56, df 1, *p*-value of 0.4543).

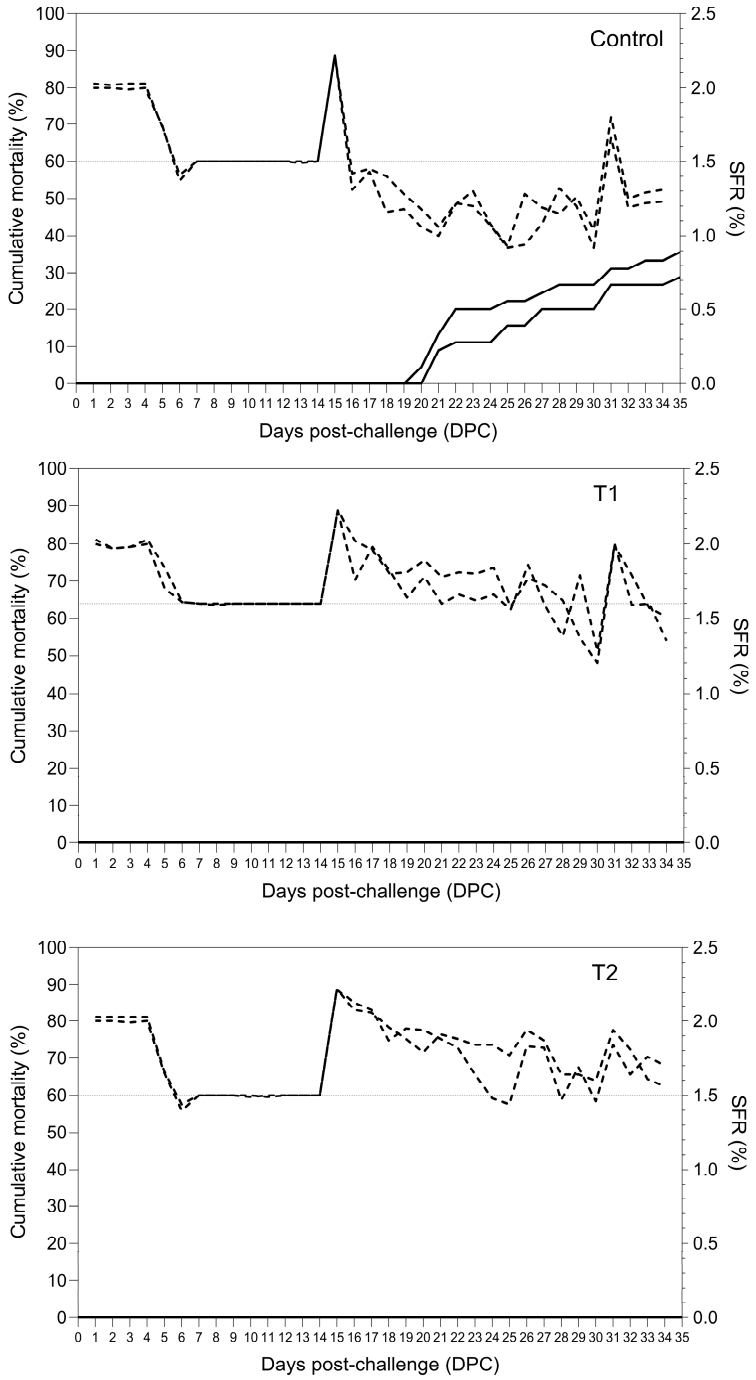


Figure 3. *Cont.*

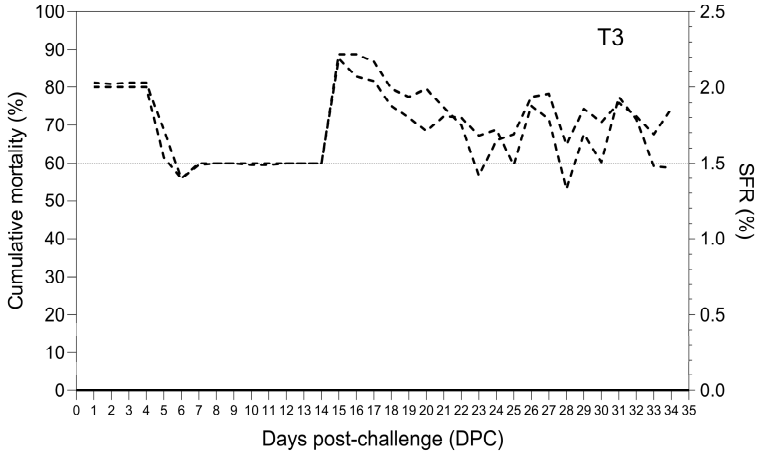


Figure 3. Cumulative mortality (%) (*P. salmonis* challenge) and specific feeding rate (SFR, % body weight per day) of duplicate tanks in the treatment study. Feed treatments were administered from DPC 5 to DPC14, and the experimental period ended on DPC35. Solid lines represent cumulative mortality, while dotted lines indicate SFR. Data are shown for the control group (no treatment), and treatment groups T1 (5 mg/kg BW/day), T2 (7.5 mg/kg BW/day), and T3 (10 mg/kg BW/day). The figure at the top represents the control group, while the subsequent figures illustrate the respective treatment groups (T1–T3).

For all the study groups, the fish maintained a relatively constant SFR of 2% before treatment. During the 10-day treatment (DPC5-DPC14), the SFR was deliberately adjusted to 1.5–1.6% to ensure that the fish received the desired FFC dose (see Figure 4). After the treatment period, the SFR (%) showed more variation among the groups, with a significant decrease observed in the control group on DPC15, four days prior to the first recorded mortality. The SFR (%) ranged from 2.22% to 1.31% between the groups at that time. As the study progressed, all treatment groups demonstrated a higher SFR (%) compared to the control group. The two-way RM-ANOVA for SFR shows there are significant differences (and also trends between groups/*p*-values between 0.1 and 0.05) in several days post-treatment.

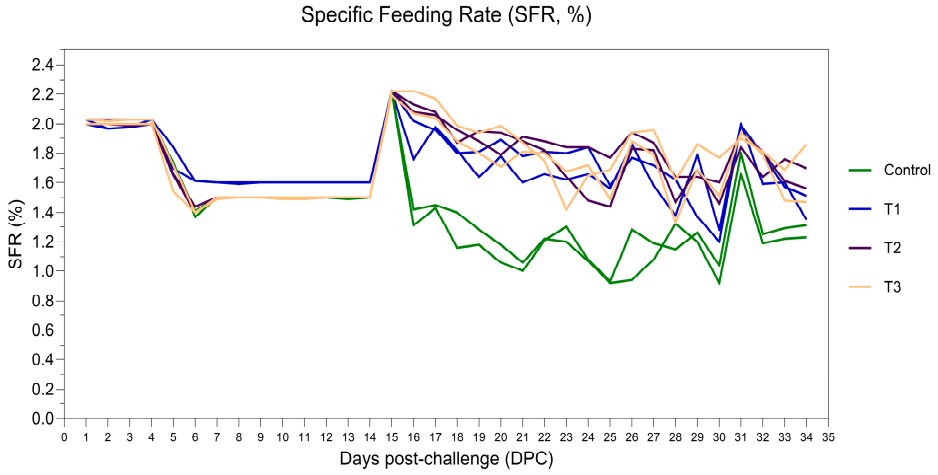


Figure 4. Specific feeding rate (SFR%) in the treatment study. Data are shown for the control group (no treatment) and treatment groups T1 (5 mg/kg BW/day), T2 (7.5 mg/kg BW/day), and T3 (10 mg/kg BW/day). During the treatment period (DPC5–DPC14), the SFR (%) was deliberately maintained at 1.5 (control, T2 and T3) and 1.6% (T1) to ensure accurate FFC dosing. Notably, the SFR (%) for the control group (green lines) showed a significant deviation from the treated groups after the treatment period.

Necropsy and SRS scores were recorded for both diseased and surviving fish (see Tables 4 and A1 in Appendix A). Diseased fish in the control group displayed pathological findings characteristic of SRS, including a severely affected liver (swollen and pale), splenomegaly, hemorrhagic pyloric caeca, and hemorrhagic visceral fat at termination. The score and frequency of findings showed no major differences among the fish groups or the replicate tanks. The most frequent findings on external evaluation were pale gills in most tanks. In addition, the control group exhibited hemorrhagic eyes. Regardless of feed treatment, pale liver, liver inflammation and splenomegaly were the most frequent findings observed during the necropsy.

Table 4. Necropsy findings in control group with diseased fish.

Frequency of Findings (SRS Score) in Mortality Group		**Control Group**	
	Duplicate tank	1	2
	Dose of FFC (mg/kg BW/day)	0	0
	Weight (g)	295	277
	SD (g)	29	29
External	Gills pale/hemorrhagic	16/16	13/13
	Eyes hemorrhagic	2/16	4/13
	Eyes exophthalmia	0/16	0/13
	Skin wounds/ulcers	0/16	0/13
Internal	Ascites	1/16	0/13
	Liver yellow/pale	16/16	13/13
	Liver node/hemorrhagic	1/16	0/13
	Liver inflammation	13/16	7/13
	Spleen pale/nodes	12/16	9/13
	Splenomegaly	16/16	13/13
	Pyloric caeca congestive/hemorrhagic	16/16	13/13
	Kidney pale	2/16	2/13
	Kidney swollen	12/16	6/13
	Visceral fat	16/16	13/13
	Feed	16/16	13/13

4. Discussion

Farmed Atlantic salmon in Chile are vaccinated against *Piscirickettsia salmonis* (SRS) as a preventative measure to control the disease. However, it is well-documented that currently available vaccines provide limited protection under field conditions. As a result, antibiotic treatments remain an essential tool for managing SRS outbreaks in Chilean aquaculture.

Our study was designed as an antibiotic challenge trial to evaluate the efficacy of three florfenicol doses under controlled conditions. This approach is particularly relevant given the limited protection offered by existing vaccines. Therefore, despite the absence of vaccinated fish in our trial, the findings remain highly applicable to real-world conditions, where antibiotic use persists due to insufficient vaccine efficacy. These results provide valuable insights into treatment strategies currently employed in the Chilean salmon farming industry. Currently, the manufacturer's recommended dose of florfenicol (FFC) is 10 mg/kg body weight per day (BW/day) for 10 consecutive days to treat SRS [30]. According to the literature, treatment doses used in the industry typically range between 10 and 40 mg/kg BW/day for 14–21 days [8,30]. The trigger for initiating treatment also varies between salmon-producing companies and sometimes even from case to case. These large variations in treatment strategies raise concerns about the potentially excessive use of antibiotics to control SRS and emphasize the need for further research to determine optimal dose, timing for initiating treatment and duration of treatment. This study, part of a larger

project focused on optimizing FFC treatment, aimed to evaluate the effectiveness of three specific doses (5, 7.5, and 10 mg/kg BW/day for 10 days) when treatment is initiated early post-challenge. The application of treatments for SRS control in a seawater production site is generally conducted/dispensed later than in this study. However, the early administration used in this challenge study provides valuable information and shows that early therapy is very effective in controlling SRS and in reducing antibiotic use. Two of the test doses are below the recommendations of the medicine producer. This deliberate selection serves to emphasize the importance of avoiding the need to exceed this recommended dose. Administering antibiotic doses below therapeutic recommendations carries several important risks, including reduced efficacy, diminished bacterial susceptibility, and the potential for resistance development [30]. While we do not recommend using a lower treatment dose than that advised by the manufacturer, it is also crucial to emphasize the importance of not exceeding the recommended dose. The same principle applies to treatment duration, which varies widely across the industry. Adhering to the recommended dose and treatment duration for managing SRS has a significant potential to reduce overall antibiotic consumption in the Chilean salmon farming industry.

A crucial finding in the present study was that all the doses tested halted the disease progression in the fish challenged with *P. salmonis* genogroup LF-89 and prevented mortality in all treated groups (T1: 5 mg/kg BW/day; T2: 7.5 mg/kg BW/day; T3: 10 mg/kg BW/day). In contrast to the control groups where mortality reached 32.2% and clinical signs were evident, there was a significant reduction in mortalities and clinical signs in all treatment groups. These results directly correlate with the effectiveness of the antibiotics and the treatment strategy employed. A typical clinical sign of SRS disease is a drop in appetite. After the treatment period in this study, the SFR (%) showed significant variation between the treated groups and the control group. The control group exhibited a notable decrease on DPC15, four days before the first recorded mortality. As the study progressed, all treatment groups maintained a higher SFR (%) compared to the control group. This suggests that the treatment helped prevent a decline in appetite (SFR%), a common clinical symptom of SRS infection. One limitation of the current study is the absence of bacterial load measurements in surviving individuals, which restricts our ability to evaluate pathogen clearance and potential subclinical infections. For future studies within this project, we have included systematic tissue sampling from sick and surviving animals to verify the causative pathogen and quantify bacterial load. This approach will strengthen our understanding of disease progression, treatment outcomes, and the risk of developing antibiotic resistance.

In this study, we aimed to use the same fish population for both the pre-study (dose determination) and the treatment study to minimize potential genetic and population-related variability that could influence the fish's resistance to the infectious agent. However, despite almost identical challenge doses, the results showed significant differences in cumulative mortality and the time to first mortality between the pre-study and the treatment study. The reason for the observed differences in mortality between the pre-study and the treatment study remains unclear. Several factors are known to influence the outcome of bacterial challenge trials. Likely contributors include variations in the challenge materials such as bacterial growth conditions and the specific growth phase at which the bacteria were harvested. In addition to bacterial load and bacterial virulence, variability in results may be influenced by factors such as fish species, age, immune status and the environmental conditions. It is reasonable to assume that fish develop more robustness as they grow larger. Fish in the pre-study group had an average weight of 159 g, while those in the treatment study groups had an average weight of 255 g, representing an increase of approximately 60%.

The absence of significant differences in mean body weight within and between groups suggests that fish size was unlikely to have influenced the mortality rates observed in the control group. At termination (DPC35), the mean weight across all groups was 469 g, with no statistically significant differences detected between treatment and control groups.

Using an IP challenge model ensures that all experimental fish are synchronously infected with the same load of bacteria. This is an advantage of controlled challenge studies over natural outbreaks for assessing treatment doses. However, applying these experimental results to commercial salmon production at sea is complex and involves considering several other factors. The infection dynamics within the fish population will fluctuate due to bacterial shedding and also the cohabitation status within the populations on the site. In commercial salmon farming, fish are treated with medicated feed once clinical signs of disease are observed, when the fish are proven positive through laboratory analysis or when SRS-related mortality has been detected. Following the detection of *P. salmonis* on a commercial production site, there may be a delay of several days before the medicated feed is delivered to the site and the fish receives the medicated treatment. As time progresses, the fish population will comprise individuals at various stages of disease progression, with portions of the population likely experiencing a reduced appetite. "Early treatment" will therefore most likely not cover the entire population when treatment is initiated. It is therefore important to document the optimal trigger for treatment and the correct FFC dose for treating a fish population consisting of fish in different stages of disease progression. This present study is part of a larger project, with a new phase recently conducted to test different treatment initiation points. In addition to early treatment (DPC5), these include starting treatment when a drop in SFR is registered, as well as when the first mortality is observed. This research is crucial for demonstrating the importance of initiating treatment early in the disease progression. Additionally, cohabitation challenges are being carried out to provide a trial that more closely represents conditions in actual commercial Atlantic salmon production.

Several bacterial factors can also influence the success rate of antibiotic treatments, one of which is the virulence of the bacterial isolate. The virulence levels can vary significantly among *P. salmonis* isolates, with multiple virulence factors contributing to their pathogenicity, ultimately affecting disease severity and treatment outcomes. These differences in virulence have been documented through mortality curve analyses in various salmonid species [31–34]. It has also been suggested that treatment failure can be related to the bacterial susceptibility to the treatment agent applied. The minimum inhibitory concentration (MIC) of FFC for the challenge isolate (genogroup LF-89) used in this study is determined to be 1 μg mL^{-1}. This value is notably higher compared to the findings of San Martin et al. [30], who reported MIC values ranging from 0.06 to 0.25 μg mL^{-1} for 87 isolates collected in Chile between 2012 and 2017. Contreras-Lynch et al. [35], on the other hand, identified two distinct sub-populations of *P. salmonis* based on MIC values for FFC. One sub-population exhibited a modal MIC of 0.0652 μg mL^{-1}, which was fully susceptible to FFC, while the other sub-population had a modal MIC of 1 μg mL^{-1}, suggesting a potential reduced susceptibility to the antibiotic. The findings imply that the test isolate used in the present study is a suitable candidate for experimental FFC treatment evaluations, as it has a MIC at 1 μg mL^{-1} for FFC, a MIC value that aligns closely with the average typically detected in operational salmon farming in Chile. However, as there are likely genovariants within the genogroup LF-89 with different virulence properties and susceptibility to antibiotics, as suggested by ADL data [19], one should be aware that this may influence the outcome of FFC treatment in the field. Considering the above, effective surveillance and understanding of the isolates present at a given site, including their specific characteristics, are crucial for implementing optimal treatment strategies. To ensure the best possible treatment outcomes

despite these bacterial differences, certain key factors must be prioritized in all cases. These include early detection of outbreaks and the prompt initiation of treatment, both of which are critical to mitigating the impact of the disease.

There is no standardized method for measuring the effectiveness of antimicrobial treatments in fish under commercial conditions. Numerous factors, ranging from environmental conditions to fish-specific variables and management practices, can significantly influence the outcome. However, the findings of Happold et al. [36], who evaluated the effectiveness of antimicrobial treatment of SRS outbreaks, using industry-generated data from 8318 cage-level production cycles stocked between 2003 and 2018, recommend that treatment for Atlantic salmon should be promptly administered to all infected cages on the farm, without interruption, immediately following the onset of an SRS outbreak. This finding aligns with the results and recommendations of the current study which show that early detection is a critical factor in controlling SRS, enabling prompt treatment and improving the likelihood of success. The result of the current work also aligns with previous field studies suggesting that antibiotic treatment success is higher if the treatment is administered when mortality associated with SRS is relatively low [37].

The current study highlights the value of conducting controlled tank experiments to answer specific questions, in this case, the effect of early onset of antibiotic treatment on SRS infection in Atlantic salmon. However, the results of this study must be compared with those of similar studies and, importantly, with field-generated data to enhance understanding and facilitate practical application. This will enhance our understanding of disease dynamics in salmon farming and provide fish producers with relevant scientific recommendations for optimizing SRS treatment strategies for better effect and a reduction in unnecessary use of antibiotics.

5. Conclusions

This in vivo study demonstrated that administering florfenicol (FFC) at doses of 5, 7.5, and 10 mg/kg/BW/day for 10 consecutive days effectively reduced mortality in Atlantic salmon intraperitoneally challenged with *Piscirickettsia salmonis* genotype LF-89, when treatment was initiated 5 days post-challenge.

The findings show that the lower doses (5 and 7.5 mg/kg/BW/day) were as effective as the recommended dose (10 mg/kg/BW/day). While we do not recommend using a lower treatment dose than specified, it is also crucial to emphasize the importance of not exceeding the manufacturers' recommendations. The positive results in this study could be related to the early on-set of treatment (DPC5). A follow-up study will therefore investigate the effect of different treatment doses and initiation points of FFC against SRS.

Responsible antibiotic stewardship is essential—not only for maintaining therapeutic efficacy and minimizing the risk of antimicrobial resistance, but also for reducing unnecessary antibiotic consumption by optimizing dosing strategies to match the actual therapeutic requirements for effective disease control.

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Conflicts of Interest: Cecilie Helen Isachsen Lie and Sverre Bang Småge are employed by the company Cermaq Group; Carlos Zarza and Linda Beate Jensen are employed by the company Skretting Aquaculture Innovation (AI); Pablo Ibieta and Pablo Ibarra are employed by the company TEKBios Ltda- Fish Trial Center.

Appendix A

Table A1. Frequency of findings (SRS score) in surviving fish at the end of the experimental treatment trial.

Frequency of Findings (SRS Score) in Surviving Fish (End of Challenge)		**T1**		**T2**		**T3**	
	Duplicate tank	1	2	1	2	1	2
	Dose of FFC (mg/kg BW/day)	5	5	7.5	7.5	10	10
	Weight (g)	436	454	477	471	464	510
	SD (g)	62	82	76	71	87	79
Externa	Gills pale/hemorrhagic	2/10	1/10	0/10	2/10	0/10	3/10
	Eyes hemorrhagic	0/10	0/10	0/10	0/10	0/10	0/10
	Eyes exophthalmia	0/10	0/10	0/10	0/10	0/10	0/10
	Skin wounds/ulcers	0/10	0/10	0/10	0/10	0/10	0/10
Internal	Ascites	0/10	0/10	0/10	0/10	0/10	0/10
	Liver yellow/pale	10/10	10/10	10/10	10/10	10/10	10/10
	Liver node/hemorrhagic	0/10	0/10	0/10	0/10	0/10	0/10
	Liver inflammation	2/10	2/10	3/10	2/10	3/10	5/10
	Spleen pale/nodes	0/10	0/10	0/10	0/10	0/10	0/10
	Splenomegaly	6/10	8/10	5/10	10/10	6/10	8/10
	Pyloric caeca congestive/hemorrhagic	0/10	0/10	0/10	0/10	0/10	0/10
	Kidney pale	0/10	0/10	0/10	0/10	0/10	0/10
	Kidney swollen	0/10	0/10	0/10	0/10	0/10	0/10
	Visceral fat	0/10	0/10	0/10	0/10	0/10	0/10
	Feed	10/10	10/10	10/10	10/10	10/10	10/10

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