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Influence of genetic adaption of rainbow trout (*Oncorhynchus mykiss*) fed with alternative protein sources based on *Arthrospira platensis* and *Hermetia illucens* on disease resistance against viral haemorrhagic septicaemia virus (VHSV)

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A R T I C L E I N F O

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A B S T R A C T

Regarding feed components, novel protein sources in the diet of carnivorous fish species are of increasing interest in aquaculture, which is caused by the fact that the use of marine resources is still being exhausted to meet consumer demand for fish. However, the renouncement of marine resources represents a decisive step towards a more sustainable aquaculture. Alternative solutions can be found in plant-based fish nutrition. Nonetheless, due to challenges related to food conversion, growth rates, fish health and welfare as well as the consequent weakening of immunocompetence against pathogens, the use of plant-based protein sources in the diet of carnivorous fish remains a subject of justified controversy. Thus, the current study aims at making use of the genetic variability of rainbow trout (*Oncorhynchus mykiss*) in order to gain new insights into their adaptability to innovative raw materials. Using a viral haemorrhagic septicaemia virus (VHSV) infection model, the particular focus is on the question how infections affect the disease resistance of differently bred and fed populations. For this purpose, genetically distinct trout populations (R3, R7, R8) were bred and fed with differently composed diets, in which 20 % fishmeal of the total composition was totally replaced by *Arthospira platensis* or *Hermetia illucens*. The infection experiment included a commercially available trout line (C9) and a diet with fishmeal as control as well. Subsequently, the trout were infected with VHSV, and tissue of the posterior intestine was investigated by means of molecular biology regarding the expression of genes encoding various inflammatory markers, antimicrobial peptides, transmembrane proteins as well as the viral load at days 0, 2 and 4 post-infection. The posterior intestine of infected animals was examined histologically by using AB-PAS and HE-staining. The results showed that the genetics played a decisive role in the disease resistance to VHSV. Diet composition, however, did not negatively affect the susceptibility to VHSV. On the contrary, an upregulation of certain antimicrobial peptides in *Arthrospira platensis* fed specimens appeared to partially improve immuno-competence. Also, no correlation between the ability of rainbow trout populations to adapt to a novel diet based on proteins of *Arthrospira platensis* or *Hermetia illucens* and pathogen resistance were observed. Therefore, *Arthrospira platensis* and *Hermetia illucens* appear to be valuable protein sources regarding the replacement of fishmeal for rainbow trout without negatively influencing the immunity and natural resistance of certain rainbow trout populations to important pathogens like VHSV.

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1. Introduction

Although marine fish stocks are slowly recovering from unsustainable exploitation due to the common fisheries policy (CFP) of the European Union, the majority of the oceans are still considered to be overfished (Moffitt and Cajas-Cano, 2014; Sumaila and Tai, 2020; Warfield, 2020). In particular, fish stocks in the Mediterranean and the Black Seas are still predominantly affected (European Commission, 2020). These circumstances are caused by a steadily increasing demand for fish worldwide (Delgado, Wada et al., 2003; Pauly and Zeller, 2016; Tacon and Metian, 2018; FAO, 2020; FAO, 2022), due not only to growing food requirements of the world's population, but also to increasing aquaculture worldwide (Moffitt and Cajas-Cano, 2014; FAO, 2020; Tacon, 2020). As a result, the demand for fishmeal is constantly increasing, while a decreasing fishmeal supply and related increasing costs threaten the sustainability of fish feed in aquaculture (Roy and Pal, 2015; Daniel, 2018). Therefore, the replacement of fishmeal in the diet of carnivorous fish with alternative protein sources appears to be an unavoidable challenge, and justifiably in the focus of scientific interest (Luthada-Raswiswi, Mukaratirwa et al., 2021). However, the use of alternative protein sources in the diet of carnivorous fish species is the subject of controversial discussion.

Therefore, it is a challenge to adequately cover the high protein and lipid requirements of carnivorous fish species. The right composition of used raw materials is also crucial for aspects of fish health and feed utilisation. Therefore, annually approximately 16.5 million tonnes of fish, which is a significant proportion of the world's fisheries production, is processed into fishmeal and fish oil. These provide proteins and lipids with adequate essential amino acid and fatty acid profiles which are required for fish species such as rainbow trout (Shepherd and Jackson, 2013; Oliva-Teles, Enes et al., 2015). Therefore, carnivorous fish species cannot be fed on alternative plant-based feed sources without restrictions (Hemre et al., 2018). Due to numerous related anti-nutritional factors and unbalanced amino acid profiles, plant-based protein sources may have adverse effects on animal health and welfare (Hardy, 2010, Daniel, 2018; Villasante et al., 2025). For example, results of a previous study on rainbow trout suggest that high levels of plant-based ingredients can also lead to increased stress responses (Sadoul, Foucard et al., 2016; Villasante et al., 2025). Nevertheless, as cost-effective ingredients, plant-based proteins are popular substitutes for fishmeal in the diet (Daniel, 2018; Ghosh et al., 2019). Therefore, soybean meal is one of the most popular sources of plant proteins used in the diet for various species of farm animals (Bakke-McKellep et al., 2000; Daniel, 2018).

However, in numerous fish species, such as rainbow trout (*Oncorhchus mykiss*) (Venold et al., 2012) or Atlantic salmon (*Salmo salar*) (Krogdahl et al., 2003) and even omnivorous fish species such as common carp (*Cyprinus carpio*) (Urán, et al., 2008) and zebrafish (*Danio rerio*) (Hedrera et al., 2013), inflammatory responses of the anterior intestine (Bruce et al., 2017), and immunosuppression due to various amounts of soybean supplementation within the feed ration have been reported (Daniel, 2018). These negative effects on fish health are due to numerous anti-nutritive factors such as glycinin, ß-conglycinin (Burrells et al., 1999; Zhou et al. 2018) or saponins (Krogdahl et al., 2015; Zhou et al. 2018) and are represented in numerous other plant-based protein sources as well. For instance, inferior feed conversion in combination with growth depression and suppressed thyroid function has been reported in rainbow trout when feeding rapeseed meal (Burel et al., 2000).

Therefore, research into other promising alternative protein sources is of crucial importance to achieve a sustainable aquaculture. Thus, insect meal and microalgae have become a focus of interest in research as raw materials for alternative protein sources in fish nutrition in recent years (Henry et al., 2015; Luthada-Raswiswi et al., 2021).

Insects are a commonly found food source in the natural consumption of many fish species such as rainbow trout (Henry et al., 2015). Their small ecological footprint, low CO_2 emission, and low water consumption, limited need for arable land, high feed conversion efficiency and their ability to grow on low-value organic by-products and convert them into valuable biomass-products makes them an attractive food source in the diet for the aquaculture industry (Henry et al., 2015; Spranghers et al., 2017). Black soldier fly larvae meal (BSFL, *Hermetia illucens*) has already been successfully applied as partial or total replacement in the diet composition of channel catfish (*Ictalurus punctatus*) (Bondari and Sheppard, 1987), Mozambique tilapia (*Oreochromis mossambicus*) (Bondari and Sheppard, 1987) and also of rainbow trout (*Oncorhynchus mykiss*) (Stamer et al., 2014; Stamer, 2015; Renna et al., 2017; Stadtlander et al., 2017). In a previous study on rainbow trout, for instance, a supplementation of 8–16 % black soldier fly meal prevented intestinal inflammation which was usually observed when feeding soybean meal. Additionally, a down-regulation of pro-inflammatory genes and improved innate immunity has been reported (Kumar et al., 2021). Therefore, the use of *Hermetia illucens* as a feed additive is especially recommended for the diet of rainbow trout if the diet contains a high proportion of plant-based proteins. Further advantages of black soldier fly larvae meal (BSFL) are known concerning the nutritional aspects of several fish species. The ingredients of *Hermetia illucens* contain high-quality proteins and unsaturated fatty acids which cover the high protein and unsaturated fatty acid requirements of fish (Henry et al., 2015). Additionally, its nutrient composition can be influenced by the ingredients of different growing substrates (Tschirner and Simon, 2015; Meneguz et al., 2018). The production is very efficient due to its potential of converting agricultural waste into animal feed (Goyal et al., 2021). Furthermore, its low trophic level and high metabolic efficiency make *Hermetia illucens* a valuable raw material. Moreover, BSFL has also proven its worth as a food additive.

Microalgae, which are easy to produce, represent a valuable and cost-effective protein source as well. They belong to the natural food spectrum of several fish species at different growth stages (Roy and Pal, 2015). The microalga *Arthrospira platensis*, a multicellular, filamentous, spiral-shaped, photoautotrophic cyanobacterium, which is commonly known as "spirulina", has gained remarkable attention in recent years (Teimouri et al., 2013). Due to its high content of unsaturated fatty acids, vitamins, minerals, proteins and a high proportion of essential amino acids (Jung et al., 2019; Zhang et al., 2020), it has been known as a valuable supplement in human and animal nutrition for years (Wu et al., 2016). However, *Arthrospira platensis* contains not only valuable ingredients. Studies also report about immunomodulatory, antioxidant and anti-inflammatory properties in the diet of humans and animals (Wu et al., 2016). Additionally, when feeding a diet that contains spirulina, the toxicity of heavy metals in some fish species can be reduced. Nonetheless, the effects of *Arthrospira platensis* appear to be very dependent on the respective fish species and the amount of *Arthrospira platensis* contained in the diet (Zhang et al., 2020). However, despite its high nutritional value and immune-enhancing properties, *Arthrospira platensis* has not yet become established in aquaculture (Hemaiswarya et al., 2011). Especially as a functional feed additive and also as a fishmeal substitute in fish and shrimp culture, *Arthrospira* has become the centre of attention only in recent years (Ragaza et al., 2020). Several researchers report about the successful use of *Arthrospira platensis* as a feed supplement in the diet of rainbow trout (Yeganeh et al., 2015; Sheikhzadeh et al., 2019), while less is known about its potential as a complete replacement for fishmeal. Recently published studies on rainbow trout indicate that a complete replacement of fishmeal by *Arthrospira platensis* is associated with reduced growth performance and effects on fillet colour and fatty acid pattern (Rosenau et al., 2021). Nonetheless, faced with a decreasing supply together with a steadily increasing demand for fishmeal, sudden changes in the feed formulation for carnivorous fish in aquaculture seem almost unavoidable (Luthada-Raswiswi et al., 2021). In this situation, the genetic potential of fish can play a crucial role in the adaption to novel protein sources. However, genetic adaptation to new environmental conditions usually represents a continuous and long-lasting process, which has hardly

taken place. Especially the replacement of fishmeal by plant-based raw materials poses a new challenge according to the breeding goals in rainbow trout, which are usually specialised in metabolising animal proteins and therefore not well adapted to plant-based protein sources (Mackensen, 2011).

Nevertheless, fish possess high heritability of important performance traits such as growth (Volckaert et al., 2012), disease resistance (Torrealba et al., 2023) or adaption to changing environmental conditions (Benfey et al., 2024).

Therefore, selection success in breeding programmes are three to five times higher compared to terrestrial animals (Gjedrem, 2012). Thus, regarding the adaption to plant-based feedings, the survival rate and growth of rainbow trout could be significantly improved after one generation of selection (Le Boucher et al., 2012). However, many fish species, including rainbow trout, have not yet undergone much breeding selection compared to agricultural livestock and hold a high potential for breeding improvement (Gjedrem, 2012, 2015). For those reasons, an adaptation to novel diets based on fishmeal substitutes in the feed has hardly taken place. Therefore, the individual variability to adapt to novel diets can be used to improve animal welfare and sustainability in trout aquaculture (Le Boucher et al., 2012). Rainbow trout from selective breeding programmes are already widely used for stocking on German aquaculture farms, despite being almost exclusively imported from abroad (Schäfer, 2020). Nonetheless, comparable studies are not yet known for rainbow trout in Germany, which is why local trout populations possess such a great breeding potential. In addition, host-pathogen interactions and their resistance mechanisms have been of particular interest in the last few decades. Virus outbreaks cause major economic damage in aquaculture and fisheries. For this reason, viral outbreaks, such as those caused by viral haemorrhagic septicaemia virus (VHSV), are considered a global problem of particular importance (Bain, 2010; Lafferty, 2015). As one of the most important *rhabdovirus* diseases in salmonid aquaculture, VHSV has become a focus of research worldwide (Quillet et al., 2001).

Viral haemorrhagic septicaemia (VHS) is caused by the viral haemorrhagic septicaemia virus. VHSV is a single-stranded RNA virus from the genus *Novirhabdovirus*, which causes a systemic infection in salmonid species. Characteristic clinical symptoms include haemorrhages of internal organs, skin and muscle and are often accompanied by apathy, darkening, exophthalmia and ascites. Acutely infected animals may show severe disease symptoms and high mortality rates (Quillet et al., 2007; Smail and Snow, 2011). There is no treatment or prophylactic measures known that can prevent or cure infected fish from these severe infections (Crane & Hyatt, 2011), which is why breeding selection of resistant fish has been in the focus of research for decades (Gjedrem, 2015; Huang et al., 2025).

In salmonid breeding, for example, attempts are being made to breed disease-resistant fish strains that have developed resistance to various diseases on the basis of intraspecific selection (Price, 1985; Fjalestad et al., 1993). Previous studies, which investigated the heritability of resistance to VHSV in rainbow trout, report on a high heritability of disease resistance (Dorson et al., 1995; Henryon et al., 2005). But less is known about the effects of *Arthrospira platensis* and *Hermetia illucens* as fishmeal substitutes on immunocompetence of rainbow trout against relevant pathogens, such as VHSV. Furthermore, fish that have survived an infection with VHSV are capable of developing disease resistance to future viral infections with this virus, such as Pacific herring (*Clupea pallasii*) (Hershberger et al., 2010) and some salmonid species (Dorson et al., 1991).

The effects of *Arthrospira platensis* and *Hermetia illucens* on intestinal health and animal welfare of site-adapted rainbow trout populations in Germany have already been investigated in a previous related study Miebach et al., 2023). Although comparable studies on *in vitro* infection experiments using VHSV have been published, no investigations on disease resistance of genetically adapted rainbow trout populations influenced by diets containing *Arthrospira platensis* or *Hermetia illucens* against VHSV have been carried out so far on living animals. Therefore, as follow-up study on intestinal health of site-adapted rainbow trout populations fed with the sustainable protein sources *Arthrospira platensis* and *Hermetia illucens* (Miebach et al., 2023), the current study investigates the effects of these fishmeal replacements in order to gain better insights into the immunocompetence of different rainbow trout populations against relevant infectious agents, based on the example of an infection with viral haemorrhagic septicaemia virus (VHSV). This issue, which has hardly been addressed in previous studies to date, is of crucial importance in order to completely exclude all negative influences of the alternative protein sources investigated to pave the way to a sustainable aquaculture without adversely affecting fish health and animal welfare.

2. Materials and methods

2.1. Ethical clearance

After approval by the Local Ethics Committee in Lublin, Poland (Approval No. 33/2020), the trout were transported to the national reference laboratory of the National Veterinary Research Institute in Pulawy, Poland where the infection experiment was carried out in accordance with international and national regulations for animal experimentation.

2.2. Rainbow trout populations and feeding trial

Rainbow trout from 27 individual matings with complete pedigree information from eight site-adapted populations from different regions in Germany (R1-R8) were bred and standardised by the Institute for Aquaculture and Water Ecology at the Georg August University of Göttingen, Göttingen, Germany. When reaching the age of a fingerling with a mean weight of 31.2 ± 9.4 g, the rainbow trout of each family were individually tagged by using passive integrated transponder tags (PIT-tags), and equally distributed among nine 200 L recirculatory aquaculture system (RAS) tanks using a communal testing design. Based on internal breeding and rearing conditions, the fish were reared in pathogen-free spring water. Additionally, previous health examinations were examined to exclude possible diseases which could negatively affect obtained results.

Afterwards, the trout were fed three isoenergetic and isonitrogenic diets containing 20 % fish meal (control group) or a diet which completely replaced the fish meal component with a microalgae (*Arthrospira platensis*) or partially defatted black soldier fly meal (*Hermetia illucens*) according to (Dietz et al., 2020, 2023) at a ratio of 1 % body weight per day. Depending on ambient water temperature (10–14 °C), the diet quantity was adjusted to the fish biomass per tank following the feeding table implemented by Stadtlander et al. (2017).

According to Dietz et al. (2023), the trout populations were acclimatised to diets until visually assessed apparent saturation per day, so that hardly any animal could be attracted by the food pellets. The achieved maximum realised feeding level of 1 % body weight for R3, R7, and R8 (around 1.2 % for C9) was used in the subsequent feeding experiment for all populations aiming to make sure that differences in growth could be correlated to nutrient utilisation rather than feed intake. Additionally, the applied feeding quantity should prevent the excess of unconsumed feed (Dietz et al., 2023). The rainbow trout were hand-fed once a day with one third of the total daily feed ration. The remaining feed was supplied by means of an automatic band feeder (Fiap GmbH, Ursensollen, Germany). The precise feed compositions are shown in Table S1.

According to Dietz et al. (2023), the feed compositions of 20 % fishmeal or it́s substitutes *Arthrospira platensis* or *Hermetia illucens* is closer to current commercial feeds due to its low percentage content than previous studies. In a related preliminary study, complete feed acceptance of the respective feeds was tested on various rainbow trout

populations and a significant increase in growth was observed in site-adapted animals (Dietz et al., 2023). The different diets were fed to trout in three replicate tanks, respectively. After 90 days of feeding (Dietz et al., 2023), the breeding values of these rainbow trout from different site-adapted populations were estimated by evaluating the diets for feed acceptance and growth trend. Based on breeding values, population R8 was selected as best performing genotype and populations R7 and R3 as least performing genotypes. In addition, a commercially available line (C9) fed with conventional fishmeal-based feed was integrated into the trial for comparison purposes. In further trials for selected populations of rainbow trout (site-adapted populations R3, R7, R8 and a commercial line C9), the nutrient utilisation (especially protein) was quantified and protein quality of the model diets was evaluated. Furthermore, effects on parameters of digestibility (Dietz et al., 2020) were examined by the Georg August University of Göttingen.

2.3. Viral strain and infection experiment

After completing the feeding experiment as described above, the trout were transported to the national reference laboratory of the National Veterinary Research Institute in Pulawy where the infection experiment was carried out in accordance with international and national regulations for animal experimentation and after approval by the Local Ethics Committee in Lublin (Approval No. 33/2020). For this purpose, after a two-day break in feeding, the trout were transferred to the laboratory animal facility to carry out the infection trial. After 24 h of acclimatisation, the trout were distributed equally among three replicate tanks. Three to six trout from each feeding group and each genetic population were placed in each tank (Fig. 1). Afterwards, trout from the populations with the highest (populations R8 and C9) and lowest breeding values (populations R3 and R7) were infected with VHSV. The VHSV strain Fi13, belonging to serogroup F1 (Enzmann and Bruchhof, 1989), was provided by the Friedrich-Loeffler-Institut (FSL), the Federal Research Institute for Animal Health, Greifswald, Germany. Subsequently, 24 h after the last regrouping, the infection with VHSV was carried out. For this purpose, the trout were removed from the holding tanks, bathed in an aerated water bath with 5×10^3 $TCID_{50}$ VHSV /mL for 2 h at 15 °C and then returned to the holding tank and kept at 12–15 °C for 29 days. The animals were checked 2–4 times per day. Moribund individuals were euthanised using a tricaine hydrochloride solution (500 mg/mL buffered MS-222 solution; Pharmaq®) added to the water tank and deceased individuals were collected. The mortality rate was documented over 29 days. At days 2 and 4 post-infection, four trout from each feeding group and genetic were killed by immersion in a solution of tricaine hydrochloride, dissected and samples of the posterior intestine were collected. Finally, due to mortality, a total number of 118 animals including population R3 (35 individuals in total), R7 (36 individuals in total) and R8 (35 individuals in total) were examined at the pre-infection period day 0 and the post-infection periods day 2 and day 4. In addition, the commercial line C9 (12 individuals in total), which was fed the conventional diet only, was analysed analogously. All fish were examined macroscopically during dissection and samples were collected from the posterior intestine approx. 0.5 cm anterior from the cloaca for histological and molecular biological analysis. The samples were transferred to reaction vessels with RNAlater and stored at −80 °C for further analysis or fixed in 4 % buffered formalin for histological examinations. Afterwards, histological changes and gene expression of inflammation parameters, antimicrobial peptides and transmembrane proteins were investigated as described below.

2.4. Histological analysis

Samples were collected from the posterior part of the intestine approx. 0.5 cm anterior from the cloaca for histological analysis and rapidly fixed in 4 % buffered formalin. After a fixation time of at least 24 h, the samples were watered for at least 6 h. Subsequently, dehydration in a series of graded ethanols and embedding of the intestine samples in paraffin blocks followed. After completing the embedding, 2 µm thick cross-sections were cut. Then, after drying on a 35-degree Celsius heating plate, the samples were deparaffinised in butyl acetate and isopropanol and stained with haematoxylin eosin (HE)- or alcian blue-PAS (AB-PAS) stainings (Yamabayashi, 1987; Fischer et al., 2008) and covered with Roti-Mount (Romeis, 1989). HE-stained sections were used to measure the diameter (mean of 2 perpendicular axes in µm per slide) and the thickness of tunica muscularis, stratum granulosum and stratum compactum (each by mean of four measurements per slide in µm). Additionally, the inflammatory cell infiltration into the lamina propria was analysed based on a predetermined score from 0 (absent), 1 (low), 2 (moderate) to 3 (high-grade infiltration of inflammatory cells) (Hamidian et al., 2018). To determine the total number of goblet cells (mean value of 10 ×10 µm of well orientated villi) in the mucosa and the filling level of goblet cell content based on predetermined scores from 1 (low grade) to 3 (high grade), AB-PAS-stained sections were analysed. Each sample was randomised and blindly evaluated.

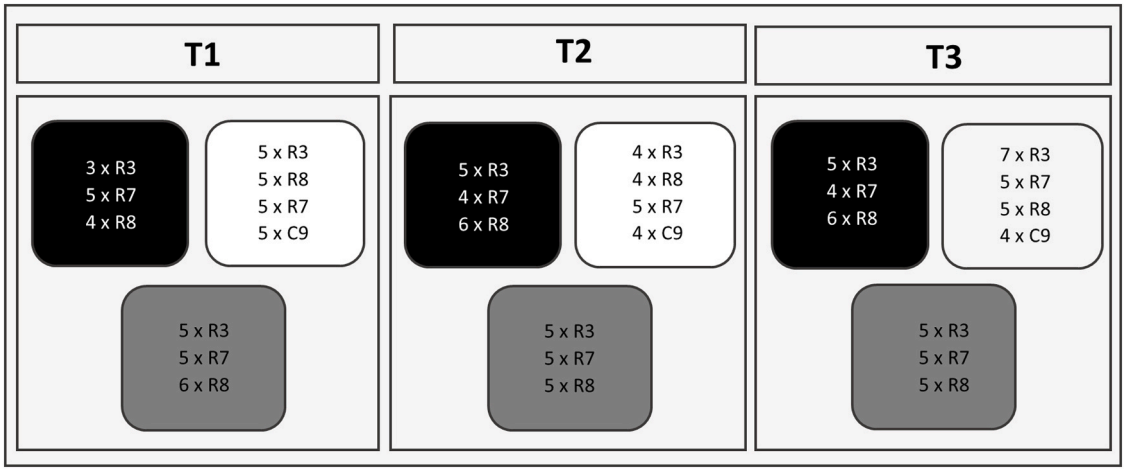

Fig. 1. Experimental set up of three tanks (T1–3) with the number of trout according to each population and diet: black = *Arthrospira platensis* diet, grey = *Hermetia illucens* diet, white = Control group, fish meal diet; R3 = regional population R3, R8 = regional population R8, C9 = commercial line C9.

2.5. Gene expression

2.5.1. RNA isolation

Samples were collected from the posterior intestines of all animals and rapidly preserved in RNAlater at a temperature of - 80 °C until further analysis. Afterwards, 50 mg of each tissue sample was removed and minced with scissors and the tissues were homogenised by using 900 μL Trizol reagent (Sigma-Aldrich, Inc., St. Louis, MO, USA), adding a 5 mm stainless steel bead and crushed in a tissue lyser (Tissue Lyser II, Qiagen GmbH, Hilden, Germany) for 5 min at 25 Hz. Total RNA was extracted from homogenised tissue according to the manufacturer's protocol (Miebach et al., 2023). Any genomic DNA remaining in the sample was digested by incubation with 2 IU DNase I (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Isolated RNA was re-suspended in 20–50 μL Ultra Pure Water depending on the amount of the isolated RNA pellet.

The synthesis of cDNA from 400 ng total RNA was then carried out using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc.). A control without reverse transcriptase was included for each sample investigated. Afterwards, the cDNA was stored at −20 °C. Then, before use in RT-qPCR analyses, the cDNA was diluted 1:20 with nuclease-free water (Thermo Fisher Scientific, Inc.).

2.5.2. Quantitative Reverse Transcription PCR (RT-qPCR)

For evaluation of the expression levels of target genes from host tissue, SYBR-Green based RT-qPCR protocols were used for quantitative determination of cDNA. An overview of the target genes investigated in the hindgut of differently fed trout populations and the start sequences (primers) used is given in Table 1. PCR reactions were performed in duplicate using Maxima SYBR™ Green 2x qPCR Mastermix (Thermo Fisher Scientific, Inc.) in a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Inc., Foster City, CA, USA). The following components were included in the prepared reaction mixture: 5 μL of SYBR green Mastermix (with 0.05 μL of ROX), 0.2 μL of the respective forward and reverse primers (sequences in Tables 1), 3.0 μL cDNA (diluted 1:20) and nuclease-free water to a final reaction volume of 10 μL. To normalise the expression of the measured target genes, the elongation factor 1 gene was used. Subsequently, the measured copy number of the target gene was divided by the normalised elongation factor 1 (EF1/100,000). The amplification programme underwent a single initial denaturation of 10 min at 95 °C, followed by 40 identical cycles of denaturation for 30 s at 95 °C, annealing at 55 °C for 30 s and subsequent elongation at 72 °C for 30 s.

2.5.3. Statistical analysis of data

Statistical analyses were performed by using the software program SigmaPlot 12.5 (Systat Software). For each experimental group, data were provided as mean ± standard error of the mean (SEM). For direct comparison, normally distributed samples were evaluated using the *t*-test, and non-normally distributed samples were evaluated using the Mann-Whitney *U* test.

When comparing more than two groups, normally distributed samples were analysed by using two-way or three-way analysis of variance (ANOVA). Samples which were not normally distributed were logarithmically transformed (log10) and either analysed by using the Kruskal-Wallis test or evaluated with a two- or three-way analysis of variance if a normal distribution was present. A p-value of < 0.05 was considered as statistically significant.

3. Results

3.1. Susceptibility of site-adapted trout populations (R3, R7, R8) with high and low breeding value estimates to VHSV after feeding fishmeal substitutes

In a previous related study, Bauer et al. (2023) investigated the susceptibility of trout from different site-adapted populations to viral or bacterial infections based on *in vitro* studies. The current study involved conducting an infection experiment to determine how trout populations with different feed utilisation react to VHSV infection and whether

Table 1
Nucleotide Sequences of the primers of target genes.

Primer and probe	Nucleotide sequence (5´-3´)	*Application*	*Accession no.*	*Efficiency*	*Reference*
Housekeeping gene:					
Onmy-EL1α	F: TGGGCTGGTTCAAGGGATGG R: CTGGAGGGGCAGACGAAGG	Real-time PCR	AF498320	91.0	(Dietrich et al., 2015)
Virus load:					
Onmy-VHSV	F: AATCCGTGCAGCTTTTTCAGG R: CAAGTGCATCCACGATCACCTTC	Real-time PCR	KF477302	107	(Kim, Kim et al. 2014)
Antiviral reaction:					
Viperin (Vig 1)	F: ACAAAGTGGCGTTCAAAATC R: ACTGTTCTCCCCAGCGTTC	Real-time PCR	NM_001124253	93.1	(Adamek et al., 2021)
Inflammatory reaction markers:					
IL1β	F: ACATTGCCAACCTCATCATCG R: TTGAGCAGGTCCTTGTCCTTG	Real-time PCR	AJ223954	91.9	(Chettri et al., 2013)
TNFα	F: GGGGACAAACTGTGGACTGA R: GAAGTTCTTGCCCTGCTCTG	Real-time PCR	AJ277604 AJ401377	91.6	(Chettri et al., 2013)
Receptors of immune cells:					
CD8α	F: GTGGCTTCCTCTTCCTCCTC R: CTGCCATTCTTGGCTGTCTT	Real-time PCR	AF178054	90.6	This paper
Antimicrobial peptides:					
Defensin β3 (omDB−3)	F: GCTTGTGGAATACAAGAGTCATCTGC R: GCATACATTCGGCCATGTACATCC	Real-time PCR	NM_001195183	97.8	(Casadei et al., 2009)
Defensin β4 (omDB−4)	F: GCAACTCTTCTAAAGAACAGT R: CGTGGGCGACACAGCATACAAATCC	Real-time PCR	NM_001195169	94.4	(Casadei et al., 2009)
Cathelicidin 2 (Cath2)	F: AAAGATTCCAAGGGGGGT R: CAAAGGGTGTGTTGTGCTGT	Real-time PCR	AY360356	97.7	(Chettri et al., 2013)
Lysozyme C	F: GAAACAGCCTGCCCAACT R: GTCCAACACCACACGCTT	Real-time PCR	X59491	90.2	(Chettri et al., 2013)
Hepcidin	F: GAGGAGGTTGGAAGCATTGA R: TGACGCTTGAACCTGAAATG	Real-time PCR	AF281354	90.4	(Chettri et al., 2013)
Transmembrane protein:					
Occludin	F: CCTGTGGAGGAAGGTGAAGA R: TGTCGTAGTTGGTGCTGGTG	Real-time PCR	NM_001190446	96.2	This paper

substituting fishmeal in their diet with *Arthrospira platensis* or *Hermetia illucens* affects their susceptibility to infection.

3.1.1. Survival rate

The results showed that 97.8 ± 6.7 % of trout from population R7 and 84.2 ± 13.6 % of trout from population R8 survived VHSV infection, while only 41 ± 23.1 % of trout from population R3 and 15.0 ± 13.2 % of trout from the C9 commercial line survived the infection. The populations with high survival rates included those with good (R8) and low (R7) feed utilisation, while those with low survival rates included populations with good (C9) and low (R3) feed utilisation. These results reveal significant differences between the populations with high and low survival rates (Fig. 2). However, an influence of the different diets on the survival rates could not be determined.

3.2. Course of viral infection

3.2.1. Viral load in the hindgut

The viral load increased significantly on days 2 and 4 post-infection compared to day 0 pre-infection (Fig. 3). In addition, genetic-specific differences were observed in trout fed the conventional fishmeal-based diet at day 2. For instance, the viral load increased notably in C9 trout (Fig. 3a) compared to R7 ($p = 0.044$; Fig. 3b) and R8 ($p = 0.032$; Fig. 3b, c). In population R3 (a, c), the viral load increased compared to population R7 ($p = 0.014$; a). This was consistent with the survival rates observed during the course of infection (population R7 had the highest survival rate, followed by R8, and populations R3 and C9 had the lowest survival rates).

These trends were also evident on day 4 p.i., though they were not significantly pronounced within the dietary groups. Instead, a cross-genetic difference was evident between populations R3 (A) and R7 (B) ($p = 0.018$). Overall, the groups fed *Arthrospira* showed the lowest expression of VHSV-specific N protein.

3.2.2. Morphology of the hindgut

The slower-growing populations R3 ($p = 0.05$) and R7 ($p = 0.049$) had a significantly smaller intestinal tubes than the faster-growing, site-adapted population R8 (Table 2). The cross-section of the hindgut was significantly increased in population R8 compared to R3 within the control diet ($p = 0.028$). A correlation was found between body size and the intestinal lumen diameter. Additionally, the intestinal tube in population R7 was significantly smaller in diameter than in population R3 and R8 within the *Arthrospira*-based diet ($p = 0.035$ and $p = 0.005$). In population R7 (pooled), the intestinal cross-section was significantly smaller after feeding *Arthrospira*-containing diet compared to the control and *Hermetia*-based diets ($p = 0.002$ and $p = 0.024$). Overall, the *tunica muscularis* of the hindgut of the investigated rainbow trout was thicker in the group fed *Arthrospira* compared to those receiving the fishmeal-based or *Hermetia*-based diets. The thickness of the *stratum granulosum* in the rainbow trout hindgut remained predominantly constant at 10–20 μm during the infection experiment. No significant alterations were detected when comparing the different populations and feeding groups.

Prior to infecting the rainbow trout populations with VHSV, the *stratum compactum* was significantly thinner in the hindgut of trout from the low-performing, site-adapted population R3 than in the hindgut of better-growing trout from the site-adapted population R8 ($p = 0.016$). During the infection experiment, the *stratum compactum* increased in thickness from day 0 to day 4, particularly in population R3 ($p < 0.001$).

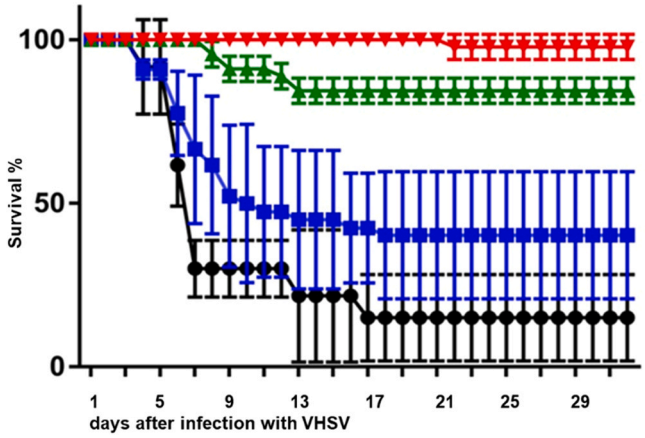

Fig. 2. Survival rate [%] of trout from different genetic populations infected with viral haemorrhagic septicaemia virus (VHSV). Mean values and standard deviations and duration of infection in days (d) are shown; red = regional population R7; green = regional population R8; blue = regional population R3; black = commercial line C9.

3.2.3. Reactions of the Lamina epithelialis mucosae

Vacuole formation was present at low to moderate levels in the hindgut, with no significant abnormalities, and remained unaffected during the infection experiment (Table 3).

The total number of goblet cells decreased during the infection experiment from day 0 pre-infection to day 2 ($p = 0.05$) and day 4 ($p = 0.044$) post-infection. An increase in goblet cell numbers was only observed on day 4 in population R3 fed the *Hermetia*-based diet (Table 3). Throughout the experiment, trout from the population R7 exhibited notably greater goblet cell filling compared to population R3 ($p = 0.004$) and population R8 ($p = 0.001$) (Table 3). With respect to the colour of the goblet cells, there were no significant alterations indicating that indicated a change in the mucin composition during the infection experiment (Table 3).

3.2.4. Inflammatory reactions in the lamina propria mucosae

During the infection experiment, increasing infiltration of inflammatory cells in the *lamina propria mucosae* was observed from day 0 to day 2 and from day 2 to day 4 (Table 2, pooled). This was particularly evident in populations R3 ($p = 0.012$; $p = 0.046$) and R8 ($p = 0.049$; $p = 0.028$) (Table 2).

The gene expression of *il1β* increased significantly on day 2 of the infection experiment, decreasing almost immediately at day 4 (Fig. 3, Table S2). Overall, the expression of *il1β* was higher in the hindgut of R8 trout than in R3 ($p = 0.001$) and R7 trout ($p = 0.001$), particularly at day 0 pre-infection and day 4 post-infection (Table S2). The expression of the gene encoding *tnfα* increased during the infection experiment from day 0 and day 2 to day 4, which was particularly evident in the intestines of trout from population R8 (Fig. 14). In trout from population R7, expression remained almost unchanged throughout the infection compared to populations R3 ($p = 0.034$) and R8 ($p = 0.005$; Table S2).

3.2.5. Immune responses - antiviral response

During the course of the infection, a significant increase in transcription of the *viperin*-encoding gene was evident from day 0 (pre-infection) to days 2 and 4 post-infection. Population R3, which exhibited the highest mortality rate among the site-adapted rainbow trout during the infection experiment, demonstrated a comparatively high *viperin* gene expression (Fig. 3).

Furthermore, a significant increase on day 2 post-infection was evident in the group of population R3 fed with *Hermetia*-based proteins compared to the control group ($p = 0.05$) and *Spirulina*-based diet ($p < 0.001$) (Table S2).

3.2.6. Immune responses - cellular response

During VHS infection, significantly lower levels of *cd8α* expression were observed on day 2 post-infection than on day 0 pre-infection ($p = 0.008$) and on day 4 post-infection ($p = 0.029$) (Fig. 4, Table S2).

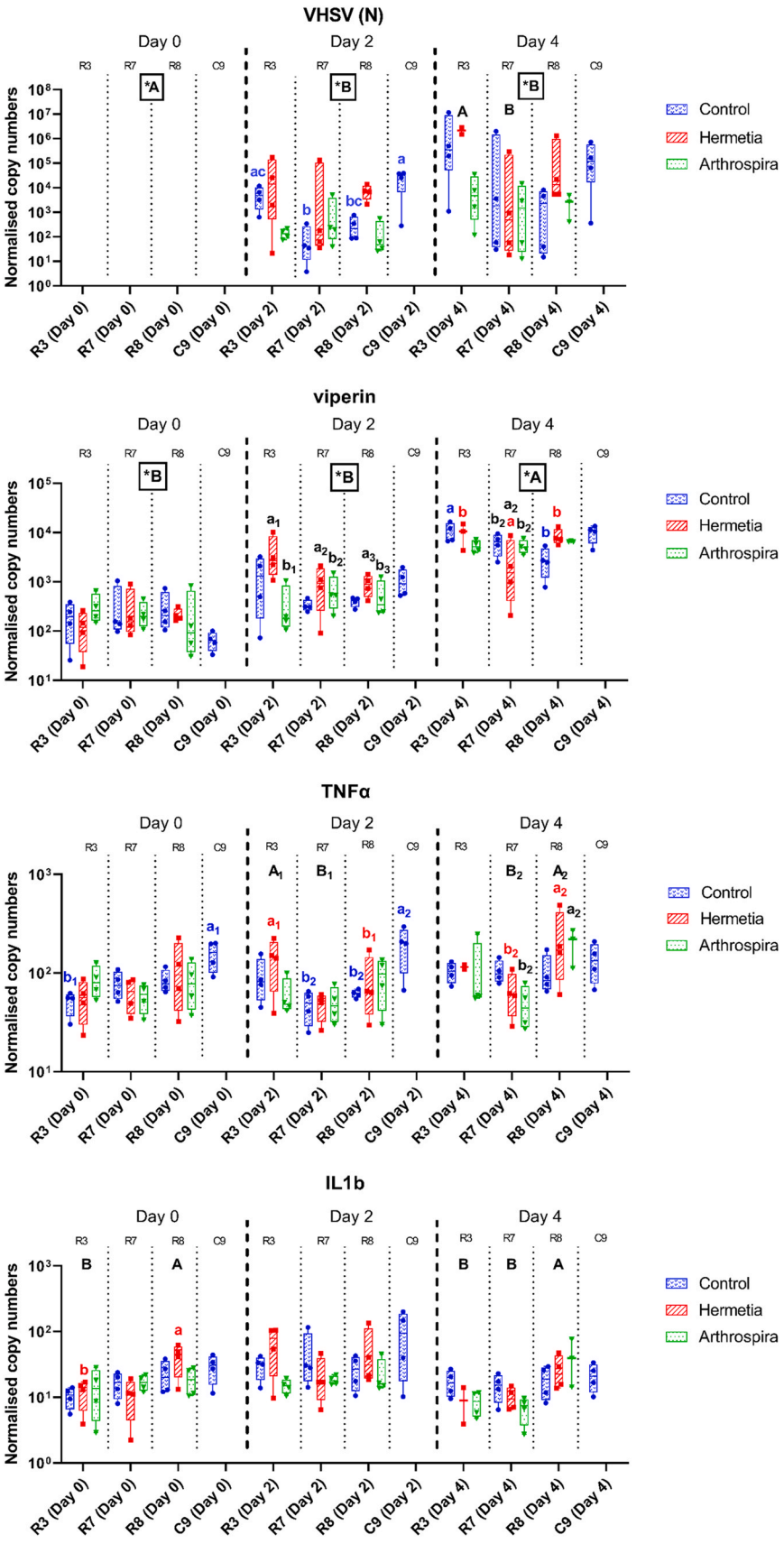

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Fig. 3. Gene expression of N protein of viral haemorrhagic septicaemia virus (VHSV), *viperin*, *tnfα* and *il1β* in the hindgut from different trout populations fed different diets under infection with VHSV; R3 = regional population R3; R7 = regional population R7; R8 = regional population R8; C9 = commercial line C9 at day 0 (pre-infection), day 2 (post-infection) and day 4 (post-infection). Asterisks: significant values between days of infection; capital letters: significant values within a day of infection; coloured letters: significant values within a diet (blue = control diet, fish meal diet; red = *Hermetia* diet; green = *Arthrospira* diet); letters marked in black: significant values between diet groups.

Table 2
Morphology of the hindgut.

	Population	Diet	Day 0	Day 2	Day 4
Cross-section [µm]	**R3** *R8D0, R8D2, R8D4	**Control**	4472.5*C8	4711.88*C8	4738.13
		Hermetia	4699.38	4777.50	4491.25
		Arthrospira	4742.50*A7	5261.25*A7	5057.50
	R7 *R8D0, R8D2, R8D4	**Control**	4605.00	6186.25*A7	5445.00*A7
		Hermetia	5140.00	4777.50	5069.38
		Arthrospira	4400.63*A3, A8	4191.25*A3, A8, C7	3607.50*C7, H7
	R8 *R3D0, R3D2, R3D4 *R7D0, R7D2, R7D4	**Control**	5316.88*C3	6209.38*C3	5288.13
		Hermetia	5006.88	5244.38	4931.67
		Arthrospira	5905.63*A7	4730.00*A7	5157.50
	C9	**Control**	5881.88	5881.88	5853.88
T. muscularis [µm]	**R3**	**Control**	58.33*A3	72.97*A3	74.10*A3
		Hermetia	60.48*A3	98.67*A3	59.24*A3
		Arthrospira	77.78*C3, *H3	86.09*C3, *H3	106.67*C3, *H3
	R7	**Control**	50.76*A7	55.31*A7	47.10*A7
		Hermetia	72.24*A7	66.95*A7	5719*A7
		Arthrospira	74.56*C7, *H7	123.01*C7, *H7	99.58*C7, *H7
	R8	**Control**	67.96*A8	37.20*A8	61.44*A8
		Hermetia	68.92*A8	64.64*A8	88.28*A8
		Arthrospira	100.34*C8, *H8	118.50*C8, *H8	116.04*C8, *H8
	C9	**Control**	55.85	70.69	68.02
Str. granulosm [µm] *D0<D4	**R3***R8D0	**Control**	11.62	15.67	16.54
		Hermetia	11.64	18.94	3.18
		Arthrospira	13.38	11.86	18.12
	R7	**Control**	15.32	10.47	10.93
		Hermetia	12.26	10.89	11.35
		Arthrospira	11.07	14.50	13.22
	R8*R3D0	**Control**	13.33	8.46	12.48
		Hermetia	15.59	11.83	17.29
		Arthrospira	13.21	14.97	15.17
	C9	**Control**	13.57	14.16	16.19
Str. compactum [µm] *D0<D4	**R3***R8D0	**Control**	4.27	5.91	7.06
		Hermetia	5.10	7.19	716
		Arthrospira	5.73	5.79	8.08
	R7	**Control**	5.57	6.05	5.76
		Hermetia	5.57	5.64	5.69
		Arthrospira	5.38	6.71	681
	R8*R3D0	**Control**	6.41	5.38	6.40
		Hermetia	5.69	5.56	5.85
		Arthrospira	6.58	6.15	773
	C9	**Control**	5.91	6.46	7.27

Table 2. Morphology of the hindgut. Significant differences to the corresponding genetic or feeding group are marked with a superscript designation of population (R3 = regional population R3, R7 = regional population R7, R8 = regional population R8, C9 = commercial line C9), timepoint of infection (D0 = Day 0, D2 = Day 2, D4 = Day 4) or feeding designation (H = *Hermetia*, *A* = *Arthrospira*, C = Control).

3.2.7. Epithelial barrier

Overall, *cathelicidin 2* expression increased during the course of infection, from day 0 to day 2 and from day 2 to day 4, in the hindgut of trout of all genetic backgrounds and feeding groups (Fig. 6). Additionally, a significant increase was observed in population R8 after feeding on a *Hermetia* protein-based diet from day 0 to day 4 ($p < 0.001$) and from day 2 to day 4 ($p < 0.001$). A significant increase in *cathelicidin 2* expression was also evident in trout from population R8 fed Spirulina proteins from day 0 to day 2 ($p = 0.035$) and from day 2 to day 4 ($p < 0.001$). Overall, trout from population R7 showed the lowest *cathelicidin 2* expression. Consequently, a significant increase in *cathelicidin 2* expression in trout from population R7 was only evident from day 0 to day 4 ($p = 0.002$). The *cathelicidin 2*-encoding gene was particularly highly expressed in trout from population R3 after they were fed the *Hermetia*-based diet, compared to the control feeding groups ($p = 0.011$) and the *Spirulina*-based diet ($p = 0.046$).

In the intestines of trout from population R3, feeding on the *Spirulina*-based diet resulted in a significant increase in *cathelicidin* expression at day 4 compared to day 0 ($p < 0.001$) and day 2 ($p < 0.001$). Overall, the expression of *defβ3* was lowest at day 2 compared to days 0 ($p < 0.001$) and 4 ($p = 0.01$). In trout from population R7, the expression of *defβ3* was lowest during the course of infection, being significantly lower than in trout from population R8 ($p = 0.006$).

The expression of *defβ4* decreased significantly overall during the infection process, particularly evident on day 4 post-infection with VHSV compared to day 0 ($p = 0.003$).

Feeding groups fed *Hermetia*-based diets were particularly affected, with a significant decrease in *defβ4* expression observed at day 2 post-infection ($p = 0.005$) (Fig. 5).

During the course of the infection, the highest expression of *defβ4* was observed in trout from population R8 compared to populations R3 ($p = 0.003$) and R7 ($p = 0.004$).

In addition, a significant increase in line C9 compared to the other regional genetics was recognisable on day 2 post-infection ($p < 0.001$), as well as an increase in *defβ4* expression between populations R8 and

Table 3
Histological examination of the hindgut.

	Population	Diet	Day 0	Day 2	Day 4
Vacuolation [Score: 0–3]	**R3**	**Control**	0.75	1.00	1.25
		Hermetia	1.25	1.75	1.00
		Arthrospira	0.75	1.33	1.50
	R7	**Control**	0.75	1.00	1.00
		Hermetia	1.50	1.25	1.25
		Arthrospira	1.50	1.25	0.75
	R8	**Control**	1.00	0.75	0.50
		Hermetia	1.25	0.75	1.00
		Arthrospira	1.50	1.00	0.75
	C9	**Control**	1.00	1.00	1.25
Cell infiltration [Score: 0–3] *D0<D2, D2<D4	**R3**^{*R7D4}	**Control**	0	1	1.5
		Hermetia	0	1.5	0.5
		Arthrospira	0.25	0	1.5
	R7$^{*R3D4, R8D4}$	**Control**	0.5	0.5	0.5
		Hermetia	0.75	0.25	0.5
		Arthrospira	0.25	0.25	0.5
	R8^{*R7D4}	**Control**	0.5	1	1.5
		Hermetia	0	0.75	1.25
		Arthrospira	0	0.75	1.25
	C9	**Control**	0.75	0.75	1.5
Number of goblet cell [mean of 10 × 100 µm] *D0>D2, D0>D4	**R3**	**Control**	9.55	8.95	8.28
		Hermetia	7.93^{*H3D4}	8.23	10.95
		Arthrospira	10.30	9.70	8.50
	R7	**Control**	10.28	9.55	8.18
		Hermetia	8.48	7.08	7.43
		Arthrospira	10.03	7.68	7.90
	R8	**Control**	8.85	8.00	8.03
		Hermetia	9.55	8.05	8.58
		Arthrospira	8.93	8.43	8.38
	C9	**Control**	8.73	7.93	8.55
Filling of goblet cells [Score: 0–3]	**R3**^{*R7}	**Control**	1.75	2	1.25
		Hermetia	1.5	1.5	1.5
		Arthrospira	1.75	2.33	1.75
	R7$^{*R3, R8}$	**Control**	1.75	2	2
		Hermetia	2.75	2.5	2.25
		Arthrospira	2	2	2.5
	R8^{*R7}	**Control**	1.25	2	1.5
		Hermetia	1.5	1.75	1.33
		Arthrospira	1.75	2	1.75
	C9	**Control**	2.25	2.25	1.75

Table 3. Histological examination of the hindgut. Significant differences to the corresponding genetic or feeding group are marked with a superscript designation of population (R3 = regional population R3, R7 = regional population R7, R8 = regional population R8, C9 = commercial line C9), timepoint of infection (D0 = Day 0, D2 = Day 2, D4 = Day 4) or feeding designation (H = *Hermetia*, *A* = *Arthrospira*, C = Control).

R3 ($p = 0.034$).

Overall, a significant increase in *hepcidin* expression was observed from day 0 to day 2 ($p < 0.001$) and from day 2 to day 4 ($p < 0.001$). However, only in the R3 population did *hepcidin* expression increase significantly from day 2 to day 4 ($p < 0.001$), but not from day 0 to day 2.

In all populations, *hepcidin* expression was higher in trout fed a *Hermetia*-based diet compared to those fed a *Spirulina*-based diet ($p = 0.005$). Within population R3, *hepcidin* expression was particularly high in trout receiving a *Hermetia*-based diet, with a significantly increase compared to the control diet ($p = 0.049$) and the *Arthrospira*-based diet ($p < 0.001$, Fig. 5). The lowest expression of *hepcidin* compared to population R3 ($p = 0.022$) and R8 ($p < 0.001$) was observed throughout the entire course of infection in population R7. In population R8, increased *hepcidin* expression was particularly evident in trout fed diets containing *Hermetia* and *Arthrospira* between day 0 and day 2 to day 4 (*Arthrospira* day 0: $p < 0.001$, day 2: $p < 0.001$; *Hermetia day 0:* $p < 0.001$, day 2: $p = 0.007$).

A significant increase in *lysozyme C* expression was observed during the course of infection, from day 0 to day 2 ($p = 0.005$) and on day 4 ($p = 0.004$; Fig. 5). Overall, the expression of *lysozyme C* increased in trout fed the *Hermetia*-based diet compared to the *Arthrospira*-based diet ($p = 0.016$). Population R8 exhibited the highest *lysozyme C* expression at all time points ($p < 0.001$). However, the transcription of this gene was not altered during the course of the VHSV infection. During the course of the infection, reduced expression of *occludin* was observed on day 2 compared to day 0 ($p = 0.011$) and day 4 ($p < 0.001$). This was particularly evident in population R7 ($p = 0.01$ day 0 to day 2; $p = 0.019$ day 2 to day 4), except for the groups fed a *Hermetia*-based diet, where the gene was significantly less expressed on day 4 compared to the previous days (Fig. 6). Population R3 was the only population that showed a significant increase in *occludin* expression from day 0 and day 2 to day 4 ($p < 0.001$, $p < 0.001$).

Prior to infection, *occludin* expression was significantly lower in the hindgut of trout from population R3 than in those from population R8 ($p = 0.014$). However, on day 4 post-infection, *occludin* expression was significantly higher in R3 trout than in R7 ($p = 0.009$) and R8 trout ($p = 0.017$, Fig. 6).

Clear upregulation of antiviral and cellular immune responses (Viperin, CD8α) was evident by day 4 post-infection. Regarding the inflammatory response, *tnfα* expression increased at day 4 post-infection, while *il1β* expression decreased from day 2 to day 4 post-infection. Furthermore, all analysed genes exhibited increased expression with respect to the epithelial barrier on day 4 post-infection (Fig. 7). Regarding the different regional trout populations (R3, R7 and R8), a clear upregulation of the expression of many genes was recognisable in

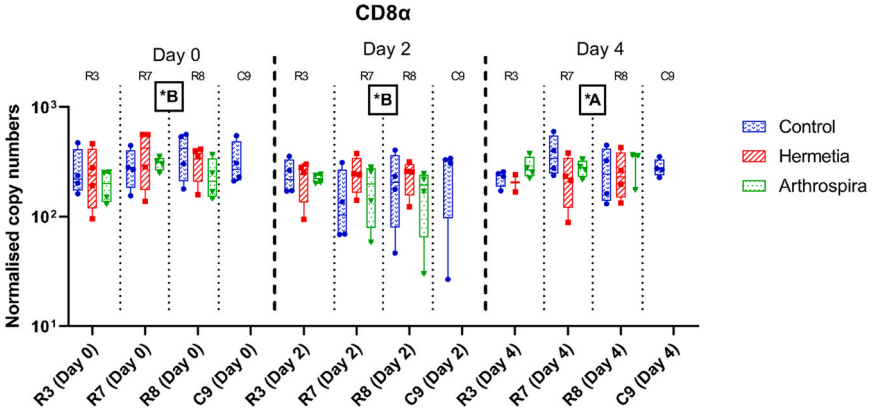

Fig. 4. Gene expression of *cd8α* in the hindgut from different trout populations fed different diets under infection with viral haemorrhagic septicaemia virus (VHSV); R3 = regional population R3; R7 = regional population R7; R8 = regional population R8; C9 = commercial line C9 at day 0 (pre-infection), day 2 (post-infection) and day 4 (pre-infection); asterisks: significant values between days of infection.

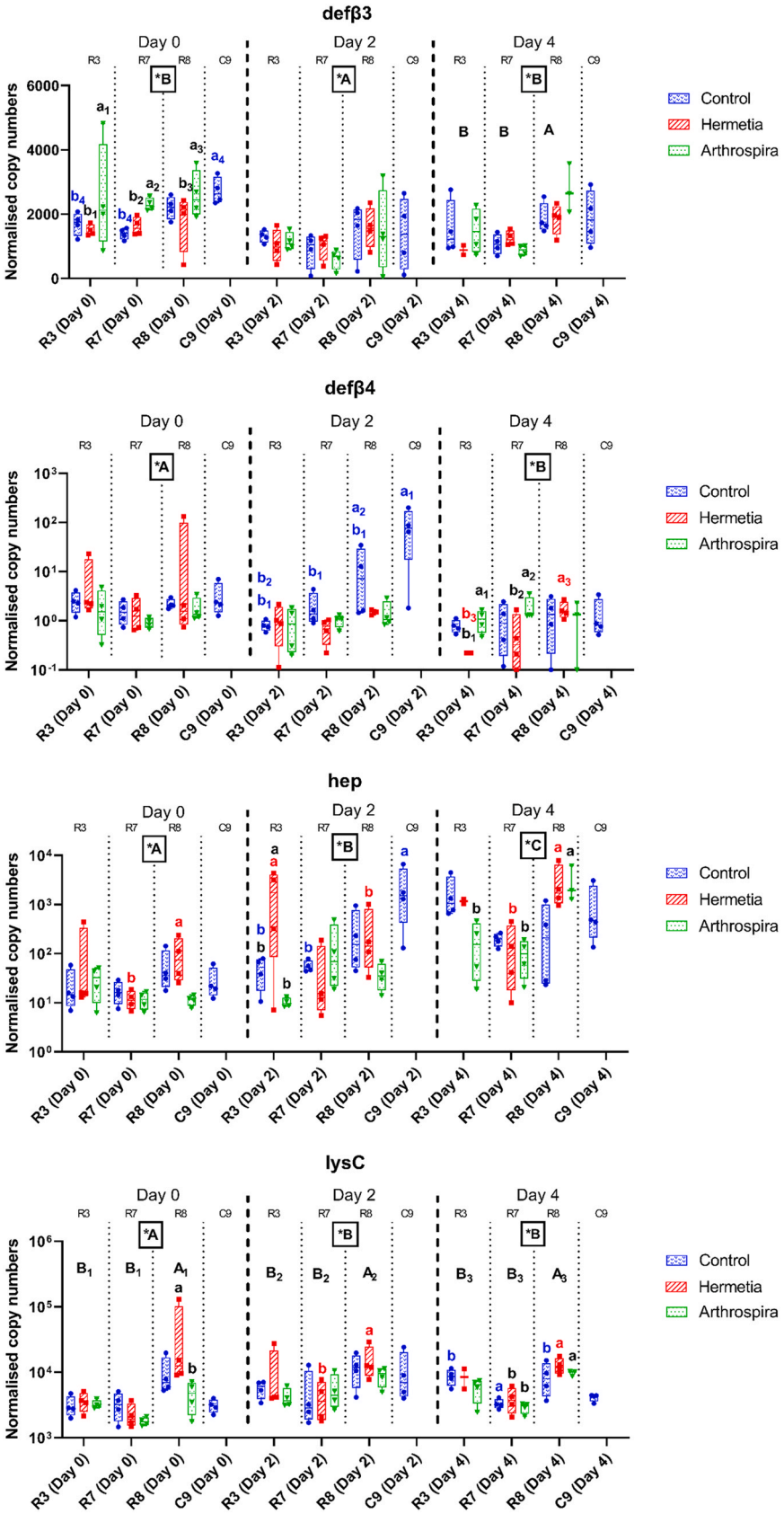

Fig. 5. Gene expression of *defensin β3, defensin β4, lysozyme C* and *hepcidin* in the hindgut from different trout populations fed with different diets under viral haemorrhagic septicaemia virus (VHSV) infection; R3 = regional population R3; R7 = regional population R7; R8 = regional population R8; C9 = commercial line C9 at day 0 (pre-infection), day 2 (post-infection) and day 4 (post-infection). Asterisks: significant values between days of infection; capital letters: significant values between populations within a day of infection; coloured letters: significant values within a diet (blue = control diet, red = *Hermetia*, green = *Arthrospira*); letters marked in black: significant values between diet groups.

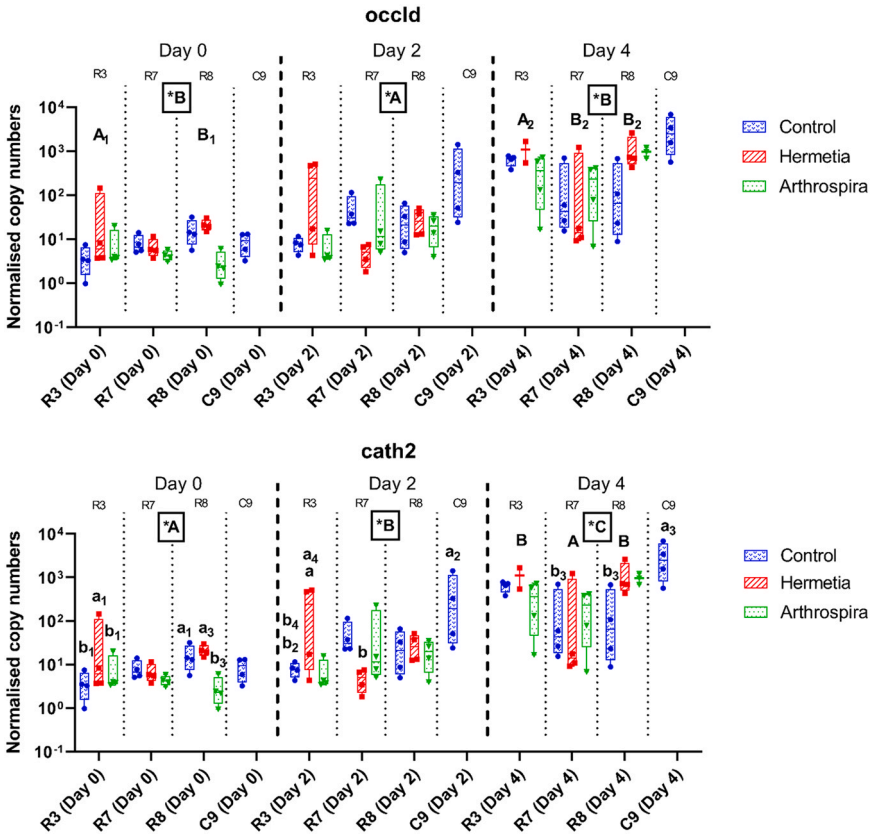

Fig. 6. Gene expression of *occludin* in the hindgut from different trout populations fed different diets under viral haemorrhagic septicaemia virus (VHSV) infection; R3 = site-adapted population R3; R7 = site-adapted population R7; R8 = site-adapted population R8; C9 = commercial line C9 at day 0 (pre-infection), day 2 (post-infection) and day 4 (post-infection). Asterisks: significant values between days of infection; capital letters: significant values between populations within a day of infection; coloured letters: significant values within a diet (blue = control diet, red = *Hermetia*, green = *Arthrospira*); letters marked in black: significant values between diet groups.

trout from population R8 under VHSV infection.

By contrast, these genes were barely expressed in VHSV-infected trout from population R7 (Fig. 8). In terms of the different feeding groups, the expression of all genes, except *defensins*, was higher in trout from all genetic groups that were fed the *Hermetia*-based diet, particularly in comparison with the *Spirulina*-based feeding groups (Fig. 9).

4. Discussion

As highlighted in the introduction, replacing fishmeal partially or completely in the diets of carnivorous fish such as rainbow trout is essential for the sustainable development of aquaculture (Luthada-Raswiswi et al., 2021). This study was therefore designed to evaluate the genetic potential of rainbow trout populations to utilise the novel protein sources from *Arthrospira platensis* and *Hermetia illucens* in their diet, and to examine how this adaptation influences disease susceptibility. Following examinations of intestinal health and *in vitro* infection experiments of primary cell cultures derived from fish that were raised and fed under the same initial conditions (Bauer et al., 2023; Miebach et al., 2023), the present infection experiment was designed as a follow-up study. Although convincing results from a previous *in vitro* study addressing the same issue are already available (Bauer et al., 2023), these have not yet been verified in living animals. However, complex interactions between pathogens and their hosts can best be studied using *in vivo* infection experiments.

4.1. Genetic background and correlation with mortality rate, gene expression and growth performance

In the current study, genetic variability played a key role in the immunocompetence of rainbow trout infected with the viral haemorrhagic septicaemia virus. This is evident when examining the survival rate of infected rainbow trout. The highest survival rate was observed in the regional population R7, followed by R8, R3 and C9. A mortality rate of only 2.2 % and 15.8 % was evident in animals from populations R7 and R8, with a virus load of approximately 1×10^4 (R7) to 1×10^3 (R8) genome copies, while 59 % of trout from population R3 and 85 % of trout from the commercial line C9 died during the infection with a virus load of approximately 1×10^6 (R3) to 1×10^5 (C9) normalised copies. The commercial line C9 showed the lowest survival rate. These results support the hypothesis that selectively bred trout populations have greater disease resistance (Buchmann, 2022). Previous studies have reported that innate mechanisms form the basis for VHSV resistance in rainbow trout (Verrier et al., 2013, 2018). According to (Verrier et al., 2012), several genes can be responsible for a host's susceptibility to viral infections. However, in rainbow trout, one main gene or locus is responsible for many phenotypes displaying different levels of susceptibilities. The most important quantitative trait locus encoding for disease resistance in rainbow trout to VHSV is located on chromosome 3 (Verrier et al., 2013). Nevertheless, the role of innate and adaptive immune mechanisms in fish for resistance to viruses has yet to be sufficiently researched (Verrier et al., 2013).

For example, hereditary resistance to *Flavobacterium columnaris* is already known in rainbow trout (Evenhuis et al., 2015). Additionally, resistance to enteric red mouth disease and viral haemorrhagic

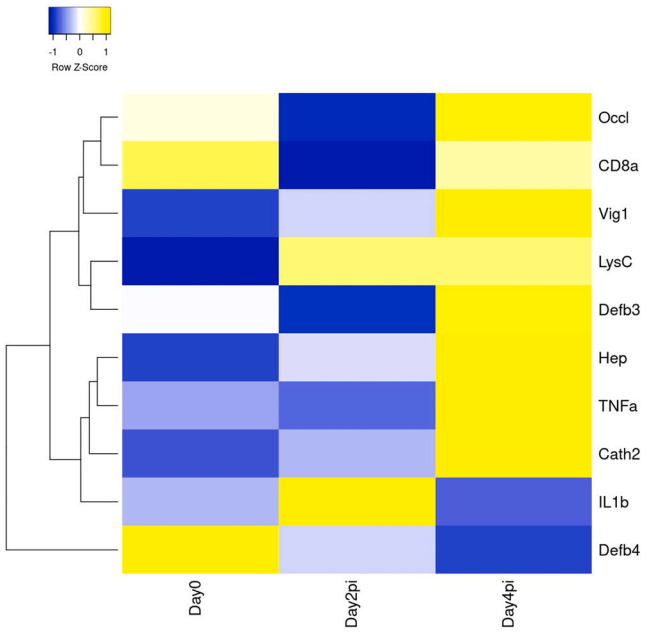

Fig. 7. Colour-coded representation of gene expression in the intestines of trout from all genetical populations (R3 = regional population R3, R7 = regional population R7, R8 = regional population R8, C9 = commercial line C9) during the course of infection with viral haemorrhagic septicaemia virus (VHSV). Day 0: pre-infection, day 2 p.i.: 2 days post-infection, day 4 p.i.: 4 days post-infection; blue: low expression, white: moderate expression, yellow: high expression of mean value.

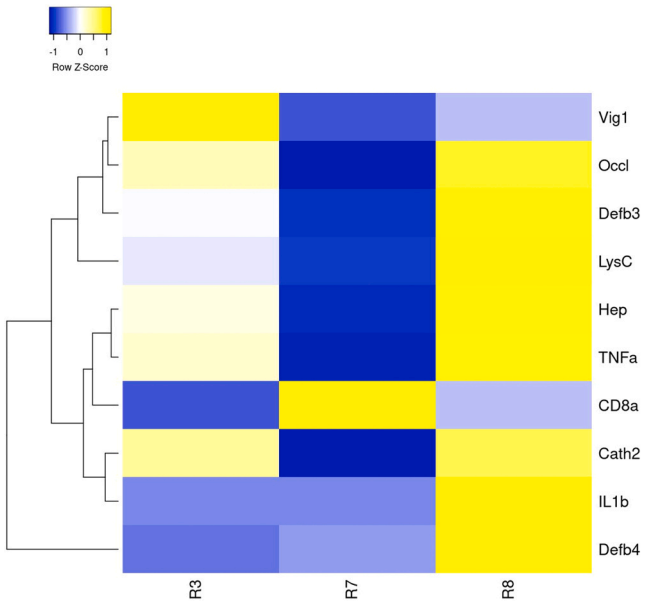

Fig. 8. Colour-coded representation of gene expression in the intestines of trout from all genetical populations (R3 = regional population R3, R7 = regional population R7, R8 = regional population R8, C9 = commercial line C9) during the course of infection with viral haemorrhagic septicaemia virus (VHSV); comparison of trout with the different genetic backgrounds: R3, R7 and R8; blue: low expression, white: moderate expression, yellow: high expression of mean value.

septicaemia has been reported in previous studies of rainbow trout strains (Henryon et al., 2005). Another previous study determined a correlation between the susceptibility of different rainbow trout genotypes to VHSV based on *in vitro* investigations of virus replication in

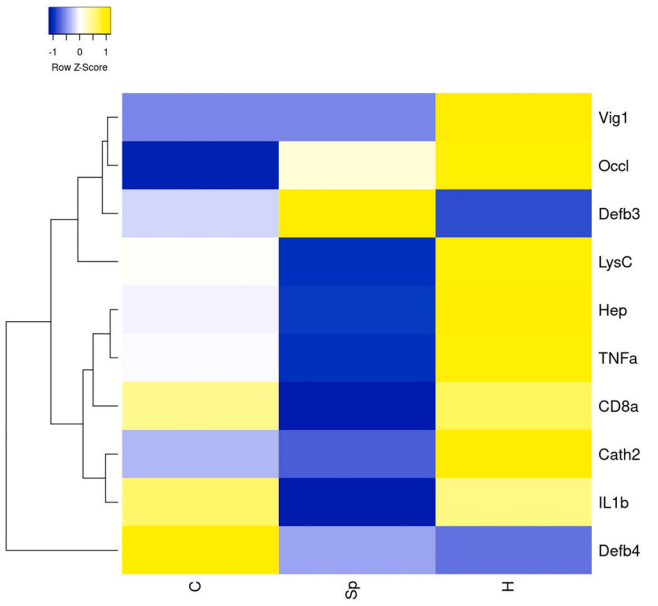

Fig. 9. Colour-coded representation of gene expression in the intestines of trout from all genetical populations (R3 = regional population R3, R7 = regional population R7, R8 = regional population R8, C9 = commercial line C9) during the course of infection with viral haemorrhagic septicaemia virus (VHSV). C: control diet, fish meal diet; Sp: *Spirulina* diet; H: *Hermetia* diet; blue: low expression, white: moderate expression, yellow: high expression of mean value.

excised fin tissue (Dorson et al., 1995). Furthermore, resistant rainbow trout genotypes harboured significantly lower viral loads in fin tissue and internal organs (Dorson et al., 1994; Quillet et al., 2007), although the influence of fishmeal substitutes on disease resistance was not considered. However, this study confirms the finding of a lower viral load in the internal organs, particularly in the intestine, in more resistant genotypes. Several studies have shown that VHSV resistance in rainbow trout is based on hereditary mechanisms (Verrier et al., 2013, 2018).

The differences in disease resistance between the genotypes under investigation are particularly evident in their reaction to VHSV. In bony fish, viperin, a virus-induced antiviral protein, was first identified as a virus-induced gene (vig1) in rainbow trout and is highly expressed in VHSV-infected leukocytes (Boudinot et al., 2000). *Viperin* is homologue to mammalian *viperin* and is regulated by complex interactions within the immune system (Wang et al., 2014). Rainbow trout viperin is induced by a rainbow trout IFN-like factor, as well as directly by VHSV without requiring ongoing protein synthesis (Boudinot et al., 1999). This indicates the presence of both IFN-dependent and IFN-independent pathways. Previous *in vivo* challenge experiments in zebrafish indicate that viperin is important for fish survival during VHSV infection. Whereas fish with lower *viperin* expression showed significantly lower survival rates and higher VHSV titers (Shanaka et al., 2023). Viperin targets the N- and P proteins of VHSV for autophagic degradation, thereby exerting its anti-VHSV function (Lu et al., 2024).

During the course of infection, a continuous increase in the expression of the antiviral protein viperin was observed. In particular, fish from population R3, which was highly susceptible to VHSV, showed a comparatively high gene expression of *viperin*. In contrast, the resistant population R8 remained at a low and R7 at an even lower expression level of *viperin*. The gene expression of the inflammatory protein interleukin 1β increased significantly at day 2 of the infection experiment and decreased almost immediately at day 4. Overall, the expression of *il1β* was increased in the hindgut of trout from the resistant population R8 compared to trout from the susceptible population R3 and the less resistant population R7, which was particularly evident at day 0 pre-infection and day 4 post-infection. Thus, a particularly strong reaction to VHSV could be observed in the resistant population R8, which

actively responded to the infection with VHSV independently from an excessive susceptibility.

The expression of the gene encoding *tnfα* increased during the course of the infection from day 0 and day 2 to day 4, which was particularly evident in the intestines of trout from the more resistant population R8. Population R8 showed by far the best immune response to VHSV, which was especially observed at day 4 post-infection. Although population R3 initially expressed particularly high levels of *tnfα* at day 2 of infection, the production was already exhausted at day 4 post-infection.

In addition, with regard to *defβ3, defβ4, il1β, cath2, tnfα, hep1, lysC* and *occludin*, fish from the largely resistant population R8 had the most intense immune response to VHSV overall. In contrast, fish from population R3, a largely susceptible population to VHSV, showed a poor to moderate reaction overall apart from an excessive expression of *viperin*. Overall, it can be concluded that population R8 responded most intensively to the infection with VHSV, this being reflected in the low mortality rate and low replication of VHSV in intestinal tissue. Population R8 was therefore able to fight the infection particularly well. When evaluating the resistant population R7 during the course of the infection, all genes analysed except *cd8α* remained low in expression, albeit accompanied by a low viral load, which indicates an overall low susceptibility to VHSV.

The correlation between disease resistance and growth of fish has been investigated in several studies and it was proven that the susceptibility was not only dependent on the investigated fish species, but also influenced by the specific pathogens (Quillet et al., 2001; Overturf et al., 2003). For example, a previous study found that size and weight of individual trout had no influence on virus replication in fish of the same age (Quillet, Dorson et al., 2001). Furthermore, a previous *in vivo* study on the susceptibility of VHSV of rainbow trout came to the conclusion that the susceptibility to VHSV infection differs significantly between rainbow trout genotypes (Quillet et al., 2001).

Indeed, the present study underlines the results of the survival rate, in which the growth rate of investigated trout populations was independent of the susceptibility to VHSV. This becomes obvious when looking at the survival rate of investigated populations, whereby trout from line C9 and population R8 with the highest ability to utilise the alternative diets showed a significant difference in VHS susceptibility. For instance, line C9 and the slowest growing population R3 exhibited the lowest survival rates (15–41 %) in the VHSV infection study. In contrast, the fast growing population R8 and the slower growing population R7 displayed significantly higher survival rates (84.2–97.8 %) when challenged with VHSV. The susceptibility to VHSV was therefore independent of the respective performance in feed utilisation of the genotypes analysed.

A previous related study investigating the intestinal health and animal welfare of trout populations fed *Arthrospira platensis*- or Hermetia illucens-based diets revealed, that differences in growth depended only on the genetic background. In that study, differences in growth dependent on the genotype of the respective population, rather than the different diets (Miebach et al., 2023). Our research data show that the ability of rainbow trout populations to utilise the fish meal substitutes *Arthrospira platensis* or *Hermetia illucens* in the diet is independent of their susceptibility to VHSV. Furthermore, the breeding potential of the VHSV-resistant rainbow trout population R8, which exhibited high growth rates across all tested diets, becomes evident. The breeding adaptation of site-adapted rainbow trout genotypes to the novel protein sources can therefore be assessed as being very successful in this study and offers great potential with regard to adaptation to novel feeds and resistance to site-relevant diseases. However, this requires further research, as it harbours great potential not only for animal welfare but also from an economic point of view.

There was no apparent influence of the diet on the survival rate. Nevertheless, our results showed a lower viral load in *Arthrospira-fed* and a higher viral load in *Hermetia*-fed groups as well as higher expression of immune-relevant genes in *Hermetia*-fed groups, especially compared to fish fed with *Arthrospira*. This could indicate that the higher expression of immune-related genes is due to the higher viral load and is not protective. *Arthrospira* does have an immunomodulatory effect and the investigated fish might therefore have had an advantage concerning the viral load.

4.2. Influence of diet composition

4.2.1. Arthrospira platensis

Previous studies showed a significant growth performance in different fish species under the influence of an *Arthospira*-based diet (Abdel-Tawwab and Ahmad, 2009; Adel, 2016) due to an elevated digestive enzyme activity, intestinal microbiota and possible increase in appetite (Adel et al., 2016). Additionally, an improved disease resistance to certain pathogens in species like Siberian sturgeon (*Acipenser baeri)* (Palmegiano et al., 2005), common carp (*Cyprinus carpio*) (Watanuki et al., 2006), tilapia (*Oreochromis niloticus)* (Ragap et al., 2012), African sharptooth catfish (*Clarias gariepinus*) (Promya and Chitmanat, 2011), hybrid red tilapia (*Oreochromis niloticus x Oreochromis mossambicus)* (El-Sheekh et al., 2014), nibbler (Girella tricuspidata) (Nakazoe et al., 1986), rohu (*Labeo rohita*) (Nandeesha et al., 2001) and great sturgeon (*Huso huso*) (Adel et al., 2016) after dietary supplementation of *Arthrospira platensis* has been reported. For instance, a 10 % supplementation of *Arthrospira platensis* significantly reduced the mortality rate of great sturgeon infected with *Streptococcus iniae* (Adel et al., 2016). Its immunomodulatory and anti-inflammatory properties in humans and mammals have already been widely reported in previous studies (Wu et al., 2016) and could explain the observed results of this study. The beneficial effects of *Arhrospira platensis* are consistent with previous studies in which the inclusion of *Arthospira platensis* in the diet obviously increased the immune defence of common carp to bacterial pathogens (Watanuki et al., 2006) and enlarged the antioxidant capacity of rainbow trout (Teimouri et al., 2019). For example, a supplementation of 1 % *Arthrospira platensis* enhanced bactericidal, phagocytic and lysozyme activities related with significant up-regulation of pro-inflammatory cytokine (IL1β and TNFα), which resulted in an improved immunity of Nile tilapia to *Pseudomonas fluorescence* (Mahmoud et al., 2018).

Specifically with regard to the composition of the intestinal mucus, one study reports positive effects on the expression of immune genes and the composition of gut microbiota when *Arthrospira platensis* is added in the diet composition of aquafeed in zebrafish (Ma et al., 2022).

Mucus of fish contains various innate immune molecules, including lysozyme and other antimicrobial peptides (Gomez et al., 2013; Sheikhzadeh et al., 2019). As a lytic enzyme, lyzozyme is antibacterially active by lytic action on the bacterial cell wall. Its presence is known in tissues, including skin, gills, liver, intestine, muscle and ovary (Palaksha et al., 2008). In the present study, the gene encoding *lysozyme C* was increased in the hindgut of rainbow trout fed the *Arthrospira*-based diet.

Similar results concerning an increased *lysozyme* expression are reported by studies on supplementation of *Arthrospira platensis* in aquafeed in common carp (*Cyprinus carpio*) (Khalil et al., 2017), Nile tilapia (*Oreochromis niloticus*) (Adel et al., 2016) and great sturgeon (*Huso huso*) (Mahmoud et al., 2018).

In addition, a better antibacterial activity in the intestine of rainbow trout is reported through increased production of lysozyme in the goblet cells through supplementation of up to 5 % in the diet. This study also shows that the immunomodulatory influences of *Arthrospira platensis* strongly depends on the amount of feeding. The best improvement related to immunological changes and disease resistance were noted at a feeding rate of only 2.5 % (Sheikhzadeh et al., 2019). Tendencies of an increased lysozyme production become particularly obvious during the infection experiment when considering the population R8 throughout the entire course of the infection. Whereas an increased production of lysozyme C was already visible in the feeding experiments in all populations, this was neither significant nor continuous (Miebach et al.,

2023). Concerning rainbow trout, there are reports of an immuno-stimulating effect when feeding 7.5–10 % *Arthrospira platensis* (Yeganeh et al., 2015). In this study, a tendential increase in IL1β was observed in the *Arthrospira*-fed animals at day 0 in population R8 and day 2 in populations R3 and R8. However, a feed-specific upregulation of *lysozyme C* or *tnfα* could not be determined.

Although the feed composition did not influence the mortality rate in the infection experiment, the *Arthrospira*-fed groups showed the lowest expression of VHSV-specific N protein overall in comparison with the other feeding groups. These results are consistent with previous *in vitro* infection experiments on fin tissue from animals in a case study (Bauer et al., 2023) in which the *Arthrospira*-feeding groups showed the lowest viral load of VHSV independently of the genetic background (Fig. 9). Furthermore, a decreased expression of some antimicrobial peptides and inflammatory markers, such as *lysozyme C, hepcidin, cathelicidin 2, cd8α, il1β* and *tnfα*, was detected during the course of infection, especially compared to the *Hermetia* feeding groups (Fig. 9). The lower expression of the VHSV-specific N protein indicates a lower viral load. Therefore, it can be assumed that the higher expression of the immune-relevant genes in *Hermetia*-fed fish has no protective effect, but was likely to have been induced by the higher virus load and could indicate an excessive immune reaction. In particular, *occludin, lysozyme C, hepcidin, tnfα, cd8α, cathelicidin 2* and *il1β* were upregulated. When comparing the results of a low expression of VHSV-specific N protein combined with an absence of an excessive immune reaction to VHS infection during the course of infection, a possible improvement in the responsiveness of the immune system can be assumed. For example, *il1β*, *defβ4*, *cathelicidin 2*, *cd8α*, *tnfα*, *hepcidin* and *lysozyme C* were expressed at particularly low levels. Only *defβ3* was upregulated within the *Arthrospira*-fed groups. However, including 20 % *Arthrospira platensis* in the diet was not effective against the highly pathogenic VHSV strain Fi13 in rainbow trout.

To achieve an adequate supplementation of *Arthrospira platensis* in the diet of rainbow trout in particular, the best possible ration quantity may still need to be investigated so that the best possible immunomodulatory qualities of the feed source can be utilised. However, this issue has to be addressed in future research, as this study primarily focused on the complete replacement of fishmeal with alternative protein sources in different genotypes and their corresponding disease resistance under the influence of the respective feeds.

Altogether, some influence of the replacement of fishmeal proteins by *Arthospira platensis* on the susceptibility of rainbow trout from different populations to a VHSV infection could be observed in this experiment.

According to Ragap et al. (2012), the oral administration of hot water extract of *Arthrospira platensis* has been shown to significantly increase the production of interferon-γ in human natural killer cells (NK). However, the mechanism of immunostimulation in fish is still unclear and needs further investigations (Wu et al., 2016). In contrast, in the related *in vitro* study (Bauer et al., 2023), the *Arthospira*-based diet was associated with lower virus load in fin tissue in the susceptible population of rainbow trout (R3), which was not reflected in the infection and mortality rate. Additionally, evidence of a possible improvement in disease resistance is provided, for example, by the *in vitro* infection of gill bioptates with *Yersinia ruckeri* in the same preliminary experiment. A lower colony number could be observed within population R8 and the *Arthrospira*-feeding groups (Bauer et al., 2023). This suggests that immunomodulation of *Arthrospira platensis* may occur, but seems not to be sufficiently strong to offer full protection from a highly virulent pathogen such as VHSV. Therefore, an improvement in an antiviral effect through feeding could not be proven.

Furthermore, the antioxidant ingredients of *Arthrospira platensis* appear to positively impact the nutritional value of the fish as a food product (Schafberg et al., 2020). Therefore, these results indicate that feeding *Arthrospira platensis* may have beneficial health effects not only on the fish itself but also on humans as final consumers. A previous study on the alternative feeding of domestic rainbow trout with *Arthrtospira* reported that consumers even may prefer the significantly more yellowish pigmented fillets when they are informed about more sustainable trout farming (Rosenau et al., 2023).

4.2.2. *Hermetia illucens*

When evaluating the feeding groups of *Hermetia illucens,* an increased expression of VHSV-specific N protein compared to the control feeding and *Arthrospira*-fed groups in the course of infection can be observed. Although the feeding composition did not influence the mortality rate, an increased expression of some antimicrobial peptides and inflammatory markers such as *lysozyme C, hepcidin, cd8α, il1β* and tnfα was detected, especially compared to the *Arthrospira* feeding groups. In addition, the epithelial barrier appears to have been particularly susceptible during the course of infection compared to the control groups and *Arthrospira* groups, as observed in an increased expression of *occludin*. Altogether, the *Hermetia*-based diet did not have a positive influence on disease resistance to VHSV. However, it is obvious concerning the survival rate that the animals were not disadvantaged in terms of immunocompetence by the *Hermetia*-based diet. Based on the expression of antimicrobial peptides and the mortality rate during the course of infection, it can be observed that the *Hermetia* feeding groups reacted excessively to an increased infection with VHSV. For instance, a significant increase of viperin was recognisable in population R3 fed with *Hermetia*-based proteins at day 2 post-infection compared to the control groups and *Arthrospira* feeding groups.

The increased expression of the N protein of VHS indicates a higher viral load, so it can be assumed that the higher expression of the immune-relevant genes in *Hermetia*-fed fish had no protective effect. This was possibly induced by the higher viral load and could indicate an excessive immune reaction. However, these results are contradictory to previous studies which report an improvement in disease resistance to various pathogens in different fish species when fed different insect meal-based diets at a percentage feeding rate of 2.5–5 % of the diet ration (Ido et al., 2015; Cho et al., 2022).

For example, dietary black soldier fly larvae meal (BSFL) is reported to improve disease resistance to *Vibrio alginolyticus* in European seabass (*Dicentrarchus labrax*) (Abdel-Latif et al., 2021). These positive effects were also reported with a diet containing 2.5 % maggot meal in black carp (*Mylopharyngodos piceus*) showing resistance to *A. hydrophila* (Ming et al., 2013) and with a diet containing 5 % housefly pupae (*Musca domestica*) showing resistance to infections with *Edwardsiella tarda* in red sea bream (*Pagrus major*) (Ido et al., 2015). These results are caused by lauric acid, which is an ingredient in insect meal, especially *Hermetia illucens*, and is suspected to improve the immune response and disease resistance in several fish species. A recently published study reports that when juvenile rainbow trout are fed a 5 % diet of *Hermetia illucens*, the lauric acid content increases antibacterial activity against *Aeromonas salmonicida*, thus contributing to improved disease resistance (Cho et al., 2022).

However, in the present study, the highly pathogenic VHSV was used to assess disease resistance in rainbow trout, which may explain the lack of protective effects observed with the inclusion of *Hermetia illucens* in the diet. On the contrary, the susceptibility to VHSV even appears to be increased in *Hermetia*-fed animals in this study. Compared to the feed composition in that previous study, this study used a 15 % higher proportion of the fishmeal substitute *Hermetia illucens*.

In contrast, investigated genes in the control diet were differently expressed. Only *defβ4*, *il1β* and *cd8α* were upregulated, while the other parameters were in the low to normal range (Fig. 9).

Overall, neither in the related study on intestinal health and animal welfare (Miebach et al., 2023) nor in the related *in vitro* study on infection trials (Bauer et al., 2023) or in the current study was a negative effect of the feed substitutes *Arthrospira platensis* and *Hermetia illucens* on fish health and defence against infection evident.

4.3. Histological morphology of the hindgut

When considering the histological changes in the hindgut, a significantly smaller cross-section of the population R3 to R8 is visible, which was caused by the different growth rate of the populations.

In addition, population R7 was significantly smaller in cross-sectional diameter when exposed to *Arthrospira* feeding compared to population R3 and R8 as well as significantly smaller compared to the control and *Hermetia* feeding groups within the same population. This is a non-replicable change that did not occur in the preliminary intestinal health trials in other studied populations (Miebach et al., 2023). Since no other alterations could be found that indicated a negative influence on intestinal health, it may be a possible adaptation to the novel feed, which was particularly well expressed in population R7.

Overall, the *tunica muscularis* in the hindgut of investigated populations was significantly thicker in *Arthrospira* groups compared to conventional fishmeal-based groups or *Hermetia* groups. The enlargement of the *tunica muscularis* in the foregut and hindgut while feeding a diet containing 20 % *Arthrospira platensis* has already been observed in the pre-trials on intestinal health and can be explained by a higher muscular activity of the intestine during digestive processing (Miebach et al., 2023).

During the course of infection, an increase in the thickness of the stratum compactum was observed from day 0 to day 4, which was particularly evident in population R3. This change can most likely be explained by the infection process with VHSV, which led to a high mortality rate, particularly in population R3. Significant size differences in the thickness of the stratum compactum between the populations were only observed between populations R3 and R8. Since the formation of vacuoles in the preliminary test was only significantly observed in the foregut during *Hermetia* feeding and especially *Arthrospira* feeding, the results correspond to the results of the pre-trials. The relevance of vacuole formation in the *lamina epithelialis* of the intestine was discussed in detail therein (Miebach et al., 2023).

4.3.1. Reactions of the Lamina epithelialis mucosae

The number of goblet cells decreased overall during the infection experiment. This may be due to possible damage to the intestinal mucosa during the course of the infection. A possible overactivity of the goblet cells with associated excessive depleting may imitate the impression of a reduced number of goblet cells. However, the filling of goblet cells was not affected during the course of infection. An increase in the goblet cell number at day 4 was only observed in population R3 fed the *Hermetia*- based diet. During the entire infection experiment, population R7 showed a particularly pronounced filling of the goblet cells compared to population R3 and R8, which indicates that mucin production is not excessively increased and is consistent with gene expression and virus content. Nonetheless, there was no influence of feeding at all. With respect to the colour of goblet cells, there were no significant alterations that would indicate a change in the mucin composition during the infection experiment or any feeding-related differences.

In the course of the infection experiment, an increasing inflammatory cell infiltration in the *lamina propria mucosae* was observed from day 0 to day 2 and from day 2 to day 4. In particular, this was evident in population R3, with the worst growth and survival rate, and in population R8, with the best growth and survival rate. Due to the increased susceptibility to VHSV, population R3 also reacted with a particularly strong immune response. Nevertheless, population R8 also responded with increased infiltration of lymphocytes and, as demonstrated by gene expression, was the most reactive of all investigated genotypes to infection with VHSV. Previous studies report an increased number of intraepithelial lymphocytes in the intestine in rainbow trout, one of the immune-stimulating effects when feeding *Arthrospira platensis*. However, these changes are independent of any diet investigated and correlate only with the VHSV infection (Sheikhzadeh et al., 2019).

The pre-trials on intestinal health and animal welfare under the influence of alternative protein sources based on *Arthrospira platensis* and *Hermetia illucens* in site-adapted rainbow trout populations shows that the results were initially contradictory with regard to intestinal health and needed to be differentiated (Miebach et al., 2023). While the use of *Hermetia illucens* as a replacement for the fish meal content in trout feed was consistently positively evaluated, the replacement of fishmeal with *Arthrospira platensis* in the feeding of genetically adapted rainbow trout showed some upregulation of investigated antimicrobial peptides and inflammatory markers. The latter could have indicated a possible reaction of the immune system to the novel feed.

However, based on the results obtained in this study, it can be conclusively ruled out that feeding investigated rainbow trout populations based on *Arthrospira platensis* and *Hermetia illucens* has no detrimental influence. Firstly, no significant differences were observed between the feeding groups prior to VHSV infection (Miebach et al., 2023). Secondly, no disadvantage in the course of infection was evident in the investigated trout populations. The observed significant differences in mortality rates and virus replication between the populations were solely due to the genotypes investigated. Further studies are needed to investigate whether there are significant differences in disease resistance to less aggressive pathogens based on feeding *Arthrospira platensis* as a complete replacement for fishmeal. Nevertheless, the genetic potential with regard to adaptation to the novel protein sources examined in this study can be considered very promising with respect to disease resistance to VHSV.

5. Conclusion

This study aimed at monitoring the influence of fishmeal substitutes on the disease resistance of different rainbow trout populations using an *in vivo* infection experiment. The results showed that the intake of the fishmeal substitutes *Arthrospira platensis* and *Hermetia illucens* had no negative effect on the susceptibility of investigated rainbow trout populations to VHSV. However, the predominant influence on the susceptibility of rainbow trout to VHSV was evident to be based on their genetic variability. Additionally, no correlation was observed between the ability of rainbow trout populations to adapt to a novel diet based on *Arthrospira platensis* or *Hermetia illucens* proteins and pathogen resistance. Therefore, *Arthrospira platensis* and *Hermetia illucens* appear to be valuable sources of protein for replacing fishmeal in rainbow trout diets without negatively affecting the immunity or natural resistance of certain rainbow trout populations to pathogens.

This study highlights the breeding potential of rainbow trout to adapt to novel feed ingredients, such as alternative protein sources, as well as their ability to exhibit site-specific disease resistance towards VHSV.

CRediT authorship contribution statement

Carsten Dietz: Investigation, Conceptualization. **Jakob Gährken:** Investigation, Conceptualization. **Dieter Steinhagen:** Supervision, Project administration, Conceptualization. **Marek Matras:** Investigation. **Jens Tetens:** Project administration, Conceptualization. **Angela Sünder:** Project administration, Conceptualization. **Simon Rosenau:** Resources, Conceptualization. **Stephan Wessels:** Project administration, Conceptualization. **Jung-Schroers Verena:** Conceptualization. **Mikolaj Adamek:** Validation, Supervision, Conceptualization. **Julia Bauer:** Supervision, Project administration, Conceptualization. **Magdalena Stachnik:** Investigation. **Michal Reichert:** Project administration, Conceptualization. **Anne-Carina Miebach:** Writing – original draft, Validation, Resources, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.102996.

Data availability

Data will be made available on request.

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