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Research Article

Tiny Worms, Big Potential: *Enchytraeus albidus* (Annelida: Clitellata) as Starter Feed for Rainbow Trout

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The aquaculture industry is seeking sustainable feed alternatives to enhance the health and growth of farmed fish during their early life stages. Live feed, such as the annelid worm *Enchytraeus albidus*, has been shown to increase growth in marine fish species. This study evaluates *E. albidus* as a potential starter live feed for rainbow trout fry (*Oncorhynchus mykiss*), focusing on growth, health, and fatty acid composition. Fry were fed one of three diets: standard dry feed (DF), live *E. albidus* (EF), or a combination (DF/EF) for 21 days, followed by a transition to DF for the following 22 days. Fry initially fed with EF exhibited higher growth rates compared to those on DF, and they maintained superior size even 3 weeks after being transitioned to DF. At the end of the experiment, the fish fed EF had a 10% higher mass than fish fed DF. No significant differences were found in health indicators across the treatment groups, which all demonstrated good health and low mortality. The fatty acid profiles differed between treatments, with lower essential fatty acid docosahexaenoic acid (DHA) levels in fish fed EF. However, despite the lower body levels, critical DHA levels seemed to be preserved in the neural tissues of the fry. These findings highlight the potential of *E. albidus* as an effective starter feed for rainbow trout fry.

Keywords: aquaculture; *Enchytraeus albidus*; fatty acids; fish health; live feed; rainbow trout; starter feed

1. Introduction

The aquaculture industry is rapidly expanding, and the per capita consumption of aquatic food has approximately doubled since 1990 [1]. Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792), a commercially important salmonid, exemplifies this trend, with annual production approaching one million metric tons globally [1]. Rainbow trout is one of the most widely studied fish species, but despite the extensive research, relatively little has focused on their feed requirements during the critical early life stages, particularly concerning the use of live feed.

Rainbow trout larvae have a fully developed digestive system at first feeding and, unlike most marine species, can consume formulated dry feed (DF) [2, 3]. However, health and welfare in salmonid aquaculture are becoming increasingly important, with growing interest in exploring alternative feed options like live insects and annelids, which could also serve as bio-carriers for oral vaccination or probiotics [4–6]. Enhancing survival and health in the early life stages of salmonids is becoming a focus, especially as losses during these stages can have significant environmental and economic impacts. For instance, in 2019, Norwegian aquaculture reported losses exceeding 60 million salmonids, including rainbow trout, during the initial freshwater phase [7]. Increasing juvenile salmonids' growth and survival will have environmental and economic benefits.

Rainbow trout fry are opportunistic predatory fish that consume various feeds. Their natural diet in the wild typically includes zooplankton, insects, crustaceans, and small fish [8].

This diversity in their natural diet underscores the potential of alternative feed sources. Hence, research in alternative sources of protein and oils has focused on insect meal, algae, vegetable oil, and by-products from agriculture and fishery [9–16]. However, some ingredients like chitin can act as antinutrients in some fish and negatively affect intestinal health despite a good protein profile [17]. Nevertheless, there is only a limited amount of research on using live feed for salmonids, and studies have mainly focused on protein from chitin-rich insects and crustaceans, which mainly showed negative or no effect on growth, but in some cases, positive health effects [18, 4]. Annelid worms do not contain much chitin (only in setae), and a recent study has demonstrated beneficial effects of the earthworm *Eisenia fetida* as an ingredient in starter feed for rainbow trout [19]. Thus, including worm meal in the fish diet (substituting fishmeal) increased both growth and health of fry. Annelid worms have also shown potential as a live feed. Studies using the annelid *Enchytraeus albidus* as live feed for European and winter flounder resulted in faster growth and higher survival compared to commercial feed [20, 21]. The white worm, *E. albidus*, has several advantages as a live feed for cultured fish, since it is easy to culture, can be mass-produced on organic waste materials, and, despite being terrestrial, can survive extended periods in water [22, 23]. The size of *E. albidus* makes it suitable for rainbow trout fry in their initial feeding stage. The macronutrient and amino acid profile of *E. albidus* also seems to meet the needs of rainbow trout [20, 23–25]. However, it is uncertain if the fatty acid profile of *E. albidus* adequately supports optimal growth and health of rainbow trout fry.

The dietary fatty acid requirements for rainbow trout are relatively easily met due to their ability to produce some amount of the essential fatty acids arachidonic acid (20:4*n*−6, ARA), docosahexaenoic acid (22:6*n*–3, DHA), and eicosapentaenoic acid (20:5*n*–3, EPA) from the precursors linoleic acid (18:2*n*–6, LA) and α-linolenic acid (18:3*n*–3, ALA) [26–28]. Therefore, for optimal growth and health, all live stages of rainbow trout need a diet containing the essential fatty acid precursors LA and ALA [29, 30]. However, the early life stages of rainbow trout also need dietary long-chain polyunsaturated fatty acids (PUFA) like DHA and EPA since the bioconversion capacity from the precursors is not expected to be sufficient for optimal growth in these life stages [31–33]. Therefore, the low DHA level and relatively lower levels of ALA in *E. albidus* than in fish meal could be a concern [25]. Conversely, phospholipid fatty acid (PLFA) catabolism tends to conserve fatty acids playing important structural and physiological roles, like DHA and EPA. In neural tissues, like brain and eye tissue, a large proportion of DHA is therefore directed to form cell membranes, and a sufficient dietary input of these fatty acids can be vital to normal function and development [28, 34, 35].

It can often be challenging for fish to change feed; therefore, it could be a concern that shifting from preying on wiggling *E. albidus* to a conventional, motionless, DF diet could negatively affect appetite, growth, or health [36–38]. This challenge should be taken into account when evaluating new feeds.

The present study aims to evaluate the suitability of the annelid worm *E. albidus* as an initial live feed for rainbow trout fry. The objectives were to (1) investigate whether substituting commercial DF with live *E. albidus* during the initial 21 days of feeding influences the growth and health of rainbow trout, (2) evaluate changes in growth and health upon transitioning from *E. albidus* to commercial feed from Day 22 to Day 43, and (3) evaluate whether rainbow trout fry's fatty acid requirements are met when fed *E. albidus*.

2. Materials and Methods

2.1. Rainbow Trout. The rainbow trout (*Oncorhynchus mykiss*) were hatched at Bryrup Dambrug (Musholm, Denmark) at 10°C and then transported to Aarhus University (2 h) in oxygenated water. The yolk sac fry was 17 days posthatch when moved to the experimental tanks, and the yolk sac was nearly gone.

2.2. Experimental Setup. The setup was a recirculation system consisting of a 210 L buffer tank with a filter system (EHEIM professional 4 + 600, pump capacity 1250 L/h) supplying 15 PP-plastic fish tanks (40 × 30 × 27.3 cm). The total water volume was 612 L, with 20%–30 % daily water exchange with oxygenated water. A water pump (boyu FP-2000, 2000 L/h) ensured water circulation with 45–48 L/h flow rates for all fish tanks. An air compressor aerated the water (95% ± 3 % dissolved oxygen saturation) and created upwelling in each fish tank. The water temperature was constant at 10.5 ± 0.4°C, the light:dark cycle was 15:9 h, and the water pH was 6.9 ± 0.2. Fish tanks were cleaned, and water quality was checked daily. O_2 and temperature were measured using a Handy Polaris 2 (Oxyguard International), pH was measured using pH strips (Dosatest, VWR), and NO_3^- and NO_2^- were measured using eSHa Quick Test strips. NO_2 was not detected, and NO_3 was never above 10 mg L^{-1}.

2.3. Feeding Experiment. A total of 900 rainbow trout fry were randomly allocated between 15 fish tanks (i.e., 60 fish per tank). These tanks were assigned to one of three dietary treatment groups: commercial DF, *E. albidus* as live feed (EF), or a combination of both (DF/EF), with five replicate tanks per treatment. In the following, dietary treatments are referred to using the abbreviations EF, DF, and DF/EF. When discussing the test organism, we use the species name *E. albidus*. The experiment consisted of two parts: In part 1 (Day 1–22), the three treatment groups were fed their assigned feed by hand feeding. In part 2 (Day 23–43), all treatment groups were fed DF using auto-feeders (EHEIM AS3581). Feeding occurred three times a day for the first 5 days and increased to four times daily thereafter. Feeding occurred between 9 AM and 7 PM, each taking 20–30 min. Uneaten food was removed daily after the last feeding.

The live feed, *E. albidus*, was mass-produced in a mixture of compost soil and heat-treated seaweed and fed oatmeal [20]. The worms were extracted by heat extraction and rinsed in tap water before use. The length of *E. albidus* ranged between 5 and 25 mm. The commercial DF was granulated Aller Organic EX trout fry feed (Aller Aqua A/S, Christiansfeld, Danmark). For feed declaration, see Table 1.

TABLE 1: Approximate content (% of dry mass) of protein, lipid, ash, wood fiber, and glycogen/starch of commercial dry feed (Aller Organic EX trout fry feed from Aller Aqua A/S, Christiansfeld, Denmark), and *Enchytraeus albidus*.

Feed contents	Dry feed 0.4 mm	Dry feed 0.5–1.0 mm	*Enchytraeus albidus*
Protein (%)	59[a]	56[a]	42.8[b]
Fat (%)	9[a]	11[a]	13.3[b]
Ash (%)	10.2[a]	11.7[a]	2.3[b]
Wood fiber (%)	1.8[a]	2.2[a]	nd
Glycogen (%)	nd	nd	19.4[b]

Abbreviation: nd, not determined.
[a]refers to values from www.aller-aqua.com.
[b]refers to values from [39].

Two different feed sizes were used: a small size (0.4 mm) for the first part (Day 1–22) and a larger size (0.5–1 mm) for the second part (Day 23–43).

2.4. Growth. Growth metrics, body length, and mass were measured on days 0, 8, 15, 22, 27, 29, 31, 36, and 43. For each measurement, 9–11 fish were randomly captured from each tank and weighed as a group (Sartorius laboratory scale model LC 4800 P). Body fresh mass was calculated as the total mass of the sample divided by the number of fish in a sample. The body length was measured by photographing the fish in a tray with a ruler (measured individually with ImageJ) before they were gently returned to their respective tanks. On days 0, 22, and 43, fish from each tank were terminated and saved for later analysis. The terminated fish were first euthanized by exposure to benzocaine (>99%, 330 mg L^{-1}) and then snap-frozen and stored at −80°C.

2.5. Health Indicators. Dead fish were counted and removed daily, and mortality was calculated as a cumulated percentage. Underfeeding or inequitable access to food can result in fin damage, which was used as a welfare indicator [40, 41]. To this end, dorsal fin damages were scored on Day 22 according to the morphological scheme by Noble et al. [41], which divided healed and active fin damages into the following four levels: Level 0: little to no evidence of fin damage, Level 1: minor evidence of fin damage, Level 2: some evidence of fin damage, Level 3: clear evidence of fin damage (Figure S1). Condition factor (CF) was used as a standard measurement of fish's nutritional status based on the mass and length of the fish [42, 43, 41]. CF was calculated using Equation (1).

$$\mathrm{CF} = \mathrm{Mass}(g)/\mathrm{Length}\,(\mathrm{cm})^3 \bullet 100. \quad (1)$$

Hepatosomatic index (HSI) was quantified as the relationship between liver and body size. The normal range of liver to body mass is 1%–2% [44, 45]. Equation (2) was used for calculating HSI.

$$\mathrm{CF} = \mathrm{Liver\ mass}\,(g)/\mathrm{Fish\ mass}\,(g) \bullet 100. \quad (2)$$

2.6. Feeding Response. The behavioral feeding response was monitored daily in each fish tank by observing the activity during the day's first feeding. Scoring was performed by the same observer throughout the experiment to ensure consistency, using a scale from 0 to 2 based on the following criteria:

0: No apparent response to the feed.

1: Low activity; few fish slowly respond and approach or eat the feed.

1.5: Immediate response and activity—several fish approach and eat the feed.

2: All fish are very active and immediately go toward the feed and eat at the surface.

During the first 22 days, fish were fed one of the three dietary treatments (EF, DF, or DF/EF). From Day 23 onward, all groups were transitioned to DF. Feeding response was recorded throughout the full 43-day period to assess whether diet or diet shift influenced feeding behavior.

2.7. Analysis of Fatty Acids. The PLFA composition of fish sampled on Day 22 was analyzed using one fish from each of five replicate tanks per treatment. Each sample included the fatty acid profile of whole fish as well as specific tissues characterized by high content of neural tissue (brain and eye). The fatty acid composition of the two food types was also analyzed. Before analysis, the samples were freeze-dried and weighed. Analysis, identification, and quantification were done as described by Waagner et al. [46]. In brief, crude lipids were extracted using a modified Bligh–Dyer single-phase method with 2:1:1 v/v/v chloroform:methanol:phosphate-buffer. The neutral lipid and PLFAs were separated using prepacked solid-phase silica columns (100 mg; Bond Elute, Agilent Technologies, Santa Clara, CA, USA). Neutral lipids were eluted with chloroform, followed by acetone and phospholipids were subsequently eluted with methanol. Both neutral lipid and phospholipid samples were turned into single-chain fatty acid methyl esters by transmethylation, as described by Dowling et al. [47]. In this study, we only focused on the PLFAs of the fish. The fatty acid methyl esters were analyzed using gas chromatography coupled with mass spectrometry (Shimadzu GCMS-QP2010 Plus with an autosampler).

2.8. Statistical Analysis. Data processing was done in Microsoft Excel version 2302. The statistical analyses were performed using R version 4.2.2, RStudio version 2022.12.0 + 353, and JMP Trial 17.2.0.

A significance level of $p < 0.05$ (adjusted with Bonferroni correction where appropriate) was applied for all statistical tests.

The growth data were analyzed using a Generalized Linear Mixed Effects Model (GLMER) in R with a gamma distribution and log as the link function. Growth was the response variable, with time (days) and treatment group as fixed effects. Random effects from fish tanks (i.e., replicates) (ID) were accounted for using the (1 | ID) specification.

For visualization purposes, separate GLMs (Gamma distribution, log link) were fitted to each treatment group and time period to illustrate growth trends (Figure 1).

The effect of diet on body mass, body length, CF, and HSI was assessed using one-way ANOVA. For variables not meeting the assumptions of normality and homogeneity of variances, such as mortality and fin damage, the nonparametric Kruskal–Wallis test was used. Post hoc analyses were conducted using Tukey's HSD or Dunn's test.

Divergences in the fatty acid compositions among dietary groups were examined through principal component analysis (PCA) using JMP software, and the principal components were further analyzed by one-way ANOVA in R.

The fatty acid datasets that met the assumptions of normality and homogeneity of variances were analyzed using one-way ANOVA. The nonnormally distributed data or data with unequal variances were tested using the nonparametric Kruskal–Wallis test.

3. Results

3.1. Growth. All three feeding treatments resulted in considerable body growth (Figure 1). In part 1 of the experiment (Day 1–22), fish fed EF grew significantly faster both in length and mass compared to fish fed DF (length: $p = 0.006$, mass: $p = 0.004$) (Figure 1A,B). Fish fed DF/EF gained mass significantly faster than fish fed DF ($p = 0.020$), while their length development was similar to fish fed DF ($p = 0.190$). In the first part (Day 1–22), the mass was 23.7% higher in fish fed EF compared to fish fed DF on Day 22 ($p < 0.001$).

In the second part of the experiment (Day 22–43), where all groups were fed DF, there were no significant differences in growth rates between treatments ($p = 0.590$), meaning that the initial growth advantage observed in fish fed EF during the first part was maintained (Figure 1C,D). Consequently, the final body mass and length were significantly greater in fish fed EF compared to those fed DF ($p = 0.040$ and $p = 0.002$, respectively) (Figure 1). These differences in mass between treatments were confirmed in a similar analysis performed on dry mass (Table S1).

3.2. Health Indicators. Mortality was low for all treatment groups with a total mortality of 2.4% for the whole experiment, and there were no significant effects between treatment groups (Table 2; $p = 0.774$). Fin damage measured on Day 22 scored below 0.07 in all treatment groups and showed no significant difference between treatment groups (Table 2; $p = 0.058$). The CFs were also similar among all treatment groups, except on Day 43, where fish fed DF had a higher *k*-factor than the other treatment groups (Table 2; $p < 0.001$). There were no significant differences in the HSI either on Day 22 or Day 43 (Table 2; $p = 0.420$, $p = 0.988$).

3.3. Feeding Behavior. In the first 18 days, appetite steadily increased in all treatments. After the transition to 0.5–1 mm DF on Day 22, appetite decreased slightly for 3 days and then returned to normal levels in all treatment groups (Figure S2).

3.4. Fatty Acid Composition of Feed. EF contained significantly lower amounts of omega-3 fatty acids ($n-3$) than DF, including less DHA and EPA (Table 3). DHA content was roughly 19 times higher in DF than in EF. However, EF had significantly higher levels of the $n-6$ essential fatty acids (LA and ARA). Moreover, PLFA contents were 2.5% of the total dry mass of EF but only 1.2% of DF (Table 3).

3.5. PLFA Composition of Fry. To examine if different feeding regimes resulted in different phospholipid profiles, we analyzed the PLFA content of whole rainbow trout fry. A PCA was conducted using the molar percentage of PLFAs of whole fish and the molar percentage of the total fatty acid content of the feeds (PLFA and neutral lipid fatty acids [NLFAs] combined). These results are shown in the PCA biplot (Figure 2), and the data used can be found in Table 3 (total fatty acids of feed) and Table S2 (PLFA of fry). The first two principal components together explained 88.6% of the total variation, with PC1 explaining 51.4% and PC2 explaining 37.2%. PC2 primarily distinguished between fish and feeds, whereas PC1 differentiated between fish based on their dietary influences on PLFA composition.

The PCA revealed a distinct clustering pattern for the different diet treatments (Figure 2). Fish fed DF and EF differed significantly on PC1 ($p < 0.001$, Table S3). Fish fed DF clustered closely together near the DF on PC1 ($p = 0.33$, Table S3), whereas fish fed DF/EF and EF were not significantly differentiated on PC1 ($p = 0.14$, Table S3) and clustered intermediate between the two feed types.

The essential fatty acid composition of whole fish is displayed in Figure 3. Fish fed EF and DF/EF had lower levels of $n-3$ PUFA than fish fed DF. Accordingly, and in conformity with the fatty acid vectors of the PCA plot, fish fed DF had significantly higher levels of EPA and DHA than fish fed EF ($p = 0.03$, $p < 0.01$) and DF/EF ($p = 0.01$, $p < 0.01$), respectively. However, the DHA:EPA ratio was the same (2.30 : 1) in EF and DF treatments. The $n-6$ essential fatty acids were more abundant in the fish fed EF than DF, mainly due to arachidonic acid (20–4 $n-6$, ARA), representing more than 10% of the composition in fish fed EF, while only representing 2% in fish fed DF (Figure 3 and Table S2).

3.6. Phospholipid DHA in Brain and Eye Tissues. To examine if fish fed EF could maintain DHA levels of important neural tissues, we analyzed the PLFA content of the rainbow trout's brain and eyes. The proportion of phospholipid DHA of brain tissue in molar percentage was significantly higher in fish fed DF than fish fed EF ($p < 0.001$) or DF/EF ($p = 0.001$), which was the same as found in whole fish (Table S2; Table S4b). Nonetheless, the actual amounts of PLFA DHA in the brain tissues (mg/g) were more similar between treatments ($p = 0.373$, Figure 4, Table S4a).

In the eye tissue, there was a significant difference in the amounts of DHA (mg/g) between treatments ($p = 0.018$) (Figure 4). All treatments seemed to conserve more DHA

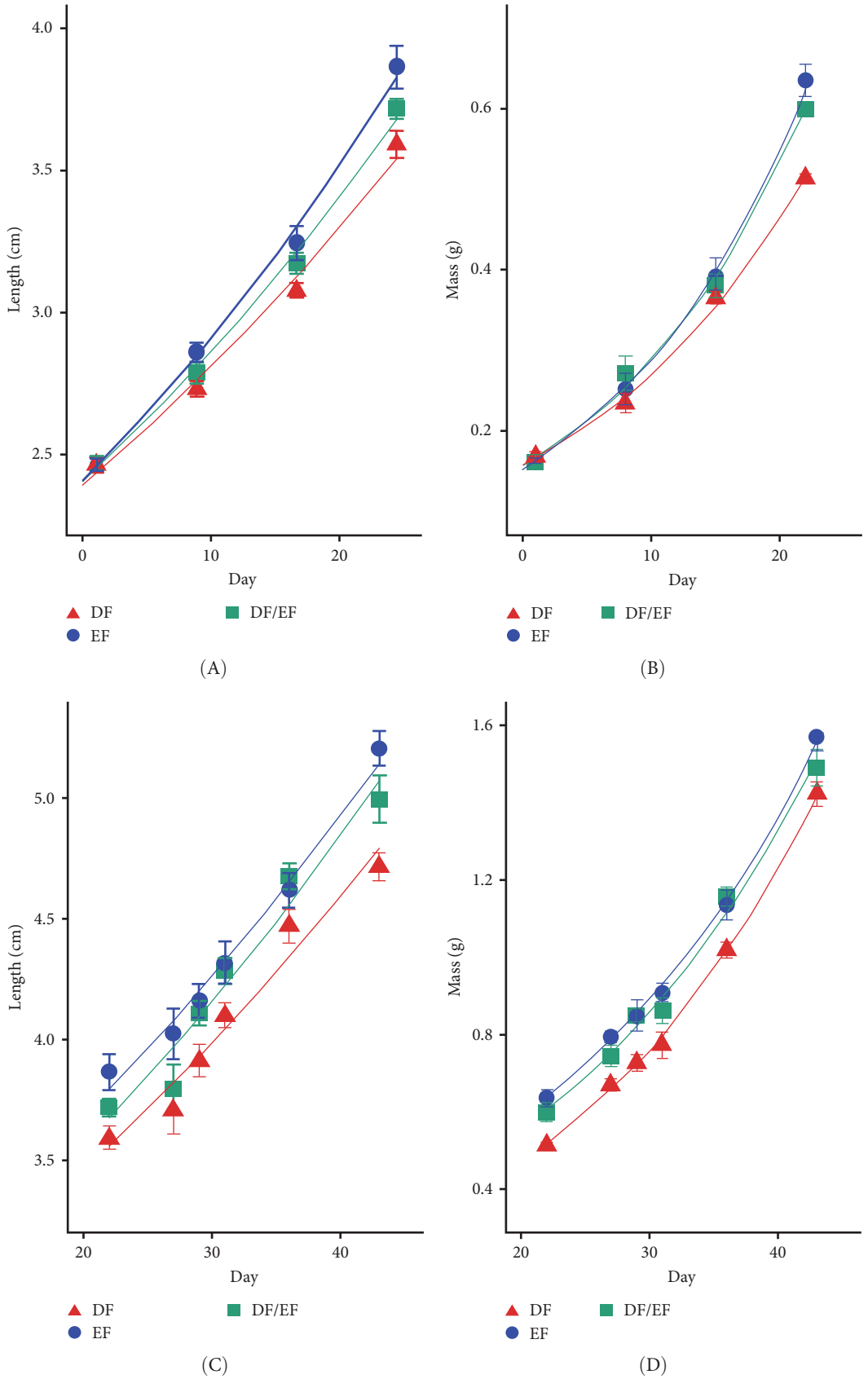

FIGURE 1: Effects of feed type on length and mass dynamics of rainbow trout fry during a two-part feeding experiment. (A) fish length from Day 1–22, (C) fish length from day 22–43, (B) mass from Day 1–22, and (D) mass from Day 22–43. Three treatment groups were tested: dry feed (▲DF), *E. albidus* (●EF), and a combination of both (■DF/EF). The experiment consisted of two parts: in part 1 (Day 1–22), the three treatment groups were fed their assigned feed, and in part 2 (Day 23–43), all were fed dry feed (DF). Lines represent generalized linear model (GLM) fit for each treatment group within each time period, using a Gamma distribution and log link function to model growth trends. Values are means ± SE, based on five replicates of 10–25 fish.

Table 2: Effects of diets on selected health indicators on Day 22 and Day 43.

Health indicators	Day	Diet treatment groups		
		Dry feed (DF)	Combination (DF/EF)	*E. albidus* (EF)
Cumulative mortality (%)	1–22	2.0 ± 0.18	2.4 ± 0.23	0.8 ± 0.10
	1–43	2.4 ± 1.16	2.4 ± 1.16	2.4 ± 0.74
Fin damage (score 0–3)	22	0.07 ± 0.02	0.02 ± 0.02	0.02 ± 0.016
Condition factor (CF)	22	1.11 ± 0.04	1.17 ± 0.03	1.10 ± 0.04
	43	1.36 ± 0.03[a]	1.30 ± 0.04[ab]	1.12 ± 0.04[b]
Hepatosomatic index (HSI)	22	1.89 ± 0.16	2.14 ± 0.18	1.85 ± 0.23
	43	1.75 ± 0.19	1.65 ± 0.10	1.49 ± 0.14

Note: Fin damage and condition factors (CFs) were observed and measured on five replicas of 25 fish each. HSI was measured on five replicates of 3 fish each. Different superscript letters indicate significant differences between means ($p < 0.05$).

Table 3: Fatty acid composition (molar percentage, mean ± SE, $N = 4$) of dry feed (DF) and *E. albidus* (EF).

Fatty acid	Fatty acid composition		Statistical significance
	Dry feed (DF)	*E. albidus* (EF)	
C14:0	7.03 ± 0.10	10.85 ± 0.20	**$p < 0.003$**
C14:1	0.00 ± 0.00	4.27 ± 1.68	N/A
C16:0	19.51 ± 0.12	6.33 ± 0.33	**$p < 0.003$**
C16:1	5.84 ± 0.03	12.44 ± 0.22	**$p < 0.003$**
C18:0	2.72 ± 0.02	3.58 ± 0.23	$p < 0.009$
C18:1	20.48 ± 0.12	7.77 ± 0.46	**$p < 0.003$**
C18:2 *n*−6 (LA)	9.45 ± 0.03	13.64 ± 0.35	**$p < 0.003$**
C18:3 *n*−3 (ALA)	2.34 ± 0.02	1.61 ± 0.14	**$p < 0.002$**
C20:1	7.76 ± 0.05	2.61 ± 0.18	**$p < 0.003$**
C20:2 *n*−6	0.00 ± 0.00	5.37 ± 0.88	N/A
C20:3 *n*−6	0.00 ± 0.00	1.64 ± 0.49	N/A
C20:4 *n*−6 (ARA)	0.20 ± 0.00	3.74 ± 0.17	**$p < 0.003$**
C20:5 *n*−3 (EPA)	14.72 ± 0.13	8.41 ± 0.60	**$p < 0.003$**
C22:2 *n*−6	0.00 ± 0.00	2.00 ± 0.19	N/A
C22:4 *n*−6	0.00 ± 0.00	1.10 ± 0.06	N/A
C22:5 *n*−3	0.27 ± 0.00	1.14 ± 0.09	**$p < 0.003$**
C22:6 *n*−3 (DHA)	9.67 ± 0.08	0.51 ± 0.08	**$p < 0.003$**
Total *n*−3 PUFA	27.00 ± 0.23	11.66 ± 0.78	**$p < 0.003$**
Total *n*−6 PUFA	9.65 ± 0.03	27.50 ± 1.39	**$p < 0.003$**
Total PUFA	36.65 ± 0.23	39.16 ± 1.43	$p < 0.006$
Total of FA (μg/mg)	67.45 ± 0.90	84.15 ± 4.66	**$p < 0.003$**
PLFA as % of diet*	1.2 ± 0.18	2.5 ± 0.26	**$p < 0.002$**

Note. ANOVA *p*-values shown in bold are statistically significant after Bonferroni correction ($p < 0.00294$). The total fatty acid content (μg/mg dry mass) and the percentage of phospholipid fatty acids in diets are shown. N/A, Not applicable, but significant difference due to missing fatty acid in dry feed diet.
Abbreviations: FA, fatty acid; PLFA, phospholipid fatty acids; PUFA, polyunsaturated fatty acids.
*assuming the average phospholipid molecule has a molecular mass of 800.

in eye tissue than in whole fish, but the fish fed EF conserved a relatively higher amount of DHA in the eye tissue. The ratio of DHA between whole fish and fish eyes was larger in the EF dietary treatment (ratio 3.20) than in the DF treatment (ratio 1.90) (Figure 4). The complete tables of eye tissue PLFA can be found in supporting information (Table S5a, b).

4. Discussion

4.1. Growth. The present study is the first to investigate the use of *E. albidus* as a starter feed for rainbow trout fry. The findings showed that feeding with EF increased the specific growth rate compared to the standard DF. Such positive effects have been observed using annelid meal as feed for rainbow trout fingerlings [19]. The cause of the enhanced growth could be connected to digestibility and good nutritional value, but it has also been proposed that the antimicrobial substances contained by annelids as part of their immune defense system could benefit the fish [48]. The growth advantage observed during feeding with EF and EF/DF persisted even after three additional weeks of feeding with DF alone, suggesting a lasting impact of the initial live

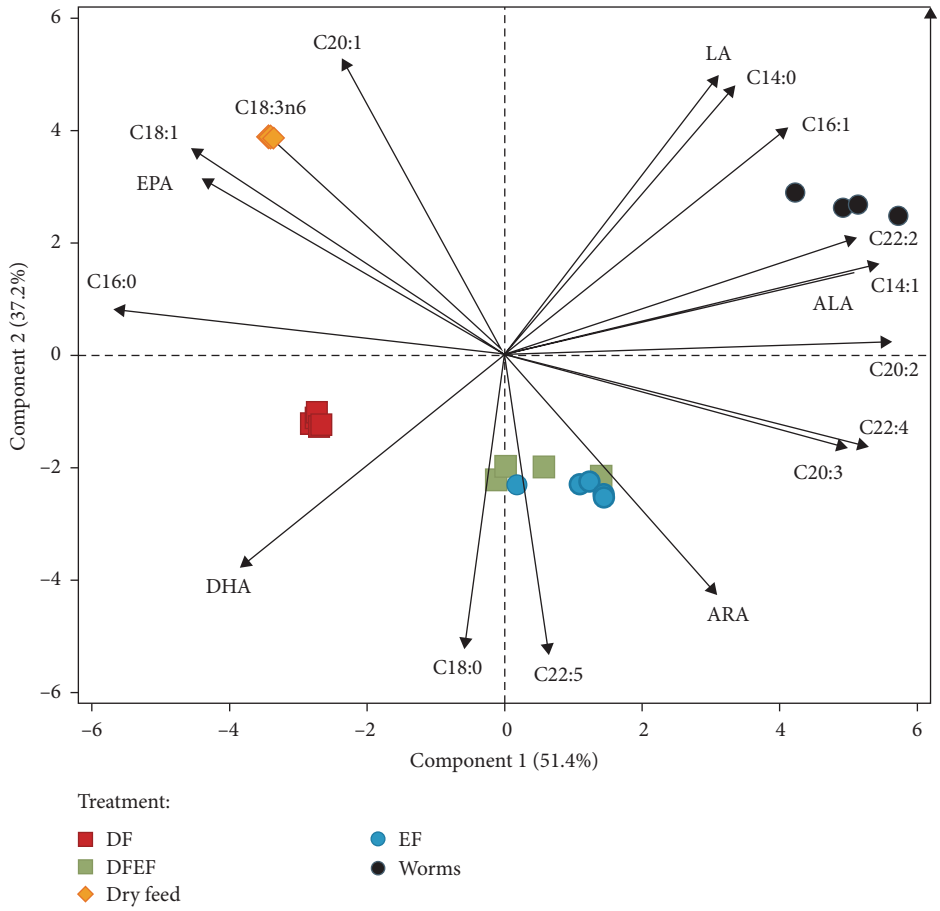

FIGURE 2: Principal component analysis (PCA) showing dietary influence on phospholipid composition in rainbow trout fry presented in biplot. The PCA shows the phospholipid fatty acid composition (molar percentage) of rainbow trout fry fed one of three diets: dry feed (DF), *E. albidus* (EF), and a combination (DF/EF). Additionally, the composition of fatty acids for the two feeds (*E. albidus*, referred to as "worms" in the figure, and dry feed) is presented. In the PCA plot, the treatment groups and feeds are visualized in a multivariate space defined by two principal components (PC1 and PC2). PC1 explains 51.4% of the total variance and PC2 37.2%. The fatty acid loadings are included as grey vectors in the background. The principal components are based on correlations between variables, and the data is normalized to ensure that the fatty acids' contribution to the PCA is based on their relative variability rather than absolute scale. Hence, a large absolute loading value has a greater impact on the principal component.

feed regimen. Notably, fish from the EF treatment maintained a 10% higher body mass and length after 6 weeks compared to those from the DF treatment. These results contradict our concern that potential growth advantages may be short-lived once transitioning to DF. For some fish species, the negative effects of the transition from live feed to DF can be mitigated by weaning strategies such as cofeeding live and DF [49, 38]. However, nothing in this study indicated that an abrupt shift from feeding EF to DF had any costs in terms of reduced growth.

4.2. Health. Mortality was low in all treatments, with no more than six out of 250 fish dying in any treatment group during the 6-week trial. The low mortality in all feeding treatments indicates that feeding with EF does not adversely affect the health of rainbow trout fry. At the end of the experiment, fish fed DF displayed a 20% higher CF than fish fed EF, which could indicate a better nutritional status in the DF treatment. However, the lower CF of EF fish more likely resulted from the more rapid growth of these fish and, therefore, is not an indication of health status. The HSI was consistent and within the normal range of 1%–2% across all treatments, indicating a healthy liver metabolism across groups [44, 45]. Fin damage is influenced by various factors, including fish health, feeding frequency, underfeeding, stocking densities, and water quality [40, 41, 50]. In our study, fin damage scores were uniformly low across all treatments. Altogether, the measured health indicators suggested that the different feeding regimes neither improved nor adversely affected the health conditions of the fish. Employing infection and stress challenges could have revealed health benefits that were not apparent under these optimal conditions, where survival was close to 100% [4, 11].

4.3. Feeding Behavior. As discussed above, rainbow trout fry readily accepted and grew on EF. The observed increase in growth may be due to factors such as higher palatability, improved digestibility, or enhanced nutritional value of EF. Low palatability of feed has been shown to lower feed intake due to lack of appetite or rejecting feed [51–53]. The feed

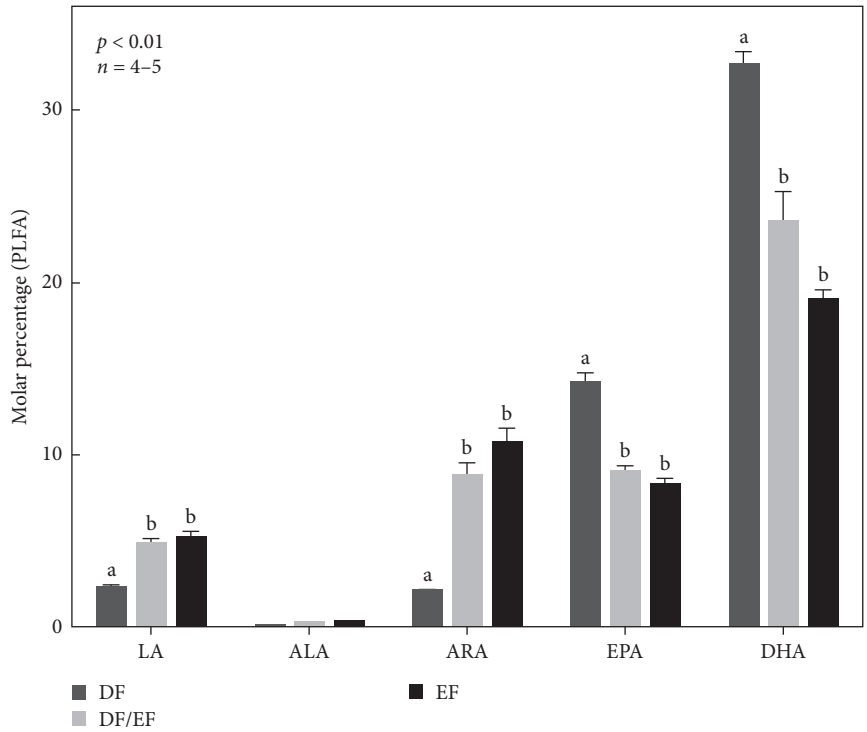

FIGURE 3: Composition of essential phospholipid fatty acids (PLFA) in whole fish on Day 22. The figure compares the phospholipid fatty acid composition (molar percentage) among fish fed one of three diets for 21 days: dry feed (■, DF), *E. albidus* (■, EF), or a combination of DF and EF (■, DF/EF). LA (linoleic acid, 18:2*n*−6), ALA (α-linolenic acid, 18:3*n*−3, ALA), ARA (arachidonic acid, 20:4*n*−6), EPA (eicosapentaenoic acid, 20:5*n*−3), DHA (docosahexaenoic, 22:6*n*−3). Values are means ± SE ($n = 4–5$). Letters indicate significant differences between treatments, with adjustments made for multiple comparisons using Bonferroni correction ($p < 0.01$).

responses of trout fry in this study were similar between treatments, which could indicate that the palatability of EF and DF was similar. However, the PCA of fatty acid composition (Figure 2) clearly shows that fish fed DF/EF had a PLFA composition similar to the EF treatment but distinct from DF. This indicates that, given the choice, trout fry prefers EF over DF. This might not be due to nutritional value but rather a result of appetite and attractiveness of EF, as factors such as prey motion have been shown to be important in triggering response in trout and salmon [36, 37, 54]. The feeding response was recorded during the first feeding each day, which, in retrospect, may not have been the optimum time. Appetite could be improved during the day's first feeding, making it harder to observe differences. Despite this, any significant variations in appetite between treatments would have manifested as noticeable trends within the initial weeks. Additionally, any adverse effects from the diet, such as stress or illness, would have likely resulted in a noticeable decline in appetite [40, 41, 50]. The low levels of fin damage in this study suggest that feeding amount and frequency were sufficient for the fish density in the tanks. Interestingly, we observed a slight drop in appetite in all treatments after transitioning to DF on Day 22. Nevertheless, the observed feeding behavior results do not imply that the fish preferred one feed type over the other.

4.4. Fatty Acid Composition of Whole Fish. Fish's PLFAs and NLFAs composition can both provide insightful information about a fish's diet. The NLFAs, which primarily serve as energy storage, are generally more responsive to dietary inputs than the PLFAs, since PLFAs are more conserved due to their essential structural and physiological roles in cell membranes [28, 35]. For this reason, the focus of this study was to investigate if the fatty acid requirements were met and how PLFA levels of essential fatty acids in EF affected differences in whole fish and neural tissues.

The immediate concern about using EF as the sole feed source for trout fry was whether the dietary levels of omega-3 fatty acids (*n*−3), including ALA and DHA, would be sufficient for growth and development. Dietary deficiencies in *n*−3 would result in retarded growth, decreased health, and increased mortality [55]. Fish fed EF had a lower proportion of DHA and slightly lower EPA than fish fed DF. Nevertheless, the phospholipid DHA:EPA ratio of whole fish was the same for both the EF and DF treatments (~2.3:1), indicating that the dietary *n*−3 requirements were met for the fish fed EF in this experiment. This pattern aligns with salmonids' ability to maintain long-chain PUFA profiles despite different diets, via diet-responsive regulation of elongase/desaturase genes coupled to selective allocation of DHA [56, 57]. In addition, the dietary phospholipid content from EF was more than twice that of DF, and higher phospholipid levels have been shown to enhance growth in the early development stages of rainbow trout by assisting digestion [58, 59].

Increased growth in rainbow trout fry and other species has been associated with higher dietary levels of DHA, EPA,

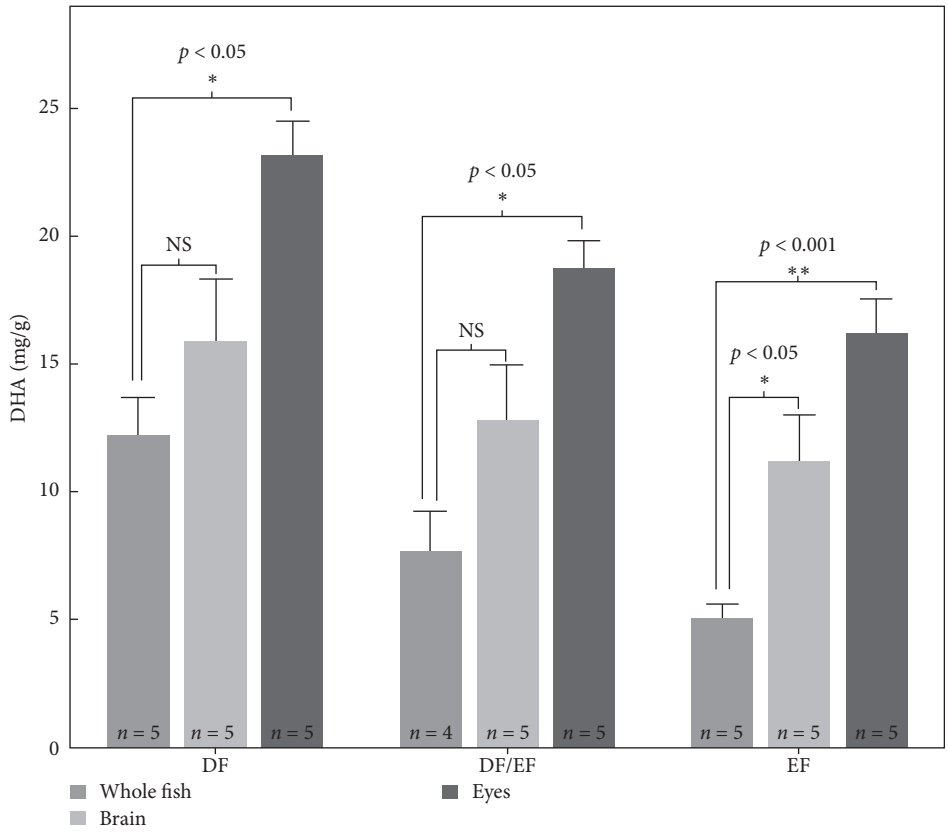

FIGURE 4: Phospholipid DHA content (mg/g) in whole fish, brain, and eye tissue. Each bar represents DHA (docosahexaenoic, 22:6*n*−3) levels in the different tissues: ■ whole fish, ■ brain tissue, and ■ eye tissue, across three dietary treatments: fish fed with dry feed (DF), *E. albidus* (EF), and a combination (DF/EF). Values are means ± SE ($n = 4$-5). NS indicated no statistically significant differences, and asterisks indicate significance $^{*}p = 0.05$, $^{**}p < 0.001$.

and ARA [60, 32]. In this study, the ARA content was 5-fold lower in fish fed DF than EF. Low levels of ARA can have a negative effect on growth and survival in other species, like gilthead sea bream larvae [61, 62]. The reason for this is that the enzyme system for elongation and desaturation of *n*−3 (EPA, DHA) and *n*−6 (ARA) fatty acids is shared between the two biosynthesis pathways, and the enzyme system prefers *n*−3 substrates over *n*−6, even when an excess of the *n*−6 precursor, LA, is being supplied through the diet [63, 60, 28]. Hence, it may be that the fish fed DF did not get a sufficient dietary ARA input, possibly contributing to the enhanced growth in rainbow trout fed EF. Notably, recent rainbow trout studies indicate that both insufficient and excessive dietary ARA can be detrimental, affecting survival and stress/immune responses, and the optimal ARA window appears life-stage dependent [64, 65].

4.5. Fatty Acid Composition of Neural Tissues. Docosahexaenoic acid (DHA) plays a critical role in normal function and development of neural tissues such as brain and eyes. Predatory fish are particularly exposed, as deficiencies have been linked to abnormal behavior and impaired ability to capture prey [66, 67]. If long-term deficiency occurs, it can result in bacterial disease and fin erosion [34]. In this study, we found a significant difference in the amount of DHA in whole fish between treatment groups. Despite this, there was no statistical difference in the amount of phospholipid DHA in brain tissue between treatment groups. On the contrary, a difference in the amount of DHA in the eyes was observed between treatments, but the fish fed EF had a larger amount of DHA in both eyes and brain relative to the whole fish than fish fed DF. This indicates an increased conservation of DHA in the neural tissues of the low DHA diet (EF treatment). Hence, our results suggest that the fish fed EF were capable of compensating and redirecting sufficient DHA to neural tissues during a period of several weeks. There are likely several mechanistic processes behind this pattern. One involves the regulation of DHA uptake across the blood–brain (and retinal) barriers via the lipid transporter Mfsd2a. This mechanism has recently been resolved in zebrafish, providing a direct route for DHA delivery to neural tissues [68]. Another mechanism is the diet-induced modulation of long-chain PUFA biosynthesis, where upregulation of genes such as elovl2 and fads2 can partially compensate for reduced DHA intake [57, 69]. Additionally, DHA is preferentially retained in neural phospholipid pools, which helps stabilize DHA levels in the brain and retina even when whole body stores fluctuate [68, 69, 57]. Together, these mechanisms support our observation of conserved neural DHA and a relatively stable whole-fish phospholipid DHA:EPA ratio

across diet treatments, and they help explain how EF-fed fish maintained neural DHA despite lower whole-body DHA.

5. Conclusion

This study confirms the potential of *E. albidus* as a compelling live starter feed for rainbow trout fry, supporting enhanced growth and health maintenance without adverse effects. Rainbow trout fry fed EF contained lower $n-3$ fatty acid levels, including DHA, but this did not result in decreased growth or adverse health effects. The significant conservation of DHA in the eye and brain tissues of fish fed with EF suggests a complex interplay between diet and physiological needs, highlighting the capability of rainbow trout fry to adapt their fatty acid metabolism in response to dietary inputs. We propose that *E. albidus* could be used as a starter feed to boost growth in rainbow trout fry.

Data Availability Statement

To enhance transparency and reproducibility, the datasets supporting the findings of this study are provided either in the Supporting Information or are publicly available in the Mendeley Data repository at https://doi.org/10.17632/pbr2r737ft.1.

Conflicts of Interest

Mathias Engell Holmstrup is an industrial PhD student between Fishlab and Aarhus University. Martin Holmstrup is a co-owner of Fishlab, which plans to commercially produce *Enchytraeus* worms. The other authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*) The following supporting information are provided: Figure S1: Morphological scheme used to diagnose and classify injuries on the dorsal fin. Figure S2: Daily feed response during the experiment. Table S1: Mass and length measurements of rainbow trout at Day 0, Day 22, and Day 43. Table S2, S4, and S5: Data on phospholipid fatty acid (PLFA) composition, in whole fish (Table S2), in brain (Table S4a and S4b), and in eye tissue (Table S5a og S5b). Table S3: Results of the TukeyHSD post hoc test comparing the effects of three different feeding treatments on fish, based on principal component scores (PC1 and PC2).

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