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## ► To cite this version:

Antony Jesu Prabhu Philip, Haohang Fang, Laurence Larroquet, Anne Surget, Alexandre Herman, et al.. Zinc nutrition at first feeding imprints a programming effect on growth and hepatic lipid metabolism in juvenile rainbow trout. *Aquaculture*, 2024, 592, 10.1016/j.aquaculture.2024.741207 . hal-04643813

**HAL Id: hal-04643813**

**<https://hal.inrae.fr/hal-04643813v1>**

Submitted on 10 Jul 2024

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# Zinc nutrition at first feeding imprints a programming effect on growth and hepatic lipid metabolism in juvenile rainbow trout

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## ARTICLE INFO

### Keywords:

Fish  
Zinc  
Copper  
Nutritional programming  
Lipid metabolism

## ABSTRACT

The objective of this study was to determine whether supplementation with deficient zinc (Zn) or/and excess copper (Cu) in the first-feeding diet of rainbow trout fry influenced the growth and physiological regulation of juvenile fish subjected to similar challenge feeds again. The feeding trial lasted for 24 weeks and had three phases. In phase I, rainbow trout fry was treated with one of four different plant-based feeds containing or exempting Zn or Cu supplementation (coded Zn-Cu-, Zn+Cu-, Zn-Cu+, Zn+Cu+) for 6 weeks. Thereafter, all groups were fed a common commercial feed for the next 12 weeks (phase II). In phase III, all groups were fed with the “challenge diet” for another 6 weeks which was the basal diet (Zn-Cu-) except that the vegetable oils were replaced by fish oil. The results demonstrated that fish fed a Zn+ diet at first feeding increased larval growth (phase-I), but it had no effect on growth at the end of phase II when fed commercial feeds. In phase III, when re-introduced to a Zn- challenge diet, the feed intake and growth of juveniles with Zn+ history significantly increased, with reduced feed efficiency. Fish growth was neither influenced by dietary Cu levels or by the dietary Cu history in any of the growth phases, but dietary Cu excess (Cu+) reduced body lipid and energy content in fry. In phase I, lower whole-body Zn concentration was observed in the fry of Zn- group compared to Zn+, while the contrary was observed in the juvenile fish as affected by dietary Zn history (phase III). In addition, Zn- dietary history showed increased levels of PUFA and higher mRNA expression of fatty acid biosynthesis genes in the liver. To conclude, early-stage dietary Zn+ history improved growth of juvenile rainbow trout, while dietary Zn- history exhibited signs of improved fatty acid biosynthesis capacity in the liver.

## 1. Introduction

Zinc (Zn) and copper (Cu) are involved in several processes of intermediary metabolism (Bjørklund et al., 2020; Blades et al., 2021a; Cunnane, 1988; Shi et al., 2020). Despite the knowledge in mammalian literature, the role of dietary Zn and/or Cu in influencing intermediary metabolism is only beginning to be understood in teleost. The transcription levels of several genes involved in the biosynthesis, transport and oxidation of fatty acids were differently regulated by dietary Zn or Cu in yellow catfish juveniles (Meng et al., 2016; Wei et al., 2018; Zheng et al., 2015). Consequently, dietary Zn deficiency and Cu excess increased hepatic lipid deposition (Meng et al., 2016; Wei et al., 2018; Zheng et al., 2015). Further, LC-PUFA biosynthesis *de novo* was improved in salmonids by a multi-micronutrient fortification containing

Zn (Giri et al., 2016; Lewis et al., 2013). Therefore, dietary need and supply of Zn and Cu are of added significance to aquaculture fish species.

Fish meal is a balanced source of trace minerals to fish. Imbalance in the supply and limitations in utilization of trace minerals were observed when salmonids were fed plant-based diets (Antony Jesu Prabhu et al., 2016a). As in mammals, trace minerals are an important group of nutrients vital for various biological processes in fish (Lall, 2022; Watanabe et al., 1997). Consequences of high inclusion of plant ingredients in salmonid feeds among others include reduced Zn and excess Cu supply (Antony Jesu Prabhu et al., 2016b). Physiologically, rainbow trout incurred higher endogenous loss of Zn leading to depletion when fed plant-based diets and the contrary was true for Cu (Antony Jesu Prabhu et al., 2015). Metabolic disturbances in cellular handling of Zn and Cu resulted in differential responses resulting in depletion or accumulation

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<https://doi.org/10.1016/j.aquaculture.2024.741207>

Received 25 January 2024; Received in revised form 22 May 2024; Accepted 8 June 2024

Available online 9 June 2024

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in tissues (Antony Jesu Prabhu et al., 2016a). As several metabolic pathways are linked to Zn or Cu, disturbances in their cellular and systemic homeostasis can influence the overall performance of the fish.

The requirement for Zn and Cu to rainbow trout are 37 mg/kg and 5 mg/kg feed, respectively (Antony Jesu Prabhu et al., 2016b; NRC, 2011). In plant-based feeds containing phytate, the dietary need for Zn to meet the requirement is suggested to be about 4-fold higher (Satoh et al., 1987; Watanabe et al., 1997). Subsequently, increased dietary Zn levels are required in present day salmonid feeds (Antony Jesu Prabhu et al., 2019; Antony Jesu Prabhu et al., 2016b; Philip et al., 2023; Sartipi Yarahmadi et al., 2022). However, as opposed to increasing dietary Zn levels, improving the availability and utilization is an ideal approach. Programming or preparing the early life of fish to sub-optimal dietary Zn to be encountered in later life is a possible way to improve Zn utilization in salmonids. Optimal Zn supply in freshwater phase improved the long term health and welfare of Atlantic salmon in the sea (Philip et al., 2023; Sartipi Yarahmadi et al., 2022). On the contrary, excess dietary Cu supplementation can increase the risk of toxic effects, especially during early life stages (Berntssen et al., 1999a, 1999b; Dominguez et al., 2019; Tseng et al., 2023). Knowledge on the significance of life-stage specific dietary requirements and the consequence of early life nutrition on later life stages is very limited in fish, more so for trace minerals. Besides methionine, zinc has been proposed to be a critical nutrient limiting the potential effects of early stage nutritional programming on the later utilization of a plant-based diets in rainbow trout (Séité et al., 2019; Yamamoto et al., 2022, 2023). In this context, we hypothesized that the metabolic capacity of fish towards utilization of dietary lipids might be altered by early nutritional history of dietary Zn or Cu.

## 2. Material and methods

### 2.1. Animal ethics statement

The experiment was approved by the ethical committee of the funding body (EU H2020 Aquaexcel TNA; grant no. AE060014) and all procedures were performed in compliance with the European Directive 2010/63/EU for the protection of animals used for scientific purposes and the French Decree no. 2013–118 for animal experimentation by trained personnel at the INRAE experimental fish farm in Donzacq (Landes, France, <https://doi.org/10.15454/GPYD-AM38>).

### 2.2. Experimental feeds

Five experimental diets were used in this experiment (Table 1). The basal diet was formulated to be completely plant based, low in Zn and Cu and devoid of Zn or Cu in the mineral premix. The diets were coded Zn-Cu- (basal diet, no Zn or Cu supplemented), Zn+Cu- (only Zn supplemented), Zn-Cu+ (only Cu supplemented) and Zn+Cu+ (Zn and Cu supplemented). The desired levels of Zn and Cu in the basal diet were 60 mg/kg and 5 mg/kg. The four experimental diets were then formulated from the basal diet through addition of 222 mg/kg ZnSO<sub>4</sub>.H<sub>2</sub>O (36% Zn) and 40 mg/kg CuSO<sub>4</sub>.5H<sub>2</sub>O (25% Cu) to the respective formulations. Diets were produced at the INRAE experimental facilities of Donzacq, using a twin-screw extruder (45 BCE, Cletral, Firminy, France). The final analysed Zn and Cu concentrations in the experimental diets were 68 or 128 mg Zn/kg and 9 or 20 mg Cu/kg feed. Further, an additional diet coded as the ‘challenge diet’ was formulated to resemble the basal diet (Zn-Cu-) except that the vegetable oils were replaced by fish oil. The detailed account of the analysed proximate and nutrient composition of the experimental diets can be found in Table S1. Besides these experimental diets, fish meal and fish oil based commercial trout diets (B Supra and Neo-Start 1, 2 & 3; Le Gouessant, France) were used during phase II of the experiment (dry matter, 93–96%; crude protein, 64–43%; crude fat, 8–16%; ash, 10–12%; energy, 22–23 kJ/g). The mean analysed Zn and Cu concentrations of the commercial feeds were 181 and 11 mg/kg, respectively.

**Table 1**  
Diet formulation.

Ingredients (%)	Zn-Cu-	Zn+Cu-	Zn-Cu+	Zn+Cu+	Challenge
Maize Gluten (Inzo)	18	18	18	18	18
Wheat Gluten (Roquette)	20	20	20	20	20
Soybean Meal	8.3	8.3	8.3	8.3	8.3
SPC (Sopropêche)	15	15	15	15	15
White Lupin (Terrena)	7.2	7.2	7.2	7.2	7.2
Dehulled peas (Primatex Sotexpro)	4.9	4.9	4.9	4.9	4.9
Whole wheat	2.9	2.9	2.9	2.9	2.9
L-lysine (Ajinomoto)	1	1	1	1	1
L-methionine (Evonik)	0.3	0.3	0.3	0.3	0.3
Attractant mix	1.5	1.5	1.5	1.5	1.5
Soy lecithin	2	2	2	2	2
Vitamin Premix	2	2	2	2	2
Dicalcium phosphate (18% P)	2.9	2.9	2.9	2.9	2.9
Fish oil (Sopropêche)					13
Rapeseed oil	4	4	4	4	
Linseed oil	5	5	5	5	
Palm oil	4	4	4	4	
Mineral premix without Cu and Zn	1	1	1	1	1
ZnSO <sub>4</sub> .H <sub>2</sub> O (36% Zn) mg/kg	–	222	–	222	–
CuSO <sub>4</sub> .5H <sub>2</sub> O (25% Cu) mg/kg	–	–	40	40	–
Analysed concentration (mg/kg diet)					
Zinc	68	128	72	130	68
Copper	9	9	20	20	9

Premixes (per kg diet): Attractant mix: glucosamine, 5 g; taurine, 3 g; betaine, 3 g; glycine, 2 g; alanine, 2 g; Vitamin premix: retinol acetate, 55,000 IU; cholecalciferol, 2500 IU; DL- $\alpha$ -tocopherol acetate, 50 IU; sodium menadione bisulfite, 10 mg; thiamin-HCl, 1 mg; riboflavin, 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 3 mg; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 10  $\mu$ g; L-ascorbyl-2-polyphosphate, 50 mg; myo-inositol, 0.3 g; choline, 1 g; Mineral premix (without Zn and Cu): CaHPO<sub>4</sub>.2H<sub>2</sub>O, 33 g; CaCO<sub>3</sub>, 2.15 g; Mg(OH)<sub>2</sub>, 1.24 g; KCl, 0.9 g; NaCl, 0.4 g; FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.2 g; MnSO<sub>4</sub>.H<sub>2</sub>O, 30 mg; NaF, 10 mg; KI, 0.4 mg; CoCl<sub>2</sub>.6H<sub>2</sub>O, 0.2 mg. All ingredients were diluted with  $\alpha$ -cellulose.

### 2.3. Experimental fish, protocol, and sampling

The first-feeding fry used in this experiment were obtained from the spring spawning INRAE-PEIMA broodstock. The eggs were spawned and incubated at PEIMA for 21 days (250°d), transferred to and incubated at Léés-athas for 32 days (200°d) and were transported to the experimental fish farm at Donzacq and randomly distributed (200 per tank) to each of the 12 tanks (50 L, flow-through freshwater, 17 °C).

The feeding trial lasted for 24 weeks and had three phases. In phase I, 2400 first-feeding rainbow trout fry (mean initial weight, 52  $\pm$  3 mg) were randomly assigned to one of the four experimental diets following a 2  $\times$  2 factorial design in triplicates for 6 weeks. At the end of phase I (6 weeks), all the fish were bulk weighed for growth, and sampled for various analyses: proximate composition (30 fish), ash and minerals (25 fish), gene expression (9 fish), histology (5 fish), and fatty acid (14 fish). In total, ninety-three fish per tank were sampled at the end of phase-I. After the sampling at the end of in phase-I, variable number of fish remained in each tank (70 to 102 fish) depending on the survival rate. The least common number of fish (and the corresponding biomass) that remained in a tank (70 fish) was chosen as initial number to go further into phase II and the fish in other tanks were sorted accordingly. In phase II, fish were fed commercial feeds (B Supra, Le Gouessant, France) up to week 9 in the same tanks, and then transferred to larger tanks (500 L, flow-through freshwater, 18 °C) and fed commercial feeds Neo-Start 1, 2 & 3 (Le Gouessant, France) until week 18. Unexpected mortalities were encountered in few tanks in phase II (between week 9 and 12) due to a bacterial infection which recovered after antibiotic treatment. At the

end of phase II (week 18), all the fish were bulk weighed and ten fish were used for whole fish and tissue samples. After the sampling at the end of phase-II, variable number of fish remained in each tank (25 to 59 fish) depending on the survival rate. The final number of fish in one of the tanks (Zn-Cu- group) was much lower than the recommended number of fish (30–35 fish) for the tanks to be used in phase III. Therefore, fish from individual tanks of control (Zn-Cu-) dietary group were pooled and then re-sorted into triplicate tanks with 33 fish in each tank. The fish in other dietary groups were also sorted in the same manner to ensure homogeneity in assortment procedures for the entire experiment. Subsequently, in phase III, thirty-three fish per tank were used and all the groups were fed the 'challenge diet' for another 6 weeks (from week 18 to 24). At the end of phase-III, fish were weighed, and samples were collected for whole-body proximate and mineral composition. In addition, samples for plasma metabolites, liver fatty acid profile and liver gene expression analysis were collected at the end of phase III. Biomass and feed intake were recorded every 3 weeks through bulk weighing, mortality was monitored daily. The fish were anesthetized before every weighing and sampling event (benzocaine; dose: 30 mg/L). The fish meant for whole body composition analyses were euthanized by an overdose of the anesthetic (benzocaine; dose: 60 mg/L). The blood sample (3 fish per tank) was drawn from the caudal vein and centrifuged at 3000 ×g for 5 min to recover the plasma. After blood sampling and euthanasia, the liver and gall bladder were dissected and weighed. Subsequently, the liver sub-samples (3 fish per tank) meant for fatty acid and gene expression analyses were collected and flash frozen in liquid nitrogen. All the samples were stored at –80 °C until analyses.

## 2.4. Analytical methods

### 2.4.1. Proximate and mineral analyses

Dry matter content of feed and whole fish samples was determined by drying until constant weight at 105 °C for 24 h. Proximate composition of diets and lyophilized whole fish homogenates were determined according to following procedures: ash by incineration at 550 °C for 10 h, protein (N × 6.25) by the Kjeldahl method after acid digestion, gross energy in an adiabatic bomb calorimeter and total lipid according to Folch et al. (1957) using dichloromethane instead of chloroform. Total lipid was separated into neutral and polar fractions using silica cartridges (Waters, Guyancourt, France) according to Juaneda and Rocquelin (1985). The ash samples were further digested in nitric acid using a microwave digestion system and subsequently used for determination of Zn and Cu by radial ICP-OES at the INRAE central laboratory of Bordeaux (USRAVE, Villenave d'Ornon, France).

### 2.4.2. Fatty acid analysis

Fatty acid methyl esters were prepared by acid-catalysed transmethylation of the lipid extract, using boron trifluoride according to Shantha and Ackman (1990) and analysed in a Varian 3900 gas chromatograph equipped with DB Wax fused silica capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm; JW Alltech, France) using helium as carrier gas (1 mL/min). The temperatures of the injector and the flame ionization detector were 260 °C and 250 °C, respectively. The thermal gradient was 100–180 °C at 8 °C/min, 180–220 °C at 4 °C/min and a constant temperature of 220 °C for 20 min. Fatty acids were identified by reference to known standards. Data were collected and processed using Chromcard for Windows (version 1.19).

### 2.4.3. Plasma analysis

Levels of lipid metabolites such as phospholipids (PL) triacylglycerol (TAG), total cholesterol (TC), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) and non-esterified fatty acids (NEFA) were determined on plasma samples at the end of week 24 using enzymatic colorimetric kits in 96-well microtiter plates (Sobioda, Montbonnot-Saint-Martin, France).

### 2.4.4. Gene expression

Total RNA was isolated from the liver using Trizol reagent (Invitrogen, Cergy-Pontoise, France). Quantitative RT-PCR was performed as described previously (Fontagné-Dicharry et al., 2018). Briefly, complementary DNA was generated from 1 mg total RNA using SuperScriptIII RT (Invitrogen) and a mix of oligo (dT)15 and random primers (Promega, Charbonnières, France). Quantitative PCR analyses were performed with 2 µL of the diluted RT reaction mixture (dilution 40) and 4 µL of master mix added with 0.4 mM of each primer (Table 2). Relative quantification of target gene transcripts was performed using elongation factor 1α (*ef1α*) as the reference gene and Zn-Cu- as the reference group, using the ΔΔCt method (Pfaffl, 2001).

## 2.5. Equations and data analyses

The following equations were used in calculating growth performance indicators presented in Table S2.

$$\text{Survival (\%)} = [\text{final fish (n)}/\text{initial fish (n)}]^* 100.$$

$$\text{Weight gain, (WG, \%)} = 100^* [(\text{final} - \text{initial mean body weight}) / \text{initial mean body weight}].$$

$$\text{Specific growth rate (SGR)} = 100[\ln(\text{final weight}) - \ln(\text{initial weight})] / \text{days}.$$

$$\text{Feed intake (FI, \%BW/d)} = 100^* (\text{Feed given (g)}) / [(\text{mean biomass, g})^* \text{days (t)}].$$

$$\text{Feed efficiency (FE)} = [(\text{final biomass} + \text{dead fish biomass}) - (\text{initial biomass})] (\text{g}) / \text{feed intake (g)}.$$

$$\text{Hepatosomatic index (HIS)} = 100^* [\text{liver weight (g)}/\text{fish weight (g)}].$$

$$\text{Bile somatic index (BSI)} = 100^* [\text{Gall bladder weight (g)}/\text{fish weight (g)}].$$

The data are presented as mean ± standard deviation (SD). The tanks were used as experimental units for data on growth, whole body proximate and mineral composition, liver lipid and fatty acid profile ( $n = 3$ ). Data on plasma lipid metabolites ( $n = 9$ ) and gene expression in the liver ( $n = 9$ ) were treated as individual observations from each fish in a mixed-effect model, wherein the tanks were considered a random factor. Two-way analysis of variance (ANOVA) was performed on all the data sets with dietary Zn and Cu supplementation as main effects along with their interaction. The direction of the effect was determined through Tukey's post-hoc analysis. The data were subjected to test of normality. Gene expression data were non-normally distributed and were hence rank transformed before ANOVA. The statistical analyses were performed in Statistica, version 13.4.0.14, TIBCO software Inc., California and the visualization of the graphs were made in GraphPad Prism 8, San Diego, California.

## 3. Results

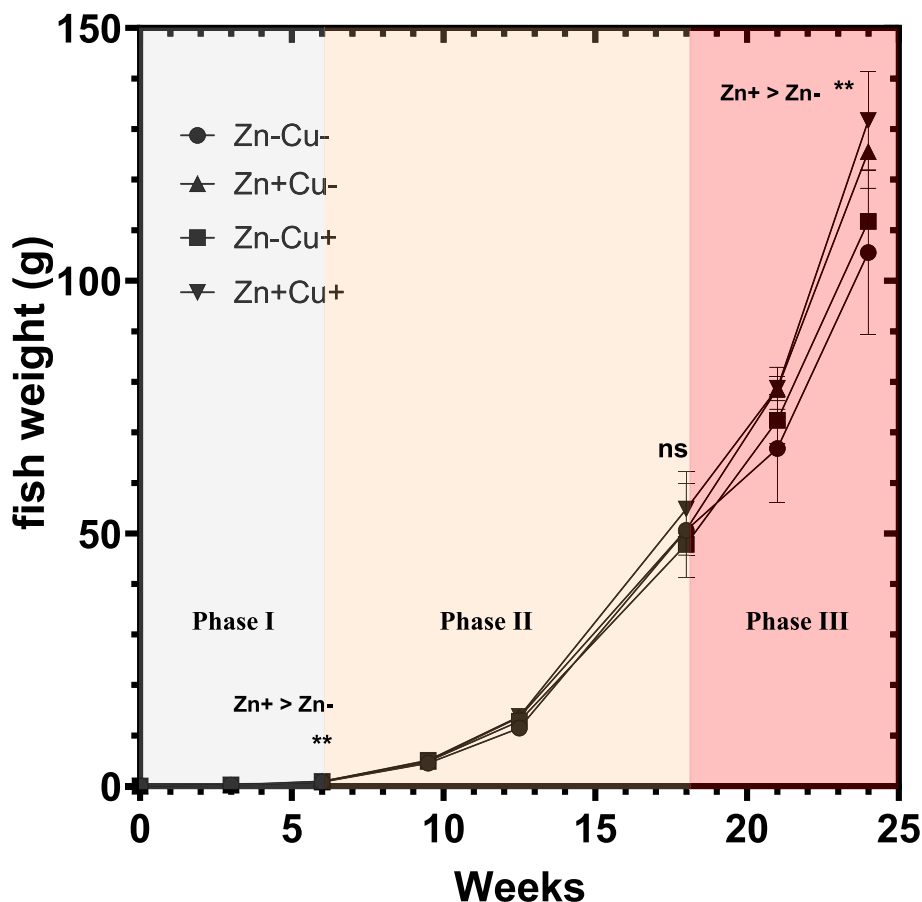
### 3.1. Growth performance

The growth of the fish during the three phases of the experiment is presented in Fig. 1. Data on growth performance indicators such as survival (%), biomass (g), mean final body weight (MFBW, g) feed intake (FI, g/d), weight gain (WG, g), weight gain percentage (WG, %), feed efficiency (FE) and specific growth rate (SGR, %/d) are presented in Table S2. Fish fed the Zn+ diets had higher MFBW, WG and SGR than those fed Zn- diets ( $p < 0.01$ ) at the end of week 6 (phase I). At week 18 (end of phase II), no significant differences were observed in the MFBW,

**Table 2**  
PCR primers used to assay gene expression by real-time quantitative polymerase chain reaction.

Gene	Forward primer sequence	Reverse primer sequence	Amplicon size	Genbank or genoscope <sup>a</sup> accession number
<i>ef1a</i>	tctctggtcgtttcgtc	accgcaggacatcctgtg	159	AF498320.1
<i>acly</i>	gcttttccacgggtgtc	gcttccgctacccaatgc	211	CA349411.1
<i>acc</i>	tggagctctacgcagacaga	ctccgggtaccaagctgtt	152	XM_036935540.1
<i>fasn</i>	tgatctgaagcccggtgca	gggtgacgttgcctggat	182	XM_021576226.2
<i>atgl</i>	ctgttccgagttcaagtc	ggagagatgggtatgggtg	174	OP393009.1
<i>mtp</i>	ctcactgaccactcccaggt	atggctccttgtttgac	152	BX860503.3
<i>apoa1</i>	cgcaggtaaccaggcttttc	aatggacctctgtgcggtca	115	AF042218.1
<i>apoa4</i>	agctgggacaggatgcaat	agacgtctctcagcacctc	148	CA363690.1
<i>apob</i>	aggttgaaccagccgcatt	gagcacggagcttggagacc	177	CA344755.1
<i>d9d</i>	gccgtccgagggttctctt	ctctccccacaggccaag	204	FP323026.1
<i>d6d</i>	agggtgctctgtaactgg	tggtgttggatgtaggg	175	AF301910.1
<i>elovl2</i>	tgtgttcccggtggatgcc	acagagtgccatttggcg	146	XM_021620134.2
<i>elovl5</i>	gaacagcttcatccatgtcc	tgactgcacatatcctctgg	149	AY605100.1
<i>ppara</i>	ctggagctggatgacagtga	ggcaagttttgcagcagat	192	AY494835.1
<i>pparβ</i>	ctggagctggatgacagtga	gtcagccatcttggagca	195	AY356399.1
<i>pparγ</i>	cccacggaaactcaccgttt	ggatctggatgacgggaag	168	CA345564.1
<i>srebp1c</i>	catgcgcagggttttctt	gatgtgtcgtgtggactg	74	XM_021624594.1
<i>hmgcs</i>	agtggcaagagagggtgtg	ttctgttggagacaggagg	279	GSONMG00010243001
<i>dhcr7</i>	gtaaccaccagaccaaga	cctctccatgcagccaac	289	GSONMG00025402001
<i>pck1</i>	acagggtgagcagatgtagg	ctagtctggaggctcaagggc	98	GSONMG00082468001

*ef1a*, elongation factor 1 $\alpha$ ; *acly*, ATP citrate synthase; *acc*, acetylCoA carboxylase; *fasn*, fatty acid synthase; *atgl*, adipose triglyceride lipase; *mtp*, microsomal transfer protein; *apoa1*, *apoa4* and *apob*, apolipoprotein A1, A4 and B; *d9d*, stearoyl-CoA desaturase; *d6d*,  $\Delta$ 6-desaturase; *elovl2*, elongation of long chain fatty acids protein 2; *elovl5*, elongation of very long-chain fatty acids like-5; *ppara*, *pparβ* and *pparγ*, peroxisome proliferator-activated receptor  $\alpha$ ,  $\beta$  and  $\gamma$ ; *srebp1c*, sterol regulatory element-binding 1c; *hmgcs*, HMG-CoA synthase; *dhcr7*, 7-dehydrocholesterol reductase; *pck1*, phosphoenolpyruvate carboxykinase 1. <sup>a</sup> <https://www.genoscope.cns.fr/trout/>; <sup>b</sup> Additionally, also GSONMG00076841001; <sup>c</sup> Additionally, also GSONMG00014864001.



**Fig. 1.** Growth increment of rainbow trout during the entire experimental period.

**F1 legend:** The increment in fish weight (g) during the experiment is plotted against experimental duration (weeks). The experimental period was divided into three phases (I, II and III). Phase I (week 0–6) rainbow trout fry were fed four experimental diets with or without supplemented Zn or Cu. Phase II (week 7–18), all the four dietary groups were fed the same commercial feed. Phase III, (week 19–24), all the groups were fed the basal diet (Zn-Cu-) during this challenge period. The data are presented as mean  $\pm$  SD ( $n = 3$ ). Data were analysed using two-way ANOVA with Zn or Cu inclusion as main factors, along with their interaction. Statistical significance of main effects is indicated in the figure as 'ns', not significant ( $p > 0.05$ ) or with \*\* ( $p < 0.05$ ), along with the nature of the effect when there is a statistical significance (Zn+ > Zn-).



WG, SGR, FI, FE and organo-somatic indices (liver and gall) of the fish between groups when fed a common commercial feed for 12 weeks (Table S2;  $p < 0.05$ ). However, increased mortality in tanks of certain groups due to a bacterial infection early in phase II resulted in interaction effects on survival ( $p = 0.05$ , tendency) and final biomass ( $p < 0.01$ ) at the end of phase II (Table S2). Impact of Zn<sup>+</sup> dietary history (Zn<sup>+</sup> > Zn<sup>-</sup>;  $p < 0.01$ ) on the final biomass, MFBW, WG and SGR appeared at week 24 when the fish were fed the 'challenge diet' for 6 weeks (Fig. 1; Table S2). The increased growth in fish with Zn<sup>+</sup> history was associated with increased FI (Zn<sup>+</sup> > Zn<sup>-</sup>;  $p < 0.001$ ), and decreased FE (Zn<sup>+</sup> < Zn<sup>-</sup>;  $p = 0.03$ ) (Table S2).

### 3.2. Body mineral composition

The proximate composition of the whole body was analysed at the end of each growth phase (Table S3). Whole body lipid (Fig. 2A;  $p = 0.01$ ) and gross energy (Fig. 2B;  $p = 0.02$ ) at the end of phase I were reduced in Cu<sup>+</sup> groups; Zn had no effect in phase I. The proximate body composition was not affected by the dietary history of neither Zn nor Cu in phase II and III (Table S3). Whole body Zn levels at the end of phase I were higher in fish fed Zn<sup>+</sup> diets (Fig. 3A), whereas no significant differences were observed at the end of phase II (Fig. 3B). Dietary Zn restriction (Zn<sup>-</sup> diet) at first feeding increased the whole-body Zn levels at the end of phase III when fed the challenge diet (Fig. 3C). The concentration of Cu in the whole body was not influenced neither by direct feeding nor by the dietary history of Cu in any of the growth phases (Fig. 3D-F). Further data on the whole-body trace mineral concentration (Zn, Cu, Mn and Fe) expressed as fresh weight or as proportion in the ash is presented in Table S4. Dietary Mn concentrations were reduced in fish fed Zn<sup>+</sup> and Cu<sup>+</sup> until end of phase II, but the effect disappeared in phase III when all the groups were fed Zn-Cu- diet.

### 3.3. Plasma lipid metabolites

Lipid metabolites such as phospholipids (PL), Triacylglycerols (TAG), total cholesterol (Chol) and non-esterified fatty acids (NEFA) were analysed in the plasma at the end of week 24 (Fig. 4). Dietary Zn<sup>+</sup> history during first feeding reduced the level of PL (Fig. 4A), total cholesterol (Fig. 4C) and HDL (Fig. 4F) in the plasma. TAG, NEFA and LDL were not affected by the dietary treatments (Fig. 4B, D and E).

### 3.4. Lipid and fatty acids in the liver

The mean total lipid content in the liver at the end of phase III was 5% across the groups, of which the polar and neutral lipids were on an average 3.5% and 1.5%, respectively (Table S5). Neither the total lipids nor their fraction was affected by the dietary Zn or Cu history. Nevertheless, the fatty acid composition of the polar and neutral lipids was influenced by Zn or Cu history at first feeding ( $p < 0.05$ ). Among the fatty acids in polar lipids (Fig. 5), the proportion of 18:3n-6 ( $\gamma$ -Linolenic acid, GLA), 18:4n-3 (Stearidonic acid, SDA) and 22:5n-3 (Docosapentaenoic acid, DPA) were higher in fish with Zn<sup>-</sup> dietary history ( $p < 0.05$ ). Whereas, 20:2n-6 (Eicosadienoic acid, EDA) and 20:3n-3 (Eicosatrienoic acid, ETE) were higher in fish with Cu<sup>-</sup> dietary history ( $p < 0.05$ ). In the neutral lipids (Fig. 6), the proportion of 12:0 (Lauric acid), 15:0 (Pentadecanoic) and total saturated fatty acids ( $\Sigma$ SFA) were reduced in fish with Cu<sup>+</sup> dietary history ( $p < 0.05$ ). The complete fatty acid profile of polar and neutral lipid fractions in the liver are presented in Tables S6 and S7, respectively.

### 3.5. Liver mRNA expression

Data on the expression of genes involved in lipid metabolic pathways are presented in Table 3 for whole fry at week 6 and in Table 4 for the liver at week 24. Fatty acid biosynthesis genes such as *acly* and *acc* were downregulated in Zn<sup>+</sup> fed fish at both week 6 and 24 ( $p < 0.05$ ), but *fasn* was not affected. Genes involved in lipoprotein assembly and transport such as *atgl* and *mtp* were downregulated in Zn<sup>+</sup> fed fish with *apoa4* at week 6 and with *apoa1* at week 24, whereas other genes were not affected. Transcription of genes related to fatty acid desaturation (*d6d* and *d9d*), elongation (*elovl2* and *elovl5*), and transcription factors (*ppara*, *ppar $\beta$* , *ppar $\gamma$*  and *srebp1*) were not affected by dietary Zn or Cu at week 6. Dietary Zn<sup>+</sup> history downregulated *atgl*, *mtp* and *apoa1*; Cu<sup>+</sup> history upregulated *apoa4*; whereas, both Zn<sup>+</sup> and Cu<sup>+</sup> histories downregulated *apob* expression at week 24. The transcription of *d6d*, *d9d*, *elovl2* and *elovl5* were downregulated by Zn<sup>+</sup> dietary history. The transcription factor *ppara* expression was unaffected, Zn<sup>+</sup> history upregulated *ppar $\beta$* , and Cu<sup>+</sup> history respectively downregulated and upregulated *ppar $\gamma$*  and *srebp1*. Expression of *hmgcs* and *dhr7* in the liver were downregulated by dietary Zn<sup>+</sup> history at week 24.

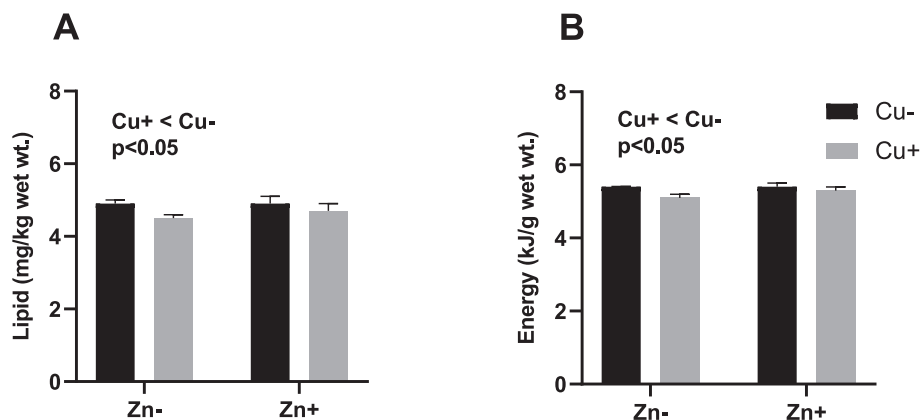
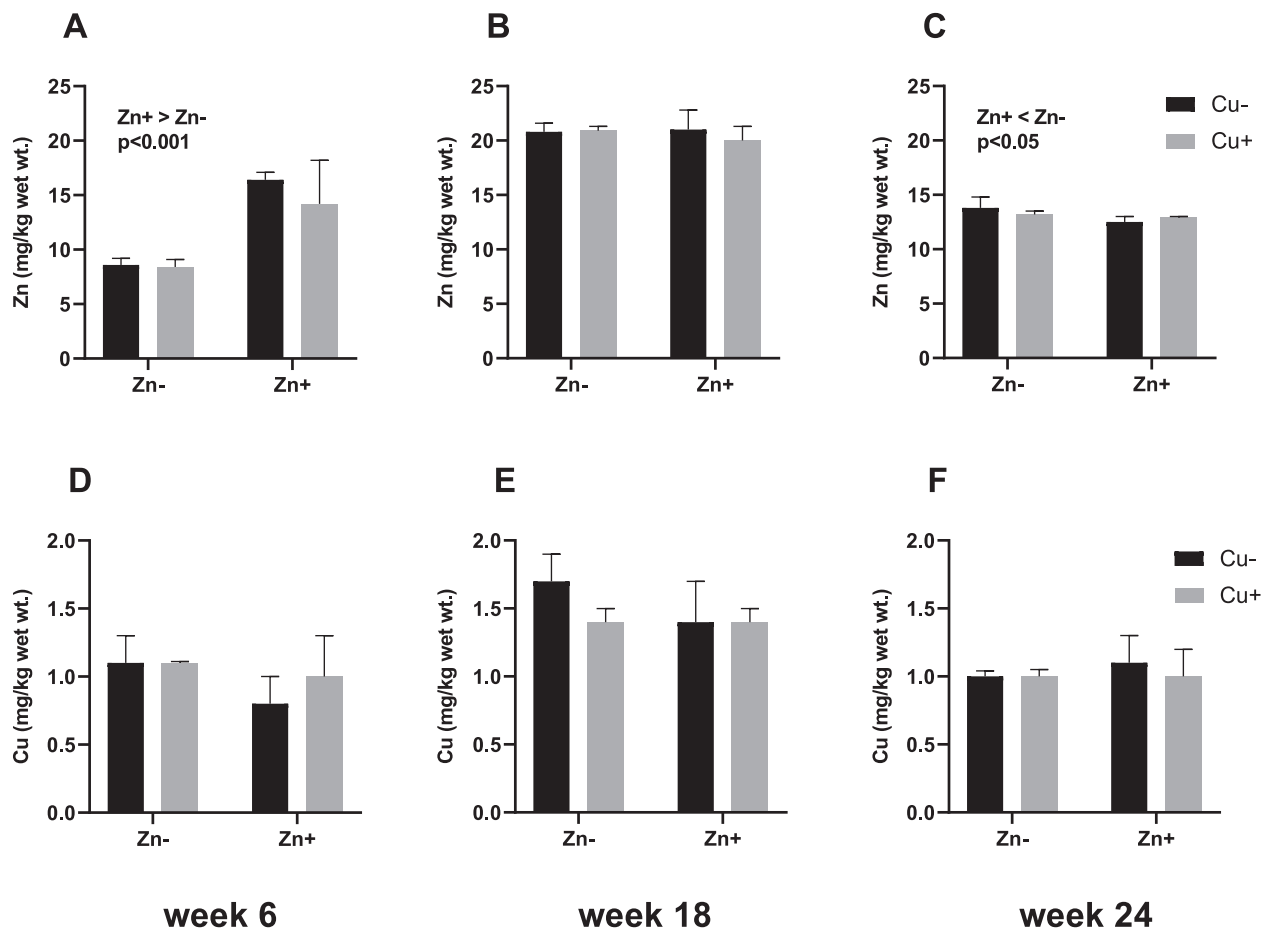


Fig. 2. Whole body lipid and gross energy content in rainbow trout fry at the end of phase I (week 6).

**F2 legend:** A. Whole body total lipid (mg/kg wet weight); B. whole body total energy (kJ/g wet weight). The data are presented as mean  $\pm$  SD ( $n = 3$ ). Data were analysed using two-way ANOVA with Zn or Cu inclusion as main factors, along with their interaction. The Zn inclusion levels Zn<sup>-</sup> and Zn<sup>+</sup> are separated along the X-axis, whereas the Cu inclusion levels Cu<sup>-</sup> and Cu<sup>+</sup> are denoted respectively as black and grey bars. Statistical significance ( $p < 0.05$ ) of main effect(s) is provided along with the nature of the effect (Cu<sup>+</sup> < Cu<sup>-</sup>).



**Fig. 3.** Whole body Zn and Cu concentration at the end of the different phases in the experiment.

**F3 legend:** A, B, C, represent data on whole body Zn concentration at the end of phase I (week 6), II (week 18) and III (week 24), respectively (mg/kg wet weight); D, E, F, represent data on whole body Cu concentration at the end of phase I (week 6), II (week 18) and III (week 24), respectively (mg/kg wet weight). The data are presented as mean  $\pm$  SD ( $n = 3$ ). Data were analysed using two-way ANOVA with Zn or Cu inclusion as main factors, along with their interaction. The Zn inclusion levels Zn- and Zn+ are separated along the X-axis, whereas the Cu inclusion levels Cu- and Cu+ are denoted respectively as black and grey bars. Statistical significance ( $p < 0.05$ ) of main effect(s) is provided along with the nature of the effect (Zn+ < or > Zn-).

## 4. Discussion

### 4.1. Growth and utilization of Zn and Cu in rainbow trout fry and juvenile

Feed intake and feed efficiency are signs of dietary nutrient limitation. Zn deficiency affected feed intake in rainbow trout fry fed unsupplemented diets or diets high in tricalcium phosphate (Satoh et al., 1992; Satoh et al., 1987). Increased feed intake and decreased feed efficiency in the Zn-Cu- group indicated that both Zn and Cu were limiting in the basal diet. Overall high survival and lack of differences among groups indicated that the Zn or Cu levels in the diets were not detrimentally deficient or toxic. Nevertheless, Zn restriction alone had a negative effect on growth and body status of Zn indicating of sub-optimal supply. Although the total Zn in the Zn- diets were above the recommended total dietary levels for rainbow trout (NRC, 2011), it proved to be sub-optimal as they could not support a higher growth rate or Zn status as with Zn+ diets. Poor availability of ingredient-bound Zn from plant-based diets being the likely possibility, however, a life stage specific higher Zn requirement for first-feeding fry also requires consideration. On the contrary, excess dietary Cu can lead to growth reduction (Berntssen et al., 1999a; Kamunde et al., 2002) and excess dietary Zn can inhibit Cu uptake and retention (Knox et al., 1984; Knox et al., 1982), neither of which were observed in this study. The growth retardation and lower whole-body Zn status in Zn- groups induced by sub-optimal Zn in phase I

appeared to be transient and reversible in phase II when fed with a commercial feed with higher Zn levels. The reoccurrence of Zn- stressor at juvenile stage only revoked the negative growth effects from the past, if any.

Nutritional programming is referred to as when a stress in early life enables the animal to adapt better to the same stressor when encountered in later life (Panserat et al., 2019). Nutritional programming through broodstock diets improved utilization of very low fishmeal and fish oil diets in gilthead sea bream (Izquierdo et al., 2015). Rainbow trout fry fed plant-based diets at first feeding had better utilization of the same when encountered as juveniles (Balasubramanian et al., 2016). Feed intake and feed efficiency are good indicators of a programming effect from an early stage nutritional intervention (Geurden et al., 2007). In the present study, fish with Zn+ dietary history seemed better adapted to counter the Zn- challenge at juvenile stage and achieve significantly higher growth. The Zn+ history fish achieved better growth when fed Zn- diets as juveniles through increased feed intake (12% more), although the feed efficiency was reduced by 5%. Similar responses in feed intake and feed efficiency have been reported in response to plant broodstock diet based selective breeding in rainbow trout (Geurden et al., 2007; Yamamoto et al., 2023). A potential role of Zn in nutritional or broodstock programming was identified through low whole body Zn status in rainbow trout fry obtained from broodstock selectively bred for better utilization of plant based feeds (Yamamoto et al., 2023). Plant-based starter diets with up to 85 mg/kg Zn enabled whole body Zn

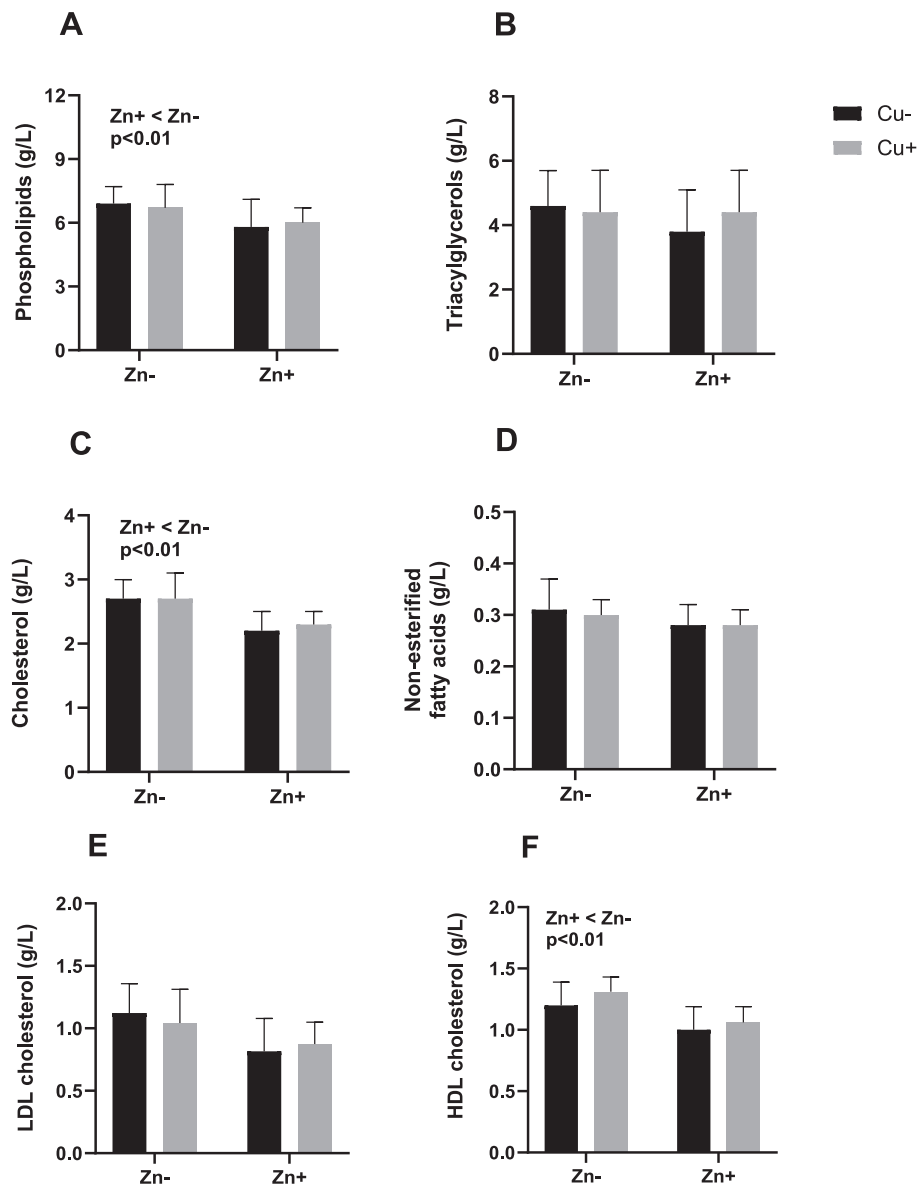


Fig. 4. Plasma lipid metabolites in juvenile rainbow trout at the end of phase III (week 24).

**F4 legend:** A. Phospholipids (g/L); B. Triacyl glycerides (g/L); C. Cholesterol (g/L); D. Non-esterified fatty acids (NEFA, g/L); E. Low density lipoprotein (LDL, g/L); F. High density lipoprotein (HDL, g/L). Data on plasma lipid metabolites were treated as individual observations from each fish in a mixed-effect model ( $n = 9$ ), Zn or Cu inclusion as main factors along with their interaction, wherein the tanks were considered a random factor. The Zn inclusion levels Zn- and Zn+ are separated along the X-axis, whereas the Cu inclusion levels Cu- and Cu+ are denoted respectively as black and grey bars. Statistical significance ( $p < 0.05$ ) of main effect(s) is provided along with the nature of the effect (Zn+ < or > Zn-).

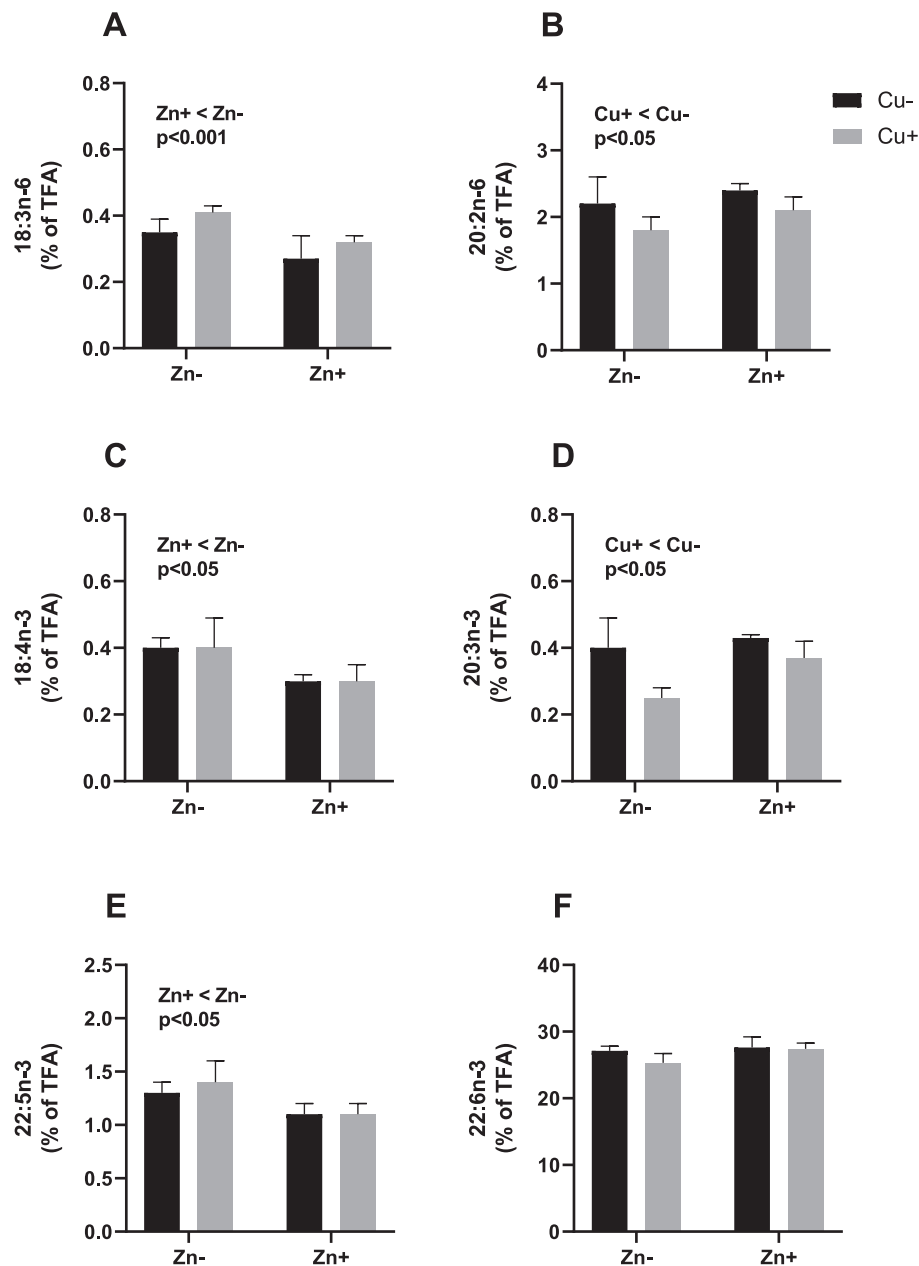
levels (17.5 mg/kg) comparable to Zn+ fry (16.4 mg/kg) in the present study. However, increased Zn supplementation did not increase the utilization of plant-based diets in juvenile stage (Yamamoto et al., 2022). However, in the present study, Zn+ history increased feed intake and growth of rainbow trout juveniles when faced with the Zn- challenge. Although the objectives of this study and that of Yamamoto et al. (2022) were different, both highlight the significance and potential of early-stage Zn nutrition for later life stage in rainbow trout. Optimal nutrition at a critical life stage can have beneficial effect in a later stage (Panserat et al., 2019). Increased dietary Zn supply during freshwater or early seawater phase in Atlantic salmon improved seawater tolerance or bone/eye health in later stage, respectively (Sartipi Yarahmadi et al., 2022). Further, increased micro-nutrient supply in freshwater phase improved health and seawater tolerance of Atlantic salmon post-smolts (Espe et al., 2020; Holen et al., 2022; Philip et al., 2022; Sissener et al., 2021; Vera et al., 2020). More recently, increased Zn and Se supply

during early seawater phase decreased cataract and vertebral anomalies in harvest size Atlantic salmon (Philip et al., 2023). In this regard, further understanding on the role of nutritional programming or selective breeding for improved zinc utilization in salmonids can have beneficial effects for growth and health.

#### 4.2. Lipid metabolic changes in first-feeding fry as affected by dietary Zn or Cu

Lipid metabolites in plasma, hepatic fatty acid composition and expression of genes involved in lipid metabolism were influenced by dietary Zn or Cu as well as their early life nutritional history. Although Zn is not a direct participant in the lipogenesis/lipolysis process (Dieck et al., 2005), insufficient Zn intake has been linked to intestinal or hepatic fat accumulation in mammals and teleost (Eder and Kirchgessner, 1993; Luo et al., 2011; Mangray et al., 2015; Zheng et al., 2015).



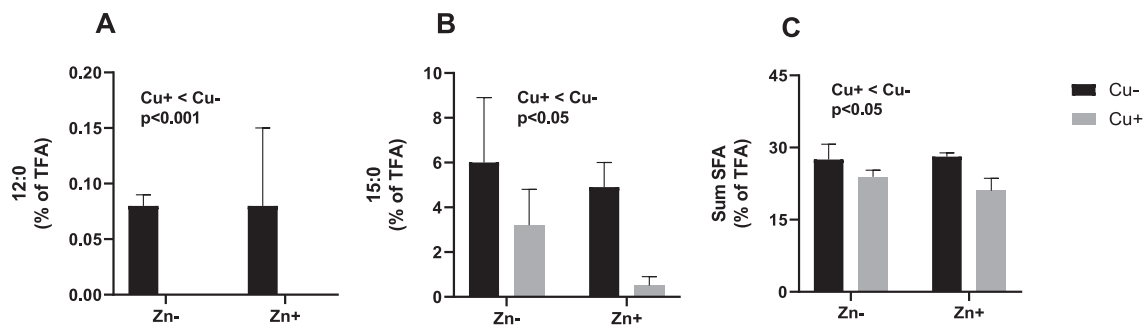


**Fig. 5.** Selected LC-PUFA composition of liver (polar lipids) in rainbow trout at the end of phase III (week 24). The complete fatty acid profile can be found in supplementary table S6.

**F5 legend:** A.  $\gamma$ -Linolenic Acid (18:3, n-6); B. Eicosadienoic acid (20:2, n-6); C. Stearidonic acid (18:4, n-3); D. Eicosatrienoic acid (20:3, n-3); E. Docosapentaenoic acid (22:5, n-3); F. Docosahexaenoic acid (22:6, n-3). The data are presented as mean  $\pm$  SD (n = 3). Data were analysed using two-way ANOVA with Zn or Cu inclusion as main factors, along with their interaction. The Zn inclusion levels Zn- and Zn+ are separated along the X-axis, whereas the Cu inclusion levels Cu- and Cu+ are denoted respectively as black and grey bars. Statistical significance ( $p < 0.05$ ) of main effect(s) is provided along with the nature of the effect (Zn+ < or > Zn- [OR] Cu+ < or > Cu-). When the interaction effect was significant (F), one-way ANOVA was performed along with Tukey's post-hoc analysis and the difference between groups were denoted using different superscript letters. Groups sharing the same letters are not statistically significant from each other.

Juvenile yellow catfish fed Zn-deficient diet accumulated intestinal fat by the upregulation of lipogenic enzyme activity and the down-regulation of lipolytic enzymatic genes (Chen et al., 2017). However, as the Zn levels in the Zn- diets were not deficient but only sub-optimal, neither an accumulation nor an increase in tissue or body lipid levels were observed. Transcriptome and proteome analysis also revealed that excessive hepatic fat accumulation in Zn-deficient rats is caused by decreased lipolysis and increased synthesis of fatty acids and TAG (Dieck et al., 2005). Similarly, the present study found that two essential fatty acids biosynthesis genes (*acly* and *acc*) (Hillgartner et al., 1995) were markedly upregulated in response to sub-optimal dietary Zn

supplementation (phase I). However, the mRNA expression level of lipid metabolic transcription factors (*ppars* and *srebp1*) were not affected by dietary Zn, in contrast to yellow catfish (Zheng et al., 2015), possibly owing to different growth stages. On the other hand, the present investigation showed that genes associated with lipoprotein assembly and transport (*mtp* and *apoA4*) were up-regulated in the Zn- treatment group (phase I). *Mtp* is required for the synthesis of apolipoproteins (Davis, 1999), whereas *apoA4* is predominantly expressed in enterocytes for chylomicron (CM) synthesis and in the liver for VLDL synthesis (Sundaram and Yao, 2012). CM and VLDL are responsible for transporting TAG from enterocytes and hepatocytes into plasma, respectively



**Fig. 6.** Selected SFA composition of liver (neutral lipids) in rainbow trout at the end of phase III (week 24). The complete fatty acid profile can be found in supplementary table S7.

**F6 legend:** A. Lauric acid (12:0); B. Pentadecanoic Acid (15:0); C. Saturated fatty acids, total (sum SFA). The data are presented as mean  $\pm$  SD ( $n = 3$ ). Data were analysed using two-way ANOVA with Zn or Cu inclusion as main factors, along with their interaction. The Zn inclusion levels Zn- and Zn+ are separated along the X-axis, whereas the Cu inclusion levels Cu- and Cu+ are denoted respectively as black and grey bars. Statistical significance ( $p < 0.05$ ) of main effect(s) is provided along with the nature of the effect (Cu+ < or > Cu-).

(Feingold, 2021). Previous investigation had demonstrated that dietary Zn-deficiency reduced the apo-B levels in rats, thereby inhibiting the CM formation as well as intestinal absorption of dietary lipids (Lee et al., 1986). In contrast, the present study found an upregulation of lipoprotein assembly and transport-related genes in the Zn- group, which may be a compensatory response to the elevated intestinal and hepatic fatty acid biosynthesis.

Excess dietary Cu can be pro-oxidant and induce lipid peroxidation both intracellular and in the membranes (Blades et al., 2021a; Poyton et al., 2016; Wagner and Heinecke, 1997). Expression of lipogenic genes was suppressed by excess Cu in mammal and teleost (Blades et al., 2021b; Meng et al., 2018), but this effect was not observed in the present study at the end of phase I. In rats, dietary Cu supplementation was not accompanied by changes in the mRNA expression of lipoprotein-related

genes in the liver and intestine (Mazur et al., 1992), which is consistent with our results. Nevertheless, dietary Cu+ intervention reduced whole body lipid and gross energy content in first-feeding fry similar to the observations in other fish species (Abdel-Hameid et al., 2017; Tseng et al., 2023). Further, Cu+ induced changes were also seen in the saturated fatty acid levels in hepatic neutral lipid fraction. Despite the Cu levels in the Cu+ diets being lower than the maximum permitted EU limits of 25 mg/kg diet for fish feeds, increased SFA/PUFA ratio in polar lipids observed in the fry, might be an indication of increased oxidation of PUFA. Nevertheless, Cu+ induced lower body lipid levels were transient and reversible, as there was no difference in whole body lipid among groups at juvenile stage, the end of phase II or III.

**Table 3**

Gene expression in whole fish at the end of phase I (week 6).

	Treatment				Statistic		
	Zn-Cu-	Zn+Cu-	Zn-Cu+	Zn+Cu+	Zn	Cu	Zn*Cu
<b>Fatty acid biosynthesis</b>							
<i>acly</i>	1.02 $\pm$ 0.25	0.84 $\pm$ 0.49	1.19 $\pm$ 0.36	0.91 $\pm$ 0.21	*	ns	ns
<i>acc</i>	1 $\pm$ 0.04	0.85 $\pm$ 0.1	1 $\pm$ 0.19	0.91 $\pm$ 0.14	*	ns	ns
<i>fasn</i>	1.03 $\pm$ 0.28	1.1 $\pm$ 0.54	1.07 $\pm$ 0.43	1.04 $\pm$ 0.35	ns	ns	ns
<b>Lipoprotein assembly and transport</b>							
<i>atgl</i>	1.02 $\pm$ 0.24	0.75 $\pm$ 0.23	1.02 $\pm$ 0.33	0.9 $\pm$ 0.18	*	ns	ns
<i>mtp</i>	1.03 $\pm$ 0.25	0.77 $\pm$ 0.23	1.02 $\pm$ 0.34	0.9 $\pm$ 0.18	*	ns	ns
<i>apoa1</i>	1.02 $\pm$ 0.25	0.9 $\pm$ 0.2	0.92 $\pm$ 0.24	0.82 $\pm$ 0.15	ns	ns	ns
<i>apoa4</i>	1.04 $\pm$ 0.31	0.75 $\pm$ 0.29	1.06 $\pm$ 0.35	0.85 $\pm$ 0.26	*	ns	ns
<i>apob</i>	1.02 $\pm$ 0.23	0.91 $\pm$ 0.32	0.89 $\pm$ 0.28	0.87 $\pm$ 0.32	ns	ns	ns
<b>Fatty acid elongation and desaturation</b>							
<i>d9d</i>	1 $\pm$ 0.14	1 $\pm$ 0.24	0.96 $\pm$ 0.18	0.96 $\pm$ 0.11	ns	ns	ns
<i>d6d</i>	1.01 $\pm$ 0.15	0.82 $\pm$ 0.29	0.99 $\pm$ 0.31	0.98 $\pm$ 0.16	ns	ns	ns
<i>elovl2</i>	1.02 $\pm$ 0.23	1.02 $\pm$ 0.37	1 $\pm$ 0.32	1.21 $\pm$ 0.2	ns	ns	ns
<i>elovl5</i>	1.02 $\pm$ 0.22	0.86 $\pm$ 0.34	1.1 $\pm$ 0.39	0.92 $\pm$ 0.24	ns	ns	ns
<b>Transcription factors</b>							
<i>ppara</i>	1.03 $\pm$ 0.28	0.95 $\pm$ 0.25	1.03 $\pm$ 0.25	1.01 $\pm$ 0.27	ns	ns	ns
<i>pparβ</i>	1.08 $\pm$ 0.46	1.02 $\pm$ 0.43	1.24 $\pm$ 0.44	1.17 $\pm$ 0.66	ns	ns	ns
<i>pparγ</i>	1.01 $\pm$ 0.2	0.84 $\pm$ 0.18	1.09 $\pm$ 0.32	1.05 $\pm$ 0.42	ns	ns	ns
<i>srebp1c</i>	1.01 $\pm$ 0.19	1.06 $\pm$ 0.44	0.98 $\pm$ 0.36	1.11 $\pm$ 0.16	ns	ns	ns

*acly*, ATP citrate synthase; *acc*, acetylCoA carboxylase; *fasn*, fatty acid synthase; *atgl*, adipose triglyceride lipase; *mtp*, microsomal transfer protein; *apoa1*, *apoa4* and *apob*, apolipoprotein A1, A4 and B; *d9d*, stearoyl-CoA desaturase; *d6d*,  $\Delta 6$ -desaturase; *elovl2*, elongation of long chain fatty acids protein 2; *elovl5*, elongation of very long-chain fatty acids like-5; *ppara*, *pparβ* and *pparγ*, peroxisome proliferator-activated receptor  $\alpha$ ,  $\beta$  and  $\gamma$ ; *srebp1c*, sterol regulatory element-binding 1c. Data presented as mean  $\pm$  SD ( $n = 9$ ). Data analysis were performed using individual observations from each fish in a mixed-effect model ( $p < 0.05$ ), Zn or Cu inclusion as main factors along with their interaction, wherein the tanks were considered a random factor.

**Table 4**  
Hepatic gene expression at the end of phase III (week 24).

	Treatment				Statistic		
	Zn-Cu-	Zn+Cu-	Zn-Cu+	Zn+Cu+	Zn	Cu	Zn*Cu
<b>Fatty acid biosynthesis</b>							
<i>acly</i>	1.3 ± 0.77	0.85 ± 0.42	1.32 ± 0.9	0.92 ± 0.34	*	ns	ns
<i>acc</i>	1.17 ± 0.39	0.77 ± 0.3	0.95 ± 0.21	0.73 ± 0.41	*	ns	ns
<i>fasn</i>	1.19 ± 0.82	0.98 ± 0.58	1.23 ± 0.67	1.2 ± 0.52	ns	ns	ns
<b>Lipoprotein assembly and transport</b>							
<i>atgl</i>	1.16 ± 0.35	0.72 ± 0.17	0.83 ± 0.23	0.71 ± 0.31	*	ns	ns
<i>mtp</i>	1.12 ± 0.2	0.81 ± 0.28	0.86 ± 0.19	0.76 ± 0.29	*	ns	ns
<i>apoa1</i>	1.04 ± 0.32	0.65 ± 0.26	0.95 ± 0.39	0.76 ± 0.62	*	ns	ns
<i>apoa4</i>	1.13 ± 0.64	1.06 ± 0.52	1.52 ± 0.85	1.42 ± 0.94	ns	*	ns
<i>apob</i>	1.07 ± 0.42	0.84 ± 0.2	0.71 ± 0.17	0.68 ± 0.42	ns	ns	*
<b>Fatty acid elongation and desaturation</b>							
<i>d9d</i>	2.22 ± 2.01	0.44 ± 0.22	1.56 ± 1.05	0.81 ± 0.69	*	ns	ns
<i>d6d</i>	1.35 ± 0.71	0.64 ± 0.36	0.97 ± 0.28	0.52 ± 0.36	*	ns	ns
<i>elovl2</i>	1.37 ± 0.72	0.71 ± 0.54	1.11 ± 0.5	0.71 ± 0.42	*	ns	ns
<i>elovl5</i>	1.2 ± 0.38	0.67 ± 0.22	0.93 ± 0.23	0.78 ± 0.35	*	ns	ns
<b>Transcription factors</b>							
<i>ppara</i>	1.12 ± 0.52	1.04 ± 0.34	1.01 ± 0.31	1.24 ± 0.82	ns	ns	ns
<i>pparβ</i>	0.94 ± 0.65	1.2 ± 0.36	0.62 ± 0.32	0.91 ± 0.5	*	ns	ns
<i>pparγ</i>	1.06 ± 0.73	0.9 ± 0.27	0.73 ± 0.16	0.68 ± 0.24	ns	*	ns
<i>srebp1</i>	1 ± 0.7	1 ± 0.4	1.44 ± 0.69	1.39 ± 0.63	ns	*	ns
<b>Cholesterol biosynthesis</b>							
<i>hmgcs</i>	1.13 ± 1.04	0.33 ± 0.24	0.71 ± 0.98	0.22 ± 0.18	*	ns	ns
<i>dhcr7</i>	1.12 ± 0.76	0.76 ± 0.25	0.86 ± 0.51	0.63 ± 0.23	*	ns	ns
<b>Glucose metabolism</b>							
<i>pck1</i>	1.16 ± 0.66	1.13 ± 0.63	0.8 ± 0.33	0.79 ± 0.54	ns	*	ns

*acly*, ATP citrate synthase; *acc*, acetylCoA carboxylase; *fasn*, fatty acid synthase; *atgl*, adipose triglyceride lipase; *mtp*, microsomal transfer protein; *apoa1*, *apoa4* and *apob*, apolipoprotein A1, A4 and B; *d9d*, stearoyl-CoA desaturase; *d6d*, Δ6-desaturase; *elovl2*, elongation of long chain fatty acids protein 2; *elovl5*, elongation of very long-chain fatty acids like-5; *ppara*, *pparβ* and *pparγ*, peroxisome proliferator-activated receptor α, β and γ; *srebp1c*, sterol regulatory element-binding 1c. *hmgcs*, HMG-CoA synthase; *dhcr7*, 7-dehydrocholesterol reductase; *pck1*, phosphoenolpyruvate carboxykinase 1. Data presented as mean ± SD (n = 9). Data analysis were performed using individual observations from each fish in a mixed-effect model (p < 0.05), Zn or Cu inclusion as main factors along with their interaction, wherein the tanks were considered a random factor.

#### 4.3. Lipid metabolic programming through early-stage Zn- dietary history

Rainbow trout juveniles with Zn- history exhibited a considerable increase in the expression of hepatic fatty acids biosynthesis genes (*acly* and *acc*) and lipoprotein assembly (*mtp*), demonstrating the programmed regulation of Zn- history in lipid metabolism. Compared to Zn+ history group, the higher level of plasma PL in the Zn- history group (phase III) may be partly contributed to the higher hepatic fatty acids biosynthesis capacity or growth. In mammals, dietary Zn deficiency suppresses hepatic *apoa1* mRNA expression and reduces plasma HDL levels (Bedi et al., 1981; Wu et al., 1998). The present study found that *apoa1*, coding for a main structural protein of HDL (Sundaram and Yao, 2012), was upregulated by dietary Zn- history, which might contribute to the adaptation of juvenile rainbow trout responding to dietary Zn-stress as first-feeding fry. The genes *hmgcs* and *dhcr7* are essential for cholesterol biosynthesis (Chittur et al., 2008). Zn-deficient rats raised plasma cholesterol level and intestinal HMGCS enzyme activity, but had no effect on hepatic HMGCS enzyme activity (Eder et al., 1999; Gebhard et al., 1983). In the present study, compared to the Zn+ history group, the Zn- dietary history groups significantly increased the plasma cholesterol and the gene expression for hepatic cholesterol biosynthesis in rainbow trout juveniles. These results indicate a programming effect of dietary Zn in cholesterol biosynthesis of rainbow trout.

Fish can acquire fatty acids from their diet, and diet generally influences tissues PUFA profiles (Ng et al., 2013; Xu et al., 2014). In addition to dietary fatty acid composition, PUFA biosynthesis capacity of fish plays a crucial role in determining the fatty acid profile of tissue

(Xie et al., 2021). The difference in the hepatic fatty acids profiles between groups in the present study can be correlated to the PUFA synthesis capacity. Several hepatic metabolic pathways involve Zn as cofactors and can influence fatty acid metabolism in mammals (Petering et al., 1977; Salgueiro et al., 2001). Zn is a cofactor for desaturase and/or elongase enzymes (Cunnane, 1988) and plasma Zn-status influenced fatty acid desaturation and/or elongation in humans (Chimhashu et al., 2018). Similarly, dietary Zn deficiency significantly impairs the desaturation capacity of linoleic acid (18:2 n-6) in rats (Cunnane et al., 1984). In mud crabs, dietary Zn increased hepatopancreas mRNA expression levels of Δ6 desaturases (*d6d* or *fad*) and Σ n-3 PUFA content than those in the low Zn diet group (Luo et al., 2021). It is well known that *d9d* is the key rate-limiting enzyme for the desaturation of saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0), whereas *d6d* and *elovl5* are essential for the conversion of dietary α-linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6) into LC-PUFA (Geerling et al., 1999; Gregory and James, 2014). Interestingly, juvenile trout with Zn- dietary history had significantly higher hepatic mRNA expression of *d9d*, *d6d*, *elovl2* and *elovl5*, despite not affected by dietary Zn at first-feeding fry stage. In addition, higher γ-linolenic acid (18:3, n-6), stearidonic acid (18:4, n-3) and DPA (22:5n-3) content of hepatic polar lipids was also observed in the Zn- history group. These results indicate that Zn restriction during the first-feeding stage can program the hepatic PUFA biosynthesis capacity when faced with sub-optimal dietary Zn levels as juveniles.

Programming effects on the metabolic regulation, utilization or adaptive response to a particular challenge at first feeding namely

glucose or carbohydrate (Geurden et al., 2007), methionine (Séité et al., 2019), PUFA (Izquierdo et al., 2015; Vagner et al., 2009) plant-based feeds (Balasubramanian et al., 2016; Yamamoto et al., 2023; Yamamoto et al., 2020) or hypoxic stress (Liu et al., 2017) are known in fish. In these studies, two different types of programming were observed, (i) a stimulus or challenge programming the metabolic regulation or utilization of the same (eg. glucose or plant-based feed); or (ii) a stimulus that programs other metabolic pathways (eg. hypoxia on glucose utilization). In the present study, (i) the improved feed intake and growth of Zn<sup>+</sup> history group when subjected to sub-optimal Zn<sup>-</sup> challenge as juveniles showed better capacity to adapt and grow under sub-optimal Zn supply. Further, the higher PUFA content and mRNA expression of genes central to desaturation or elongation in the liver showed a programming effect of early-stage Zn<sup>-</sup> history on PUFA biosynthesis capacity.

## 5. Conclusion

To conclude, in plant-based feeds, dietary Zn restriction (Zn<sup>-</sup>) at first feeding reduced the growth of rainbow trout fry and dietary Cu excess (Cu<sup>+</sup>) reduced body lipid and energy content. These effects were temporary and reversible when fed optimal levels. Nevertheless, when re-subjected to the Zn<sup>-</sup> challenge as juveniles, early-stage Zn<sup>-</sup> dietary history induced growth retardation but exhibited signs of improved PUFA and cholesterol biosynthesis capacity in the liver.

## Funding and acknowledgement

This study was financed by AQUAEXCEL2020, a Horizon 2020 research infrastructure project under the TNA grant #AE060014. In addition to the AQUAEXCEL2020 TNA funding, this study was further supported from IMR, Norway and Nofima, Norway in the form of base funding to the lead researcher (Antony J Prabhu Philip). We are thankful to the contributions of technical staff at INRAE (i) Donzac experimental station and (ii) laboratories at St-pee-sur-Nivelle. Frédéric Terrier, Anthony Lanuque and Franck Sandres (diet preparation and fish rearing), and Lucas Bouakaz, Elisabeth Plagnes-Juan, Marius Bidon and Alexia Soisson (molecular analyses).

## CRedit authorship contribution statement

**Antony Jesu Prabhu Philip:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **HaoHang Fang:** Writing – review & editing, Writing – original draft, Formal analysis. **Laurence Larroquet:** Validation, Resources, Methodology, Formal analysis, Data curation. **Anne Surget:** Validation, Methodology, Formal analysis, Data curation. **Alexandre Herman:** Methodology, Formal analysis, Data curation. **Stéphanie Fontagné-Dicharry:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

None.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2024.741207>.

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