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Dietary electrolyte balance of Atlantic salmon (*Salmo salar*) freshwater feeds: Impact on osmoregulation, mineral metabolism and performance in seawater

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ABSTRACT

Dietary electrolyte balance is the equilibrium of monovalent cations and anions that influence the acid-base balance of the feed (dEB = Na + K − Cl, mEq kg^{-1}). Dietary electrolytes/minerals can influence the physiological changes during smoltification in Atlantic salmon. In this context, we aimed to study if the dEB of the freshwater feeds can be used to pre-adapt the hypoosmotic functionality and the associated effects on mineral metabolism. The dEB of commercial freshwater Atlantic salmon feeds in Norway varied from −9 to 400 mEq kg^{-1} feed. Three experimental feeds were formulated to study incremental levels of dEB reflecting the low (L-dEB, −50 to 0), median (M-dEB, 200–250) and high (H-dEB, 350–400). Triplicate groups of Atlantic salmon parr (36 g) were fed one of the three feeds for 8 weeks in freshwater at 12 °C. The fish were transferred to full strength seawater in indoor tanks and fed a commercial diet for 6 weeks. Growth was not differentially affected by dEB levels, neither in the freshwater phase nor in the seawater. Plasma electrolytes (Na+ and Cl−) and gill mRNA expression of sodium potassium ATPase (NKA a1b, seawater isoform) were significantly lower in L-dEB fed fish. In the intestine, carbonate precipitates 24 h after seawater transfer was higher in fish fed both L-dEB and H-dEB feeds compared to the M-dEB fed fish. Whole body and plasma mineral levels were significantly affected by dEB levels in freshwater feeds. Interestingly, the carryover effect of dEB in freshwater feeds was significant after 6 weeks in seawater for plasma and whole-body Zn status, with the H-dEB fed fish showing significantly increased body Zn status compared to L-dEB and M-dEB fed fish. The study revealed that mineral metabolism and intestinal response to seawater transfer can be pre-adapted by modulating the electrolyte and/or mineral balance in freshwater feeds in Atlantic salmon. Further, dEB did not affect long term development of cataract or vertebral deformities.

1. Introduction

Atlantic salmon has an anadromous life cycle migrating between freshwater and marine environments. Smoltification, the physiological metamorphosis of the salmon parr into smolt largely determines the success of transition from life in freshwater to seawater (Langdon, 1985). The smoltification process is under the regulation of various endocrine and environmental factors (Zaugg, 1982; Brauer, 1982; McBride et al., 1982). Among the environmental factors, dietary minerals have a role to play in the development of hypoosmotic ability of fish moving to seawater (Zaugg, 1982). Salmonids fed a mineral enriched diet show improved salinity tolerance and survival upon seawater transfer (Basulto, 1976; Pellertier and Besner, 1992; Zaugg et al., 1983). The underlying mechanism was identified to be salt-induced structural and molecular re-modelling of the gills into a partial seawater phenotype with enhanced expression of ion transporters in

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the gills (Eroldo et al., 2005; Harpaz et al., 2005; Pellertier and Besner, 1992; Zaugg et al., 1983; Basulto, 1976; Perry et al., 2006). The intestine is equally important in salt and water balance, especially in saltwater fish (Grosell, 2010; Whittamore, 2012; Wilson et al., 2002). Insufficient preparedness of the intestine to the change from freshwater to seawater coinciding with an altering osmotic challenge affected the seawater performance in Atlantic salmon (Vargas-Lagos et al., 2018). However, little is known about the impact of dietary salt induced regulation of osmoregulatory responses in the intestine. Given the increasing evidence on the osmoregulatory importance of mineral precipitation in the intestine of marine teleosts (Perry et al., 2011; Salter et al., 2018), and in Atlantic salmon (McKay et al., 2020), its dietary regulation is of significance in Atlantic salmon aquaculture.

Dietary electrolyte balance (dEB = Na + K − Cl, mEq kg^{-1} of diet) is the state of equilibrium between the monovalent cations and anions that influence the acid-base balance in the feed (Sauvant et al., 2004). The dEB characterizes the acidifying or alkalising potential of the feed, wherein potassium (K^+) and sodium (Na^+) are alkalising, while chloride (Cl^-) is acidifying. In the past, dEB calculated from the mineral data of commercial fish feeds in Europe ranged between 200 and 400 mEq kg^{-1} (Tacon and De Silva, 1983). Lack of more recent data does not allow us to understand how the evolution of feed composition in the past 40 years has impacted the dEB of fish feeds. Although data on Na and K can be found in the literature, data on feed chloride levels are seldom reported. Therefore, it is required to understand the dEB levels in modern day Atlantic salmon feeds, and more so with the renewed interest in feed based smolt production strategies. In fish, literature on dEB is very limited and shown to affect acid-base homeostasis (Chiu et al., 1984), amino acid metabolism and requirements (Chiu et al., 1988; Chiu et al., 1984), feed intake, growth, nutrient and energy utilization (Saravanan et al., 2013) and digestion (Magnoni et al., 2017). However, influence of dEB on micro-mineral metabolism has not been studied. Differential regulation of intracellular electrolyte levels in freshwater- and seawater-adapted coho salmon indicated that electrolyte homeostasis, osmoregulation and mineral metabolism in salmonids are closely related (Marini and Kerstetter, 1982). Further, mild cataracts often described as 'osmotic cataracts' are associated with osmotic imbalance (Bjerkås and Sveier, 2004). Therefore, a study was designed to investigate the effect of dEB of freshwater feed on direct effects to Atlantic salmon in freshwater and the impact of dEB history on short- and long-term effects on nutrient metabolism, osmoregulation and production disorders after seawater transfer. It was hypothesized that change in dEB can affect the mineral metabolism of Atlantic salmon smolt and bring about unintended short- and long-term effects.

2. Material and methods

2.1. Formulation and production of experimental feeds

Our preliminary data from the Norwegian fish feed surveillance program suggested that commercial Atlantic salmon feeds in Norway might have a large variation in dEB (Sissener et al., 2013). To validate this, samples of 13 commercially available freshwater Atlantic salmon feeds were analysed for electrolytes (Na^+, K^+ and Cl-) to calculate the dEB of these feeds; other macro-minerals (P, Ca and Mg) were also analysed. The dEB (mEq kg^{-1}) was calculated based on the following formulae, $dEB = (Na^+ + K^+) - (Cl^-)$, where Na^+, K^+ and Cl^- were expressed in mEq kg^{-1} feed (Sauvant et al., 2004). Three experimental feeds were formulated to study incremental levels of dEB (mEq kg^{-1}) reflecting the low (L-dEB, −50 to 0), median (M-dEB, 200–250) and high (H-dEB, 350–400) range observed in commercial feeds. The basal feed mix contained 25% fish meal and 10% fish oil along with other macro- and micro- ingredients to satisfy the nutrient requirements and resemble the commercial feeds for Atlantic salmon at this life stage (Table 1). The M-dEB diet was produced from the basal mix with a calculated dEB content of 281 mEq kg^{-1}. The L-dEB and H-dEB diets were formulated to have a dEB of 0 and 450 mEq kg^{-1}, respectively, which was achieved by adding respectively 1.6% $CaCl_2$ and 0.9% Na_2CO_3 to the basal mix. The feeds were produced as extruded sinking pellets in 2 mm and 3 mm diameter to accommodate for the change in pellet size as the fish grew. The experimental feeds were formulated and produced at Skretting ARC, Stavanger, Norway. The analysed proximate composition, mineral concentration and dEB of the diets are provided in Table 2.

Table 1
Ingredient composition of the experimental diets.

	L-dEB	M-dEB	H-dEB
Wheat[a]	16.5	18.9	17.6
Wheat gluten[b]	3.91	3.50	3.73
Soy protein concentrate[c]	26.5	26.5	26.5
Pea protein concentrate[d]	9.00	9.00	9.00
Fish meal[e]	25.0	25.0	25.0
Rapeseed oil[c]	4.57	4.56	4.56
Fish oil[f]	10.0	9.97	9.99
Water	0.99	0.72	0.88
Taurine[g]	0.13	0.13	0.13
L-Lysine[h]	0.64	0.64	0.64
L-Threonine[i]	0.07	0.06	0.06
Calcium chloride	1.62	0.00	0.00
Sodium carbonate	0.00	0.00	0.90
Vit B6[i]	0.01	0.01	0.01
Vit B12[i]	0.01	0.01	0.01
NRC vitamin premix[i]	0.10	0.10	0.10
Mineral premix[i]	0.10	0.10	0.10
Yttrium premix[j]	0.10	0.10	0.10
Astaxanthin 10%[k]	0.05	0.05	0.05
Other microingredients[l]	0.70	0.71	0.71

[a] Fiskå mølle, Forus, Norway.
[b] Cargill Nordic AS, Søborg, Denmark.
[c] European Commodity Company S.A., Luxembourg.
[d] AM Nutrition, Stavanger, Norway.
[e] Norsildmel, Bergen, Norway.
[f] P/F Havsbrun, Fuglafjørdur, Faroe Islands.
[g] Orffa, Bornem, Belgium.
[h] Meihua Group International Trading, Hong Kong.
[i] Trouw Nutrition Netherlands BW, Putten, Netherlands, propriety of Skretting ARC.
[j] Trouw Nutrition Netherlands BW, Putten, Netherlands, the mix provides yttrium oxide in a concentration of 100 mg kg^{-1} feed, while the product also contains sepiolite (E562, 472.1 g kg^{-1} yttrium mix), calcium (109.1 g kg^{-1}), magnesium (64.9 g kg^{-1}), sodium (2.8 g kg^{-1}), potassium (4.5 g kg^{-1}), iron (6.8 g kg^{-1}), moisture (149.2 g kg^{-1}).
[k] Divi's laboratories Europe B·V, Basel, Switzerland.
[l] The contents and concentration are not disclosed as it contains commercially relevant information.

2.2. Study design, rearing conditions and sampling

The study was conducted according to the guidelines of the Norwegian State Commission for Laboratory Animals and the experimental protocols were approved by The National food safety authority (identification number: ID 15465). The study involved a feeding trial with three phases and was part of a larger experiment described in detail elsewhere (Sissener et al., 2021). In brief, it consisted of first a freshwater feeding period (Phase 1), followed by a seawater period in tanks (Phase 2) and a sea-cage period (Phase 3). In the first phase Atlantic salmon parr were fed different dEB levels for 10 weeks; the second phase of the trial was in seawater comprising of an acute 24 h challenge to seawater transfer and short term rearing in land based tanks for 6 weeks while being fed a commercial diet; the third phase was to study the effect under commercial-like open net pen conditions over a period of 14 weeks in the sea. All the phases of the feeding experiment were conducted at Matre research station of the Institute of Marine Research, Norway.

Table 2
Analysed proximate and nutrient composition of the experimental diets.

	2 mm pellet			3 mm pellet		
	L-dEB	M-dEB	H-dEB	L-dEB	M-dEB	H-dEB
Dry matter (%)	93.0	93.0	94.0	92	93.0	93
Crude protein (%)	45.0	46.0	46.0	44	46.0	45
Crude fat (%)	19.0	19.0	19.3	18.7	19.0	18.7
Ash (%)	7.2	6.2	7.0	7.1	6.1	7.1
Energy (kJ g^{-1})	21.4	21.8	21.7	21.3	21.7	21.7
dEB (mEq kg^{-1})	-25	237	383	−46	217	382
P (g kg^{-1})	10	10	11	9.7	9.9	9.4
Ca (g kg^{-1})	13.0	8.7	8.9	12.9	8.6	7.8
Mg (g kg^{-1})	2.1	2.1	2.2	2	2.1	2.1
Na (g kg^{-1})	3.3	3.3	6.8	3.1	3.2	6.9
K (g kg^{-1})	11	11	11	10.3	10.6	11.1
Cl (g kg^{-1})	16	6.7	6.9	15.8	6.9	7.2
Zn (mg kg^{-1})	131	138	135	134	138	132
Mn (mg kg^{-1})	36	38	42	43	34	30
Cu (mg kg^{-1})	8.6	8.8	8.7	8.6	8.9	9.4
Fe (mg kg^{-1})	140	150	167	152	180	150
Se (mg kg^{-1})	0.94	0.96	0.95	0.9	0.93	0.81

dEB, dietary electrolyte balance, calculated as the difference between the monovalent cations and anion (Na + K – Cl) in mEq kg^{-1}; L-dEB, low dEB feed; M-dEB, medium dEB feed; H-dEB, high dEB feed.

2.3. Freshwater feeding trial (Phase 1)

Atlantic salmon parr (Aquagen strain, ~15 g) were individually tagged with microtransponders (Glass tag 2, 12 mm, TrackID AS, Stavanger, Norway) and acclimatized for 3 weeks before the start of the study. All the fish were individually recorded for length, weight and were distributed in 9 tanks of 1 cubic meter with 140 fish each, supplied with freshwater at 12 °C. The fish were acclimatized to the trial conditions for 2 weeks while being fed a commercial diet. After the acclimation period (mean weight, 36 ± 4.3 g), the fish were randomly assigned one of the three experimental feeds in triplicate groups and fed one of the experimental diets to apparent satiation twice a day for 10 weeks. The fish were maintained under continuous light before the feeding experiment. During the experiment, the photoperiod was changed to a 12 L:12D for initial 6 weeks, followed by a 24 L:0D photoperiod for latter 4 weeks to induce the parr-smolt transformation. The water flow was adjusted so that the oxygen saturation of the outlet water was always above 80%. The temperature was kept constant at 12 °C. Fish were fed during the light period in LD12:12, and continuously under the LD24:0 regime. At the end of the 10-week feeding period, 12 fish per tank were randomly sampled and anesthetized by overdose of tricaine methyl sulfonate (FINQUEL MS-222, 0.1 g/l), identified for the registered the pit tag, recorded for length, weight and scored for cataract according to Waagbø et al. (1996). Out of the 12 fish, six fish were dissected for sampling of tissues, viz. plasma, bile and gill filaments and the rest were pooled and homogenized for body composition and mineral analyses. The rest of the fish in each dietary group were divided accordingly to be used for seawater phase either on land-based tanks (Phase 2) or open net pens (Phase 3).

2.4. Seawater transfer in land-based tanks (Phase 2)

The seawater phase in tanks was performed in common garden along with other dietary groups as described in Sissener et al. (2021), however with an exception that this study was performed only on fish transferred to seawater at 12 °C. The acute seawater challenge was performed by transferring 24 fish from each dietary group to one of the triplicate tanks (1-m diameter) maintained with full strength seawater (34 ppt) at 12 °C, without any prior acclimation. All the three dietary groups were maintained in 'common garden' within each tank and feeding was withheld for the 24 h duration of this challenge period. Four fish per tank (12 per dietary group) were anesthetized as described before and sampled for plasma, gill, and carbonate precipitates in the intestinal lumen, 24 h after seawater transfer. Plasma and gill were collected as individual samples, while the intestinal carbonate precipitates were pooled per tank.

Further, a total of 72 fish per dEB group (24 per tank) from the freshwater phase were distributed to each of the two land-based tanks (3 m dia.) with full strength seawater (34 ppt) maintained at 12 °C and kept in common garden along with fish from other dietary groups as described elsewhere (Sissener et al., 2021). The fish were maintained in these common garden tanks for a period of 6 weeks and fed a commercial diet (Skretting Supreme). At the final sampling from this phase, 10 fish per diet (5 per tank) were used for plasma sampling and, another 5 where homogenized for pooled whole fish samples. All these ten fish were recorded for weight and length and were scored for cataract as described above and X-rayed for vertebrae deformities.

2.4.1. Sea cage rearing (Phase 3)

At the end of Phase 1, besides seawater transfer in tanks, 30 fish per tank (90 per diet group), were transferred to three open net pens in a common garden set up and reared on a commercial diet (Skretting Supreme). Detailed description of the sea cage transfer procedure can be found in Sissener et al. (2021). In brief, fish destined for the sea cages were vaccinated (PharmagMicro 6) 3 weeks prior to SW transfer. Before transfer to net pens, the fish were held for five days (1st to 5th Nov, 2018) on land based tanks (1 × 1 m) with seawater at 9 °C. Phase 3 of the trial (sea cage) was terminated with a final sampling on February 5th, 2019 i.e. 14 weeks after SW transfer. Weight and length of all fish were recorded individually with PIT-tag, cataract scoring was conducted on 50 fish per diet group and liver was weighed for 18 fish per diet group.

2.5. Sampling and analytical methods

Length and weight of the fish were done individually on anesthetized fish during the start of the trial and at each sampling. The total length was recorded manually on a measuring board while the weight was recorded automatically using the PIT-tag reader. The fish were then scored for cataract, euthanized, sampled for blood from the caudal vein using a pre-heparinized syringe and dissected for tissue sampling as described in detail elsewhere (Sissener et al., 2021). Plasma was separated from the blood through centrifugation at 14200*g* for 2 min at 4 °C; the recovered plasma was then stored at −80 °C until further analyses. Gill filaments from the two front gill arches on the right side were sampled for gene expression analysis before SW transfer and 24 h after SW transfer (4 fish per tank; 12 per diet group). The tissue samples were flash-frozen in liquid nitrogen and then stored at −80 °C until. The liver and visceral mass were dissected out and weighed for the calculation of hepato- and viscero-somatic indices, respectively. The gall bladder was then removed intact, held over an Eppendorf tube and pierced with a scalpel knife to collect the bile into the tube. The collected bile was then placed on ice and later stored at −20 °C until analyses. The visceral mass was cleared of the fat and the GIT was uncoiled to examine for the presence and sampling of white ellipsoidal or string like 'carbonate precipitate' structures from the gut. The proximate and mineral composition of the feeds, homogenized fish, plasma, bile or carbonate precipitate samples were performed according to the methods followed in Antony Jesu Prabhu et al. (2019). Chloride in feeds were analysed at a commercial laboratory (ALS Laboratory Group Norway AS, Oslo) as per the AOAC approved method (AOAC, 1974). Plasma chloride was analysed using Sherwood Chloride Analyser 926 (Sherwood Scientific Ltd., Cambridge, UK). Plasma cortisol was quantified by enzyme-linked immunosorbent assay (ELISA) following and a detailed description of the methodology has been described in detail elsewhere (Sissener et al., 2021). Total RNA was extracted from the gill filament samples, cDNA synthesized through reverse transcription and qPCR amplification of the target genes. Primers for expression analysis of sodium/potassium-transporting ATPase subunits were based on

Kolarevic et al. (2014). The primer sequences were, alpha-1 submit (NKA α1a, GenBank no. AY692142) forward: GGCCGGCGAGTCCAAT and reverse: GAGCAGCTGTCCAGGATCCT; subunit beta-1 (NKA α1b, GenBank no. NM_001124460) forward: CTGCTACATCTCAACCAA-CAACAT and reverse: CACCATCACAGTGTTCATTGGAT. The primer sequences of the three housekeeping genes β-actin, elongation factor 1αb (Ef1αb), and ribosomal protein L13 (Rpl13) were as described elsewhere (Antony Jesu Prabhu et al., 2020) and normalized gene expression was calculated using the reference genes as described and verified by Olsvik et al. (2005).

2.6. Data analysis and statistics

The complexity of the study design and varying number of replicates in different phases of the feeding experiment meant more than one data analysis method had to be adopted. To begin with, all data were tested for normality and homogeneity of variance before analysis of variance (ANOVA) and non-normally distributed data were rank transformed and subjected to Kruskal-Wallis test of non-parametric analysis. Standard one-way ANOVA was used for all data available on a tank basis/pooled samples from each tank, while nested ANOVA (with tank as the random factor) was used for data measured on individual fish. The ANOVA in all cases was followed by Tukey's HSD post-hoc multiple comparison analysis in case a significant difference between treatments was detected ($p < 0.05$). The statistical analyses were conducted with Statistica (Version 11; Statsoft, Tulsa, OK, USA) and the graphical presentations were made using GraphPad Prisim v8, California, USA.

3. Results

3.1. Electrolyte balance of commercial freshwater Atlantic salmon feeds

Thirteen freshwater Atlantic salmon feeds commercially available in Norway were analysed for macro-minerals (P, Ca and Mg), electrolytes (Na, K and Cl) and the dEB calculated (Fig. 1). The dEB ranged from −9 to 399 mEq kg^{-1} feed with a median of 232.7 mEq kg^{-1} feed and a CV of 48%. The variation in dEB resulted from high variation observed in Na and Cl concentrations among the feeds; the CVs of Na, K and Cl were respectively 75%, 12% and 75%. Among the macro-minerals analysed, Ca concentrations showed high variation ranging from 6.4 to 31 g kg^{-1} feed, with a CV of 37%. Phosphorus concentration had a CV of 21% and Mg had the lowest CV of 14% among the macro-minerals analysed.

3.2. Growth performance

Growth and other performance indicators measured (weight gain, specific growth rate and organosomatic indices) were not affected by the dietary treatments throughout the study period (Table 3). Atlantic salmon grew from an initial weight of 36 ± 4.3 g (mean ± S.D.) to a final weight of 321 ± 57 g (mean ± S.D.) across the three treatments. Neither the direct effect of different dEB in freshwater phase, nor the dietary dEB history in the seawater phase (both tanks and sea cages) was statistically significant.

3.3. Phase 1: freshwater feeding with different dEB levels

3.3.1. Plasma electrolytes

Plasma Cl^- and Na^+ concentrations increased with increasing dEB, while plasma K^+ was unaffected by the dEB (Table 4). Atlantic salmon fed the H-dEB diet had significantly higher plasma Cl^- and Na^+ concentrations compared to salmon fed L-dEB.

3.3.2. The mRNA expression of sodium potassium ATPase in the gills

At the end of phase 1, NKA α1b had 2-fold higher expression than NKA α1a in the gills of Atlantic salmon. The dEB affected NKA α1b mRNA expression, but not of NKA α1a (Fig. 2). Fish fed M-dEB diet had significantly higher NKA α1b mRNA expression compared to L-dEB fed fish.

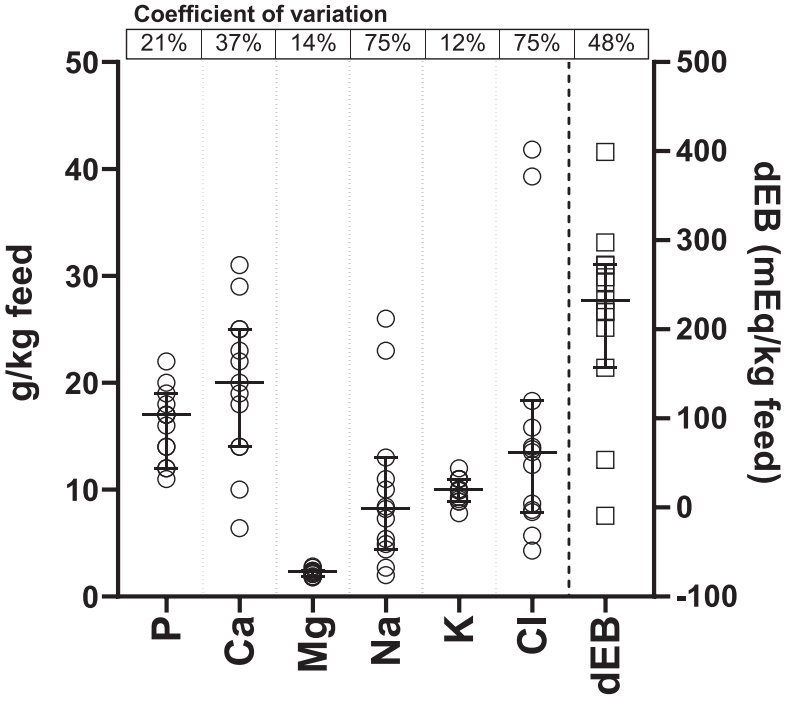

Fig. 1. Mineral concentration and dietary electrolyte balance (dEB) of commercial Atlantic salmon feeds used in Norway for freshwater phase (n = 13). Of the 13 feeds analysed, eight were obtained from the Norwegian fish feed surveillance program 2019 and the rest were obtained from Skretting ARC, Norway. The percentage values on top of the graph correspond to the respective coefficient of variation.

Table 3
Growth and other performance indicators of Atlantic salmon throughout the experimental period.

	L-dEB	M-dEB	H-dEB	p-value
Phase 1: Freshwater				
Final weight, g	80.1 ± 12.3	80.1 ± 11.2	83.1 ± 11.6	n.s.
Weight gain, g	43.5 ± 9.6	43.2 ± 8.4	47.1 ± 8.9	n.s.
Condition factor	1.28 ± 0.06	1.26 ± 0.05	1.27 ± 0.07	n.s.
Specific growth rate, % d^{-1}	1.17 ± 0.17	1.15 ± 0.14	1.25 ± 0.16	n.s.
Liver index	0.81 ± 0.09	0.88 ± 0.17	0.84 ± 0.12	n.s.
Visceral index	6.61 ± 0.34	6.14 ± 0.52	6.45 ± 0.42	n.s.
Heart index	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	n.s.
Phase II: Seawater, in tanks				
Final weight, g	142 ± 21	140.4 ± 21	149 ± 23	n.s.
Condition factor	1.18 ± 0.08	1.17 ± 0.06	1.21 ± 0.06	n.s.
Liver index	1.02 ± 0.14	1.11 ± 0.28	1.13 ± 0.21	n.s.
Heart index	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	n.s.
Phase III: Seawater, cages				
Final weight, g	330 ± 58	307 ± 65	317 ± 52	n.s.
Weight gain, g	294 ± 59	281 ± 69	281 ± 52	n.s.
Condition factor	1.2 ± 0.06	1.24 ± 0.06	1.2 ± 0.07	n.s.
Specific growth rate, % d^{-1}	1.37 ± 0.06	1.34 ± 0.04	1.35 ± 0.05	n.s.
Liver index	1.23 ± 0.3	1.28 ± 0.13	1.35 ± 0.2	n.s.

Mean initial weight, 36 ± 4.3 g with no significant differences between diet groups and no tank effects. Data presented as mean ± S·D. The sample size was n = 3 (phase 1 and 3); n = 2 (phase 2).

3.3.3. Tissue mineral concentrations

Macro- and micro-mineral concentrations in the plasma and bile of Atlantic salmon fed different dEB levels were analysed (Table 4). In the plasma, concentration of P, Ca and Fe were unaffected, while Mg and Zn increased with increasing dEB, being significantly higher is H-dEB compared to L-dEB fed fish. On the contrary, concentrations of Mn and Cu declined with increasing dEB, with significantly higher concentrations in L-dEB than H-dEB fed fish. Plasma Se was significantly higher in H-dEB fed salmon compared to M-dEB.

Atlantic salmon fed the H-dEB diet had the highest levels for all analysed minerals in the bile, either significantly or with a tendency (Table 4). Among macro-minerals, the bile concentrations of Ca and Mg were significantly higher in H-dEB fed fish than both the other groups. In the case of P and K, salmon fed H-dEB were significantly higher than M-dEB group, but not different from L-dEB. Bile Na^+ concentration was significantly higher in Atlantic salmon fed L-dEB and H-dEB feeds in comparison to M-dEB. Among micro-minerals, significantly higher level of Cu was found in the bile of H-dEB fish, and a tendency for a similar effect in other micro-minerals such as Mn ($p = 0.05$), Se ($p = 0.06$) and Zn ($p = 0.16$), except for Fe.

3.3.4. Proximate body composition and mineral concentration

The proximate composition and mineral concentration of the whole body at the end of the FW feeding (Phase 1) is presented in Table 5. The crude protein content was affected by dEB, being higher in L-dEB fed fish compared to M-dEB group. There were no significant differences between the diet groups in DM or lipids. Of the analysed macro-minerals (P, Ca, Mg, Na and K), none were significantly affected by dEB. Among micro-minerals, body Zn and Se levels were affected by dEB, while others were not affected. Whole body Zn was significantly higher in H-dEB fed salmon compared to salmon fed L-dEB, exhibiting a dose response in relation to increasing dEB levels. Whole body Se level was significantly lower in salmon fed M-dEB compared to other two groups, although the differences were marginal.

Table 4
Plasma and bile mineral concentration of Atlantic salmon fed different dEB levels before seawater transfer.

	L-dEB	M-dEB	H-dEB	p-value
Plasma[1]				
Na + (mmol l^{-1})	153 ± 4.4^a	156 ± 0.89^{ab}	162 ± 2.2^b	**0.02**
K+ (mmol l^{-1})	3.8 ± 0.95	3.4 ± 0.31	3.9 ± 0.4	n.s.
Cl- (mmol l^{-1})	122 ± 0.7^a	125 ± 4.1^{ab}	128 ± 0.9^b	**0.04**
P (mmol l^{-1})	12 ± 1.3	11 ± 0.6	13 ± 0.5	n.s.
Ca (mmol l^{-1})	2.6 ± 0.13	2.6 ± 0.03	2.7 ± 0.07	n.s.
Mg (mmol l^{-1})	0.41 ± 0.02^a	0.47 ± 0.01^{ab}	0.52 ± 0.03^b	**0.002**
Cu (μmol l^{-1})	10.0 ± 0.4^b	9.0 ± 0.5^a	9.6 ± 0.2^a	**0.03**
Fe (μmol l^{-1})	29 ± 20	17 ± 2.7	22 ± 4.7	n.s.
Zn (μmol l^{-1})	126 ± 16^b	178 ± 8.7^a	195 ± 9.5^a	**0.0009**
Mn (μmol l^{-1})	2.1 ± 0.06^b	1.5 ± 0.19^a	1.6 ± 0.16^a	**0.006**
Se (μmol l^{-1})	1.2 ± 0.11^{ab}	1.1 ± 0.05^a	1.3 ± 0.09^b	**0.04**
Bile[2]				
Na + (mmol l^{-1})	321 ± 14^b	290 ± 4.6^a	336 ± 6.8^b	**0.003**
K+ (mmol l^{-1})	9.3 ± 0.6^{ab}	9.0 ± 0.8^a	11.0 ± 1.1^b	**0.03**
Cl- (mmol l^{-1})	–	–	–	–
P (mmol l^{-1})	0.5 ± 0.11^{ab}	0.4 ± 0.03^a	0.62 ± 0.02^b	**0.02**
Ca (mmol l^{-1})	11 ± 0.24^a	10 ± 0.55^a	13 ± 0.37^b	**0.0008**
Mg (mmol l^{-1})	1.2 ± 0.05^a	1.1 ± 0.14^a	1.5 ± 0.07^b	**0.006**
Cu (μmol l^{-1})	98 ± 15^a	109 ± 17^a	188 ± 4.1^b	**0.0003**
Fe (μmol l^{-1})	6.9 ± 2.0	7.1 ± 0.95	8.6 ± 0.7	n.s.
Zn (μmol l^{-1})	3.2 ± 1.4	3.4 ± 1.1	5.5 ± 1.7	n.s. ($p = 0.16$)
Mn (μmol l^{-1})	0.9 ± 0.1	0.95 ± 0.2	1.4 ± 0.2	n.s. ($p = 0.05$)
Se (μmol l^{-1})	1.5 ± 0.36	1.5 ± 0.47	2.3 ± 0.18	n.s. ($p = 0.06$)

Data presented as mean ± SD (n = 3).
[1] Each 'n' is the mean of 2 pooled samples from 3 fish each.
[2] Each 'n' is obtained by analyzing a pooled sample from 6 fish. Values within a same row with different superscripts are statistically significant. Chloride in the bile samples were not analysed due to lack of enough sample.

3.4. Acute seawater challenge

3.4.1. Plasma cortisol

Cortisol concentration in plasma was measured before and 1 h after SW transfer and was not affected by dietary dEB at either time points (Fig. 3). Cortisol levels 1 h after seawater transfer was elevated compared to the pre-transfer concentrations in all the groups. The plasma cortisol levels were however not different between dietary groups due to high individual variations and presence of tank effects within groups.

3.4.2. Plasma mineral concentration 24 h after seawater transfer

Plasma mineral concentrations 24 h after SW transfer are presented in Table 6. The dEB levels of FW feeds did not have an impact on any of the plasma macro-minerals, however, plasma Na^+ tended to be higher in the M-dEB group ($p = 0.08$). The micro-minerals Mn and Se were significantly lower in plasma of salmon from M-dEB group. The concentration of plasma Zn in salmon with L-dEB history was significantly lower compared to the other two groups and increased in a dose response manner with increasing dEB.

3.4.3. Carbonate precipitate in the intestinal lumen

The intestinal carbonate precipitates (ICP) formed in the gut lumen of Atlantic salmon after seawater transfer and their estimated quantity over a 24 h period is presented (Fig. 4). The quantity of the ICP per unit body weight was influenced by the dEB of the FW feeds. The M-dEB fed salmon had significantly low amount of ICP compared to salmon fed L-dEB or H-dEB. The mineral composition of Atlantic salmon ICP is presented in Table 7. The ICP were composed predominantly of Ca and Mg

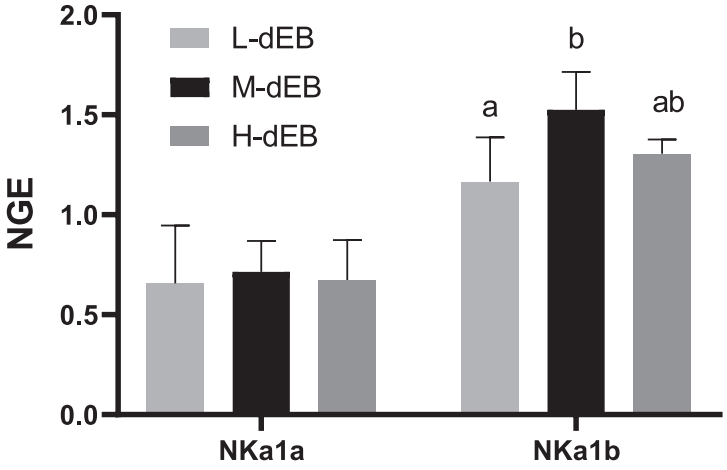

Fig. 2. The mRNA expression of sodium potassium ATPase isoforms namely NKa1a (A) and NKa1b (B) in the gills of Atlantic salmon fed the different dEB diets before transfer to seawater. Data analysed using nested ANOVA from 12 data points (4 fish per tank, 3 tanks per treatment) with tanks as random variable.

Table 5
Proximate body composition and mineral concentration of Atlantic salmon fed different dEB levels in freshwater.

	Initial	L-dEB	M-dEB	H-dEB	p-value
Proximate composition					
Dry matter (%)	30.4 ± 0.5	31.3 ± 0.5	31.1 ± 0.1	31.1 ± 0.4	n.s.
Protein (%)	16.4 ± 0.07	17.4 ± 0.1^{b}	17.1 ± 0.1^{a}	17.2 ± 0.2^{ab}	**0.04**
Lipid (%)	11.7 ± 0.8	12.3 ± 0.5	12.2 ± 0.2	12.8 ± 0.8	n.s.
Mineral concentration					
P (g kg^{-1})	4.7 ± 0.5	5.0 ± 0.6	4.9 ± 0.7	4.9 ± 0.5	n.s.
Ca (g kg^{-1})	3.8 ± 0.9	4.5 ± 1.3	4.3 ± 1.4	4.3 ± 0.8	n.s.
Mg (g kg^{-1})	0.3 ± 0.01	3.0 ± 0.04	2.9 ± 0.02	3.0 ± 0.02	n.s.
Na (g kg^{-1})	0.7 ± 0.01	0.74 ± 0.04	0.73 ± 0.02	0.75 ± 0.03	n.s.
K (g kg^{-1})	4.1 ± 0.02	4.2 ± 0.2	4.1 ± 0.07	4.2 ± 0.2	n.s.
Zn (mg kg^{-1})	39 ± 2.2	24.2 ± 1.4^{b}	28.1 ± 3^{ab}	37.1 ± 7.3^{a}	**0.03**
Mn (mg kg^{-1})	1.3 ± 0.4	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.3	n.s.
Fe (mg kg^{-1})	12.4 ± 1.8	8.0 ± 0.5	8.1 ± 0.4	7.7 ± 0.4	n.s.
Cu (mg kg^{-1})	1.2 ± 0.2	0.93 ± 0.05	0.98 ± 0.19	0.95 ± 0.09	n.s.
Se (mg kg^{-1})	0.2 ± 0.01	0.2 ± 0.003^{ab}	0.19 ± 0.003^{a}	0.2 ± 0.006^{b}	**0.03**

Data presented as mean ± S·D (n = 3). Each 'n' represents data obtained by analyzing a pooled sample of 5 fish. Values within a same row with different superscripts are statistically significant.

in the ratio of 2:1, with residues of other macro- and micro-minerals. Unlike the quantity, the content of Ca, Mg or their ratio were not influenced by the change in the dEB. However, the concentration of P, K, Zn and Se were significantly higher in the ICP from the M-dEB fed fish compared to either of the dEB treatments.

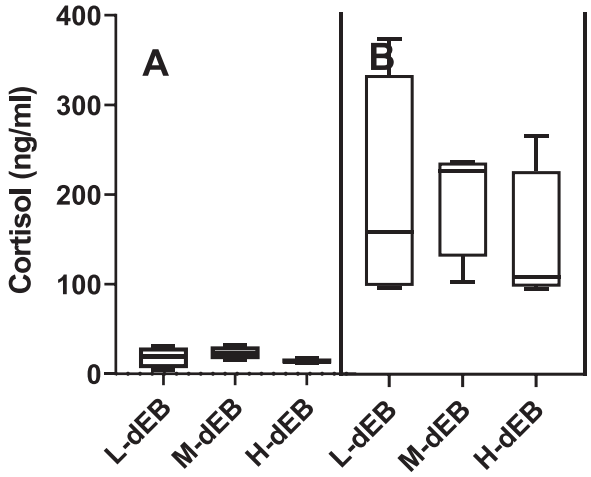

Fig. 3. Plasma cortisol concentration in Atlantic salmon fed the different dEB diets before (A) and 1 h after (B) transfer to seawater. Data analysed using nested ANOVA from 12 data points (4 fish per tank, 3 tanks per treatment) with tanks as random variable.

3.5. Seawater rearing on land-based tanks and open net pens

3.5.1. Plasma mineral levels and body composition

The plasma concentration of most analysed minerals were significantly affected by the dEB history of the FW feed (Table 8). The concentrations of K, P and Ca were significantly lower in salmon that received the L-dEB feed in FW compared to salmon fed the H-dEB diet (K, $p < 0.05$; P and Ca, $p < 0.01$). The concentration of Mg showed a tendency to be higher in salmon with H-dEB dietary history compared to M-dEB ($p = 0.05$). Among micro-minerals, concentration of Zn, Mn and Se were significantly lower in salmon with L-dEB dietary history compared to both other groups for Zn ($p < 0.01$) and Se ($p < 0.01$), and only with H-dEB for Mn ($p < 0.05$).

Data on whole body proximate and mineral composition is presented

Table 6
Impact of dEB history on plasma mineral concentration in Atlantic salmon 24 h after seawater transfer.

	L-dEB	M-dEB	H-dEB	p-value
Na^+ (mmol l^{-1})	166 ± 2.3	171 ± 1.1	167 ± 3.5	n.s.(p = 0.08)
K^+ (mmol l^{-1})	3.6 ± 0.2	3.9 ± 0.1	3.5 ± 0.6	n.s.
Cl^- (mmol l^{-1})	127 ± 2.4	128 ± 1.5	126 ± 1.9	n.s.
P (mmol l^{-1})	12.0 ± 0.6	11.9 ± 0.6	12.8 ± 0.4	n.s.
Ca (mmol l^{-1})	2.9 ± 0.05	2.9 ± 0.02	2.9 ± 0.07	n.s.
Mg (mmol l^{-1})	0.6 ± 0.03	0.6 ± 0.05	0.6 ± 0.03	n.s.
Zn (μmol l^{-1})	147 ± 16^a	173 ± 16^b	184 ± 12^b	**0.01**
Mn (μmol l^{-1})	1.0 ± 0.06^b	0.7 ± 0.1^a	1.0 ± 0.14^b	**0.005**
Se (μmol l^{-1})	1.3 ± 0.08^b	1.1 ± 0.1^a	1.4 ± 0.06^b	**0.0001**

Data presented as mean ± S.D. (n = 2). Each 'n' represents the mean of 4 individual fish. Values within a same row with different superscripts are statistically significant.

in Table 9. The crude protein content of L-dEB group was significantly lower in comparison with M-dEB. The DM and crude lipid content of the fish were not significantly different, although a tendency for lower lipid concentration appeared in the M-dEB group (p = 0.09). Among the minerals, whole body K and Zn were significantly different between the groups. Salmon fed the H-dEB feed had significantly higher levels of K and Zn compared to the M-dEB and both groups, respectively.

3.6. Prevalence and development of production disorders

3.6.1. Cataract score

Atlantic salmon fed different dEB feeds were scored for cataract at three different time points in this study (Fig. 5). At the end of phase 1, cataract scores were significantly affected by dEB of freshwater feeds (Fig. 5A). Fish fed L-dEB diet had higher score (p = 0.009, Kruskal-Wallis test) compared to M-dEB and H-dEB fed fish. However, there were no significant effect of freshwater dEB history on the cataract score after 6 weeks in seawater on land (Fig. 5B) or after 14 weeks in open net pens (Fig. 5C).

3.6.2. Vertebral deformities

There were no significant differences (Chi square, P > 0.05) in the occurrence of fish with one or more deformed vertebra, or regarding type of deformity (Table 10). Nevertheless, the number of fish with one or more deformed vertebrae and total number of deformed vertebrae recorded decreased with increasing dEB.

4. Discussion

While knowledge on the optimal dEB for Atlantic salmon is lacking,

Table 7
Mineral composition of carbonate precipitates formed in the gut lumen of Atlantic salmon 24 h after seawater transfer.

	L-dEB	M-dEB	H-dEB	p-value
Ca (g kg^{-1})	27.1 ± 4.4	27.2 ± 7.3	29.3 ± 5.5	n.s.
Mg (g kg^{-1})	13.9 ± 4.2	12.7 ± 4.2	14.8 ± 4.3	n.s.
Ca/Mg ratio	2.0 ± 0.3	2.2 ± 0.1	2.1 ± 0.3	n.s.
P (g kg^{-1})	0.6 ± 0.2^b	1.4 ± 0.2^a	0.6 ± 0.1^b	**0.003**
Na (g kg^{-1})	1.5 ± 0.1	1.6 ± 0.4	1.6 ± 0.1	n.s.
K (g kg^{-1})	0.7 ± 0.1^b	1.3 ± 0.1^a	0.7 ± 0.1^b	**0.004**
Zn (mg kg^{-1})	3.3 ± 0.7^b	6.1 ± 1.2^a	4.0 ± 0.8^b	**0.04**
Mn (mg kg^{-1})	4.0 ± 0.6	4.9 ± 0.2	4.4 ± 1.3	n.s.
Se (mg kg^{-1})	0.11 ± 0.02^b	0.21 ± 0.06^a	0.09 ± 0.02^b	**0.03**

Data presented as mean ± S.D. (n = 3). Each 'n' represents a mean of 2–3 pooled samples obtained from 4 fish each. The concentrations are presented on as is basis. Values within a same row with different superscripts are statistically significant.

Table 8
Impact of dEB history during freshwater phase on plasma mineral concentration in Atlantic salmon 6 weeks after seawater transfer.

	L-dEB	M-dEB	H-dEB	p-value
Na (mmol l^{-1})	189 ± 0.2	188 ± 3.4	187 ± 1.4	n.s.
K (mmol l^{-1})	4.5 ± 0.14^a	4.9 ± 0.18^{ab}	5.2 ± 0.15^b	**0.04**
P (mmol l^{-1})	7.2 ± 0.4^a	9.8 ± 0.1^b	10.0 ± 0.4^b	**0.005**
Ca (mmol l^{-1})	2.6 ± 0.004^a	3.0 ± 0.02^b	2.9 ± 0.03^b	**<0.001**
Mg (mmol l^{-1})	0.73 ± 0.12	0.65 ± 0.004	0.94 ± 0.04	n.s. (p = 0.05)
Zn (μmol l^{-1})	112 ± 9.7^a	188 ± 3.4^b	201 ± 15^b	**0.006**
Mn (μmol l^{-1})	0.42 ± 0.02^a	0.6 ± 0.08^a	0.79 ± 0.09^b	**0.03**
Se (μmol l^{-1})	0.64 ± 0.1^a	1.2 ± 0.04^b	1.3 ± 0.1^b	**0.01**

Data presented as mean ± SD (n = 2), 'n' is the mean of 2 pooled samples from 2 fish each. Values within a same row with different superscripts are statistically significant.

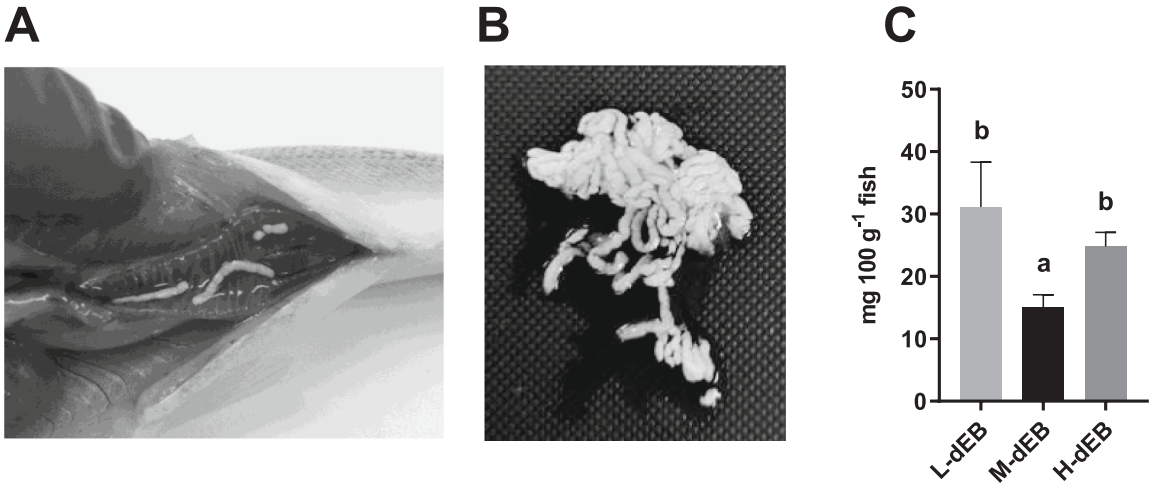

Fig. 4. Carbonate precipitate formed in the gut of Atlantic salmon after 24 h in seawater (A–B) and their quantity presented as mg 100 g^{-1} fish (mean ± SD) collected from three pools of 4 fish each, on as is basis (C).

Table 9
Impact of dEB history during freshwater phase on body composition and mineral concentration of Atlantic salmon post-smolt 6 weeks after seawater transfer.

	L-dEB	M-dEB	H-dEB	p-value
Proximate composition				
Dry matter (%)	32.0 ± 0.2	31.5 ± 0.3	32.1 ± 0.4	n.s.
Protein (%)	17.7 ± 0.13^{b}	18.4 ± 0.17^{a}	18.0 ± 0.09^{ab}	**0.03**
Lipid (%)	9.1 ± 0.8	6.8 ± 0.8	8.5 ± 0.4	n.s.(p = 0.09)
Mineral concentration				
P (g kg^{-1})	4.7 ± 0.4	4.5 ± 0.5	4.9 ± 0.8	n.s.
Ca (g kg^{-1})	4.2 ± 0.8	3.9 ± 1	4.4 ± 1.6	n.s.
Mg (g kg^{-1})	0.34 ± 0.01	0.32 ± 0.02	0.35 ± 0.15	n.s.
Na (g kg^{-1})	1.0 ± 0.02	1.0 ± 0.3	1.0 ± 0.2	n.s.
K (g kg^{-1})	3.9 ± 0.04^{ab}	3.7 ± 0.05^{a}	4.0 ± 0.07^{b}	**0.02**
Zn (mg kg^{-1})	31.4 ± 0.9^{a}	32.8 ± 1^{a}	36.6 ± 1.2^{b}	**0.03**
Mn (mg kg^{-1})	1.3 ± 0.3	1.4 ± 0.3	1.5 ± 0.5	n.s.
Se (mg kg^{-1})	0.18 ± 0.007	0.18 ± 0.004	0.18 ± 0.004	n.s.

Data presented as mean ± S.D. (n = 2). Each 'n' represents the mean of 2 pooled samples. The data are presented on wet weight basis. Values within a same row with different superscripts are statistically significant.

commercial freshwater feeds for Atlantic salmon in Norway had dEB levels ranging from −9 to 400 mEq kg^{-1}. The optimal dEB established in terrestrial monogastric animals (pig and poultry) and in a freshwater fish species tilapia range between 150 and 250 mEq kg^{-1} (Saravanan et al., 2013). Historic data on the dEB of commercial fish feeds in the EU also ranged between 200 and 400 mEq kg^{-1} (Tacon and De Silva, 1983). The commercial feeds analysed in this study revealed high and strikingly similar variation (CoV, 75%) in Na^+ and Cl^- levels, yet at a respective ratio of 1:2. Dietary common salt (NaCl) is known to induce SW tolerance in salmonids (Basulto, 1976; Salman and Eddy, 1987; Perry et al., 2006). The higher Cl^- to Na^+ ratio confirmed that NaCl was not the only contributor to the Cl^- load and certainly not responsible for lowering the dEB, as NaCl is dEB inert (dEB of NaCl = 0). Therefore, the lower dEB in two of the commercial feeds had most likely originated from the use of $CaCl_2$, as evident from the subsequent high variation in Ca levels (CV, 37%). Besides, recent data from the Norwegian fish feed monitoring program revealed extreme high variation in dietary Na (range, 0.9 to 23 g kg^{-1}; CoV, 98%; n = 85) and Ca (range, 2.9 to 27 g kg^{-1}; CoV, 60%; n = 85) levels, while data on Cl- was not available (Sele et al., 2021). It is therefore likely that the dEB is altered, but to what extent and their impact requires further attention.

Though NaCl and $CaCl_2$ can lower the osmotic load in the feed, their influence on dEB is different and hence the impact on the osmoregulatory responses in fish seem to differ. Feeding NaCl enriched diets increased the abundance of chloride cells in the gills, branchial expression or activity of NKA and plasma ion levels (Perry et al., 2006; Pellertier and Besner, 1992; Salman and Eddy, 1987). On the contrary, low expression of NKa1b in the gills and low plasma ions (Na^+ and Cl^-) were observed in Atlantic salmon fed low dEB levels. Along with the gills, the gut is an important organ for osmoregulation (Grosell, 2010). In saltwater, excess divalent cations (Ca^{2+} and Mg^{2+}) entering the gut through drinking are precipitated by HCO_3^- secreted in the epithelial cells and excreted as ICP. The ICP are milky white and are made of

Table 10
Impact of dEB history during freshwater phase on the occurrence (%) of vertebra deformities in Atlantic salmon at the end of the period in seawater tanks.

	L-dEB	M-dEB	H-dEB	p-value
Prevalence				
Total fish sampled	60	60	60	–
Total number of fish with DV	8	7	5	n.s.
Fish with DV, %	13	12	8	n.s.
total nb. of DV	24	20	22	n.s.
Mean DV per fish	3.0	2.9	4.4	n.s.
Type of deformity (%)				
Compression	5.1	9.8	6.7	n.s.
Compression & fusion	1.7	1.6	0	n.s.
Compression, fusion & internal shift	1.7	0	0	n.s.
Hyper-radiodense	3.4	0	1.7	n.s.
Internal shift	1.7	0	0	n.s.
Total deformity	13.6	11.5	8.3	n.s.

DV, deformed vertebrae.

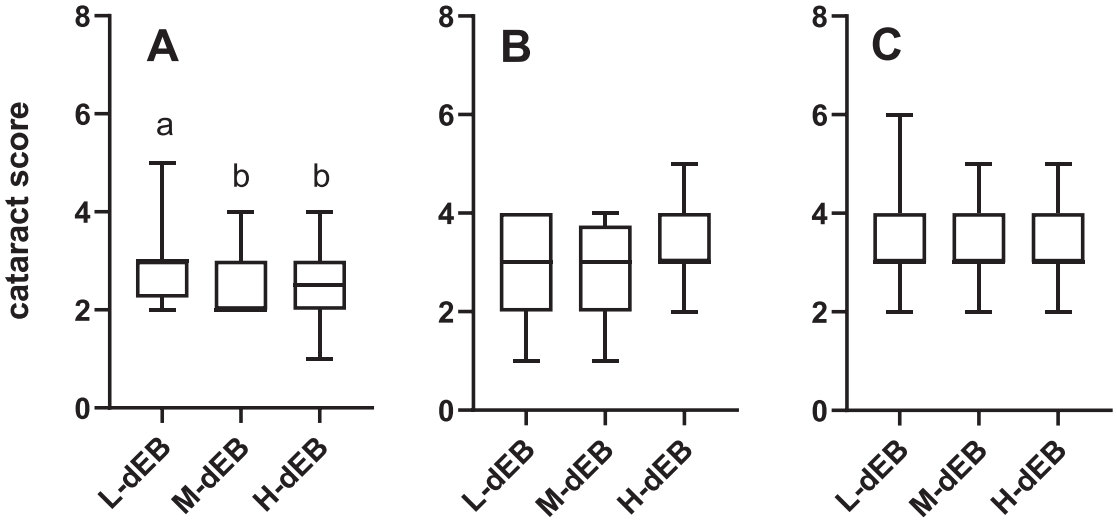

Fig. 5. Cataract score of Atlantic salmon smolt before transfer to seawater (A, n = 36 per diet), after 6 weeks in sweater on land (B, n = 20 per diet), and after 14 weeks in open net pens (C, n = 50 per diet).

calcium magnesium carbonate (Perry et al., 2011; Wilson et al., 2002; Foran et al., 2013). In the present study, we have demonstrated that the abundance of ICP in the gut 24 h after SW transfer was increased by both L-dEB and H-dEB feeds compared to the M-dEB. The precipitation rate, form and mineral composition of ICP in the gut varies with fish species (Salter et al., 2018). In Atlantic salmon, no previous report exists on the occurrence or description of ICP. The precipitation process also enables elimination of oxalate as calcium oxalate, thereby reducing the risk of nephrocalcinosis (Whittamore, 2020). Increased HCO_3^- secretion in response to salinity increase (Ruiz-Jarabo et al., 2017; Walsh et al., 1991) can increase ICP formation in the gut (Foran et al., 2013; Wilson et al., 2002). Further, the rate of precipitation in the first 24 h after SW transfer was influenced by the dEB of the freshwater feeds. In the L-dEB fed fish, it is likely that intestinal HCO_3^- secretion and calcium sensing receptor (CsR) were influenced by dietary $CaCl_2$ as in gilthead seabream in vitro (Gregório et al., 2019; Gregório and Fuentes, 2018). Whereas, in the H-dEB fed fish, carbonate ions directly derived from dietary Na_2CO_3 could be have increased the formation of ICP in this group. The milky white amorphous ICP in Atlantic salmon gut after SW transfer contained Ca and Mg in the ratio of around 2:1 (w/w) or 1:1 (molar ratio), similar to reports in marine fish (Perry et al., 2011; Salter et al., 2018; Walsh et al., 1991; Foran et al., 2013). Change in dEB did not influence the composition of Ca and Mg or their ratio in the precipitates but influenced the concentration of other minerals such as P, K, Zn and Se, although at trace amounts. Whether the increased ICP is an outcome of change in dEB or a direct impact of the specific salts ($CaCl_2$ or Na_2CO_3) used in the respective diets remain inconclusive and requires further study.

Trace element interactions and the antagonistic effects of macro-mineral sources on micro-mineral utilization in fish are well documented (Antony Jesu Prabhu et al., 2016; Watanabe et al., 1997). Luminal interactions with other minerals are significant in altering the uptake, absorption, tissue distribution of Zn (Glover and Hogstrand, 2003). The negative impact of L-dEB on tissue and body Zn status can be explained by the well-known antagonistic effect of Ca-salts on Zn uptake and availability (Porn-Ngam et al., 1993; Satoh et al., 1992; Satoh et al., 1987; Antony Jesu Prabhu et al., 2014). On the contrary, the reason for improved Zn status in H-dEB fed fish is less clear. The dEB induced differences in the acid-base balance of the chyme in the stomach might be a reason for the observed differences in Zn metabolism. Decreased acid secretion in the stomach is associated with impaired Zn absorption and plasma Zn status in humans and animal models (Farrell et al., 2011). The dEB of the feeds can alter the acid secretion in the stomach and has been shown to improve protein digestibility when fed high dEB levels in tilapia (Saravanan et al., 2013). Further, the role of Na^+ dependent mechanisms in the uptake of amino acids can be a plausible reason for improved protein digestibility (Subramaniam et al., 2020; To et al., 2019), which could indirectly influence Zn uptake via amino acid uptake pathways (Prabhu et al., 2018; Glover and Hogstrand, 2002). The concurrent contrary effects in plasma concentrations of Mn and Cu also point towards altered Zn absorption by change in dEB, as Zn has antagonistic effects on Cu and Mn absorption (Clearwater et al., 2002; Knox et al., 1984; Knox et al., 1982). Further, H-dEB also helped to maintain body Cu homeostasis by 2-fold increased biliary Cu excretion. The direct impact of altered dEB in feeds on mineral metabolism in FW was expected, however, the persistent effect after 6 weeks in SW eludes a plausible explanation. Given the substantial increase in plasma and whole-body Zn levels with H-dEB fish and the importance of tissue Zn status in fish health, the possibility of exploring the use of dEB as a tool to improve dietary Zn availability and fish health needs consideration.

Cataracts and vertebral deformities are production disorders that result in poor animal welfare. Dietary electrolytes can affect osmotic balance in the lens, which in turn can influence occurrence or severity of cataract in salmonids (Chiu et al., 1984; Bjerkås and Sveier, 2004). Dietary NaCl at 5% inclusion before and during smoltification reduced cataract frequency and severity after SW transfer in Atlantic salmon (Bjerkås and Sveier, 2004). In the present study, although, salmon fed L-dEB had a higher cataract score before SW transfer, it was only marginal, and no further effect was observed after SW transfer. Therefore, altering the dEB of FW feeds does not affect cataract formation or severity in Atlantic salmon in SW. Bone health also seemed unaffected by dEB treatments as there were no significant differences in occurrence or type of vertebral deformities. Vertebral compressions reflect on-going pathology and are most relevant with respect to the dietary treatment effects at the end of FW feeding stage. It therefore remains to be known if dEB variation during FW phase affects bone health over long term.

To conclude, neither an impact of direct feeding of dEB altered feeds nor its history impacted growth in Atlantic salmon at any stage in the study. Altering the dEB of FW feeds affected osmoregulatory responses in the gills and intestine before and 24 h SW transfer, respectively. Micro-mineral metabolism especially of Zn, Cu and Mn were altered by dEB of FW feeds, even six weeks after SW transfer. Incidence of cataracts and vertebral deformities over long term were not affected by change in dEB of the FW feeds. Given the substantial divide in Zn status of Atlantic salmon fed contrasting dEB levels, and the extreme variation in the mineral levels and dEB of commercial FW Atlantic salmon feeds, impact of dEB on smolt quality regarding health status upon challenge requires further attention.

CRediT authorship contribution statement

Antony J. Prabhu Philip: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Per Gunnar Fjelldal:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Sofie C. Remø:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Chandrasekar Selvam:** Formal analysis, Data curation, Investigation, Methodology, Writing – review & editing. **Kristin Hamre:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Marit Espe:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Elisabeth Holen:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Kaja H. Skjærven:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Vibeke Vikså:** Funding acquisition, Investigation, Writing – review & editing. **Saravanan Subramanian:** Funding acquisition, Investigation, Writing – review & editing. **Johan W. Schrama:** Investigation, Methodology, Writing – review & editing. **Nini H. Sissener:** Conceptualization, Data curation, Project administration, Funding acquisition, Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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