

## Histidine nutrition and genotype affect cataract development in Atlantic salmon, *Salmo salar* L.

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### Abstract

The aim of this study was to investigate effects of dietary levels of histidine (His) and iron (Fe) on cataract development in two strains of Atlantic salmon monitored through parr-smolt transformation. Three experimental diets were fed: (i) a control diet (CD) with 110 mg kg<sup>-1</sup> Fe and 11.7 g kg<sup>-1</sup> His; (ii) CD supplemented with crystalline His to a level of 18 g kg<sup>-1</sup> (HD); and (iii) HD with added iron up to 220 mg kg<sup>-1</sup> (HID). A cross-over design, with two feeding periods was used. A 6-week freshwater (FW) period was followed by a 20-week period, of which the first three were in FW and the following 17 weeks in sea water (SW). Fish were sampled for weighing, cataract assessment and tissue analysis at five time points. Cataracts developed in all groups in SW, but scores were lower in those fed high His diets ( $P < 0.05$ ). This effect was most pronounced when HD or HID was given in SW, but was also observed when these diets were given in FW only. Histidine supplementation had a positive effect on growth performance and feed conversion ratio ( $P < 0.05$ ), whereas this did not occur when iron was added. Groups fed HD or HID had higher lens levels of His and *N*-acetyl histidine (NAH), the latter showing a marked increase post-smoltification ( $P < 0.05$ ). The HD or HID groups also showed higher muscle concentrations of the His dipeptide

anserine ( $P < 0.05$ ). There was a strong genetic influence on cataract development in the CD groups ( $P < 0.001$ ), not associated with tissue levels of His or NAH. The role of His and His-related compounds in cataractogenesis is discussed in relation to tissue buffering, osmoregulation and antioxidation.

*Keywords:* anserine, Atlantic salmon, cataract, histidine, iron, *N*-acetyl histidine.

### Introduction

Cataract has been described in European Atlantic salmon aquaculture for more than two decades, and continues to cause significant production losses (Wall 1998; Midtlyng, Breipohl, Ahrend, Bjerkås, Waagbø & Wall 2000; Menzies, Crockford, Breck & Midtlyng 2002). Cataracts appear as both permanent and reversible lens opacities. The latter, 'osmotic cataracts', are considered a result of disturbances in the osmotic homeostasis of the lens and are often related to rapid environmental changes (Bruno & Raynard 1994; Bjerkås & Bjørnstad 1999; Breck & Sveier 2001). Infestation with eye flukes, *Diplostomum* spp. and intraocular infections, as well as environmental and toxicological factors may cause irreversible lenticular damage in salmonids (Ashton, Brown & Easty 1969; Sharif, Richards & Sommerville 1980; Fraser, Duncan & Tomlinson 1990; Krise & Smith 1993; Cullen, Monteith-McMaster & Sivak 1994; Laycock, Schirmer, Bols & Sivak 2000). Genetically linked predisposition in trout and Atlantic salmon have been demonstrated (Kincaid 1989; Wall &

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Richards 1992; Breck, Bjerkås, Campbell, Arnesen, Haldorsen & Waagbø 2003). Cataracts in reared Atlantic salmon are most frequently reported in yearling (S1) and sub-yearling (S0) smolts 2–3 and 8–10 months after transfer from fresh water (FW) to sea water (SW), respectively, related to elevated water temperatures and rapid growth (Wall 1998; Wegener, Laser, Ahrend, Breck, Bjerkås, Gloeckner, Midtlyng & Breipohl 2001). Rapid growth does not seem to be the only cataract-inducing factor, as growth rates in FW in periods with elevated water temperatures greatly exceed those observed in SW, whereas only mild FW cataracts prior to transfer have been reported (Waagbø, Sveier, Breck, Bjørnstad, Maage & Bjerkås 1998).

Nutritional linked cataracts in salmonids have mainly been reported in trout species, related to sub-optimal levels of riboflavin (Hughes, Riis, Nickum & Rumsey 1981), methionine (Poston, Riis, Rumsey & Ketola 1977; Cowey, Cho, Sivak, Weerheim & Stuart 1992) and tryptophan (Poston & Rumsey 1983; Akiyama, Mori & Murai 1986). Cataracts induced by zinc deficiencies have been described in both trout and salmon species, caused by low dietary concentrations or as a secondary consequence of reduced availability due to formation of chelates in the intestine or competitive uptake of other minerals (Ketola 1979; Shearer, Maage, Obstvedt & Mundheim 1992; Maage & Julshamn 1993). In general, these nutrient-related cataracts were seen more often when salmonid farming was in its infancy.

Some severe occurrences of cataracts in reared salmon coincided with the omission of mammalian blood meal (BM) as a protein source in the salmonid feed (Crockford 1998; Wall 1998; O. Breck unpublished data). Mammalian BM, especially rich in histidine (His), was banned as a feed ingredient within the EU in the early 1990s due to the occurrence of bovine spongiform encephalopathy. Dietary studies performed with Atlantic salmon have confirmed that supplementation with BM or crystalline His reduces the incidence of severe cataracts (Breck *et al.* 2003).

The aims of this study were to (i) investigate cataract development and growth in Atlantic salmon fed two dietary levels of His, (ii) investigate the effect of adding extra iron to the His-enriched diet, (iii) study the relative importance of feeding the adjusted diets in FW and/or in SW, (iv) compare growth and cataract development in two different strains of Atlantic salmon subjected to the

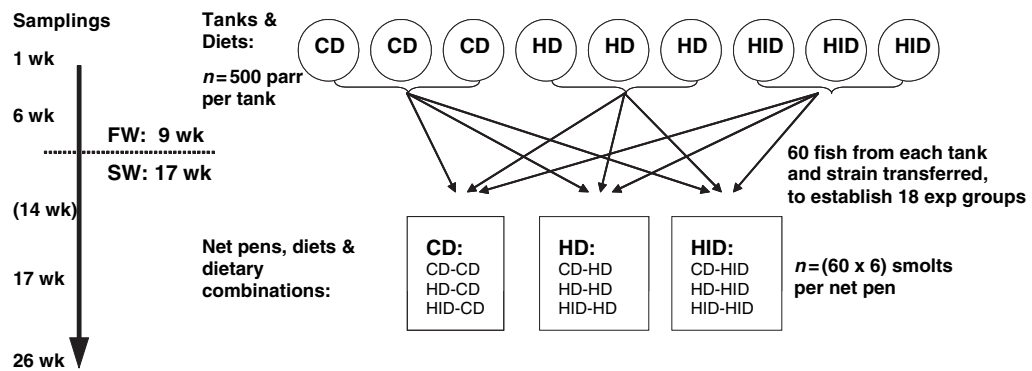
different diets and feeding regimes and (v) investigate tissue levels of His and His derivatives in relation to life stage, feeding regime and cataract development.

## Materials and methods

### Animals, diets and study design

The 26-week feeding trial performed in 2001 consisted of a 9-week FW period (4 April to 7 June) in the Marine Harvest Norway freshwater facility, Glomfjord, Nordland, followed by a 17-week SW period (until 2 October) at Gildeskål Research Plant, Nordland, Norway (Fig. 1). These sites had previously experienced outbreaks of cataracts in Atlantic salmon parr and smolt, respectively. The trial was conducted according to the guidelines of the Norwegian State Commission for Laboratory Animals.

In March nine experimental circular tanks (diameter 3 m, depth 1.5 m,  $n = 500$  fish per tank) were established in the hatchery, each containing a 50:50 mixture of two strains [Aqua-Gen strain, Norway (strain A) and MOWI strain, Norway (strain B)] of Atlantic salmon, *Salmo salar* L., parr, hatched in February 2000. Previously, both strains had been reared under similar conditions in two standard outdoor tanks and received the same commercial diet (Skretting, Norway). The fish were randomly selected from the two tanks by dip netting after reduction of water level and crowding of the fish, and strain B was labelled by removal of the adipose fin concurrent to measuring length and weight, after anaesthesia of both groups in a  $100 \text{ mg L}^{-1}$  solution of MS 222 (tricaine methane sulphonate). This method of anaesthesia was used during the FW period, whereas  $40 \text{ mg L}^{-1}$  of benzocaine (in a propylene glycol solution) was used in the SW phase. The nine tanks continued on a common diet until the commencement of the trial at the beginning of April. The mean overall start weight of the parr was  $44.7 \pm 8.9 \text{ g}$  ( $n = 1100$ ), whereas the weight for strains A and B were  $47.9 \pm 8.0 \text{ g}$  ( $n = 537$ ) and  $41.2 \pm 8.4 \text{ g}$  ( $n = 563$ ), respectively. Fresh water with a mean temperature of  $12.7 \text{ }^\circ\text{C}$  (April  $11.0 \text{ }^\circ\text{C}$ , May  $14.4 \text{ }^\circ\text{C}$ ) was supplied to the tanks throughout the FW period and natural light conditions were used. The parr were fed according to in-house feeding tables (calculated daily rations, based on fish size, biomass, water temperature plus



**Figure 1** Schematic diagram showing the design of the histidine (His) and iron feeding trial with Atlantic salmon. In fresh water (FW) triplicate tanks were fed one of three experimental diets (CD, control diet; HD, high His diet; HID, high His and iron diet) for 6 weeks. Then equal combinations of the tank populations (three diets and two strains) were allocated to three tanks by group tagging the fish, and the mixed populations fed one of the three diets (nine dietary combinations of the two strains; i.e. 18 experimental groups). The fish were kept in FW for another 3 weeks before full smoltification and were then transferred to their respective net pens in sea water (SW). Fish were examined for cataract at week 1 ( $n = 75$  fish per strain from each three tanks) and week 6 ( $n = 12$  fish per strain and tank). In SW (weeks 14, 17 and 26) all remaining individuals per experimental group were examined (max  $n = 48$ –60). Six fish per experimental group were collected for tissue analysis per sampling (weeks 3, 6, 17 and 26), whereas in week 1, six fish per strain from each three tanks were collected.

a calculated excess, ensuring satiation feeding) by use of automated feeders.

Each of three tanks were randomly selected and the fish in these units received one of three extruded pelleted diets manufactured by Biomar Ltd at the Biomar Technology Centre in Brande, Denmark: (i) a control diet (CD) based on Norse LT 94 fish meal, wheat, wheat gluten, corn gluten and fish oil, (ii) the CD supplemented with crystalline His (*L*-histidine monohydrochloride monohydrate, Sigma-Aldrich, Dorset, UK) (high histidine diet, HD) and (iii) the HD with added Fe (iron sulphate, Roche Vitamins, Belfast, UK) (high histidine and iron diet, HID). The feed composition and final concentrations of selected macro- and micronutrients are shown in Table 1. In this study, the CD represents the diet with the lowest His content, although the actual level,  $11.7 \text{ g kg}^{-1}$ , is far beyond NRC (1993) recommendations of  $7 \text{ g kg}^{-1}$ . Diets with high fish meal contents were chosen, to ensure the requirements of other essential amino acids in Atlantic salmon were met, with the potential for rapid growth and low feed conversion ratio (FCR).

In the middle of May, shortly before scheduled transfer to SW, 60 smolts per strain were randomly selected from each of the nine FW tanks and divided into three new tanks (Fig. 1). The dietary groups were individually marked on the ventral surface prior to mixing, by use of an Ink Jet syringe (Hart & Pitcher 1969). The fish in the three tanks were starved for 2 days and then fed one of the three experimental diets. Thus, within each tank,

containing 180 fish of each of the two strains, 60 individuals per strain either received changed diets or continued on the feed they had received for 6 weeks previously. By use of this cross-over design, 18 experimental SW groups were established (nine dietary combinations  $\times$  2 strains).

A representative number of smolts from the FW tanks were subjected to a SW challenge test prior to scheduled transfer time (week 6), by moving them to test units, exposing them to SW ( $30$ – $32 \text{ g L}^{-1}$ ) and monitoring plasma chloride levels after 48 h (Radiometer CMT 10 chloride titrator; Radiometer, Copenhagen, Denmark) as described by Breck & Sveier (2001). The test results showed suboptimal SW adaptation status (data not shown) and the three dietary groups were kept in FW and tested again at 1-week intervals. Three weeks later, at the beginning of June, the fish were considered well adapted to SW, based on both their performance in the SW challenge test and their external appearance (data not shown).

On 7 June, the three dietary groups were transferred to the adjacent SW research plant. The fish were put into three  $4 \text{ m}^2$  square net pens (depth 3 m) and the dietary regimes from the FW site were continued. The units were fed *ad libitum*, using automatic feeders combined with hand feeding and visual appetite control. In addition, a feed waste control system developed on site was used during the meals, collecting and re-feeding all spilled pellets and thereby ensuring feeding to satiation with knowledge of exact feed intake. Mortality

**Table 1** Feed ingredients and selected macro- and micronutrients in the experimental diets<sup>a</sup>

	Diet		
	CD	HD	HID
Gross composition (g kg <sup>-1</sup> )			
Norse LT 94 fish meal	500	500	500
Wheat	148.5	148.5	148.5
Fish oil	191	191	191
Wheat gluten	100	100	100
Corn gluten	30	30	30
Mineral premix	2	2	2
Vitamin premix	8.5	8.5	8.5
Mono sodium phosphate	16	16	16
Carophyll pink	0.5	0.5	0.5
Iron sulphate	0	0	0.36
L-histidine <sup>b</sup>	0	9	9
Analysed content			
Moisture (g kg <sup>-1</sup> )	70	75	72
Protein (g kg <sup>-1</sup> )	486	478	488
Fat (g kg <sup>-1</sup> )	232	234	229
Gross energy (MJ kg <sup>-1</sup> )	21.1	21.0	21.4
Minerals			
Iron (mg kg <sup>-1</sup> )	114	110	221
Amino acids (g kg <sup>-1</sup> diet)			
Histidine	11.7	17.6	18.1
Cysteine	6.2	5.9	5.9
Methionine	11.8	11.9	11.5
Aspartic acid	38.5	39.3	38.2
Threonine	18.5	18.2	18.5
Serine	23.8	22.9	22.5
Glutamic acid	89.7	86.1	88.0
Proline	27.7	27.0	27.7
Glycine	25.2	25.1	25.3
Alanine	27.0	26.3	25.5
Valine	22.0	24.1	24.2
Isoleucine	17.9	16.2	18.9
Leucine	36.8	40.5	37.1
Phenylalanine	20.2	20.2	20.4
Lysine	29.9	31.0	31.7
Arginine	24.9	24.1	24.9

<sup>a</sup> Analysis carried out by Biotechnological Institute, Kolding, Denmark.

<sup>b</sup> L-histidine monohydrochloride monohydrate (Sigma-Aldrich, Dorset, UK).

baskets were pulled up from each trial unit daily, followed by identification, recording and removal of mortalities. The mean salinity at the SW site was 31.9 g L<sup>-1</sup> (range 30.4–33.1) and the mean temperature (1 m depth) was 11.0 °C. The temperature for the whole period ranged between 7.7 and 13.0 °C, the lowest monthly mean temperature being recorded in June (9.1 °C) and the highest in September (12.2 °C).

Disease outbreaks did not occur in any of the trial units in FW or SW and the overall mortality was low and similar between the dietary groups (data not shown). A light infestation of sea lice, *Lepeophtheirus salmonis*, during the SW phase necessitated a bath treatment with pyrethroids (Betamax, Novartis, Horsham, UK) in September.

Lice burdens before and after the treatment were similar between the trial cages.

No differences in behaviour or appetite between the three net pens were reported during the SW period. Low stocking densities were maintained and the research facility was located in open coastal waters with good currents and water exchange, ensuring high water quality with good oxygen saturation throughout the trial.

### Sampling procedures and monitoring of growth performance

The fish were sampled for weighing, cataract monitoring and tissue collection at five time points; at start of the trial (3 April, week 1), at the initially scheduled SW transfer time (14 May, week 6), 4 weeks after actual transfer (10 July, week 14, weighing and cataract screening only), 3 weeks later (1 August, week 17), and at the end of the trial (1 October, week 26) (Fig. 1). The weights of approximately 60 fish per strain from each of the nine tanks were recorded at the start; 12 fish per strain and tank were measured in May; whereas all individuals were measured at the SW sampling points (max. 60 per experimental group). The same fish were screened for cataracts except in April, when 75 individuals per strain, randomly selected from three tanks, were examined.

Tissue specimens were collected from six individuals per experimental group (18 experimental groups with respect to diet and strain), except at the first sampling in April, when six fish per strain from each three tanks were sampled. Tissues were analysed in triplicate per experimental group: blood haemoglobin (Hb), aqueous humour osmolality and pH, lens buffering capacity and concentration of free amino acids, white muscle concentration of free amino acids, and liver iron and zinc concentrations. All tissues were frozen on dry ice immediately following collection and stored at -70 °C. Analyses of lenses, white muscle, liver and aqueous humour were carried out on each pooled samples per experimental group (strain and diet) based on six fish in April and two fish at the other samplings.

Calculation of the biological feed conversion ratios (FCR) were based on amount of feed given and the total biomass gain in each trial unit between the SW sampling points:  $FCR = (\text{kg feed given}) \times (\text{kg final live weight biomass} + \text{kg dead and sampled fish} - \text{kg biomass at starting point})^{-1}$ .

### Cataract examination

For examination of lens opacities a slit lamp (Kowa SL-14 with 16× magnification, Kowa, Tokyo, Japan) was used, under darkened conditions and after anaesthesia. At each sampling, frequency and severity of the cataracts were recorded, the latter scored on a scale from zero (no changes) to 4 per eye (complete cataracts), in accordance with a standard scoring method described by Wall & Bjerkås (1999). Individual cataract scores are given as the sum of both eyes, ranging from 0 to 8. Mean scores of all examined individuals (both affected and unaffected) within the experimental groups are given, if not stated otherwise.

### Analysis of muscle, lens and liver samples

Determinations of *N*-acetyl histidine (NAH) and free His levels in lens tissue at four tissue sampling points (weeks 1, 6, 27 and 26) were made by a modification of the method of O'Dowd, Cairns, Trainor, Robins & Miller (1990). Lenses were homogenized in 80% (v/v) ethanol and centrifuged at 2000 *g* for 20 min. The supernatants were dried in a Termaks incubator (40 °C, normal atmosphere) (Termaks, Bergen, Norway), dissolved in phosphate buffer (pH 2.0) and filtered through a membrane filter (0.45 µm). An isocratic reverse phase HPLC was performed, using a 4.6 mm ID × 250 mm column with a silica-based packing (ZORBAX SB-C18, Agilent Technologies AS, Kolboth, Norway) and a Waters 600 E pump (Waters Corporation, Milford, MA, USA). A 0.1 M phosphate buffer (pH 2) was used as eluting solvent, with a flow rate of 0.6 mL min<sup>-1</sup>. NAH and His were detected by UV absorbance (Waters 486–Tuneable Absorbance Detector, Waters Corporation) at 210 nm, using external standards.

For determination of free amino acids in muscle and lens (April and August), tissue samples were prepared as described above. After complete drying, samples were dissolved in running buffer (Lithium Citrate Loading Buffer, 80-2038-10, Biochrom Ltd, Cambridge, UK) and amino acid content was analysed by use of a Biochrom 20 Plus Amino Acid Analyser (Biochrom Ltd) based on low pressure ion-exchange chromatography. Different gradient elution systems were used for identification of either complete free amino acid profiles or, in a shortened version, the profile of basic amino acids only (Mike Davies, Biochrom

Ltd, personal communication). After post-column ninhydrin derivatization, colorimetric detection was made at 570 and 440 nm (Waters 486, Waters Corporation).

Differences in the analysed concentrations of free His were found between the two methods described. The HPLC method (Fig. 4) gave higher values than the ion-exchange chromatography (Table 3). This is due to the HPLC method not separating baseline His from 1-methyl-histidine, the latter being found at low levels in the fish lens. Hence, the degree of overestimation when using the HPLC method is higher when analysing very low concentrations of His.

Zinc and iron levels in pooled liver samples were analysed using a flame atomic spectrometer (PerkinElmer AAG-3300, PerkinElmer Analytical Instruments, Shelton, CT, USA), as described by Andersen, Lorentzen, Waagbø & Maage (1997).

Lens and aqueous humour buffering capacity was measured by titration using a method adapted from Castellini & Somero (1981). Following dissection from the eye, lenses were washed in saline (0.9% NaCl) and rolled on filter paper to remove adhering tissue and saline. After recording its weight the lens was homogenized in 1 mL saline. To eliminate the contribution of bicarbonate buffering 1 µL conc. HCl was added to the homogenate. The pH was then raised by titration with NaOH solution. The buffering capacity, defined as the number of micromoles of NaOH required to raise the pH by one unit per gram of tissue (wet weight), was determined over the range pH 6–7.

Osmolality (mmol kg<sup>-1</sup>) of aqueous humour samples was measured using a vapour pressure osmometer (Vapro<sup>®</sup> Vapor Pressure Osmometer, Wescor Inc., Logan, UT, USA).

### Blood samples and analysis

After anaesthesia, blood samples were collected from the caudal vein by 1 mL heparinized syringes. Blood Hb was measured in accordance with a cyanomethaemoglobin method (Sandnes, Lie & Waagbø 1988).

### Sampling of lens and aqueous humour

Collection of aqueous humour was made with a 1-mL tuberculin syringe and a 25-gauge needle, whereas the lens was carefully dissected out after opening the cornea by an incision along the limbus.

## Statistics

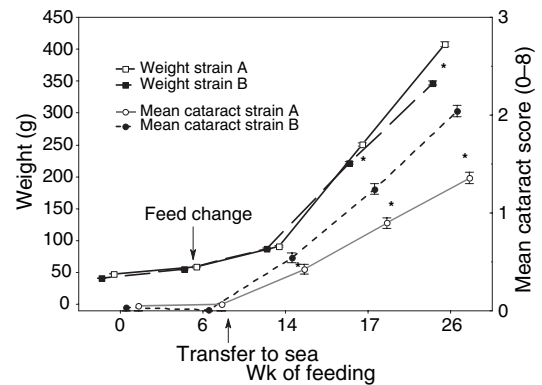
Statistical analyses of biological data were performed by use of ANOVA and the Tukey unequal *n* HSD *post hoc* test, in addition to the Student's *t*-test. For cataract scores, Kruskal–Wallis ANOVA median by rank was used to test overall differences between strains and diets at each sampling point or differences between dietary groups within SW units. The Mann–Whitney *U*-test was used *post hoc* to study differences between individual dietary groups. The effects of SW diets on cataract, growth, FCR and various tissue analyses data were statistically analysed as described above, although the SW cages were not replicated. This was considered justifiable due to the highly standardized rearing conditions at the SW experimental units, but should be kept in mind when evaluating type and magnitude of observed differences related to SW diets. Statistical data analysis software system (version 6, StatsSoft Inc. 2001, StatSoft Scandinavia AB, Uppsala, Sweden) was used for statistical analyses and differences were considered significant at  $P < 0.05$ . Data are presented as mean  $\pm$  SEM.

## Results

### Cataract

Low mean cataract scores were recorded both at the start (score 0.04) and after 6 weeks in FW (mean scores 0.03, 0.07 and 0.01 for groups receiving CD, HD and HID, respectively), with no differences seen between the two strains. There was a cataract prevalence of approximately 4% at both screenings and all affected fish had a cataract severity of score 1. The FW cataracts mainly appeared as pinpoint sized dots in the anterior or equatorial cortex. In SW high frequencies of cataract developed in all groups, with an overall frequency of 80% at the final sampling in October. Between the 18 SW experimental groups, the frequency of fish affected in October varied between 98% (strain B salmon receiving CD in both FW and SW) and 60% (strain A fed HID in FW and HD in SW).

Mean cataract scores at the end point in the three SW units were 3.01, 0.99 and 1.14 for CD, HD and HID, respectively (pooled strain data). Thus, a considerable positive effect of feeding HD in SW was indicated ( $P < 0.01$ ), whereas additional supplementation of Fe seemed to have a minor negative effect. The preventative effect of His was

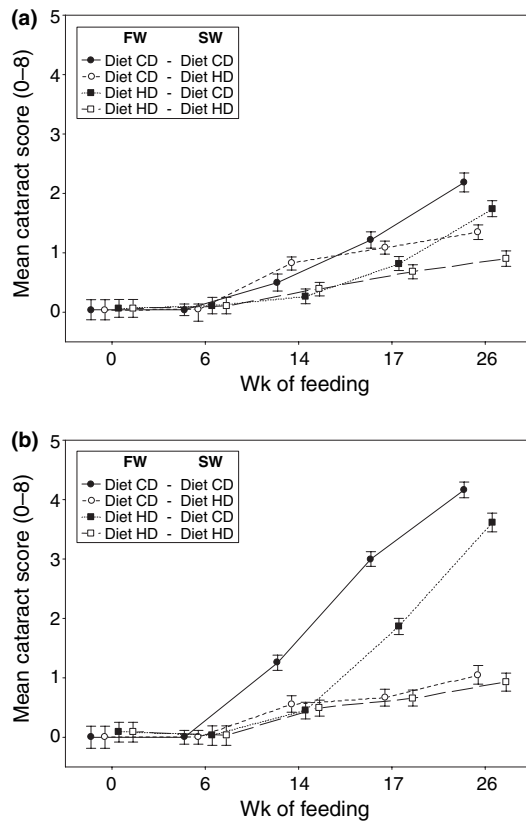


**Figure 2** Development of body weight and cataract in the two strains of Atlantic salmon throughout the trial. Significant differences at individual sampling points are denoted by an asterisk. Data are given as mean  $\pm$  SEM. Each value based on pooled data from all dietary groups for each strain. For weight data  $n = 537$ – $563$  and  $n = 108$  for week 1 and week 6 samplings in fresh water (FW), respectively, whereas  $n = 376$ – $461$  for the seawater (SW) sampling points. For cataract data  $n = 82$ – $108$  and  $376$ – $461$  for the FW and SW sampling points, respectively.

seen in both strains, but considerable differences in cataract susceptibility were demonstrated (Fig. 2, pooled dietary groups and Fig. 3, four selected dietary groups of the two strains). The development of cataract when feeding CD in SW was far more severe in strain B compared to strain A, whereas dietary His supplementation in SW reduced the severity to similar levels in the two strains. An additional positive effect of giving HD in FW, when continuing on HD in SW, was only seen in strain A, whilst the cataract-reducing effect of feeding HD in FW only was more pronounced in strain B (Fig. 3).

The development of cataract in SW for the nine dietary groups (representing the different possible combinations of diets) within strain B is presented in Table 2. The addition of Fe in SW slightly increased the mean cataract score. This was not seen in strain A (data not shown).

The cataracts that developed during the SW period generally resembled those described earlier in farmed salmon from Norway (Wall 1998; Breck & Sveier 2001). The severity was rather low, with approximately 5% of the salmon scoring above 5 at the final sampling. Anterior or posterior superficial areas of the cortex were primarily affected in early stages, whereas opacities at later sampling points were more extended and located in deeper cortical and perinuclear regions, indicating a further devel-



**Figure 3** Cataract development in Atlantic salmon strains A (a) and B (b) for four selected feeding groups during the trial period. Data presented as mean  $\pm$  SEM. Significant differences in mean cataract scores between groups receiving similar freshwater (FW) diet and different seawater (SW) diets were found at week 26 for strain A, within groups which received control diet (CD) or high histidine diet (HD) in FW and either changed diets or continued on the same feed. In strain B, these differences were found at all SW samplings, except week 14, where a difference was only present in groups starting on CD in FW, with the group changing to HD in SW developing less cataracts than the one continuing on CD ( $P < 0.05$ ). Significant differences between groups of similar SW and different FW dietary background were found at the week 14 and 17 samplings ( $P < 0.05$ ) in strain A between groups receiving HD in SW and in strain B for groups fed the control diet (CD) in SW.

opment of the early changes, but no new cataract formation in the younger lens tissue. Early posterior cortical changes often appeared as a speckled or dusty hemisphere. At the October sampling, a layer of unaffected outer cortex was found in fish lenses from all dietary groups, most frequently however, in groups on HD or HID (data not shown). Interestingly, in July the screening for permanent opacities was to some degree rendered difficult in all groups due to a frequent occurrence of anterior diffuse opacities in the polar region, often with a distinct

**Table 2** Weight and cataract development in the nine dietary groups of Atlantic salmon strain B, obtained from the samplings at weeks 14 and 26<sup>1</sup>

Diets		Weight (g) <sup>2</sup>		Mean cataract score (0–8) <sup>3</sup>	
Sea water	Fresh water	Week 14	Week 26	Week 14	Week 26
CD	CD	88.1 <sup>bcd</sup>	334 <sup>acd</sup>	1.25 <sup>a</sup>	4.16 <sup>a</sup>
	HD	87.4 <sup>bcd</sup>	344 <sup>abc</sup>	0.45 <sup>b</sup>	3.61 <sup>a</sup>
	HID	82.6 <sup>bcd</sup>	322 <sup>a</sup>	0.39 <sup>b</sup>	3.65 <sup>a</sup>
HD	CD	84.0 <sup>bcd</sup>	367 <sup>bd</sup>	0.56 <sup>b</sup>	1.05 <sup>bc</sup>
	HD	79.3 <sup>bc</sup>	344 <sup>abc</sup>	0.49 <sup>b</sup>	0.93 <sup>bc</sup>
	HID	82.8 <sup>bcd</sup>	358 <sup>b</sup>	0.49 <sup>b</sup>	0.82 <sup>b</sup>
HID	CD	98.3 <sup>a</sup>	358 <sup>b</sup>	0.45 <sup>b</sup>	1.19 <sup>bc</sup>
	HD	92.4 <sup>ad</sup>	343 <sup>abc</sup>	0.29 <sup>b</sup>	0.98 <sup>bc</sup>
	HID	89.2 <sup>acd</sup>	362 <sup>bd</sup>	0.41 <sup>b</sup>	1.23 <sup>c</sup>

CD, control diet; HD, high histidine diet; HID, high histidine and iron diet.

<sup>1</sup> Group mean values within columns not sharing same superscript differ significantly ( $P < 0.05$ ).

<sup>2</sup> One-way ANOVA and Tukey unequal n HSD *post hoc* test ( $n = 46–59$  per group).

<sup>3</sup> Kruskal–Wallis ANOVA median by rank and Mann–Whitney *U*-test ( $n = 34–55$  per group).

vertical suture line. Such changes are generally considered to be reversible and related to osmotic disturbances, and only a few individuals had similar changes at later screening points.

## Growth

At the 6-week FW sampling point, the mean weights were 60.0, 54.0 and 57.8 g for fish receiving CD, HD and HID, respectively, the HD group being significantly smaller than the others ( $P < 0.05$ ) (pooled strain data). At the last sampling in October, the mean weights (0.95 confidence interval) in the three SW units were 361 (351–371), 386 (276–396) and 386 g (376–395) for CD, HD and HID, respectively, representing an increased weight of 6.9% in HD and HID, compared with CD ( $P < 0.01$ ). Similar trends in weight differences were seen in both strains (data not shown). Calculation of FCR for the two last SW periods (weeks 14–17 and weeks 17–26 samplings) showed a 10% decrease when the CD was His supplemented, from 0.74 to 0.67 and from 0.95 to 0.85, respectively. There were no differences between HD and HID in FCR. Data on weight development in SW for strain B in each of the nine dietary groups (Table 2), indicate that there were no additional positive effects on growth from feeding HD or HID in FW, as was also observed in strain A (data not shown).

Endpoint weight was significantly affected by both strain and diet (diet:  $P = 0.01$ , strain:  $P < 0.01$ , interaction: n.s., two-way ANOVA). At start of the trial, strain A was approximately 17% larger than strain B. This difference was lower at the intermediate samplings, but showed a similar magnitude at end of the trial (Fig. 2). Strain A had lower mean cataract scores throughout the SW phase of the trial (Fig. 2). When final weights were categorized with cataract scores 0–1 and  $>1$  the data indicated a higher final weight in the higher cataract category ( $>1$ ), irrespective of dietary treatment or strain ( $0.1 > P > 0.05$ ). For example, for strain A the range of the mean final weights for the diets ranged between 379 and 406 g for cataract scores 0–1 compared with 401–430 g for cataract scores  $>1$ .

### Lens and muscle amino acid

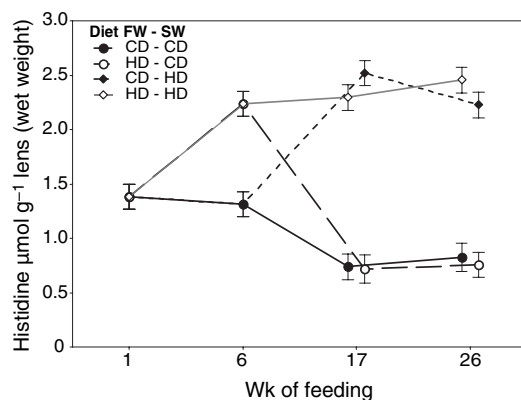
There were no significant differences between strains with respect to the levels of free His or His-containing compounds in muscle or lens tissue at either of the sampling points (data not shown). With the exception of taurine, this was also the case for the other free amino acids analysed. Thus, all mean values are based on combined data from both strains. Changes with time of lens tissue levels of free His and NAH in selected dietary groups are shown in Figs 4 & 5, respectively. During the six first weeks of FW feeding free His remained at a constant level in the pre-smolt group fed low His,

while it reached a significant twofold higher level in the high His group. In the same period there was a general decrease in the NAH levels in each dietary group, reaching a significantly lower level in the group fed CD ( $P < 0.05$ ).

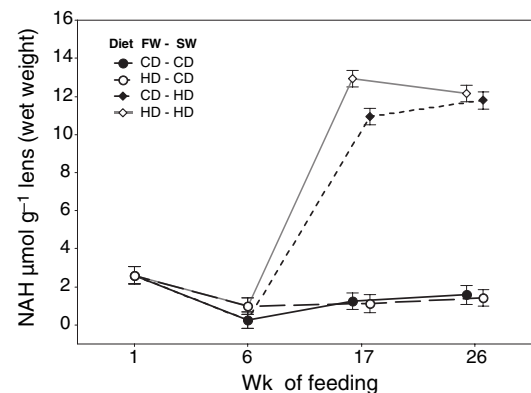
Continued feeding of the HD after the initial 6 weeks did not lead to further elevation of lens free His levels. The fish fed the CD continuously experienced a decrease in lens free His after transfer to SW, while a cross-over from HD to CD resulted in a 70% drop in His down to CD levels. NAH remained at FW levels in fish fed CD in SW. However, in fish receiving HD in the SW feeding period, there was almost a 50-fold increase in lens NAH levels.

The free amino acid profiles of the lens and muscle tissue were analysed at the starting point and at the August sampling, the latter only including groups fed either CD or HD in both FW and SW. Table 3 shows that muscle His, as well as the His-derivatives anserine (Ans) and carnosine were higher in August in the group continuously fed a high His diet (HD-HD), whereas a number of the other amino acids, especially glycine, alanine and hydroxyproline, were significantly higher in the group continuously receiving CD (CD-CD), resulting in a total free amino acid concentration comparable with that of the HD group.

The corresponding data from whole lens is presented in Table 4, showing that the total amount of free amino acid in both groups nearly doubled when they were transferred to SW. There



**Figure 4** Levels of lens histidine (mean  $\pm$  SEM;  $n = 6$  pooled samples) in Atlantic salmon given four selected feeding regimes throughout the trial, based on pooled strain data (FW, fresh water; SW, sea water; CD, control diet; HD, high histidine diet). Dietary regimes were changed at week 6 and the fish were transferred to SW at week 9.



**Figure 5** Levels of lens *N*-acetyl histidine (NAH; mean  $\pm$  SEM;  $n = 6$  pooled samples) in Atlantic salmon given four selected feeding regimes throughout the trial, based on pooled strain data (FW, fresh water; SW, sea water; CD, control diet; HD, high histidine diet). Dietary regimes were changed at week 6 and the fish were transferred to SW at week 9.



**Table 3** Free amino acids in muscle at start and in August, in groups of Atlantic salmon fed either control (CD) or high (HD) histidine diets throughout the trial<sup>1</sup>

Amino acid ( $\mu\text{mol g}^{-1}$ muscle)	Initial sampling FW 3 April	Sampling 1 August SW (week 17)	
		CD-CD <sup>2</sup>	HD-HD
Histidine (His)	0.69 <sup>a</sup>	0.08 <sup>b</sup>	2.7 <sup>c</sup>
Anserine (Ans)	10.11 <sup>a</sup>	9.73 <sup>a</sup>	16.07 <sup>b</sup>
Carnosine	0.08 <sup>a</sup>	0.06 <sup>a</sup>	0.60 <sup>b</sup>
<i>N</i> -acetyl histidine <sup>3</sup>	n.d. <sup>4</sup>	n.d.	n.d.
3-Methylhistidine	n.d.	n.d.	n.d.
1-Methylhistidine	0.01 <sup>a</sup>	0.09 <sup>b</sup>	0.11 <sup>b</sup>
$\beta$ -Alanine	0.42 <sup>a</sup>	0.65 <sup>b</sup>	0.20 <sup>c</sup>
Methionine	0.12 <sup>a</sup>	0.07 <sup>bc</sup>	0.09 <sup>ac</sup>
Glycine	7.38 <sup>a</sup>	4.80 <sup>b</sup>	1.39 <sup>c</sup>
Hydroxyproline	0.55 <sup>a</sup>	1.12 <sup>b</sup>	0.36 <sup>a</sup>
Alanine	3.12 <sup>a</sup>	3.21 <sup>a</sup>	2.04 <sup>b</sup>
Phenylalanine	0.12 <sup>a</sup>	0.12 <sup>a</sup>	0.18 <sup>b</sup>
Cysteine	n.d.	n.d.	n.d.
Tryptophan	n.d.	n.d.	n.d.
Serine	0.96 <sup>a</sup>	0.70 <sup>b</sup>	0.14 <sup>c</sup>
Glutaminic acid	0.53 <sup>a</sup>	0.57 <sup>a</sup>	0.29 <sup>b</sup>
Arginine	0.05 <sup>a</sup>	0.09 <sup>b</sup>	0.09 <sup>b</sup>
Lysine	0.27 <sup>a</sup>	0.39 <sup>b</sup>	0.32 <sup>b</sup>
Ornithine	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>
Tyrosine	0.12 <sup>a</sup>	0.19 <sup>b</sup>	0.18 <sup>b</sup>
Leucine	0.24 <sup>a</sup>	0.22 <sup>a</sup>	0.18 <sup>a</sup>
Isoleucine	0.14 <sup>a</sup>	0.13 <sup>a</sup>	0.11 <sup>a</sup>
Valine	0.31 <sup>a</sup>	0.30 <sup>a</sup>	0.26 <sup>a</sup>
Proline	0.28 <sup>a</sup>	0.16 <sup>a</sup>	0.13 <sup>a</sup>
Threonine	0.51 <sup>a</sup>	0.37 <sup>b</sup>	0.29 <sup>c</sup>
Aspartic acid	0.01 <sup>a</sup>	0.03 <sup>b</sup>	0.02 <sup>ac</sup>
Taurine	2.25 <sup>a</sup>	1.03 <sup>b</sup>	0.93 <sup>b</sup>
Sum free AA (excl. Ans and His)	18.2	14.4	7.9
Sum free AA (incl. Ans and His)	28.3	24.1	26.7

AA, amino acids; FW, fresh water; SW, sea water; CD, control diet; HD, high histidine diet.

<sup>1</sup> Different letter within rows denotes significant differences ( $P < 0.05$ ). Each value represents mean of six muscle samples (three pooled samples per strain).

<sup>2</sup> Shows diet given in fresh water (before hyphen) and sea water (after hyphen).

<sup>3</sup> Based on the HPLC method.

<sup>4</sup> Not detectable.

were significantly higher levels of His and NAH in the high His group. Carnosine and Ans were not detected in the lens in any group. The sum of free amino acids showed a similar trend as in muscle, with higher levels of almost all amino acids in the CD group compensating elevated His and NAH in the HD group. In the lens, however, glutamine, leucine, phenylalanine and glycine contributed mostly on a molar basis. Again, this resulted in no significant difference in the total free amino acid content in the CD and HD groups. The drop of taurine levels observed in muscle when fish went from FW to SW was not seen in the lens. Strain B

**Table 4** Lens content of free amino acids at starting point, and in August, in groups of Atlantic salmon fed either low (CD) or high (HD) histidine diets throughout the trial<sup>1</sup>

Amino acid ( $\mu\text{mol g}^{-1}$ lens)	Initial sampling FW 3 April	Sampling, 1 August SW (week 17)	
		CD-CD <sup>2</sup>	HD-HD
Histidine	1.31 <sup>a</sup>	0.35 <sup>b</sup>	2.38 <sup>a</sup>
Anserine	n.d. <sup>3</sup>	n.d.	n.d.
Carnosine	n.d.	n.d.	n.d.
<i>N</i> -acetyl histidine <sup>4</sup>	2.6	1.3	12.9
3-Methylhistidine	n.d.	n.d.	n.d.
1-Methylhistidine	0.05 <sup>a</sup>	0.28 <sup>b</sup>	0.10 <sup>c</sup>
$\beta$ -Alanine	n.d.	0.10 <sup>5</sup>	n.d.
Methionine	1.09 <sup>a</sup>	1.96 <sup>b</sup>	1.87 <sup>b</sup>
Glycine	1.05 <sup>a</sup>	2.00 <sup>b</sup>	0.74 <sup>a</sup>
Hydroxyproline	n.d.	0.23 <sup>a</sup>	0.13 <sup>b</sup>
Alanine	0.56 <sup>a</sup>	1.07 <sup>b</sup>	0.46 <sup>a</sup>
Phenylalanine	1.01 <sup>a</sup>	3.24 <sup>b</sup>	1.86 <sup>c</sup>
Cysteine	n.d.	n.d.	n.d.
Tryptophan	0.27 <sup>a</sup>	0.54 <sup>b</sup>	0.17 <sup>c</sup>
Serine	0.44 <sup>a</sup>	0.65 <sup>b</sup>	0.20 <sup>c</sup>
Glutaminic acid	0.87 <sup>a</sup>	2.16 <sup>b</sup>	1.38 <sup>c</sup>
Glutamine	1.37 <sup>a</sup>	2.45 <sup>b</sup>	0.10 <sup>c</sup>
Arginine	0.05 <sup>a</sup>	0.06 <sup>a</sup>	0.02 <sup>b</sup>
Lysine	0.12 <sup>a</sup>	0.26 <sup>a</sup>	0.08 <sup>a</sup>
Ornithine	n.d.	0.15 <sup>a</sup>	0.10 <sup>a</sup>
Tyrosine	0.41 <sup>a</sup>	2.35 <sup>b</sup>	1.13 <sup>c</sup>
Leucine	1.10 <sup>a</sup>	2.45 <sup>b</sup>	0.74 <sup>c</sup>
Isoleucine	0.27 <sup>a</sup>	0.73 <sup>b</sup>	0.11 <sup>c</sup>
Valine	0.75 <sup>a</sup>	1.98 <sup>b</sup>	0.88 <sup>a</sup>
Proline	0.15 <sup>a</sup>	0.23 <sup>b</sup>	0.11 <sup>a</sup>
Threonine	0.44 <sup>a</sup>	0.39 <sup>a</sup>	0.26 <sup>b</sup>
Aspartic acid	0.17 <sup>a</sup>	0.61 <sup>b</sup>	0.26 <sup>a</sup>
Asparagin	0.07 <sup>a</sup>	0.15 <sup>b</sup>	0.05 <sup>a</sup>
Taurine	1.05 <sup>a</sup>	1.02 <sup>a</sup>	0.73 <sup>a</sup>
Sum free AA (excl. NAH)	12.6	25.4	13.9
Sum free AA (incl. NAH)	15.2	26.7	26.8

AA, amino acids; NAH, *N*-acetyl histidine; FW, fresh water; SW, sea water; CD, control diet; HD, high histidine diet.

<sup>1</sup> Different letter within rows denotes significant differences ( $P < 0.05$ ). Each value represents mean of six tissue samples (three pooled samples per strain).

<sup>2</sup> Shows diet given in fresh water (before hyphen) and sea water (after hyphen).

<sup>3</sup> Not detectable.

<sup>4</sup> Based on the HPLC method.

<sup>5</sup> Only detectable levels in strain B samples.

had generally higher lens taurine levels at the August sampling, being significantly different from strain A ( $P < 0.05$ ) in the salmon fed the CD.

### Lens and aqueous humour buffering and osmolality

The buffering capacity measurements in lens homogenates from the final sampling in October were  $49.4 \pm 0.8$  and  $52.2 \pm 1.6$   $\mu\text{mol NaOH}$  per gram for fish fed CD and HD, respectively. Non-significant differences were found ( $P = 0.10$ , two-sided *t*-test,  $n \geq 4$ ).

The aqueous humour buffering capacities were  $0.23 \pm 0.006$ ,  $0.19 \pm 0.003$  and  $0.20 \pm 0.004$   $\mu\text{mol NaOH per mL}$  for fish fed CD, HD and HID, respectively ( $n = 5-6$ ). The buffering capacities in aqueous samples from fish fed the CD were significantly higher than those from fish fed the HDs ( $P < 0.05$ ).

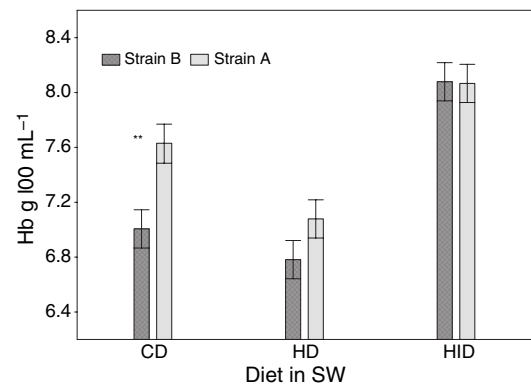
Aqueous humour osmolality was measured both in FW (May) and SW (August and October). There was a general increase in osmolality following the transfer from FW to SW from a range of 310–314  $\text{mOsmol kg}^{-1}$  in May to a range of 320–324 and 322–335  $\text{mOsm kg}^{-1}$  in August and October, respectively. No apparent differences between the feed groups were seen in FW or in the first tissue sampling after SW transfer. By October, the aqueous osmolality of the fish receiving the CD was approximately 10  $\text{mOsm kg}^{-1}$  higher than the high His groups ( $P < 0.05$ ).

### Blood Hb

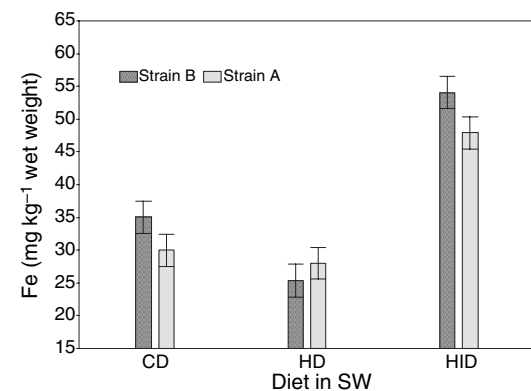
Blood Hb levels in May were significantly influenced by both strain and diet ( $P < 0.05$ ). Mean Hb of individual strains, based on data from all dietary groups were  $9.1 \pm 0.09$  and  $8.7 \pm 0.07$   $\text{g } 100 \text{ mL}^{-1}$  for strains A and B, respectively, whereas the mean for the three diets, based on pooled strain data were  $8.5 \pm 0.1$ ,  $9.0 \pm 0.1$  and  $9.1 \pm 0.1$   $100 \text{ mL}^{-1}$  for CD, HD and HID, respectively. Blood Hb measurements in August are presented in Fig. 6. A lower Hb was seen in the groups receiving the HD, whereas the supplementation of both His and Fe (HID) resulted in significantly higher Hb levels than in the CD group. In October a similar trend was seen, but there were no differences between the strains within the diets. From August to October there was a general increase in blood Hb by 4–12% (data not shown).

### Liver iron and zinc

No significant differences were found between the strains during the trial. At the start, liver iron was  $50.3 \text{ mg kg}^{-1}$  (pooled strain data). Six weeks later, fish fed HD or HID had significantly higher liver iron levels than fish fed the CD, at 53.3, 62.9 and  $40.0 \text{ mg kg}^{-1}$ , respectively ( $P < 0.05$ ). Liver iron in October was reduced in all dietary groups. Similar to blood Hb, the highest liver iron values were found in salmon receiving the HID and the lowest values in the fish fed HD (Fig. 7). Liver zinc



**Figure 6** Blood haemoglobin (Hb) levels in Atlantic salmon strains B and A in August (mean  $\pm$  SEM). Each value represents mean of 18 fish per SW diet and strain. The FW dietary background had no effect on Hb levels at week 17, and therefore the data from six fish per FW diet were combined. Double asterisk denotes significant differences ( $P < 0.05$ ) between strains (FW, fresh water; SW, sea water; CD, control diet; HD, high histidine diet; HID, high histidine and iron diet).



**Figure 7** Liver iron content in Atlantic salmon liver (mg Fe per kg wet weight) at the week 26 sampling (mean  $\pm$  SEM). Each value represents mean of 18 fish per SW diet and strain. The FW dietary background had no effect on liver Fe levels in October and therefore the data from six fish per FW diet were combined. No significant differences were found between strains (SW, sea water; CD, control diet; HD, high histidine diet; HID, high histidine and iron diet).

from the individuals sampled in October varied between 23.4 and 24.9  $\text{mg kg}^{-1}$  (w/w). There was a non-significant trend of fish fed HD to have the lowest liver zinc values (data not presented).

### Discussion

This study shows that dietary His levels had a major influence on cataract development in salmon smolts

in SW, with the largest effect related to the nutrition after transfer from FW to SW. Especially in strain B, fish receiving the diets supplemented with His in SW showed a dramatically reduced cataract severity. Cataract development in SW was also to some extent affected by feeding HD in FW prior to transfer. This effect was more pronounced when the groups were given the CD in the second feeding period, but a significant difference was also found in strain A, when the fish continued on HD in SW. Interestingly, the groups that were fed HD in FW and continued on HD or CD in SW had a similar cataract development until week 14 of the trial. Hence, our data indicate the ability of the fish to utilize or benefit from increased tissue levels of His or His compounds already built up in the body prior to the period when cataracts develop. However, it is also evident that fish from strain A that received CD in FW and continued on HD in SW needed time to build up sufficient tissue levels, as this group developed slightly more severe cataracts than the group continuously fed HD.

Similar low mean cataract scores were found in the two strains when feeding His-supplemented diets (HD) throughout the whole trial. The actual reductions in mean cataract scores compared with the CD groups were different between the strains, suggesting a higher sensitivity to dietary His in strain B. The mild but consistent cataracts seen in both strains fed HD throughout the study indicate that the aetiology of these changes may not be directly related to dietary His levels. Whether, with suboptimal lens His concentrations, these changes induce secondary damage and further cataract development, cannot be ascertained from our study. Alternatively, initial cataractogenesis may have also been related to His nutrition prior to the initiation of the experiment in FW.

The effect of dietary His content and feeding regimes on lens His and NAH levels are clearly demonstrated, outlining different developmental patterns between the two compounds, possibly related to hormonally induced physiological changes appearing in Atlantic salmon undergoing parr-smolt transformation (smoltification). The influence of HD on lens NAH concentrations before transfer to SW seemed limited, whereas up to 50-fold increases in lens concentrations were seen in later stages, given high dietary His levels. As the NAH increase coincided with final stages of smoltification, is it reasonable to hypothesize that the building up of lens NAH levels is part of the

pre-adaptation of Atlantic salmon to a marine environment. From the present study it is not possible to say whether SW exposure itself is a precondition for the increased synthesis of NAH. However, data from another feeding trial indicate a build up of NAH in salmon undergoing the parr-smolt transformation, prior to SW exposure (O. Breck & R. Waagbø unpublished data).

Lens His concentration during smoltification stabilized close to  $2.5 \mu\text{mol g}^{-1}$  lens in the HD group. In SW, lens His represented only 20% of NAH levels on a molar basis in the high His dietary groups. The actual SW transfer date (7 June) represented a 3-week postponement. A third FW sampling and cataract monitoring point closer to the time when the trial population was found to be fully smolted, could have given more precise information on possible correlations between physiological status and lens NAH concentrations, in addition to determining the role of SW exposure in NAH synthesis.

The role of elevated NAH and His levels in the lens in preventing cataracts could be explained by the general characteristic of imidazol-containing compounds as good tissue buffering agents (Ogata, Konno & Silverstein 1998) and antioxidants (Babizhayev 1989; Wade & Tucker 1998). The buffering capacities recorded in this study were comparable with those previously measured in the muscle of a variety of fish species (Castellini & Somero 1981; Ogata 2002). The very high protein content of the lens will contribute a large part of the buffering capacity of a whole lens homogenate. Nevertheless, we did record a higher level of buffering capacity (although not significant;  $P = 0.10$ ) in the lenses of fish fed the high His diet. This could reflect the significantly higher molar concentrations of free NAH and His in the lenses, compared with fish fed the CD. However, the somewhat increased buffering capacity and osmolality of the aqueous humour from fish fed on the CD may be related to pathological leakage of small-sized intracellular crystallins through the lens capsule, as has been described in human cataractous lenses (Maisel 1963; Sandberg 1976).

*In vitro* studies with goldfish, *Carassius auratus* (L.), lenses have indicated an organ-specific role of NAH related to water homeostasis (Baslow 1998). NAH has been found in the brain, lens and occasionally heart of poikilothermic vertebrates, including rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Baslow 1965; Yamada, Tanaka,

Sameshima & Furuichi 1993), whereas skeletal muscle, with the possible exception of Nile tilapia, *Oreochromis niloticus* (L.), is devoid of the compound (Yamada, Tanaka, Sameshima & Furuichi 1992). Our trial has demonstrated surprisingly high levels of NAH in the lenses of fish fed high dietary concentrations of His. It is possible that NAH might be of special importance for lens osmoregulation under SW conditions, by contributing to lens adaptation to increased aqueous osmolality. The osmolality in aqueous humour showed an increase from about 312–323 mOsm kg<sup>-1</sup> after transfer to SW. As the cornea of fish has very low water permeability (Edelhauser, Russel Hoffert & Fromm 1965), any changes in aqueous osmolality most probably reflect changes in plasma osmolality. To counteract loss of water and lens cell shrinkage caused by an increase in aqueous osmolality, lens cells must increase intracellular osmolality. Interestingly, the sum of free amino acids in the salmon lenses of both dietary groups almost doubled following transfer to SW and in the high His group this increase was primarily accounted for by NAH. In the low His group, however, a similar increase was achieved by a general increase in almost all free amino acids in the lens, whereas NAH was maintained at a low level. Assuming a lens water content of approximately 40% (data not shown), the sum of the molar concentrations of amino acids, including NAH, increased by approximately 29 mM, which is much higher than the measured increase in aqueous osmolality following SW transfer. It is possible that the overall lens osmolality is regulated by increased amino acid concentrations, concurrent with decreased levels of other osmolytes, as the fish is transferred from FW to SW.

In muscle tissue there was a small decrease in the total amount of amino acids at the August sampling compared with the initial FW sampling. In the group continuously fed CD, the sum of amino acids, Ans and His excluded, was about 6 µmol g<sup>-1</sup> higher than in salmon fed HD, primarily caused by increased concentrations of the non-essential amino acids glycine, alanine and hydroxyproline. Similar findings were made by Bjerkås, Bjørnstad, Breck & Waagbø (2001), analysing whole eye free amino acid concentrations in cataractous vs. normal Atlantic salmon. They speculated that there was an activated lens protease activity in fish with cataracts (Duncan, Williams & Riach 1994).

The nearly doubled concentration of Ans in muscle samples from fish fed the high His diet

compared with the CD may represent an important buffering factor enabling the salmon to better withstand pH changes in white muscle tissue induced by burst swimming in SW. Ogata *et al.* (1998) have shown increased levels of muscle Ans and reduced levels of muscle His in masu salmon, *O. masou* (Brevoort), smolt vs. parr, and correlated these findings to measured differences in muscle-buffering capacities. It is important to note that in our study, as seen with lens NAH, elevated muscle Ans levels were only observed in the salmon fed the HD. The reduction in muscle non-essential amino acids following increased levels of imidazole compounds has been described in masu salmon (Ogata & Murai 1994), as well as in yellowtail, *Seriola quinqueradiata* Temminck & Schlegel (Ogata 2002). Ogata (2002) suggested a physiological mechanism by which white muscle selectively accumulates imidazole compounds and maintains the total amino acid pool by down-regulating the level of non-essential amino acids. This is in accordance with our findings in Atlantic salmon muscle, as well as in lens tissue of this species. Both Ans and NAH are believed to keep His 'trapped' in the respective tissues and prevent this essential amino acid from being catabolized or used in protein synthesis (Yamada, Tanaka, Sameshima & Furuichi 1994; Baslow 1998).

Inclusion of mammalian BM in feed to Atlantic salmon was shown to mitigate cataract formation (Breck *et al.* 2003) and, as a major constituent in BM, iron may play a role in the aetiology. Increasing the dietary Fe concentration in the SW diets from 110 to 220 mg kg<sup>-1</sup> in the present study had, however, a minor negative effect on cataract development in one of the strains. Waagbø, Bjerkås, Hamre, Berge, Wathne, Lie & Torstensen (2003) found that elevated dietary Fe induced more severe cataracts in Atlantic salmon based, however, on a much higher inclusion level (74 mg kg<sup>-1</sup> vs. 1228 mg kg<sup>-1</sup>).

Our data suggest a growth-promoting effect of feeding high His diets (HD and HID) in SW, compared with CD. Weight differences between diets in favour of HD and HID were also seen among fish classified in the low cataract group (score 0–1), indicating a growth-enhancing role of His not related to visual abilities (data not shown). Mean weights within the dietary groups in October were in most cases higher in more severely affected individuals (score > 1) (data not shown), apparently contradicting the general expectation that

cataracts impair vision, feed uptake and thereby growth (Breck *et al.* 2003). A possible explanation relates to cataract development in early stages being probably positively correlated to growth, as also reported in a study with individually labelled salmon post-smolts (Breck & Sveier 2001). As the cataracts in most groups in our trial did not develop into severe forms affecting feed uptake, a trend of higher weights in affected fish was still present at the last sampling.

Given that all diets were well above general NRC (1993) recommendations of 7 g His per kg, the observed positive effect of crystalline His on growth and biological FCR was surprising. Elevated post-prandial plasma total free amino acids, as well as free His levels were observed in groups of parr force fed diets supplemented with crystalline His compared with fish fed control feed (R. Waagbø & O. Breck unpublished data). Similar effects have been seen after addition of crystalline lysine (Campbell 1999). Studies in humans have suggested that oral or intravenous administration of L-histidine may have an impact on plasma levels of other amino acids (Hamblin & Holton 1972). A number of feeding experiments with salmonids using diets supplemented with single or mixtures of crystalline amino acids have been conducted (Yamada, Simpson, Tanaka & Katayama 1981; Cowey & Walton 1988; Schuhmacher, Wax & Gropp 1997). Higher as well as lower FCRs and growth rates have been reported and some authors have questioned the use of crystalline forms, due to their more rapid and disproportional absorption, possibly leading to an imbalance of free amino acids at sites of protein synthesis and consequently elevated catabolism (Fauconneau, Gray & Houlihan 1995). Further work is needed to confirm our findings and elucidate whether elevated dietary His levels affect FCR and growth via general amino acid metabolism or by improving the physiological condition of different tissues. The relative importance of the dietary form of His should also be investigated further.

The increased blood Hb levels observed in both FW and SW in salmon fed HID reflected the high dietary iron content. Interestingly, in FW the supplementation of His to the CD led to increased Hb levels, whereas in SW, a trend of lower Hb levels in HD vs. CD was found. Blood Hb did not seem to correlate with either growth performance or cataract development as fish on both HD (lowest Hb) and HID (highest Hb) seemed to have improved growth and less severe cataracts than fish

on CD (intermediate Hb). The dietary FW treatment did not influence Hb levels in August or October (data not shown). All Hb values are considered within the normal range of Atlantic salmon (Sandnes *et al.* 1988).

Liver Fe levels within the dietary groups were significantly lower in SW than in FW ( $P < 0.05$ ). In October, salmon fed the HID had 57% higher liver iron levels than fish fed the CD, reflecting the difference in dietary iron concentration. In the group fed HD, the liver iron concentration was 18% lower than in fish fed the CD. His is known to chelate with minerals like iron and zinc and will enhance their absorption (Ashmead & Zunino 1992). The reduced liver iron levels found in October in the group fed HD suggest that absorbed iron might have been distributed to other organ systems in fish fed high His levels. Alternatively, excess dietary His may also induce excretion of iron into the urine, as seen with copper and zinc (Aoyama, Mori, Hitomi-Ohmura & Yoshida 1992). Liver iron concentrations in all SW groups must be considered low, although the three diets contained Fe levels above the general recommendation of 60–100 mg kg<sup>-1</sup> for Atlantic salmon (Andersen, Maage & Julshamn 1996). Our liver iron values were considerably lower than those obtained in feeding studies with Atlantic salmon parr (Andersen *et al.* 1996) and smolt (Andersen *et al.* 1997), using similar dietary iron levels.

To summarize, our findings illustrate the challenging complexity of His nutrition in Atlantic salmon, related to: genetics and changes in environment; and physiological status of anadromous fish species. Elevated dietary His levels reduced cataract formation in Atlantic salmon post-smolts. This effect was probably related to high levels of His and NAH building up in the lens, possessing buffering and antioxidative characteristics, in addition to NAH possibly being of importance in lens water homeostasis. The build up of higher lens concentrations of NAH seems to be coupled to the smoltification process of Atlantic salmon. The differences in cataract susceptibility and growth performance observed between the two strains were not related to different lens or muscle levels of His compounds. Our findings suggest a revision of the dietary His recommendation for intensive farming of salmon, using the concentration of tissue-specific His containing compounds as indicators of sufficient His status. A successful and sustainable growth of the aquaculture industry depends on the increasing use

of non-marine vegetable protein sources, generally lower in His content. This necessitates special care in formulating feeds for the salmon farming industry.

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