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# Effects of vegetable feed ingredients on bone health in Atlantic salmon

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## Summary

The aim of the present study was to examine if dietary inclusion of vegetable lipids (VL) and proteins (VP) influenced markers of bone health in Atlantic salmon. Triplicate groups were fed one of four different diets; 100% fish protein (FP) and fish lipids (FL) (FPFL), 80% VP and 35% VL (80VP35VL), 40% VP and 70% VL (40VP70VL), or 80% VP and 70% VL (80VP70VL) for 12 months on-growth in sea water. Fish were analyzed for vertebral bone mineralization (mineral content, as % of bone dry weight), vertebral deformities (radiology), vertebral bone mRNA expression of factors involved in mineralization (*bone gla protein, bgp*) and growth regulation (*igf-I and growth hormone receptor*), as well as plasma vitamin D metabolites. The fish grew from 0.35 to 4 kg during the experimental period. At the end of the experiment, significantly lower prevalence of fish with one or more deformed vertebrae was observed in the 80VP70VL group (11%) compared to the other groups (33–43%). There was a significant higher relative expression of *igf*-I mRNA in vertebral bone of fish fed the 80VP70VL diet compared to control fish (FPFL), while the other genes studied were unaffected. Elevated plasma 25-hydroxyvitamin $D_3$ recorded in the marine feed group is discussed as a predictor for later development of bone deformities. In conclusion, the present study shows that high inclusion levels of vegetable lipids and proteins may have a positive effect on bone health in Atlantic salmon postsmolts.

## Introduction

Marine feed resources are a limiting factor for further expansion of the salmon farming industry and it is essential that new feed resources become available (Tacon, 2004; Pike, 2005). Vegetable protein (Espe et al., 2006) and lipid (Torstensen et al., 2005) sources are therefore being included into salmon diets at an increasing rate. At present, the main challenges of these replacements have been the low marine polyunsaturated fatty acid composition (Menoyo et al., 2007; Bell and Waagbø, 2008), unbalanced essential amino acid composition (NRC, 1993; Halver and Hardy, 2002), and high levels of antinutrients (Francis et al., 2001) in such diets. The impact of vegetable feed ingredients on growth (Waagbø et al., 1991; Torstensen et al., 2000, 2005; Mundheim et al., 2004; Espe et al., 2006; Pratoomyot et al., 2008), feed utilization (Opstvedt et al., 2003), and product quality (Waagbø et al., 1993; Mundheim et al., 2004; Torstensen et al., 2005; Menoyo et al., 2007), has been extensively studied in Atlantic salmon. There has also been some work on fish health (Oxley et al., 2005; Seierstad et al., 2005, 2009; Waagbø, 2006, 2008; Hemre and Sandnes, 2008). However, the knowledge on the impact of plant ingredient based diets on fish health and welfare is still limited, especially on bone health aspects such as mineralization and development of bone anomalies. Vertebral deformities is a common problem in salmon farming (Waagbø et al., 2005; Witten et al., 2005; Fjelldal et al., 2007a; Sullivan et al., 2007a; Waagbø, 2008), where it results in down-grading losses during harvest (Michie, 2001) and constitutes an ethical dilemma related to fish welfare (Hansen et al., 2010; Fjelldal, P.G., Yurtseva, A.,Berg, A., 2009, Unpubl. data).

The present experiment was designed to study the impact of vegetable feed ingredients on the development of vertebral deformities in Atlantic salmon, during an 1 year on-growth period in seawater. Triplicate groups of salmon were fed complete diets; a normal marine diet, or diets with three different inclusion levels of vegetable lipids and proteins. Gene expressional levels in vertebral bone of *bone gla protein* (also named *osteocalcin, bgp*), *igf-I* and *growth hormone receptor*, and vertebral bone mineral content was monitored to assay possible effects on bone physiology. Finally, plasma vitamin D metabolites was examined in fish fed the marine diet and the complete vegetable diet, since these dietary groups represents a dramatic change in vitamin D supply, and since changes in the vitamin endocrine system previously has been related to development of bone deformities (Fjelldal et al., 2009).

## Material and method

### Fish stock, rearing conditions, and experimental design

Triplicate groups of Atlantic salmon postsmolts (355 g) were fed one of four different diets; 100% fish protein and lipid (FPFL), 80% vegetable protein (VP) and 35% vegetable lipid (VL) (80VP35VL), 40% VP and 70% VL (40VP70VL), or 80% VP and 70% VL (80VP70VL) for 12 months in sea water tanks (volume: 12–10 $m^3$, salinity: 34.9 g $L^{-1}$, water supply: 52 L $min^{-1}$). Each tank contained 500 fish (strain: AkvaGen AS, Tingvoll, Norway) at the start of the experiment (1500 fish per dietary group), the temperature was stable at 8.9 ± 0.1°C throughout the study, and the oxygen saturation of the outlet water was never < 80%. For a detailed description of the fish stock, rearing conditions, experimental design and diet composition see Torstensen et al. (2008).

In Feb, after 8 months of exposure to the experimental diets, part of the population from the marine control group (FPFL; previously marked by fin marking) and the vegetable group (80VP70VL) were exchanged between respective triplicates (crossed-over), to examine short- term responses to changes in diet. A sampling conducted 1 month (27 days) therafter of theses groups, included analysis of plasma vitamin D metab-

olites [25OHD$_3$ and 1,25(OH)$_2$D$_3$], as well as plasma calcium and phosphorous.

#### Sampling

Whole vertebral columns were dissected and prepared for radiology at the start of the experiment in June 2006 (n = 30, random sample of 30 fish before the start of the experiment), and in Nov 2006 (n = 120; 30 per dietary group) and June 2007 (n = 150; 30 in the 80VP35VL and 40VP70VL groups and 45 in the FPFL and 80VP70VL groups). Vertebrae number 43 was dissected for RNA extraction (n = 18; 9 per dietary group) in July 2006 and June 2007, and for measurement of mineral content (n = 60; 30 per dietary group) in June 2007 from the FPFL and 80VP70VL dietary groups.

#### Radiology

Dissected vertebral columns were radiographed by using a portable X-ray apparatus (HI-Ray 100, Eickenmeyer Medizintechnik für Tierärzte e.K., Tuttlingen, Germany) and 30 × 40 cm film (FUJIFILM IX 100, FUJIFILM Corp., Tokyo, Japan). The film was exposed for 50 mAs and 72 kV (90 cm), developed using a manual developer (Cofar Cemat C56D, Arcore (MI), Italy) with Kodak Professional manual fixer and developer (KODAK S.A., Paris, France), and digitalised by scanning (Epson Expression 10000 XL, Seiko Epson Corp., Nagano-Ken, Japan). Each fish were evaluated for vertebral deformities, and the location and type of deformity was recorded according to Fjelldal et al. (2007a).

#### Mineral content

Vertebrae number 43 were defatted in acetone and chloroform baths, dried overnight at 100°C, and then incinerated for 13.5 h in a muffle furnace (Mod. L40, Nabertherm GmbH, Bremen, Germany) (115°C for 0.5 h, 540°C for 5 h, and 750°C for 8 h). The dry and ash (mineral) weights of each individual were weighed to nearest $10^{-2}$ mg. The mineral content was calculated as follow:

$$\text{Mineral content} = (\text{mineral weight})\,(\text{dry weight})^{-1}$$

#### RNA isolation and RT-PCR

After dissection, samples were immediately frozen on liquid nitrogen. Total RNA were extracted from each sample using the FastRNA Pro Green Kit (Qbiogene). RNA used for RT-PCR was DNAse treated according to manufactures recommendations (Invitrogen). The amount and quality of was verified by the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). All gene expressional level analysis reactions were run on the 7900 HT Fast Real-Time PCR system. The PCR conditions was 50°C for 2 min followed by 95°C for 10 min, the reactions thereafter proceeded through 40 cycles of 95°C for 15 s followed by 60°C for 1 min. Primers used for the real-time analysis are shown in Table 1. The efficiency of targets in relation to reference [*elα* (Olsvik et al., 2005)] was determined using a standard curve method together with a validation experiment (ABI User Bulletin #2 for ABI 7700 sequence detections system). In the validation experiment 500, 250, 125, 62.5 and 31.1 ng of RNA was used for cDNA synthesis and the slope of log input amount of RNA vs delta Ct is included in Table 1. All genes used displayed approximately equal efficiency between target and reference. The relative expression level was calculated using the Comparative Ct method (ABI User Bulletin #2 for ABI 7700 sequence detection system). In all experiments no-template controls and standard curve were run together with the samples.

#### Plasma vitamin D metabolite analysis, calcium and phoshorous

The vitamin D metabolites, 25-hydroxyvitamin D$_3$ (25OHD$_3$) and 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$; calcitriol] were analysed by use of liquid chromatography-tandem mass spectroscopy according to a modified method (Fjelldal et al., 2009), based on (Kissmeyer et al., 2000; Kissmeyer and Sonne, 2001). The UPLC/MS/MS system (Waters, Milford, MA, USA) and running conditions are described by Fjelldal et al. (2009). Plasma samples were protein precipitated, solid phase (Isolute™ MF C$_{18}$ SPE cartridge) extracted and eluted with heptan:2-propanol (93:7), evaporated under N$_2$, and finally reconstituted in 200 $\mu$l methanol : 1 M ammonium acetate:water (500:2:500) before injected into the LC system. External calibration curves (ranging from 0.6 to 24 nmol L$^{-1}$) were applied based upon standard solutions of the two metabolites treated in the same way as the samples, while a similar amount of deuterised 25OHD$_3$ was used as internal standard in all samples and standards. An in-house fish plasma was used in the quality control.

#### Statistics

Data were analysed using Statistica® (version 8.0, Statsoft, Tulsa, USA). Significant differences in gene expressional levels were tested with a two-way ANOVA with diet and time as the dependent factors. Significant ANOVAs were followed by Bonferronis multiple comparison test to locate any differences between diets or over time. Significant differences in vertebral

Table 1
Relative gene expression (mean ± SE) in vertebral bone of *bgp*, *igf-I*, and *ghr* in Atlantic salmon fed a 100% fish protein and lipid diet (FPFL; n = 18, 9 per date) or a replacement diet with 80% vegetable protein and 70% vegetable lipid (80VP70LP; n = 18, 9 per date) for 1 year in seawater (June 2006 to June 2007)

| | FPFL[1] | | 80VP70VL[1] | | P-value | |
|---|---|---|---|---|---|---|
| Parameter | July 06 | June 07 | July 06 | June 07 | Date | Diet |
| *bgp* | 0.82 ± 0.16 | 1.07 ± 0.19 | 0.60 ± 0.12 | 1.02 ± 0.13 | 0.036 | 0.374 |
| *Igf-I* | 1.52 ± 0.18$^{ab}$ | 0.94 ± 0.13$^{b}$ | 1.93 ± 0.12$^{a}$ | 1.24 ± 0.23$^{b}$ | 0.0006 | 0.039 |
| *ghr* | 2.58 ± 0.39$^{a}$ | 0.88 ± 0.12$^{c}$ | 2.04 ± 0.29$^{ab}$ | 1.03 ± 0.06$^{bc}$ | 0.000007 | 0.451 |

[1]Different lower case letters indicate significant differences (Bonferronis test, P < 0.05) within a parameter with 'a' as the highest value.

bone mineral content between diets were tested with a one-way ANOVA. Chi-square tests were used to test dietary group differences in the prevalence of deformed fish within sampling points (level of significance Bonferroni adjusted to $P < 0.0125$, Sokal and Rohlf, 1995). Differences in vitamin D metabolites were tested by use of a nested ANOVA (fish nested in tanks) and Tukeys *post hoc* test.

## Results

### Mortality

Mortality was negligible (< 1%) in all dietary groups during the entire experimental period (Torstensen et al., 2008).

### Somatic growth

For details on somatic growth, see Torstensen et al. (2008). The group fed the highest inclusion level of vegetable proteins and lipids grew slower (SGR, % $day^{-1}$) compared to the other groups during the first 3 months of the experiment (June–September 2006), while the growth rate was equal between the groups during the rest of the experiment.

### Vertebral bone mineralization and gene expression

The expressional level of *igf*-I in vertebral bone was significantly higher (two-way ANOVA, $P < 0.05$) in the fish fed the 80VP70VL diet compared to the fish fed the FPFL diet (Table 1). There were no dietary effects on the other studied genes. However, there was a significant effect (two-way ANOVA, $P < 0.05$) of date on the expressional levels of *bgp*, *igf-I* and *ghr* in vertebral bone, with significantly higher (Bonferronis test, $P < 0.05$) levels of *igf-I* in July 2006 compared to June 2007 in the fish fed the 80VP70VL diet, and of ghr in July 2006 compared to June 2007 in the fish fed the FPFL diet (Table 1).

Mean (±SE) mineral content in the bone of the vertebrae in June 2007 was not significantly different (one-way ANOVA, $P = 0.1$) between the marine control diet (57.2% ± 0.12, $n = 30$) and the high vegetable inclusion diet (57.4% ± 0.10, $n = 30$).

### Vertebral deformities

In June 2007, there was a significant lower prevalence (Chi-square, $P < 0.0125$) of fish with one or more deformed vertebrae in the high vegetable inclusion diet 80VP70VL compared to the other diets (Table 2). The prevalence of deformed vertebrae in the deformed fish was not significantly different (one-way ANOVA, $P > 0.05$) between groups (pooled mean 6.5% ± 0.9 S.E., $n = 45$). However, there was a tendency to less severely deformed fish in the high vegetable inclusion diet compared to the other diets (Table 2). There were no increase over time in the prevalence of fish with one or more deformed vertebrae in the 80VP70VL group, while the other groups showed an increase in this parameter (Fig. 1).

The predominate location for vertebral deformities at the termination of the experiment in June 2007 was in the most cranial and caudal parts of the vertebral column (Fig. 2, radiographic example in Fig. 3a,b), and 67% of the deformed vertebrae suffered from ankylosis and compression, while 33% were compressed.

### Vitamin D metabolites

Plasma $25OHD_3$ and $1,25(OH)_2D_3$ were analysed in the marine control and the high vegetable dietary groups in Feb 2007, 1 month after change in diet (cross-over). Fish contiously fed on the marine control diet showed significantly elevated plasma $25OHD_3$ concentrations compared to fish contiously fed the vegetable diet (Fig. 4). Fish crossed-over from the marine to vegetable diet reduced the plasma $25OHD_3$ concentrations to vegetable diet level during the 27 days, while vica

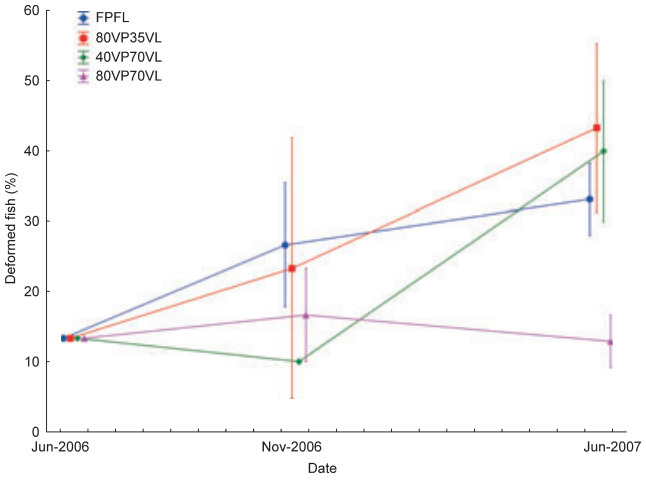


Fig. 1. Changes in prevalence (%) of one or more deformed vertebrae in Atlantic salmon evolving during the experimental period when reared for 12 months in sea water and fed diets containing 100% fish protein and lipid (FPFL), 80% vegetable protein (VP) and 35% vegetable lipid (VL) (80VP35VL), 40% VP and 70% VL (40VP70VL), or 80% VP and 70% VL (80VP70VL). The values are means ± SE (bars) of three replicate tanks per treatment

Table 2
Number (n) of radiographed fish and of fish with one or more deformed vertebrae (V), 1-2 deformed V, 3-10 deformed V and 11-18 deformed V at the end of the experiment in June 2007 in Atlantic salmon fed diets containing 100% fish protein and lipid (FPFL), 80% vegetable protein (VP) and 35% vegetable lipid (VL) (80VP35VL), 40% VP and 70% VL (40VP70VL), or 80% VP and 70% VL (80VP70VL) for 12 months in sea water

| | Diet[1] | | | |
|---|---|---|---|---|
| Category | FPFL (n) | 80VP35VL (n) | 40VP70VL (n) | 80VP70VL (n) |
| Analysed fish | 45 | 30 | 30 | 45 |
| Fish with ≥ 1 deformed vertebrae | 15 (33%)[a] | 13 (43%)[a] | 12 (40%)[a] | 5 (11%)[b] |
| 1–2 deformed vertebrae | 6 | 8 | 6 | 4 |
| 3–10 deformed vertebrae | 8 | 4 | 5 | 1 |
| 11–18 deformed vertebrae | 1 | 1 | 1 | |

[1]Different lower case letters indicate significant differences (Chi square, $P < 0.0125$) within a category with 'a' as the highest value.

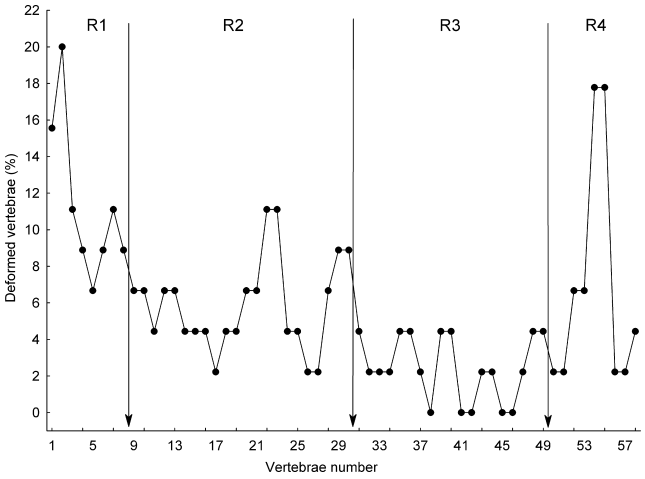


Fig. 2. Location of vertebral deformities at the end of the experiment in June 2007. Prevalence (%) of deformed vertebrae in different regions (R) of the vertebral (V) column in the deformed individuals (all groups pooled; n = 45). R1 = cranial trunk (V1-8), R2 = caudal trunk (V9-30), R3 = tail (V31-49), R4 = tail fin (V50-58) (Kacem et al., 1998)

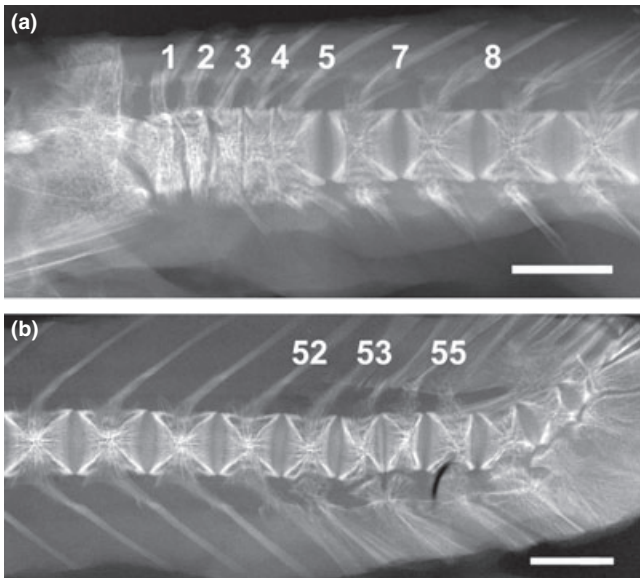


Fig. 3. Lateral radiograph of deformed vertebral bodies taken at the end of the experiment in June 2007 from the cranial trunk (V1-8) (a) and tail fin (V50-58) (b) regions of the vertebral column. The vertebra number is indicated on the corresponding neural arches. Scale bar = 1 cm

versa crossed-over fish did not increase vitamin D status. For the active vitamin D metabolite plasma $1,25(OH)_2D_3$, no significant differences were observed between the dietary groups (Fig. 4).

## Discussion

The results of the present study clearly demonstrated that a high inclusion level of vegetable proteins and lipids had no negative effects on the recorded bone health parameters in Atlantic salmon postsmolts, since the prevalence of vertebral deformities was stable over time and lowest at the termination of the experiment in the fish fed the high vegetable (80VP70VL) diet, and there were no significant effects on bone mineralization of the different diets.

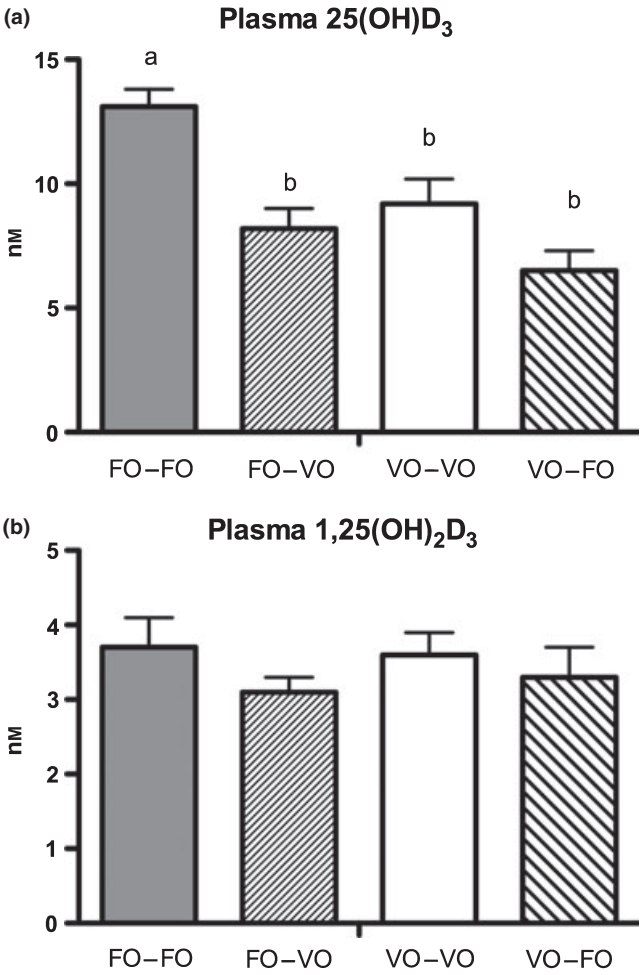


Fig. 4. Plasma vitamin D metabolites ($25OHD_3$ and $1,25(OH)_2D_3$; calcitriol) after 9 months exposure in sea water in salmon fed diets containing 100% fish protein and lipid (FPFL; here FO) or 80% VP and 70% VL (80VP70VL; here VO). A part of the tank populations were transferred to respective opposite diets (crossed-over from FO to VO and from VO to FO) after 8 months and sampled 27 days after (i.e. 9 months). n = 9 per dietary group treatment (columns represent mean values; bars = standard error)

### The prevalence of vertebral deformities

The prevalence of deformed fish increased from 13% to 33–43% during the experimental period in the FPFL, 80VP35VL and 40VP70VL diets, while it was stable over time and ranged between 11 and 16% in the 80VP70VL diet. Other studies have shown an increase over time in the prevalence of vertebral deformities during on-growth in seawater (Fjelldal et al., 2007a, 2009), and Fjelldal et al. (2009) found that salmon fed a diet (approximately 20VP50VL) with extra mineral supply during the first period in seawater had a prevalence of deformed fish of 15% at transfer to seawater, and 36% 1 year later, based on radiology. However, fish fed the same diet but with a normal mineral supply showed a prevalence of 73% after 1 year in seawater. Other radiological studies on farmed Atlantic salmon have shown occurrences of vertebral deformities ranging between 3.8 and 20% during the parr stage (Fjelldal et al., 2007a; Sullivan et al., 2007a,b), between 3.3 and 46% in post smolts (Fjelldal et al., 2009; Wargelius et al., 2009), and between 12 and 73% in harvest size salmon (Witten et al., 2006; Fjelldal et al., 2007a, 2009). The 80VP70VL group had a lower weight gain during the first 3 months of the experiment compared to the other groups (Torstensen et al., 2008). This was the first period after transfer to seawater,

which in earlier studies has shown to be a critical period for a normal development of the vertebral column (Fjelldal et al., 2006, 2007b, 2009). Slower growth in this period may have had a positive effect on the structural integrity and chemical composition of the vertebral bodies, and contributed to a more optimal bone health in the 80VP70VL group.

On the other hand, the results of the present study may also lead us to speculate if there are some component(s) in marine feed sources that contributes to the development of vertebral deformities during on-growth in seawater, irrespective of the difference in somatic growth. Firstly, substitution of fish oil with vegetable oil in the feed have shown to reduce the levels of dioxins and dioxin-like PCBs in farmed Atlantic salmon (Berntssen et al., 2005), and rainbow trout, (*Oncorhynchus mykiss* (Oo et al., 2007). Dioxins are known to interrupt skeletal development during early life stages in zebra fish (*Danio rerio*) (Henry et al., 1997), rainbow trout (Hornug et al., 1999), Japanese medaka (*Oryzias latipes*) (Kim and Cooper, 1999), and fathead minnow (*Pimephales promelas*) (Olivieri and Cooper, 1997). However, the impact of dioxins on bone growth and development at later stages in the life cycle remains to be studied. Since the 80VP70VL and 40VP70VL dietary groups, both high in vegetable lipids, developed a prevalence of vertebral deformities at 11 and 40%, respectively, the presently reported deformities are most probably not induced by dioxins.

Secondly, marine ingredients are among the few natural sources of vitamin D, like the classical cod liver oil used as a natural vitamin D supplement for humans. While vitamin D is essential to human bone health, the role of the vitamin D endocrine system in fish bone growth and development is more uncertain (Lock et al., 2010). For adult salmon reared in calcium rich seawater, vitamin D has been suggested to play a role in the phosphorous homeostasis. Recently, we observed elevated levels of plasma vitamin D metabolites in salmon fed normal level of mineral supply compared to fish fed extra dietary mineral supply, where the former fish group developed considerable higher incidences of bone deformities at later stages (Fjelldal et al., 2009). Plasma phosphorous, but not calcium was also lower in the normal mineral supplemented group. The presently observed elevated plasma 25OHD3 and higher occurrence of vertebral deformities in salmon fed the complete marine diet, compared to the vegetable group, supports the idea that plasma levels of vitamin D metabolites may be used to predict the risk for bone deformities in Atlantic salmon.

Elevated or toxic dietary vitamin D has been considered as a risk factor for bone deformities in fish (Graff et al., 2002a,b), but since extremely high dietary levels of vitamin D did not impact bone health in theses studies, it has been suggested that the risk may be related to the potential active metabolites in the marine ingredients. The bioavailability of the vitamin D metabolites from marine sources are not known, and consequently it is difficult to sort out whether the vitamin D endocrine system is activated by the given conditions (rapid growth or lack of phosphorous / mineral supply) or pushed by elevated dietary intakes and uptakes of vitamin D metabolites in fish fed the marine diets. However, since the intermediate replacement groups also developed bone deformities at a similar incidence as the marine diet (not analysed for vitamin D metabolites), the former suggestion is most probable. Interestingly, fish crossed-over from the marine to the high vegetable diet reduced plasma 25OHD3, while fish crossed over from vegetable diet to marine diet did not show elevated plasma 25OHD3. This further supports the idea that the relationship between D-vitamin metabolites and the development of bone deformities acts through an activation of the vitamin D system.

#### The location and severity of the deformities

The vertebral column of Atlantic salmon can be sub-divided into 4 main regions; the cranial trunk (V1 $\rightarrow$ 8), caudal trunk (V9 $\rightarrow$ 30), tail (V31 $\rightarrow$ 49), and tail fin (V50 $\rightarrow$ 58) regions (Kacem et al., 1998). The vertebrae of the cranial and caudal trunk regions posses ribs, while the vertebrae of the tail and tail fin regions posses haemal arches. In the present study, the predominate locations for vertebral deformities were in the cranial trunk and tail fin regions of the vertebral column, and there was a low occurrence of deformities in the tail region, and intermediate occurrence in the caudal trunk region. This is in agreement with the findings of Fjelldal et al. (2009), who found that a group of salmon that had the same occurrences of vertebral deformities as the FPFL, 80VP35VL and 40VP70VL diets at the same stages of the production cycle had no development of deformities in the tail region over time from seawater transfer until harvest size. On the other hand, deformities that develop in the cranial and caudal trunk regions have shown to be present at the parr stage (Fjelldal et al., 2007a; Sullivan et al., 2007a), while deformities in the tail and tail fin regions have shown to develop later in ontogeny (Fjelldal et al., 2007a, 2009), and in some cases after transfer to seawater. Thus, it is possible that the deformities in the present study were initiated before the onset of the experiment, and that the 80VP70VL diet had a therapeutic effect and reduced the extent of the disorder, maybe through a reduction in growth rate during the early seawater phase. In the FPFL, 80VP35VL and 40VP70VL dietary groups, some of the deformed fish had more than 10 deformed vertebrae. Such severe cases with more than 10 deformed vertebrae imply impaired fish welfare, as it will reduce the growth performance of the fish (Hansen et al., 2010; Fjelldal, P.G., Yurtseva, A.,Berg, A., 2009, Unpubl. data).

#### Gene expressional levels

The vertebral gene expression of igf-I was significantly higher in the fish fed the 80VP70VL diet compared to the fish fed the FPFL diet. In Atlantic salmon vertebral bone, induction in gene expression of *igf-i* and its receptor has been associated with increased ECM production and increased size of the vertebrae (Wargelius et al., 2005; Nordgarden et al., 2006). This may imply that the vertebral bodies of the fish fed the 80VP70VL diet were better 'equipped' for a normal development compared to those of the fish fed the FPFL diet. The vertebral bone gene expression of *ghr* and *igf-I* was significantly higher in July 2006 compared to June 2007 in the FPFL and 80VP70VL dietary groups, respectively. The weight gain (% day$^{-1}$) was 0.88–1.00% in June–September 2006, and 0.35–0.44% in February–June 2007. Thus, the higher expression of ghr and igf-I in the early part of the experiment may have been related to higher growth rate in this period.

## Concluding remarks

The combination of high inclusion levels of both vegetable lipids and proteins had a positive effect on bone health in Atlantic salmon postsmolts, when compared to lower inclusion

levels of vegetable lipids or proteins, or a pure marine diet. This may be related to slower growth during the early seawater phase in the fish fed the highest inclusion levels.

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