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Inadequate phosphorus nutrition in juvenile Atlantic salmon has a negative effect on long-term bone health

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ABSTRACT

In order to study the short-term effects of dietary P levels during juvenile rearing on mineral status (bone ash content), and its long-term effects on the development of vertebral deformities (radiography and external examination), triplicate groups of Atlantic salmon juveniles (1.3 g) were fed blue whiting meal (67%) based diets added 0 (0P), 3 (3P) and 6 (6P) g inorganic P kg^{-1} (15, 18 or 21 g kg^{-1} total P) for 77 days (18.3 g), and then followed up on a common commercial diet for 432 days (1927 g). At the termination of the period on the experimental feeds the vertebrae of the fish fed the 0P diet had a significantly lower ash weight than those fed the 3P and 6P diets, while there was no difference in the occurrence of radiological deformed fish. 252 days later, the 0P (31.7 ± 5.5%) dietary group had a significant higher occurrence of radiological deformed fish than the 6P dietary group (9.4 ± 5.6%), while the 3P dietary group displayed an intermediate level (19.7 ± 2.8%). At termination, 432 days after the termination of the experimental feeds, the 0P dietary group (5.9 ± 0.7%) had a significantly higher prevalence of externally deformed fish compared to the 3P (3.5 ± 0.9%) and 6P (2.0 ± 0.4%) dietary groups. This was mainly caused by a higher level of deformities in the caudal region (V31–58) of the vertebral column in the 0P group. There were no effects on mortality or growth of the present diets.

The results show that inadequate P nutrition in a short period during the juvenile stage can predispose Atlantic salmon to develop vertebral deformities following seawater transfer.

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1. Introduction

Phosphorus is an essential nutrient for fish and has many metabolic roles, i.e. as a constituent of bone, scales, ATP, cell membranes, and nucleic acids (Skonberg et al., 1997), and has to be supplied via the diet as the P content in the water is low and the absorption from the water is inefficient (Lall, 1991; 2002). Excess dietary P is harmful for the environment (Mente et al., 2006), while low dietary P can induce bone deformities in Atlantic salmon (Baeverfjord et al., 1998), and then raise concerns regarding welfare (Hansen et al., 2010) and down-grading losses (Michie, 2001). The P requirements for coldwater species have been suggested to be 6 g available P kg^{-1} dry feed (NRC—National Research Council, 1993). The specific P requirement in Atlantic salmon reared in freshwater was found to be higher and about 10 g kg^{-1} available P to support growth and optimal mineralization (Åsgård and Shearer, 1997). This is in line with the study by Albrektsen et al. (2009), showing that in addition to the 3 g kg^{-1} available P in blue whiting meal, another 6 g kg^{-1} (9 g kg^{-1} available P in total) is needed to support optimal mineralisation in Atlantic salmon postsmolts. Fjelldal et al. (2009a) further found that fast growing under-yearling postsmolts of Atlantic salmon fed a diet with 6.3 g available P kg^{-1} dry feed (12.2 g total P) during the first period in saltwater developed a higher prevalence of vertebral deformities compared to fish fed a diet with 9.3 g available P kg^{-1} dry feed (16.7 total P). Although Helland et al. (2005) found that a low P diet (9.2–9.5 g kg^{-1} total P) had no effect on the development of vertebral deformities in Atlantic salmon parr (8.4 g) after 30 days of feeding when compared to a high P diet (13.7–13.9 g kg^{-1} total P), the long-term effects of juvenile feeds marginal in P have never been tested; vertebral deformities may develop a period after the point at which they are induced (Grini et al., 2011). In salmon farming, juveniles are reared under continuous light in heated water to enhance growth. Both temperature (Grini et al., 2011; Ytteborg et al., 2010a) and photoperiod (Fjelldal et al., 2005) affects bone development in Atlantic salmon, and the combination of high temperature, low available dietary P and continuous light can be detrimental for normal bone growth (Fjelldal et al., 2011).

Both marine and vegetable protein sources are used in the production of commercial salmon feeds. The marine sources are fish meals based on species such as herring (*Clupea harrengus*), sprat (*Sprattus sprattus*), European anchovy (*Engraulis encrasicolus*), sandeels (*Ammodytes*), Atlantic horse mackerel (*Trachurus trachurus*), capelin (*Mallotus villosus*), Atlantic mackerel (*Scomber scombrus*)

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and blue whiting (*Micromesistius poutassou*). The total P content in fishmeal is high and expected to provide adequate amounts of available P (9–15 g total P kg^{-1} diet) at high inclusion levels of marine ingredients in the diet. As the digestibility values of P from fishmeal show great variation, ranging from 20 to 60% (Lall, 1991; Riche and Brown, 1996) it is difficult to predict that adequate P is available to meet dietary P needs. It has been reported that high quality low temperature (LT) fishmeal produced from blue whiting reduce growth, feed efficiency and bone mineralization in rapidly growing underyearling Atlantic salmon postsmolts (Albrektsen et al., 2009). In this study, total and available P was 15.0 and 3.2 g kg^{-1}, respectively, in a diet based on 57.4% blue whiting meal, which indicates that P availability in blue whiting meal is very low and inadequate to meet dietary P requirement. Fish meal produced from blue whiting has been recommended not to be used in commercial salmon diets during weaning and early juvenile stages, mostly due to its high natural vitamin A content and potential toxicological risk in fish at early developmental stages as reported by Dedi et al. (1995, 1997); Ørnsrud et al. (2002) and Lall and Lewis-McCrea (2007). In the study by Albrektsen et al. (2009) it was concluded that the high vitamin A content in blue whiting meal cannot explain the reduced growth performance of seawater transferred salmon, and that improved growth can easily be obtained by supplementation of soluble, highly available P salts to meet dietary P requirement. There is however no literature on how Atlantic salmon juveniles respond to blue whiting meal based diets.

To study the long-term effects of dietary P during juvenile rearing on the vertebral column, triplicate groups of Atlantic salmon juveniles (1.3 g) were fed blue whiting meal based (67%) diets added with 0, 3 and 6 g inorganic P kg^{-1} (15, 18 and 21 g kg^{-1} total P) for 77 days, and followed up for 432 days (1927 g) on a common commercial diet with regular recordings of vertebral deformities (radiography and palpation). The ash weight of the vertebral column at the termination of the period on different feeds was measured to evaluate possible dietary effects on bone mineralization.

2. Materials and methods

2.1. Diets

A commercial LT quality fishmeal produced from blue whiting at Egersund fish meal factory in Norway was used for the production of a basal diet according to the ingredient composition shown in Table 1. Phosphorus from the basal fishmeal (21 g kg^{-1}) accounted for a total P level of about 15 g kg^{-1} in all diets. The experimental diets were supplemented with graded levels of inorganic P obtained from KH_2PO_4 (22.5% P), providing 0, 3 and 6 g P kg^{-1} and total dietary P of 15.6, 17.8 and 21.1 g kg^{-1}. The small dietary differences due to variable P inclusion were balanced by graded inclusion of K_2CO_3, wheat gluten and wheat to balance dietary potassium and protein levels, while keeping all other ingredients constant (Table 1). All feed ingredients were grounded and homogenized through a 1 mm sieve mesh prior to extrusion of the diets. The analysed contents of nutrients, energy and minerals of the experimental diets are shown in Table 2. All chemical analyses were carried out in duplicate by a laboratory accredited by the Norwegian National Accreditation body. Total phosphorus (P) was determined by a spectrometric method (ISO 6491–1998), while NaOH extractable P (soluble P) was determined following incubation of ingredient samples (0.4 g) in 40 mL of 1 N NaOH for 16 h according to a procedure described in Ruban et al. (2001) and modified by Hua et al. (2005).The chemical contents of the diets showed that the intended contents of nutrients and expected levels of P were achieved. All diets were isoenergetic (dry matter based), mean value; 23.9 ± 0.1 MJ kg^{-1}, and the protein and lipid energy accounted for 55 and 39%, respectively, of total energy in the diets. The experimental feed mixtures were extruded on a TX-52 co-rotating, fully intermeshing, twin-screw extruder (Wenger Manufacturing Inc., Sabetha, KS). The wet extrudate was produced at 1.0 mm dies and cut at the die surface by a 6 blade knife assembly. The steam/water ratio and knife speed were adjusted during extrusion in order to produce pellet ranging from 1 to 1.5 mm. The pellet was dried (6.5% water) and coated with fish oil in a rotating coating reel (Model SU 145L, Susemihl GmbH, Neu-Ansprach, Germany), before sieving into pellet sizes of 1.0 (5 kg), 1.2 (5 kg) and 1.5 (20 kg) mm particle sizes.

Table 1
Diet composition.

Dietary total P (g P kg^{-1})	15	18	21
Added inorganic P (g P kg^{-1})	0	3	6
Ingredient content g kg^{-1}			
Fish meal (LT)[a]	677.6	673.8	670.2
Fish oil[b]	145	146	146
Wheat gluten meal[c]	10	15	20
Wheat meal[d]	121	112	104
KH_2PO_4 (22.5% P)	0	13.4	26.8
K_2CO_3	13.4	6.8	0
Soyalecithin	3	3	3
Inositol	0.3	0.3	0.3
Betafin	4	4	4
Carophyll pink	0.6	0.6	0.6
Vitamin mixture[e]	20	20	20
Mineral mixture without P[f]	5	5	5

[a] LT fishmeal, SILFAS, N-5892, Bergen, Norway. Protein: 718 g kg^{-1}, Lipid: 78 g kg^{-1}, ash: 140 g kg^{-1}, moisture: 72 g kg^{-1}.
[b] Norsalmoil, Norsildmel AL, N-5141 Fyllingsdalen, Norway.
[c] Wheat gluten meal: Protein: 800 g kg^{-1}, Lipid: 14 g kg^{-1}, ash: 9 g kg^{-1}, moisture: 87 g kg^{-1}.
[d] Wheat meal: Protein: 124 g kg^{-1}, Lipid: 19 g kg^{-1}, ash: 14 g kg^{-1}, moisture: 133 g kg^{-1}.
[e] Provided per kg of feed: vitamin D3, 3000 I.E.; vitamin E, 136 mg; thiamin, 20 mg; riboflavin, 30 mg; pyrodoxine–HCl, 25 mg; vitamin C, 200 mg; calcium pantothenate, 60 mg; biotin, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B12, 0,05 mg; menadion bisulphite, 20 mg.
[f] Provided per kg of feed: magnesium 500 mg; potassium, 600 mg; zinc, 120 mg; iron, 60 mg; manganese, 30 mg; copper, 6 mg.

2.2. Fish stock, rearing conditions and experimental design

On February 29th 2008, 4500 Atlantic salmon start feeding fry (Aquagen strain) were randomly allocated between 9 square, gray and covered tanks ($1 \times 1 \times 0.25$ m) at the Institute of Marine Research, Matre (Western Norway), and start fed with a commercial start feeding diet (NUTRA ST 0.5, Skretting AS, Fontaine-les-Vervins, France). The fish were fed continuously 24 h per day and the photoperiod was 24 h continuous light. The pellet size was increased to 0.75 mm (NUTRA ST 0.75, Skretting AS, Fontaine-les-Vervins, France) on

Table 2
Proximate composition, energy and mineral contents of experimental diets.

Dietary total P (g P kg^{-1})	15	18	21
Added inorganic P (g P kg^{-1})	0	3	6
Ingredient content			
Protein, g kg^{-1}	528	508	516
Lipid, g kg^{-1}	223	216	219
Moisture, g kg^{-1}	54	85	73
Ash, g kg^{-1}	116	115	120
Gross energy[a], MJ kg^{-1}	22.6	21.8	22.1
Minerals (analysed values)			
Ca, g kg^{-1} diet	21.3	19.2	20.5
P, g kg^{-1} diet	15.6	17.8	21.1
Soluble P, g kg^{-1} diet	6.3	8.9	11.7
Zn, mg kg^{-1} diet	198	184	192
Dietary Ca/P ratio	1.37	1.08	0.97

[a] Gross energy values are calculated according to the following caloric values (MJ kg^{-1}): protein 23.6; lipid, 39.5; carbohydrate, 17.1.

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