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Utilization of acid hydrolysed phosphorous from herring bone by-products in feed for Atlantic salmon (*Salmo salar*) start-feeding fry

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ABSTRACT

The aim of this study was to test the efficacy of a new marine P ingredient from the bone fraction of herring by-products by evaluating the dietary impacts on growth, mineralization and skeletal development in Atlantic salmon fry. Five experimental diets were produced from a fish meal based diet, only differing in the dietary P source and level; low P control (5.5 g/kg soluble P), intermediate P (6.5 g/kg soluble P) and high P (8.0 g/kg soluble P) from either fish bone hydrolysate (FBH1, FBH2) or from NaH_2PO_4 (NaP1, NaP2). The diets were given for 168 days from start of feeding (0.17 g fish) until 24 weeks of feeding (33 g). Weight and specific growth rate did not reveal diet dependent differences except in the initial 10 week feeding period where fish fed FBH1 showed lower growth as compared to fish fed the Na–P diets. Mineralization of fish evaluated by whole body and bone ash and mineral contents showed diet dependent differences that were related to dietary P level, but not to P source. Fish fed the low P diet showed clear P deficiency signs with significantly reduced tissue ash and mineral content, reduced whole body Ca:P ratio and morphological deviation from the normal. Histological evaluation of the vertebrae after 19 weeks of feeding (15 g) revealed stagnation in cartilage development, with accumulation of mature chondrocytes in fish fed low P control and FBH1 diet. FTIR showed that fish fed FBH1 had lower mineralization and increased cross binding in the vertebral end plates. Fish fed FBH2 resembled the positive NaP1 control. Real time qPCR analyses confirmed the histological results, by showing up-regulating of *col10a1* (a marker for mature cartilage) in fish fed low P diet and FBH1. *Osteocalcin* (a marker for mineralization) was also activated in fish fed the low P diet and FBH1, possibly indicating a compensatory regulation in response to inadequate P. The dietary impacts on histology, FTIR and qPCR analyses in the FBH1 fed fish disappeared at 24 weeks of feeding (33 g). In conclusion, the results showed that P solubilized from bone fraction of herring can be efficiently utilized for growth, mineralization and bone development in Atlantic salmon fry. However, in the initial weeks of feeding, the FBH was a less efficient P source compared to NaP. Overall, the data suggest that P from fish bone hydrolysate is more suited for fish of minimum 15 g size.

Statement of relevance: The phosphate (P) rock reserve is a limited resource worldwide. Increased efficiency in the utilization of P and improved recycling of P from waste and manures are examples that could reduce the industry's vulnerability to the limited P supply while also reducing the negative environmental impacts. Recycling of P from fishery offal and development of new available P ingredients to farmed fish will significantly improve the sustainability of the aquaculture industry and reduce the environmental loss. In this paper we show that P hydrolysed from herring bone by-product is as efficient and good for salmon fry as commercially available NaP-salts.

1. Introduction

Phosphorus (P) is essential for normal growth and bone development in all organisms, with main functions in the formation of bones and teeth. The dietary need for this mineral in fast growing, cold water salmonids is high, i.e. 0.6% available P in feed for Atlantic salmon (*Salmo salar*) (Norwegian Research Council, NRC, 2011). There is a limit to the phosphate rock reserves worldwide that are economically feasible to recover, with the inevitable consequence of increased production cost (Van Vuuren et al., 2010). Increased efficiency in the utilization of P and improved recycling of P from waste and manures are examples that could reduce the industry's vulnerability to the limited P supply while also reducing the negative environmental impacts. Fishmeal has traditionally been the main natural source of P in salmon feeds. However, with limited marine resources to meet the increased protein need in

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the aquaculture, the fishmeal in the salmon diet has been significantly reduced and today accounts for only 18% (Ytrestøyl et al., 2014). Plant proteins with 60–80% non-available P as phytate have increased, and as a consequence, the need for additional P supplements has also increased from 20 to 24% the last years (Ytrestøyl et al., 2014). Recycling of P from fishery offal and development of new available P ingredients to farmed fish will significantly improve the sustainability of the aquaculture industry and reduce the environmental loss.

Fish by-products and trimmings are increasingly used as ingredient in fish meal production and it is expected that this resource will account for 40% of the produced fish meal by 2020 (Shepherd and Jackson, 2013). Marine by-products contain a large portion of bone and therefore high amounts of minerals such as P. The amount of P available for fish is however low because P in the bones is present as insoluble mineral complexes (i.e. hydroxyapatite) that is poorly digestible for the salmon, as discussed in Albrektsen (2015) and Albrektsen et al. (2016). Due to general low retention of dietary P in salmon (~30%), about 60–80% of P is released into the environment (Ytrestøyl et al., 2014), contributing to waste of valuable resources and environmental pollution. However, by using a new process technology, about 85–90% of P present in hydroxyapatite can be solubilized through hydrolysis in a strong acid (Albrektsen et al., 2010, 2013). It has been shown that solubilized P is equally available as crystalline and highly available mono-NaP salts when used in smolt diets (Albrektsen et al., 2013; 2015).

Many experiments have shown that sub-optimal levels of dietary P reduce the mineral content of bone in fish (e.g. Roy et al., 2002; Lall and Lewis-McCrea, 2007). In salmon, limited availability of P during the early juvenile stages negatively affect bone development and results in weaker bone structures and deformities (Baeverfjord et al., 1998). The basic foundation for normal bone development in salmon and other vertebrates is established during the early developmental stages, where skeletal formation and growth result from tightly regulated processes in the production and mineralization of extracellular matrix (ECM). In Atlantic salmon, the vertebrae formation occurs through two well characterized mechanisms; compact bone of the amphicoel and trabeculae is formed directly through intramembranous ossification by the osteoblasts, whereas the cartilaginous template in the arches is replaced by bone through endochondral ossification (Witten et al., 2006, 2009). Cartilage and bone cellular activity largely depends on the interaction with the ECM regulating their growth factor activity (Waddington et al., 2003; Gentili and Cancedda, 2009; Pihlajaniemi et al., 2009), while collagen spacing, arrangement and crosslinking of the ECM are important for mineralization and formation of crystals during the process (Gerstenfeld et al., 1993; Pornprasertsuk et al., 2004; Pornprasertsuk et al., 2005; Yamauchi and Katz, 1993). The resulting mechanical strength of bone is therefore highly dependent on the bone architecture; e.g. the mineral content, the crystallinity and crosslinking of the ECM, the relationship to the non-mineral phase of the bone, and also to a balanced activity between osteoblasts (bone cells) and chondrocytes (cartilage cells) (Ytteborg et al., 2010a; Pedersen et al., 2011; Hannesson et al., 2015; Totland et al., 2011; Currey, 2003). The activity of the enzymes alkaline phosphatase (ALP) and tartrate resistant acid phosphatase (TRACP) has been used as markers for osteoblast and osteoclast activities, hence as markers of bone formation and bone resorption, respectively (Ytteborg et al., 2010a, 2010b; Witten, 1997). Active metabolites of vitamin D further have important functions related to maintaining normal Ca and P balance in the body by increasing the intestinal absorption and by regulating bone resorption (Lock et al., 2009). Thus, bone quality is controlled by a variety of factors; including molecular regulatory mechanisms, environmental conditions and availability of minerals, that may easily be disturbed by sub-optimal conditions.

The aim of this experiment was to evaluate the efficiency of using P from herring by-products as dietary P source to Atlantic salmon fry compared to a commercial, crystalline mono-NaP salt. The experiment included a low P control diet and two graded dietary levels of soluble P for each of the two P sources. The diets were formulated suboptimal to the assumed dietary P requirement to give the opportunity to evaluate dietary P requirement in Atlantic salmon fry based on soluble P. The effects of the two P sources were thoroughly evaluated in regard to providing normal growth and bone development.

2. Material and methods

2.1. Production of the new marine P ingredient from by-products

New marine P ingredients were produced from herring by-products (head and backbone) collected at Norway Pelagic AS, Liavåg, Norway. Briefly, bone was separated from fish muscle and treated according to Albrektsen et al. (2016)prior to freezing at −20 °C. The raw bone material was thawed before hydrolysis in a process where fish bone was mixed with water (1:5 w/w) in 100 l plastic barrels, added 95% H_2SO_4 (5% w/w) and hydrolysed for 18 h to dissolve the minerals from hydroxyapatite. The water-soluble fraction was separated from the solids by sieving (100 μm). The solid phase was washed twice with cold water to collect the remaining water-soluble material. Thereafter, pH of the water-soluble fraction was adjusted with 25% NH_3 solution (pH 2.8) and left to precipitate overnight. Floating lipids were removed from the solution and the clear liquid was pumped into a kitchen kettle and sterilized by heating to 90 °C for 5 min before separation and removal of the precipitate by sieving (Jesma sievt, 80 μm cloths). The water-fraction was concentrated by evaporation (33% Brix) and spray dried (Niro Atomizer, Denmark) into a fine powder (20–60 μm particle size). A new analytical method that distinguishes between soluble and insoluble P in ingredients and feeds has been developed and experimentally tested in Atlantic salmon (Albrektsen, 2015; Albrektsen et al., 2016). This method offers an advantage in defining P digestibility based on the assumption that the chemical form and solubility of P are the main criteria for intestinal P absorption (Groote, 1986; Nordrum et al., 1997). The fish bone hydrolysate (FBH) ingredient produced from herring contained 10.9% total P and 9.2% soluble P. This new marine ingredient further contained 33.9% protein, 0.8% lipid, 31.8% ash and 97.3% dry matter. The ingredient also contain 32.5% $(NH_4)_2SO_4$ originating from sulfuric acid and NH_3-solution used for acid hydrolysis and pH adjustment, respectively, during processing of the bones.

2.2. Experimental diets

Five different experimental diets were produced (EWOS Innovation, Dirdal, Norway): one low P control diet, two test diets with two different dietary levels of P: FBH1 and FBH2 based on P hydrolysed from herring bones, and NaP1 and NaP2 based on a crystalline mono-Na–P salt (NaH_2PO_4) used for direct comparison of P levels and P sources. The diets were formulated as described in Table 1. The basal low P control diet contained 42% LT fish meal (Karmsund Fish Meal AS, Norway) with 2.09% total P and 1.02% soluble P, meaning that the fish meal provided 80% of dietary soluble P. Wheat gluten and wheat were used as plant protein and binder, respectively. Wheat generally contains low P levels (<0.25% soluble P) and wheat gluten is also a very efficient protein source for salmon. All diets were balanced for the amino acids Met, Thre, Lys and Arg to meet the basal requirement in Atlantic salmon fry. The oil used consisted of 75% fish oil and 25% rapeseed oil. The diets were extruded in a Wenger X-85 single-screw extruder with die size 1.5 mm ϕ. The extruded diets were produced at EWOS Innovation (Dirdal, Norway) and transported to Nofima's Feed Technology Centre in Bergen (Norway). The diets were granulated and sieved into 4 pellet sizes of about 0.6, 1.0, 1.2 and 1.7 mm before manually coated with the oil. The protein content in diet FBH1 and FBH2 were corrected for the non-protein N contribution by analyzing TVN, TMA-N, TMAO-N and NH_3-N.