



## Evaluation of supplemental fish bone meal made from Alaska seafood processing byproducts and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*)

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### ABSTRACT

We report performance of rainbow trout (*Oncorhynchus mykiss*) fed a balanced dietary mix of plant proteins supplemented with either fish bone meal (FBM) derived from Alaskan seafood processing byproducts or dicalcium phosphate. Seven experimental diets were formulated to contain two levels of dicalcium phosphate or two levels of two different kinds of FBM in all plant-protein (APP) based diets as follows: Diet 1, FM based; Diets 2 and 3, APP diets with low or high dicalcium phosphate; Diets 4 and 5, APP diets with 4% or 8% low phosphorus FBM; and Diets 6 and 7, APP diets with 2.7% or 5.4% high phosphorus FBM. The limiting amino acids methionine and lysine, as well as the amino sulfonic acid taurine, were added to the APP diets at appropriate concentrations. Triplicate groups of juvenile rainbow trout (average weight 31.5 g) were fed one of the seven experimental diets for 12 weeks. Negative effects on growth performance and feed utilization were observed in the fish fed both kinds of FBM or the low level of dicalcium phosphate supplemented to APP diets. Fish fed high levels of dicalcium phosphate showed growth performance and feed utilization that was not significantly different from the control group indicating that FM may be completely replaced by plant proteins with appropriate dietary supplements. Significantly decreased ash levels and increased lipid content were observed in fish fed diets supplemented with FBM or dicalcium phosphate at low levels. Whole-body phosphorus level was not significantly different between controls and fish fed APP diets supplemented with dicalcium phosphate whereas phosphorus levels were significantly lower in FBM fed fish. The apparent digestibility coefficients (ADC) of protein and phosphorus were lower in the control diet than diet 3. The digestibility of the two kinds of FBM was not different. Therefore, the results indicate that total replacement of dietary FM solely by plant proteins could be possible in rainbow trout with no apparent reduction in growth performance or feed utilization when a balanced mix of plant protein ingredients is properly supplemented with highly available inorganic phosphate, limiting amino acids and taurine. Maintenance of the body Ca/P ratio close to 1 was also found in rainbow trout although the differences in diets were considerable. Alaskan FBM could be used in fish feeds as a supplemental calcium source but not as the primary source of phosphorus because of the low bioavailability of phosphorus to fish in these diets.

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### 1. Introduction

Fish meal (FM) has been the most important protein source in aquafeeds for several decades especially for carnivorous fish species because it is an excellent source of high quality protein, essential nutrients, attractants and potentially unidentified growth factors

(Hardy, 1996). However, world FM production is not expected to increase yet increasing demand for aquafeed protein, unstable supplies and high prices seen with the dramatic expansion of aquaculture make it prudent to search for alternative protein sources or proper combination of those (Hardy and Tacon, 2002; Gatlin et al., 2007). For this reason, replacement of FM by alternative protein sources has long been of interest and will increasingly be important for the development of low-cost highly efficient aquafeeds (Lee et al., 2002; Gatlin et al., 2007; Pham et al., 2008).

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FM replacement by plant proteins has been considered an attractive way to achieve low-cost aquafeed formulations (Gatlin et al., 2007; Gaylord and Barrows, 2009; Lim and Lee, 2009). However, total FM replacement solely by plant-protein sources has not been successful especially in carnivorous fish species. Recent studies indicated that an all-plant protein based diet for rainbow trout was possible with a balanced combination of plant protein ingredients, specifically corn gluten, soy protein concentrate and wheat gluten, together with supplementation of limiting nutrients, such as lysine, methionine, taurine and dicalcium phosphate (Gaylord et al., 2007; Gaylord and Barrows, 2009).

The major obstacle in using the plant proteins has been the presence of anti-nutritional factors, such as phytic acid, saponins, and trypsin inhibitors found in crude preparations of the plant proteins (NRC, 1993; Masumoto et al., 2001). Most plant proteins used in fish diets contain phytic acid in the range of 5 to 30 g kg<sup>-1</sup> (Reddy, 2002) and over 70% of their total phosphorus in these sources is bound as phytate which cannot be digested or absorbed by monogastric animals including fish (Lall, 1991; Lim and Lee, 2008). Furthermore, phytic acid can chelate other divalent and trivalent cations besides phosphorus, such as calcium, magnesium, zinc, copper and iron resulting in significantly decreased bioavailability of these minerals (Wise, 1983).

The Alaskan fisheries harvest roughly 2 million metric tons of fish annually through one of the largest capture fisheries on Earth, Alaskan or walleye pollock (*Theragra chalcogramma*). The processing of these fish into human food creates a large amount of processing byproducts that can be made into a high quality protein meal, useful as an alternative to industrial fishery whole fish FMs in aquafeeds (Smiley et al., 2003; Li et al., 2004; Foster et al., 2005; Hardy et al., 2005). Fish bone is largely composed of calcium phosphate mineralized cartilage models. Bone meal is recovered from fish meal operations in Alaska by sieving protein meal and sizing the bone fragments by grinding. A promising minor

protein source, and one that is currently underexploited, fish bone meal (FBM) made from seafood processing contains high levels of phosphorus (6.5–9.7%) and calcium (13–18%) with crude protein measuring over 38% and could be used in fish feeds as a supplemental source of P and Ca when high levels of plant proteins are used.

Little information is available on the use of FBM in fish feeds as a supplement or substitution ingredient for FM. The FBM used as a dietary ingredient in a study (Toppe et al., 2006) on Atlantic cod was produced by separation of the bone fraction from a commercial FM. The study reported a promising result suggesting that almost 45% of dietary FM protein can be replaced by the FBM containing 56% crude protein. Vielma et al. (1999) reported that supplementation of a FBM (herring bone meal) in a low phosphorus basal diet increased the growth rate of rainbow trout. Therefore, the present study was conducted to examine the supplemental effects of FBM, produced from Alaskan seafood processing waste, on the growth performance and bioavailability of both phosphorus and calcium in diets formulated with all plant-proteins for rainbow trout. We also investigated the supplemental effects of inorganic phosphorus added in supplement to the all plant-protein diets for total FM replacement.

## 2. Materials and methods

### 2.1. Experimental ingredients and diets

Seven experimental diets (Table 1) were formulated to contain two kinds of fish bone meal (FBM) derived from Alaskan seafood processing byproduct in all plant-protein (APP) based diets as follows: Diet 1, FM based; Diets 2 and 3, APP diets with low or high dicalcium phosphate; Diets 4 and 5, APP diets with 4% or 8% low phosphorus FBM (LFBM); and Diets 6 and 7, APP diets with 2.7% or 5.4% high phosphorus FBM (HFBM). Two limiting amino acids, L-methionine

**Table 1**

Composition of the experimental diets (% dry matter).

Ingredients	Diets						
	1 (FM)	2 (LDPC)	3 (HDPC)	4 (LFBM <sub>L</sub> )	5 (LFBM <sub>H</sub> )	6 (HFBM <sub>L</sub> )	7 (HFBM <sub>H</sub> )
Anchovy meal	45.00	–	–	–	–	–	–
Corn gluten, yellow	10.00	20.00	21.00	20.00	20.00	20.00	20.00
Soy protein concentrate	2.00	24.30	23.50	23.30	21.00	23.50	22.50
Wheat gluten meal	0.00	4.00	4.50	3.00	4.00	3.41	3.50
Dicalcium phosphate	–	1.80	3.00	–	–	–	–
LFBM <sup>a</sup>	–	–	–	4.00	8.00	–	–
HFBM <sup>b</sup>	–	–	–	–	–	2.65	5.30
Wheat flour	24.7	25.46	23.56	25.26	22.56	26.00	24.26
Fish oil	15.10	18.60	18.60	18.60	18.60	18.60	18.60
TM Salts <sup>c</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mix <sup>d</sup>	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Vitamin C (stay-C)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Permapel (binder)	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60	0.60
L-lysine	–	1.64	1.64	1.64	1.64	1.64	1.64
DL-methionine	–	0.50	0.50	0.50	0.50	0.50	0.50
Taurine	–	0.50	0.50	0.50	0.50	0.50	0.50
<i>Chemical compositions (%)</i>							
Moisture	4.94	8.08	6.40	5.97	6.07	7.14	6.78
Protein	48.0	51.0	46.8	46.4	48.9	52.6	48.4
Lipid	20.6	21.6	22.6	22.0	22.4	21.8	22.3
Ash	8.48	4.44	5.49	4.17	6.22	4.29	5.08
Energy (kcal/kg)	5621	5759	5705	5741	5624	5758	5684
Calcium	2.50	0.48	0.70	0.72	1.40	0.70	1.30
Phosphorus	1.60	0.83	1.00	0.74	1.10	0.69	0.96
Ca/P ratio	1.56	0.58	0.70	0.97	1.27	1.01	1.35

<sup>a</sup> LFBM: low phosphorus fish bone meal. This product contains 40% protein, 4.0% lipid and 42% ash.

<sup>b</sup> HFBM: high phosphorus fish bone meal. This product contains 39% protein, 3.0% lipid and 47% ash.

<sup>c</sup> Composition of trace mineral premix (mg/kg): Zn (as ZnSO<sub>4</sub>·7H<sub>2</sub>O), 75; Mn (as MnSO<sub>4</sub>), 20; Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O), 1.54; I (as KIO<sub>3</sub>), 10.

<sup>d</sup> Composition of vitamin premix (mg/kg of premix, unless otherwise listed): D-calcium pantothenate, 26,840; pyridoxine (pyridoxine HCl), 7700; riboflavin, 13,200; niacinamide, 55,000; folic acid, 2200; thiamine (thiamine mononitrate), 8800; biotin, 88; vitamin B12, 5.5; menadione sodium bisulfate complex, 2.75; vitamin E (DL α-tocopherol acetate), 88,000 IU; vitamin D3 (stabilized), 110,000 IU; vitamin A (vitamin A palmitate, stabilized), 1,650,000 IU.

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