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Post-prandial changes in plasma mineral levels in rainbow trout fed a complete plant ingredient based diet and the effect of supplemental di-calcium phosphate

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ABSTRACT

Post-prandial changes in plasma mineral levels and utilisation of minerals in rainbow trout fed complete plant ingredient based diets with or without supplemental di-calcium phosphate (DCP) were studied over an 8 week period. Three diets were used: diet M was FM and fish oil (FO) based diet (control); diets VP0 and VP+ (V diets) were completely based on plant derived protein and lipid sources. One of the V diets (VP+) was supplemented with DCP to supply 5 g kg^{-1} dry matter available phosphorus (P); while the other diet (VP0) was not supplemented with DCP. Change in dietary protein source significantly affected the post-prandial pattern in plasma levels of P ($p < 0.05$), Ca ($p < 0.007$), Mg ($p < 0.001$) and Zn ($p < 0.03$). Area under the curve analysis indicated that compared to VP0, DCP supplementation in VP+ improved plasma levels of P ($p < 0.01$) and K ($p < 0.05$); Cu ($p < 0.002$), Se ($p < 0.009$) and Zn ($p < 0.001$) levels were reduced while Ca, Mg and Fe levels were unaffected ($p > 0.05$). Based on measurement of apparent digestibility, growth and whole body composition analyses, mineral balances were established showing that supplementation of DCP led to significant increase in whole body P concentration and P retention in VP+, comparable to fish fed diet M with significantly ($p < 0.05$) reduced faecal and non-faecal P losses. There was improved post-absorptive retention (as % of available intake) of Ca ($p < 0.05$), Mg ($p < 0.05$) and K ($p < 0.05$) in VP+ compared to VP0. Utilisation of Cu ($p < 0.05$) and Zn ($p < 0.01$) was negatively affected. DCP supplementation to complete plant ingredient based diet increased the post-prandial plasma levels, whole body concentration and utilisation of macro-minerals (P, Ca, Mg and K) whereas that of micro-minerals especially Zn and Cu were negatively affected.

1. Introduction

Ensuring an adequate dietary supply of minerals to farmed fish is essential for proper somatic and skeletal growth, health and final flesh quality. Mineral composition of edible as well as non-edible portions in farmed fish is linked to the mineral composition of the feeds (Carpene et al., 1998; Fallah et al., 2011; Fuentes et al., 2010; Lall, 1995). Fish meal (FM) is a major source of minerals and trace elements (Julshamm et al., 1978) and especially of phosphorus (P) to farmed fish (Kaushik, 2001; Lall, 2002). The substitution of FM by plant protein sources which is well under way for farmed fish (Kaushik and Hemre, 2008; Tacon and Metian, 2008) inevitably calls for measures to ensure an adequate supply of minerals in an available form to meet the physiological demands of fish. The bioavailability of minerals supplied by the diets to meet such physiological demands is markedly influenced by efficiency with which the body uptakes and utilises the dietary minerals (Lall, 2002; Watanabe et al., 1997a).

Post-prandial plasma levels of minerals can serve as an indicator of absorption through dietary intake and bio-availability (Navarro and Wood, 2003). As already pointed out by Rodehutscord (1996) with regard to phosphorus, in order to assess the mineral status using circulating levels of minerals as indicators, knowledge on post-prandial changes as affected by dietary factors is necessary. However, data on time course of changes in the post-prandial plasma mineral levels are scarce in fish. Secondly, in diets containing high levels of plant-protein ingredients and low levels of FM, supplementation with mono- or di-basic inorganic P salts has been found to be an efficient strategy to increase the levels of available P supply (Kaushik

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et al., 2004; Ketola, 1975; Watanabe et al., 1997b). Many studies have focused on the adverse effects of tri-calcium phosphate and calcium phytate on the utilisation of other minerals (Gatlin and Phillips, 1989; Hardy and Shearer, 1985; Satoh et al., 1987, 1989, 1992a, 1993). But, only a few (Kousoulaki et al., 2010; Vielma and Lall, 1998) have studied the effect of mono- or di-basic P salts, which are the commonly used supplements under both experimental and practical conditions. The objective of this study was to test the effects of change in dietary protein sources and of DCP supplementation on the post-prandial patterns of plasma mineral levels and mineral balance in rainbow trout.

2. Materials and methods

2.1. Diets

Three diets (M, VP0 and VP+) were formulated with two different basal ingredient compositions (Table 1). Diet M was formulated to contain fish meal (FM) and fish oil (FO) as protein and lipid sources and served as a control. Diets VP0 and VP+ (collectively addressed as V diets) were based on plant ingredients (with no FM or FO). The V diets were supplemented with a mineral pre-mixture at 10 g kg^{-1} diet to meet all the essential mineral requirements of rainbow trout (NRC, 2011), except for available P. To one of the V diets (VP+), additional supplementation of di-calcium phosphate, DCP at 22 g kg^{-1} diet was made to supply adequate levels of available P as per NRC (2011). For measurement of the apparent digestibility of minerals, the same diets were prepared with the incorporation of 10 g kg^{-1} chromic oxide (Cr_2O_3) as the inert marker by replacing an equivalent amount of whole wheat.

Table 1
Ingredient and analytical composition of the experimental diets.

	M	VP0	VP+
Ingredients (%)			
Norwegian herring meal, (CP 70; Sopropêche, France)	62.6		
Corn gluten meal (CP 60; Inzo, France)		18.0	18.0
Wheat gluten (CP 70; Roquette, France)		20.0	20.0
Soybean meal (CP 48; Inzo, France)		6.1	6.1
Soy protein concentrate (Estrilvo; CP 70; Sopropêche, France)		15.0	15.0
White lupin meal (Terrena, France)		5.0	5.0
Extruded peas (Aquatex, Sotexpro, France)		3.8	3.8
Rapeseed meal (Primor 00; Sud Ouest Aliment, France)		5.2	5.2
Whole wheat	24.6	4.2	2.0
Soy lecithin (Louis François, France)		2.0	2.0
L-Lysine (Eurolysine)		1.3	1.3
L-Methionine (Evonik, Germany)		0.3	0.3
CaHPO4·2H20 (18% P; 22% Ca)			2.2
Attractant mix[a]		1.5	1.5
Mineral premix[b]		1.0	1.0
Vitamin premix[c]	1.0	1.0	1.0
Fish oil (southern hemisphere, Sopropêche, France)	11.9		
Rapeseed oil (Daudruy, France)		6.2	6.2
Linseed oil (Daudruy, France)		6.2	6.2
Palm oil (Daudruy, France)		3.1	3.1
Analysed proximate composition			
Dry matter (DM), %	91.8	91.4	93.1
Crude protein, % DM	49.2	51.1	49.9
Crude lipid, % DM	19.6	21.7	22.2
Crude ash, % DM	9.0	3.1	4.9
Energy, kJ g^{-1} DM	22.9	24.7	24.4
Analysed mineral composition			
P, g kg^{-1} DM	14.1	6.1	10.3
Ca, g kg^{-1} DM	16.0	4.5	10.8
Ca/P ratio	1.1	0.7	1.0
Mg, g kg^{-1} DM	2.1	1.7	1.8
K, g kg^{-1} DM	11.0	5.4	5.1
Fe, mg kg^{-1} DM	176.3	244.3	246.6
Mn, mg kg^{-1} DM	10.5	77.8	79.7
Cu, mg kg^{-1} DM	4.3	16.1	17.4
Se, mg kg^{-1} DM	1.3	0.4	0.4
Zn, mg kg^{-1} DM	58.9	85.7	84.7

[a] Attractant mix (g kg^{-1} mixture): glucosamine, 5 g; taurine, 3 g; betaine, 3 g; glycine, 2 g; and alanine, 2 g.

[b] Mineral premix (g or mg kg^{-1} diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60% Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g; copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Mn), 0.3 g; dibasic calcium phosphate (20% Ca, 18% P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30% Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g (UPAE, INRA).

[c] Vitamin premix (IU or mg kg^{-1} diet): DL-a tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg (UPAE, INRA).

2.2. Fish, feeding and rearing condition

Rainbow trout juveniles (78.1 ± 0.6 g, IBW) were randomly distributed into nine experimental units, each of 500 L (35 fish $unit^{-1}$) and acclimatised to the rearing conditions two weeks prior to the start of the experiment. Triplicate groups of fish were hand fed twice a day to visual satiation for a period of 8 weeks (6 days a week). The experiment was carried out in a flow through rearing system at the INRA experimental fish farm at Donzacq (Landes, France). Water temperature was 17.5 ± 0.5 °C and flow rate was maintained at 50 L min^{-1} during the course of the study. The mineral concentrations in the water (mg L^{-1}) were analysed to be P, <0.2; Ca, 41.7 ± 6.3; Mg, 19.2 ± 0.8; K, 1.8 ± 0.1; Fe, <0.02; Mn, <0.02; Cu, <0.008 and Zn, <0.007.

2.3. Weight and tissue sampling

A total of 15 fish were sampled at the start of the study for whole body composition analysis. Fish from each experimental unit were bulk weighed at the start, after 4 weeks and at the end of the 8 week growth trial. Feed was withheld for 24 h before every weighing. At the end of the trial, six fish from each replicate were withdrawn, anaesthetised (benzocaine, 30 mg L^{-1}) and euthanized subsequently by a sharp blow to the head; liver and viscera were dissected and weighed for calculating hepato- and viscero-somatic indices (HSI and VSI). The carcass along with liver and viscera were immediately frozen and kept at −20 °C awaiting analyses.

2.4. Post-prandial mineral absorption study

After the end of the growth trial and samplings, the remaining fish (n = 25 per replicate) were used for the post-prandial mineral absorption study. The fish were not fed for 24 h and were subsequently fed a single ration (55 g per tank of 25 fish, equal amount to all groups) at 09:00 h in the morning. Following this single meal, blood samples were collected from the caudal vein into heparinized syringes at 8 time points (0, 1, 2, 4, 6, 9, 12 and 24 h). At each time point, a total of 18 fish (6 per treatment) were anesthetised in a solution of benzocaine (30 mg L^{-1}) prior to sampling of blood. A sample size of 6 fish per time point was used to reduce variation arising from possible differences in feed intake, as this was not monitored for individual fish within each tank. Six fish from one of the triplicate tanks were withdrawn at successive time points for sampling of blood; thus, each tank was sampled a maximum of three times over the entire 24 h period. This sampling protocol provided a window of a minimum of 4 h between two successive samplings from the same tank. This was done in order to minimise handling stress and any subsequent bias in plasma mineral levels due to stress arising from frequent and repeated sampling from the same tank. All the fish used for blood collection were independent samples and no repeated sampling was made on the same fish. Plasma was recovered from centrifuged (3000 *g* for 5 min) blood samples, immediately frozen and stored at −20 °C until mineral analysis.