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Effect of a microbial phytase on the growth performance, digestibility and retention in a high plant meal inclusion diet for Atlantic salmon (*Salmo salar*)

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Abstract
The effect of supplementation of a novel phytase (OptiPhos®; Huvepharma; 750 OTU) to high plant meal inclusion diets for Atlantic salmon (*Salmo salar*) was assessed. Diets were prepared without phytase, with phytase and with supplemental inorganic phosphorus (MCP). After 61 days significant increases in growth, FCR and protein efficiency were seen in diets supplemented with phytase and with MCP over the negative control ($P < 0.05$). Addition of phytase significantly increased digestibility of phytate and phosphorus (P) as well as retention over other diets ($P < 0.05$). Phytase addition significantly decreased faecal P losses while increasing metabolic loss over the negative control ($P < 0.05$). Addition of MCP showed significant increases in metabolic P loss ($P < 0.05$). In conclusion, the addition of phytase (OptiPhos®; Huvepharma) resulted in performance improvements comparable to inorganic P dosing, while significantly improving the reduction of effluent wastes from plant meal-based diets.

Keywords Phytase · Phytate · Salmon · Phosphorus

Introduction

Aquaculture continues to be one of the fastest growing food sectors, accounting for roughly half of global fish consumption (FAO 2021). Though the growth of aquaculture has significantly contributed to fish production, the reliance on fishmeal in aquafeeds has raised concerns about the sustainability of the industry (Deutsch et al. 2007; Naylor et al. 2000; Merino et al. 2012).

Fishmeal traditionally has been the main protein source used in feeds (Luthada-Raswiswi et al. 2021) as its high protein content, amino acid profile and palatability make it suitable for piscivorous fish species (Rolland et al. 2015). However, due to the increased price of fishmeal and the inconsistency of global supply, plant proteins have gained significant

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interest (Burel et al. 2000; Carter and Hauler 2000; Chou et al. 2004; Hansen et al. 2007; Opstvedt et al. 2003; Øverland et al. 2009). Plant-based proteins are an interesting alternative due to their high availability, competitive pricing and nutritional profile (Gatlin et al. 2007; Hardy 2010) and have shown promise in several species (Lazzarotto et al. 2018; Egerton et al. 2020; Hartviksen et al. 2014; Taylor et al. 2019; Vera et al. 2020; Hansen and Hemre 2013; Kaushik et al. 2004; Torrecillas et al. 2017).

Despite noted success in aquafeeds, antinutritional factors present in plant materials present a constraint to their use (Kumar et al. 2012). Phytate is a free form of inositol hexakisphosphate (IP6) and a polyanionic molecule with six phosphate groups and is one of the major antinutrients in plant-based ingredients. Phytate has been shown to adversely affect the absorption and digestion of minerals in fish (Papatryphon et al. 1999), and its excretion has been shown to be environmentally damaging (Baruah et al. 2004). Despite negative effects, phytate acts as a potential source of phosphorus (P) within feeds, consisting of two-thirds of the total P in plant-based ingredients (Singh 2008). Current sources of inorganic P, such as monocalcium phosphate (MCP), are non-renewable and expensive (Mullaney et al. 2000; Lee et al. 2020), and so accessing phytate-bound P has garnered a large amount of interest to maximise the nutritive values of plant-based ingredients as well as reduce the negative effects associated with their use.

The use of phytase supplementation is now established in the aquaculture industry (Adeola and Cowieson 2011; Kumar et al. 2012), and though characterisation of phytases in vitro are widely available, a broad range of factors have been shown to influence the efficacy of phytases in vivo. Factors such as environmental temperature, pH, dose, fish species, complementary dietary ingredients and phytase source have all been shown to influence efficacy (Greiner and Konietzny 2006; Cao et al. 2007; Dersjant-Li et al. 2015; Lee et al. 2020; Kumar et al. 2012).

Previous in vivo studies have shown several phytases to be effective additions to plant-based feeds for Atlantic salmon (*Salmo salar*), though these are limited. Direct experimental investigation on the specific effect of phytases on performance, digestibility and retention is limited to six studies. The phytase most commonly used is a hybrid 6 phytase produced by *Aspergillus niger* (Storebakken et al. 1998; Sajjadi and Carter 2004a; Sajjadi and Carter 2004b; Carter and Sajjadi 2010), with only two studies looking at alternate phytase sources; *Citrobacter braakii* 6-phytase produced by *Aspergillus oryzae* (Greiling et al. 2019) and an *Escherichia coli* 6 phytase produced by *Pichia pastoris* (Denstadli et al. 2007).

The current trial aims to assess the efficacy of a previously unstudied *Escherichia coli* phytase (OptiPhos®; Huvepharma) on the growth performance, digestibility and retention in Atlantic salmon (*Salmo salar*) in order to establish characterisation of in vivo effects within a high plant ingredient-based diet.

Materials and methods

Animal trials

The trial was conducted at the experimental facilities of GIFAS (Gildeskal Forskningsstasjon AS, Inndyr, Norway). The experiment was directed by trained personnel (accredited according to FELASA function A: carrying out procedures on animals) and conducted by trained technical staff (accredited according to FELASA function B: designing procedures and projects; function C: taking care of animals) according to the EU guidelines on protection of animals used for scientific purposes (Directive 2010/63/).

Experimental diet preparation and test product

A basal diet was formulated with moderate levels of marine-derived proteins and high levels of plant protein sources (Table 1). The trial comprised of 3 experimental diets: a positive control treatment (PC) consisting of a basal formulation supplemented with monocalcium phosphate (MCP); a negative control diet (NC), without MCP supplementation; and a phytate dosed diet containing 750 OTU/kg feed phytase (OptiPhos®; Huvepharma). All diets were supplemented with crystalline amino acids to guarantee balanced amino acid supply. To allow for apparent digestibility recording, a small batch of each diet was supplemented with 500 mg/kg of yttrium oxide.

Diets were manufactured by extrusion (pellet size 4.5 mm) at SPAROS facilities, by means of a pilot-scale twin-screw extruder (CLEXTRAL BC45, France) with a screw diameter of 55.5 mm and temperature ranging 108–113 °C. Upon extrusion, both batches of extruded feeds (PC and NC formulations) were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). Following drying, pellets were allowed to cool at room temperature, and subsequently, the test enzyme and oil were applied by coating under vacuum conditions (DINNISEN, PG-10VCLAB, Netherlands). For the post-extrusion coating procedure of enzyme and oils, the target amount of test enzyme (OptiPhos 8000L) was diluted in 2.5% demineralized water, emulsified with the oil on a high-shear mixer (Silverson L5T, UK) and sprayed onto the pellets under vacuum (760 mbar) for approximately 3 min. The PC and NC control diets without enzyme supplementation were also coated with the oils, using the same procedure. Representative samples of each diet were taken for proximate composition analysis and quantification of supplemental enzyme. Throughout the duration of the trial, experimental feeds were stored in cold storage at room temperature.

Animals and experimental setup

The trial was conducted at Gildeskal Forskiningsstasjon AS, Inndyr, Norway (GIFAS). A total of 984 Atlantic salmon (*S. salar*) were selected from GIFAS' production stock and were randomly allocated to 12 square 5 × 5 × 5 m experimental sea cages with a volume of 125 m^3 (67.0319°N, 14.0281°E, Inndyr, Norway). Fish were subjected to a freshwater bath at transfer as a prophylactic measure against seawater, and no mortalities were observed in association with the transfer to experimental cages. Fish were placed in quarantine for 3 weeks for observation and acclimation to new experimental conditions (temperature: 10 ± 1 °C; dissolved oxygen: > 8.2 mg/l). During this period, fish were fed a 4.5-mm commercial salmon diet (BIOMAR EFICO Enviro 940) by hand in two daily meals according to the manufacturer feeding table at approximately 1.5% biomass daily.

Performance data

Triplicate groups of 82 post-smolt Atlantic salmon with a mean initial body weight (IBW) of 163 ± 14 g were fed one of three experimental diets (Table 1) over 61 days. During the trial period, cages were subjected to natural temperature, water quality and photoperiod conditions (temperature 12.8 ± 1.3 °C; salinity: 32.4 ± 0.5 ppt; dissolved oxygen: > 5.9 mg/l). Fish were hand fed to visual satiety over 2 meals per day (0800 and 1400 h). Apparent satiation was considered to occur when animals lost interest in the feed.

Table 1 Formulation and composition of experimental diets for Atlantic salmon

Ingredients	NC	PC	PHY-750
Fishmeal LT70 (South American)[1]	10.00	10.00	10.00
Krill meal[2]	4.20	4.20	4.20
Soy protein concentrate[3]	18.00	18.00	18.00
Pea protein concentrate[4]	12.50	12.50	12.50
Wheat gluten[5]	12.00	12.00	12.00
Corn gluten[6]	5.00	5.00	5.00
Rapeseed oil[7]	4.50	4.50	4.50
Wheat meal[8]	10.85	8.65	10.85
Fish oil[9]	9.95	9.95	9.95
Rapeseed oil[10]	9.95	9.95	9.95
Vitamin and mineral premix[11]	1.50	1.50	1.50
Monocalcium phosphate[12]	-	2.00	-
Carophyll pink 10%[13]	0.05	0.05	0.05
L-Lysine[14]	0.50	0.50	0.50
L-Threonine[15]	0.25	0.25	0.25
DL-Methionine[16]	0.70	0.70	0.70
Optiphos 8000L (OTU/kg feed)[17]	0.00	0.00	750.00
Yttrium oxide*	0.05	0.05	0.05
Dry matter (DM) (%)	94.7 ± 0.0	94.6 ± 0.0	94.7 ± 0.0
Crude protein (% DM)	49.8 ± 0.0	49.8 ± 0.0	49.8 ± 0.0
Crude fat (% DM)	23.5 ± 0.0	23.5 ± 0.1	23.5 ± 0.0
Ash (% DM)	5.7 ± 0.0	6.7 ± 0.0	5.7 ± 0.0
Gross energy (kJ/g DM)	23.9 ± 0.0	23.9 ± 0.0	23.9 ± 0.0
Total phosphorus (% DM)	0.80 ± 0.01	1.17 ± 0.01	0.81 ± 0.02
Phytate-P (% DM)	0.54 ± 0.01	0.55 ± 0.00	0.54 ± 0.01
Calcium	0.76 ± 0.02	1.09 ± 0.02	0.77 ± 0.02
Yttrium oxide (mg/kg DM)	516 ± 3	518 ± 8	518 ± 2
Phytase (OTU/kg)	< 13	20	795

[1]Peruvian fishmeal: 71% crude protein (CP), 11% crude fat (CF), EXALMAR, Peru

[2]QRILL meal: 55.6% CP, 25.4% CF, Aker Biomarine, Norway

[3]Soycomil P: 65% CP, 0.8% CF, ADM, The Netherlands

[4]Nutralys F85F: 78% CP, 1% CF, ROQUETTE, France

[5]VITEN: 85.7% CP, 1.3% CF, ROQUETTE, France

[6]Corn gluten meal: 61% CP, 6% CF, COPAM, Portugal

[7]Defatted rapeseed meal: 34% CP, 2% CF, SORGAL, Portugal

[8]Wheat meal: 10.2% CP; 1.2% CF, Casa Lanchinha, Portugal

[9]SAVINOR, Portugal

[10]Henry Lamotte Oils GmbH, Germany

[11]PREMIX Lda, Portugal: Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 7.5 mg; sodium chloride, 400 mg; excipient wheat middlings

Table 1 (continued)

[12]MCP: 22.6% P, 16% Ca, Fosfitalia, Italy

[13]Carophyll Pink 10% CWS (astaxanthin), DSM Nutritional Products, Switzerland

[14]Biolys 54.6% Lysine, EVONIK DEGUSSA GmbH, Germany

[15]L-Threonine 98%, EVONIK DEGUSSA GmbH, Germany

[16]DL-Methionine 99%, EVONIK DEGUSSA GmbH, Germany

[17]OptiPhos 8000L (9100 OTU/g), HUVEPHARMA NV, Belgium

*Yttrium oxide was incorporated in feeds used for digestibility measurements

Animals were weighed individually at the start of the trial, and batch weights were carried out after day 61.

Digestibility data

At the end of the growth performance trial and following all associated sampling, the remaining fish were used to determine the apparent digestibility coefficients (ADC) of P and phytate. Diets containing 0.5% yttrium oxide (Table 2) were fed to groups of the remaining fish (average weight 390 g), following identical procedures as the performance period. Fish were fed for 1 week to allow for adaptation to the feeds, and then faeces were collected via stripping, approximately 4 h following a meal. Twelve fish per replicate were stripped after light anaesthesia by applying gentle pressure to the ventral abdominal area, just posterior to the pelvic fins and moving posteriorly to the anal opening. Faeces from each fish were combined into a plastic container and stored frozen at − 20 °C prior to subsequent analysis.

Biological sampling

To assess whole-body P and bone ash content, whole fish from the initial stock ($n=6$) and whole fish ($n=18$) at the end of the trial were sampled and stored at − 20 °C for subsequent analysis. Fish vertebrae were freed from soft tissue with a toothbrush and finally washed with deionized water. The vertebral samples were defatted by extraction with chloroform–methanol (2:1) as described by Folch et al. (1957). Vertebral samples were collected at the start ($n=6$) and at the end of the trial ($n=6$) and frozen at − 20 for analysis of bone ash.

Table 2 Performance data for high plant inclusion diets in Atlantic salmon ± SD with varying levels of phosphorus sources (NC, negative control; PC, inorganic phosphorus dosed diet; PHY-750, phytase dosed diet). SGR, specific growth ratio; FCR, feed conversion ratio; FI, feed intake; PER, protein efficiency ratio. Bold *p* values and different superscript letters denote statistical differences ($P<0.05$)

Diet	NC	PC	PHY-750	Test statistic	*P*
Initial bodyweight (g)	163 ± 1	163 ± 2	163 ± 1	$H_{(2)}=2.220$	0.896
Final bodyweight (g)	379 ± 8[a]	397 ± 6[b]	398 ± 6[b]	$F_{(2,8)}=7.741$	**0.022**
SGR (%BW day^{-1})	1.38 ± 0.04[a]	1.46 ± 0.01[b]	1.47 ± 0.02[b]	$F_{(2,8)}=8.710$	**0.017**
FCR (kg feed/kg gain)	1.03 ± 0.02[b]	0.94 ± 0.02[a]	0.93 ± 0.01[a]	$F_{(2,8)}=31.269$	**0.001**
FI (%BW day^{-1})	1.34 ± 0.01	1.28 ± 0.04	1.28 ± 0.01	$H_{(2)}=5.083$	0.079
PER (kg gain/kg protein)	2.07 ± 0.03[a]	2.27 ± 0.06[b]	2.28 ± 0.03[b]	$F_{(2,8)}=26.701$	**0.001**

Analytical methods

Feed samples were ground prior to analysis of proximate composition. Frozen whole-body samples were minced and mixed, and a representative sample was used for determination of moisture content. Whole-body minced samples and frozen faeces samples were freeze-dried and homogenized with a laboratory mill prior to analysis. Analysis of feed, whole body and faeces was carried out with analytical duplicates following the methodology descried by AOAC (2006).

Analysis of dry matter, total ash, crude protein, crude lipid, gross energy, total P, calcium, phytate-P and yttrium were performed at the University of Porto, Portugal, using the following methods: Dry matter was assessed after drying at 105 °C for 24 h. Total ash was analysed by combustion (550 °C during 6 h) in a muffle furnace (Nabertherm L9/11/8170, Germany). Crude protein ($N \times 6.25$) was analysed by a flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection using a Leco N analyser (Model FP-528, Leco Corporation, USA). Crude lipid was determined by petroleum ether extraction (50–60 °C) using a Saxtec™ 2055 Fat Extraction System (Foss, Denmark). Gross energy was measured in an adiabatic bomb calorimeter (Werke C2000 basic, IKA, Germany). Total P and calcium were measured according to the ISO 27085–2009 by ICP AES methodology (ISO 2009). Phytate phosphorous content in feeds and faeces was determined by a colorimetric method involving a wet washing step followed by phosphorous measurement with a 1-amino-2-naphthol-4-sulfonic acid-molybdate reagent in a microplate reader at 660 nm (Brooks et al. 2001).

Phytase activity in the feeds was measured by Biovet, Bulgaria. Activity was measured a unit of phytase activity (OTU) being defined as the amount of enzyme that catalyses the release of 1.0 micromole of inorganic phosphate per minute from a 5.1 mM sodium phytate in pH 5.5 citrate buffer at 37 °C, measured as the blue molybdate complex colour at 820 nm.

Evaluation criteria

Performance parameters were calculated as follows, where FBW represents final mean body weight (g) and IBW represents initial mean body weight (g).

$$\text{Specific growth rate (SGR, \%/day)}: \ (\text{LnFBW} - \text{LnIBW}) \times 100/\text{days}$$

$$\text{Feed in take(\%BW/day)}: \ (((\text{crude feed in take}/(\text{IBW} + \text{FBW}/2))/\text{days}) \times 100$$

$$\text{Feed conversion ratio(FCR)}: \ \text{crude feed in take}/\text{weight gain}$$

$$\text{Protein efficiency ratio(PER)}: \ \text{wet weight gain}/\text{crude feed in take}$$

$$\text{Protein retention}: \ 100 \times (\text{FBW} \times \text{final body P content} - \text{IBW} \times \text{initial body P content})/\text{P intake}$$

Digestibility parameters were calculated as follows, where FBW represents final mean body weight (g) and IBW represents initial mean body weight (g).

$$\text{Apparent digestibility coefficient(ADC, \%)}: \ 100 - \left(100 \times \left(\frac{\%\textit{yttrium in diet}}{\%\textit{yttrium in faeces}}\right) \times \left(\frac{\textit{nutrient in faeces}}{\%\textit{nutrient in diet}}\right)\right)$$

$$\text{Daily P gain}: (\text{final body P content} - \text{initial body P content})/(\text{IBW} + \text{FBW})/2)/\text{days}$$

$$\text{Daily P intake}: \text{P intake}/(\text{IBW} + \text{FBW})/2)/\text{days}$$

$$\text{Daily faecal P losses}: \text{daily P intake} \times (100 - \text{ADCP})/100$$

$$\text{Daily metabolic P losses}: \text{daily P intake} - (\text{daily P gain} + \text{daily faecal P losses})$$

Statistical analysis

All data were analysed using the SPSS statistical software (IBM; V21). All data were initially tested for departures from normality using the Shapiro–Wilk test and for homogeneity using the Levene's test. One-way analysis of variance (ANOVA) was used to examine performance, whole body and bone composition, phosphorous retention, digestibility and P balance. Where significant differences were found ($P < 0.05$), a post hoc Tukey's HSD was performed. All percentage data were subjected to arcsine square root transformation prior to analysis (Dytham 1999). Where normality or homogeneity testing failed, a non-parametric Kruskal-Wallace test was used. Where type II errors occurred, the means and confidence intervals were graphically analysed to illustrate the data under normal assumptions.

Results

Performance

Animal health remained high throughout the experiment with only one mortality recorded from diet NC. No external lesions or apparent abnormalities in the internal organs were observed, and the remainder of the test animals were recorded with no apparent health issues.

Fish fed diets PC and PHY-750 showed significant increases compared to diet NC in specific growth rate (SGR), final bodyweight (FBW), feed conversion ratio (FCR) and protein efficiency ratio (PER) (Table 2). No significant differences were seen in feed intake (FI) (Table 2).

Digestibility

Digestibility of dry matter was not affected by dietary treatments (Table 3). Significant differences were seen between all treatments in P digestibility (Table 3). Diet PHY-750 showed significantly higher digestibility of phytate than both control diets (Table 3).

Body content

No significant differences in body P were seen between diets (Table 3). Diets PC and PHY-750 showed significantly higher bone ash than diet NC (Table 3).

Phosphorus retention

Diet PHY-750 showed significantly higher phytase retention than treatments NC and PC (Table 3).

Phosphorus balance

No significant differences in P gain were seen between diets ($H_{(2)} = 3.518$, $P = 0.172$, Fig. 1). Diet PHY-750 showed significantly lower faecal losses than diet NC. ($H_{(2)} = 7.385$, 0.0025, Fig. 1). Significant differences in metabolic P losses were seen between all treatments ($F_{(2,8)} = 147.119$, $P = 0.001$, Fig. 1).

Discussion

The antinutritional properties of plant materials have long been recognised, and the use of supplementary ingredients is long established in aquafeeds (Carter et al. 1994; Bedford 2000; Cao et al. 2007; Rao et al. 2009). The inclusion of alternative plant meals to replace fishmeal products in Atlantic salmon is widely recognized (Naylor et al. 2009), but research carried out on action of specific phytases is limited ((Storebakken et al. 1998; Sajjadi and Carter 2004a; Sajjadi and Carter 2004b; Greiling et al. 2019; Denstadli et al 2007; Carter and Sajjadi 2010). In the present study, the effect of a previously uninvestigated microbial *E. coli* phytase (Optiphos, Huvepharma) on growth performance, digestibility and retention in Atlantic salmon was assessed. Applications of phytase were shown to be successful in achieving improved performance, digestibility and retention compared to inorganic phosphorus dosing.

Performance

Growth improvement from phytase addition has been seen in other phytases, though existing literature specifically targeting the effect of phytase on growth is limited. Across all

Table 3 Digestibility and biological sampling performance data for high plant inclusion diets in Atlantic salmon ± SD with varying levels of phosphorus sources (NC, negative control; PC, inorganic phosphorus dosed diet; PHY-750, phytase dosed diet). Bold *p* values and different superscript letters denote statistical differences ($P < 0.05$)

Diet	Initial	NC	PC	PHY-750	Test statistic	*P*
Digestibility						
Dry matter (%)	-	82.9 ± 0.2	83.1 ± 0.1	82.8 ± 0.1	$F_{(2,8)} = 3.765$	0.087
Phosphorus (%)	-	39.3 ± 1.8^{a}	64.8 ± 1.7^{b}	69.5 ± 2.0^{c}	$F_{(2,8)} = 232.352$	**< 0.001**
Phytate (%)	-	35.4 ± 1.0^{a}	34.4 ± 2.5^{a}	41.1 ± 2.0^{b}	$F_{(2,8)} = 10.793$	**0.010**
Body content						
Body phosphorus (%)	0.44 ± 0.00	0.30 ± 0.01	0.31 ± 0.03	0.33 ± 0.01	$H_{(2)} = 3.524$	0.172
Bone ash (%)	5.84 ± 0.44	4.88 ± 0.24^{a}	8.16 ± 0.37^{b}	8.12 ± 0.24^{b}	$F_{(2,8)} = 126.471$	**< 0.001**
Retention						
Phosphorus	-	24.4 ± 2.0^{b}	20.5 ± 4.7^{b}	36.6 ± 1.9^{a}	$F_{(2,8)} = 21.740$	**0.002**

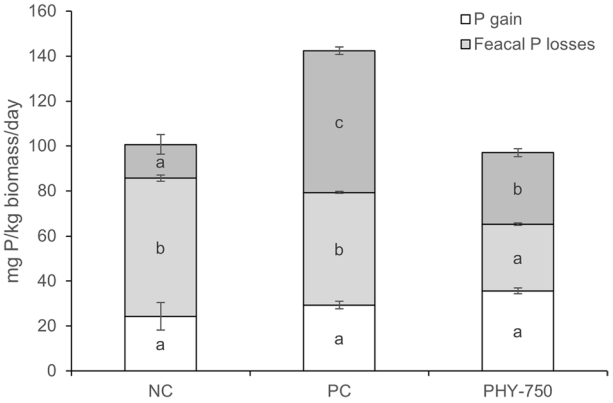

Fig. 1 Daily phosphorus balance in Atlantic salmon. Error bars = ± SD. Different superscript letters denote statistical differences ($P < 0.05$)

studies available in Atlantic salmon, additions of phytase to diets showed reduced performance than were seen in the present trial. Previous studies investigating the effect of microbial phytases on performance required postproduction inclusion levels in diets of at least 2000 FTU *A. niger* phytaseto result in significant performance improvement over the basal diet (Sajjadi and Carter 2004b), and additions of *P. pastori phytase* at 1900 FTU showed no significant performance improvement over a fishmeal control or basal diet (Denstadli et al 2007). Though lower inclusion levels of phytase were effective in the present study, the variable capacity of phytase to increase the availability of micro- and macronutrients in different plant ingredients has been noted in salmonids, and the differences in the ingredients used in studies may have some impact on the availability of phytate bound-P (Cheng and Hardy 2002, Sajjadi and Carter 2004a; Denstadli et al. 2006, 2007).

Salmon trials addressing the effects of *A. niger* phytase on growth have shown a great deal of variation in feed formulation, varying from 41.1 to 48.4% protein, 16.4 to 21.3% lipid and 0.62 to 0.78% phosphorus (Table 4). Though the levels seen in these trials meet recommendations for species, variations in ingredient composition can have direct impacts on growth and variation in the availability of phosphorus present in ingredients is also important to consider (Einen and Roem 1997; Storebakken et al. 1998).

The feed formulations most comparable to the current study were conducted by Sajjadi and Carter (2004b, Table 4), in which dosing of 2000 FTU of *A. niger* phytase resulted in a 6.56% improvement in bodyweight over a non-dosed control. Though the phytase test diet showed similar levels of macronutrients and phosphorus, levels of plant-based proteins were lower and phytase additions were much higher. No comparisons were made to inorganic dosing, and temperatures were higher than the current study at 15.1 °C. Despite the lower temperatures, higher plant ingredient inclusions and lower FTU dosing of phytase in the current study, performance was comparable with a 4.53% improvement in bodyweight over the negative control.

The improved action of OptiPhos to function in a high plant-based protein diet at lower temperatures may be a result of the characteristics of different phytases. The optimal conditions for OptiPhos activity is 58 °C and a pH of 3.4 compared to 65 °C and a pH of 2.0 in *A. niger* phytase (Dersjant-Li et al. 2015). This increased activity at higher pH, closer to the active pH of the stomach of salmon (Krogdahl et al. 2015), and lower optimal temperature may account for the increased performance seen.

Table 4 Comparison of basal diet composition of experimental diets assessing the efficacy of phytase in Atlantic salmon

Composition	Present study	Carter and Sajjadi 2010	Sajjadi and Carter 2004a	Sajjadi and Carter 2004b	Denstadli et al 2007	Storebakken et al 1998	Greiling et al. 2019
Crude protein (% DM)	49.8	41.1	477	48.4	52.7	43.9	51.6
Crude fat (% DM)	23.5	18.1	164	21.3	19.5	26.8	21.6
Ash (% DM)	5.7	6.8	9.34	7.3	7.7	8.2	5.5
Gross energy (kJ/g DM)	23.9	20.01	21.74	22.67	-	26.7	-
Total phosphorus (% DM)	0.81	0.62	-	0.78	1.1	3.5	0.596
Phytase (OTU/kg)	795	4000	2000	2000	1878	43.9	2767
Phytase source	Optiphos	*A. niger*	*A. niger*	*A. niger*	*P. pastori*	*A. niger*	*A. oryzae*
Plant-based ingredients (%)	54.8	71.4	55	48.2	54.3	57.73	89.74
Non-plant-based ingredients (%)	14.2	9.5	18.5	31	25	15	8.54
Minerals and vitamins	1.5	0.6	0.8	0.6	0.4	4.07	1.18
Oil	24.4	15	14.2	17.1	20.3	24.07	9.72

Digestibility

The effect of phytase on the digestibility of P in diets has been shown to be influenced by a broad variety of factors such as the source and concentration of available proteins, the source and concentration of phytate, the digestibility of the protein source, the mineral levels as well as the calcium, P and phytase inclusion rates (Selle et al. 2000; Sugiura et al. 2001). Increasing P digestibility with the inclusion of phytase has been seen in Atlantic salmon (Storebakken et al. 1998; Carter and Sajjadi 2010) as well as other salmonids (Brown 1993; Cain and Garling 1995; Rodehutscord and Pfeffer 1995; Riche and Brown 1996; Lanari et al. 1998; Vielma et al. 1998; Forster et al. 1999; Sugiura et al. 2001). Digestibility of phytate was seen in all diets which is expected with high plant meal inclusions; plant phytases have shown to be active in pH levels like those seen in the salmon stomach, and residual phytate digestion may be a result of intrinsic phytase activity (Nys et al. 1999).

The application of phytase resulted in significant improvement in P digestibility over both the negative control and MCP dosing, suggesting active increases in P digestibility were a direct action of phytate breakdown as evidenced by the significantly higher phytate digestibility seen in the test diet. Investigations into the efficacy of *A. niger* phytase showed inclusions of 1000 FTU were seen to produce similar P digestibility values as additions of 750 FTU in the current study (Carter and Sajjadi 2010). Though digestibility values seen in this study were similar to activity levels at 1000 FTU, differences in collection methods of faeces for analysis may mean that actual results may be more distant in direct comparison. The current study collected faeces via stripping as opposed to the settlement collection used by Carter and Sajjadi (2010). Stripping of faecal matter often underestimates the digestibility of minerals due to undigested material, whereas settlement can result in overestimation of digestibility as a result of leaching of the sample (Storebakken et al. 1998). Despite potentially reduced ADC's, lower inclusions of phytase resulted in improved digestibility of phosphorus and phytase.

Body content

Bone ash and P are indicators of the P status of fish (Rodehutscord 1996; Vielma and Lall 1998) as rapid growth is still seen in initial feeding periods of P deficient diets (Åsgård and Shearer 1997). P deficiency initially results in the use of bone bound P for metabolic processes (Baeverfjord et al. 1998), and growth is only inhibited once whole-body P content falls below a critical threshold (Nordrum et al. 1997). Bone ash in the current study was significantly higher at 750 OTU dosing of phytase and MCP dosed diets, suggesting sufficient P availability as seen in other studies conducted with rainbow trout (Vielma et al. 2002) and Atlantic salmon (Sajjadi and Carter 2004). Reductions in bone P in diet NC were not reflected in whole-body P content, and a reduction in bone ash content was seen in diet NC, evidencing supplementation of P deficiency from bone bound P.

Phosphorus retention and balance

P retention efficiency has been shown to increase with the addition of phytase (Vielma et al. 2002; Sajjadi and Carter 2004), which was reflected in the retention in the current experiment. P retention efficiency was significantly higher in diets containing 750 OTU of phytase, showing improved P utilisation. P losses through effluent release has significant environmental impact, resulting in pollution of aquatic ecosystems through

eutrophication (Milián-Sorribes et al. 2021), and improvement of retention can reduce negative environmental effects by reducing P release through greater utilisation.

Promotion of the reduction of waste has been a focus of the industry (FAO 2021), and assessment of the specific utilisation of P can give insights into the efficacy of products. The higher digestibility (the "Digestibility" section) and retention of P in diets containing 750 OUT of phytase resulted in significantly lower faecal losses. Faecal release accounts for most effluent loadings (Islam 2005), and active reduction of effluent waste by phytase inclusion can reduce pollution associated with farms. Despite low digestible P in treatment NC, P gain was closer to treatments PC and PHY-750, which further supports the active use of bone—bound P (Sugiura et al. 2004). Metabolic P loss was seen highest in treatment PC, at over double the amount seen in phytase dosed treatments, which may be a result of the higher inclusion levels of P included in diet PC. Though higher faecal wastage was seen, higher metabolic losses were also present, suggesting that higher levels of P were required to achieve similar performance to phytase dosed feed. This may also have been influenced by P source solubility as MCP has been shown to have a higher solubility in water (> 85%) (Morales et al. 2018). Higher metabolic losses noted may be a result of leaching into the water column, whether from excess P in faecal matter or from feeds, resulting in higher predictions of metabolic use.

Conclusion

The inclusion of phytase (OptiPhos®, Huvepharma) at 750 OTU proved to be an effective strategy to enhance P utilisation in high plant content feeds. Phytase supplementation has been shown to increase nutrient and mineral availability (Kumar et al. 2012) and enhance protein and amino acid digestibility (Storebakken et al. 1998; Sugiura et al. 1998). This reduction of the negative effects of phytate and promotion of essential nutrient availability in turn promotes increased growth and efficiency of feeds, and results seen in the present study collaborate previous experiments. Supplementation resulted in an enhanced digestibility and retention compared to inorganic P dosing, while achieving comparable growth performance at lower temperatures used in alternative studies with other phytases. The benefits associated with phytase use make it a valuable nutritional tool to reduce waste effluents as well as reduce the use of expensive inorganic P sources. Action to reduce the release of P into the environment is one of the major issues facing aquaculture production as environmental regulation expands to meet environmental degradation (Sugiura 2018). Continued development of products able to increase bioavailability and utilisation of previously phytate-bound P can ensure effective production practises meet environmental and regulatory requirements (Munguti et al. 2021). Though OptiPhos was found to enhance P utilisation in high plant content feeds, further investigation into appropriate dose responses of specific phytases in similar feed formulations, as well as comparisons between different phytase sources would be advantageous.

Author contribution Conceptualization: Daniel Arana Braidi; Methodology: Robert Serwata; Validation: Daniel Arana Braidi; Formal analysis: David Terrey; Investigation; David Terrey; Resources: David Terrey; Writing – Original draft: David Terrey; Writing – Review & Editing: David Terrey; Visualization: David Terrey; Supervision: Robert Serwata; Project administration: David Terrey; Funding acquisition: Daniel Arana Braidi.

Data Availability Data sets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval The trial was conducted at the experimental facilities of GIFAS (Gildeskal Forskningsstasjon AS, Inndyr, Norway). The experiment was directed by trained personnel (accredited according to FELASA function A: carrying out procedures on animals) and conducted by trained technical staff (accredited according to FELASA function B: designing procedures and projects; function C: taking care of animals) according to the EU guidelines on protection of animals used for scientific purposes (Directive 2010/63/).

Competing interests This study was funded by Huvepharma. David Terrey has received research support from Huvepharma. Robert Serwata and Daniel Arana Braidi receive a salary from Huvepharma where they hold the position of Global Product Manager. The sponsor was not involved in the final decision to submit the article for publication. Financial support for the conduct of research and preparation of the article was provided by Huvepharma. The research was designed and carried out blind at an independent contract research organisation (SPAROS, Portugal). Data was handled and analysed by an independent data analysis company (DT Biostat Ltd.) and prepared for publication independent of the funding source. The sponsor was not involved in the final decision to submit the article for publication.

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