

Abstract

Reduction of phosphorus levels in aqua feed without impacting growth, feed efficiency and health is key to the develop a sustainable aquaculture. This is currently being achieved by the use of low-ash fishmeal and highly available phosphorous supplementation. However, phytase enzymes can efficiently release phosphorus which is locked up as phytate and is found in high vegetable inclusion salmon feeds and can significantly help reduce eutrophication.

The objective of the present study was to measure the efficiency of a 6-phytase (OptiPhos®) using three different concentrations: 250, 500, 750 (OTU/kg) in feed for a growth performance trial utilising rainbow trout (*Oncorhynchus mykiss*). Feed was formulated with 48.4% crude protein, 22.3% crude fat and 22.3 MJ/kg gross energy. The enzymes were applied post pelleting using a vacuum coater. The fish were separated in five treatments, one Positive Control (PC) with 1.14 % P (1.7 % MCP in feed formula); 0.48 % phytate-P, a Negative Control (NC) with 0.75% P, without any MCP addition. And three phytase treatments at 250, 500 and 750 OTU/kg feed. The growth performance indicators Body Weight (BW), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and Feed Intake (FI) were recorded during the trial. The BW varied between 58.0 and 69.2 g, and the SGR varied between 1.78 and 1.98 %/d. Fish fed with MCP, 500 OTU, and 750 OTU in formula showed a significantly higher BW and SGR compared with the NC, and 250 OTU (P<0.05). Fish fed diet with 250 OTU showed a significantly higher BW, SGR compared to the NC (P<0.05). The FI varied between 1.42 and 1.47 (% BW/d) and FCR varied between 0.93 and 0.96. Any dietary treatments had no significant effect on feed intake and FCR (P>0.05).

Exogenous enzyme (OptiPhos®) added at 500 OTU/kg feed and 750 OTU/kg feed can significantly reduce the usage of inorganic phosphorus in trout feeds and be used as a tool to minimize the excess of phosphorus discharged to the environment or allow higher biomasses in compliant with the current local phosphorus discharge regulations.

Introduction

A lower use of finite marine-harvested resources is a sustainability challenge facing the future growth of the aquaculture industry. Plant base ingredients and by-products are promising sources of protein and energy for aquaculture feeds. However, high dietary inclusion levels of plant proteins generally depress growth and feed efficiency. The poor growth performance commonly found in fish fed plant-protein rich diets is generally related to the lower biological value (essential amino acid imbalance, impaired phosphorus availability, presence of anti-nutritional factors, higher carbohydrate fraction) of the plant-protein sources. The use of feed enzymes has shown the opportunity to enhance production economics resulting from the upgrade of the nutritional value of vegetable ingredients, a greater flexibility of ingredient use by the feed formulators and more important, a reduced environmental impact minimizing the excess of phosphorus discharged to the environment that allow higher biomasses to be farmed. The present trial assessed the efficacy of graded supplementation doses (0, 250, 500, 750 FTU/kg feed) of phytase OptiPhos 8000 L on the growth performance of of rainbow trout.

Material & Methods

The product under testing was a phytase enzyme (OptiPhos 8000L) in a liquid form produced by Huvepharma.

The trial comprised 5 experimental diets (Tables 1 and 2).

Table 1. Formulation of experimental diets.

| Ingredients, % | PC | NC | NC-P250 | NC-P500 | NC-P750 |
|--|-------|-------|---------|---------|---------|
| Fishmeal 70 LT ¹ | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 |
| Fish protein concentrate ² | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Krill meal ³ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Soy protein concentrate ⁴ | 13.20 | 13.20 | 13.20 | 13.20 | 13.20 |
| Pea protein concentrate ⁵ | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| Wheat gluten ⁶ | 9.65 | 9.30 | 9.30 | 9.30 | 9.30 |
| Corn gluten ⁷ | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Guar meal ⁸ | 11.80 | 11.80 | 11.80 | 11.80 | 11.80 |
| Soybean meal ⁹ | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Rapeseed meal ¹⁰ | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Wheat bran ¹¹ | 2.50 | 4.55 | 4.55 | 4.55 | 4.55 |
| Fish oil ¹² | 16.95 | 16.85 | 16.85 | 16.85 | 16.85 |
| Vitamin & Mineral Premix ¹³ | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| Soy lecithin ¹⁴ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Guar gum ¹⁵ | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Antioxidant ¹⁶ | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Monocalcium phosphate ¹⁷ | 1.70 | | | | |
| Calcium carbonate ¹⁸ | | 0.10 | 0.10 | 0.10 | 0.10 |
| L-Lysine ¹⁹ | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL-Methionine ¹⁹ | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| L-Taurine ¹⁹ | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Test phytase (FTU/kg) ²⁰ | | | 250 | 500 | 750 |
| Yttrium oxide [*] | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |

Table 2. Composition of experimental diets.

| | PC | NC | NC-P250 | NC-P500 | NC-P750 |
|---------------------------|-------------|-------------|-------------|-------------|-------------|
| Dry matter (DM) (%) | 94.5 ± 0.2 | 94.4 ± 0.1 | 94.5 ± 0.3 | 94.5 ± 0.1 | 94.6 ± 0.1 |
| Crude protein (% DM) | 48.4 ± 0.0 | 48.4 ± 0.0 | 48.4 ± 0.1 | 48.4 ± 0.0 | 48.5 ± 0.0 |
| Crude lipid (% DM) | 22.2 ± 0.1 | 22.3 ± 0.1 | 22.3 ± 0.0 | 22.2 ± 0.1 | 22.3 ± 0.1 |
| Crude ash (% DM) | 8.2 ± 0.0 | 8.5 ± 0.0 | 8.5 ± 0.1 | 8.5 ± 0.0 | 8.4 ± 0.1 |
| Gross energy (kJ/g DM) | 22.3 ± 0.1 | 22.3 ± 0.0 | 22.3 ± 0.0 | 22.3 ± 0.0 | 22.3 ± 0.0 |
| Total phosphorus (% DM) | 1.14 ± 0.01 | 0.75 ± 0.03 | 0.76 ± 0.01 | 0.75 ± 0.01 | 0.75 ± 0.01 |
| Phytate phosphorus (% DM) | 0.48 ± 0.00 | 0.48 ± 0.00 | 0.48 ± 0.00 | 0.48 ± 0.00 | 0.48 ± 0.00 |
| Phytase activity (FTU/kg) | 107 ± 6 | 119 ± 6 | 342 ± 6 | 668 ± 4 | 855 ± 23 |
| Yttrium oxide (mg/kg DM) | 269 ± 10 | 230 ± 6 | 222 ± 4 | 223 ± 6 | 226 ± 11 |

* Yttrium oxide was only incorporated in a fraction of feeds used for digestibility measurements.

Diets were manufactured by extrusion (pellet size 3.0 mm) by extrusion (pellet size 3.0 mm) by a pilot-scale twin-screw extruder (CLEXTRAL BC45, France) with a screw diameter of 55.5 mm and temperature ranging 111-114°C. All batches of extruded feeds were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). After, pellets were allowed to cool at room temperature, and subsequently the test enzyme, at the various doses, and oil were applied by coating under vacuum in a DINNISEN Pegasus vacuum mixer (PG-10VCLAB).

Regarding the post-extrusion coating procedure of enzyme and oil, 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, United Kingdom) and sprayed onto the pellets under vacuum (760 mbar) for 3 minutes. The PC and NC control diets without enzyme supplementation were also coated using the same procedure. It was added 1.7% MCP to the Positive Control (PC).

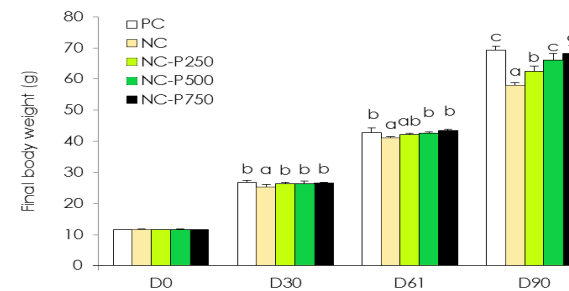
Quadruplicate groups of 35 rainbow trout, with a mean initial body weight (IBW) of 11.7 ± 0.5 g were fed one of the five experimental diets for 90 days. Fish were grown in fiberglass circular tanks (volume: 300 L) supplied with flow-through freshwater, with temperature ranging 13.9 ± 0.2°C and dissolved oxygen levels kept above 7.6 mg/L. Fish were hand fed 3 times per day (09:00, 14:00 and 17:00h) during weekdays and twice a day during weekends (10:00 and 17:00) to visual satiety. Utmost care was taken to avoid feed wastage and allow quantification of feed intake. Anesthetized fish (20 µl/L of AQUI-S™, New Zealand) were individually weighed at the start of the trial and group weighed at day 30, day 61 and day 90.

Results

At the end of the trial (90 days of feeding), fish showed a 6-fold increase of their initial body weight (Fig 1). SGR ranged from 1.78 to 1.98 %/day. In comparison to the negative control treatment (NC), both PC and all phytase supplemented diets led to significant increase of final body weight and SGR. Moreover, fish fed the PC diet and those supplemented with phytase at 500 and 750 FTU/kg showed a significantly higher FBW and SGR than those fed the diet with phytase at 250 FTU/kg. FCR values were low (0.92 - 0.96) suggesting good feeding practices. FCR, feed intake and PER values were not affected by dietary treatments (P>0.05).

Fig 1. Changes on final body weight of trout fed the various diets.

Bars are means ± standard deviation (n=4). Different superscripts denote a statistical difference (P<0.05).



Conclusion

The overall growth performance can be considered as satisfactory for rainbow trout of this size. Fish fed diet supplemented with MCP and OptiPhos®, led to a significant increase of final body weight and SGR compared with the NC. Fish fed diets supplemented with OptiPhos® at 500, and 750 FTU/kg achieved a performance, in terms of final body weight and growth rate, as the one observed in fish fed the PC diet. FCR, feed intake, and PER values were not affected by dietary treatments.



Daniel Arana Braid
Global Product Manager
Aquaculture at
Huvepharma
Daniel.Arana@huvepharma.com



Natalia Soares
Global Product Manager
Enzymes at Huvepharma
Natalia.Soares@huvepharma.com



Robert Serwata
Global Product Manager
Nutrition at Huvepharma
Robert.Serwata@huvepharma.com