



Extruded fish feed with high residual phytase activity and low mineral leaching increased *P. mesopotamicus* mineral retention

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ABSTRACT

This study attempts to provide valuable information about optimization of extrusion process in order to have a fish feed with maximum residual phytase activity, minimum mineral leaching and good mechanical characteristics. Also, the effects on mineral retention of extruded feed obtained in optimal condition, using a juvenile *Piaractus mesopotamicus* model was determined. In order to study the simultaneous effect of blend moisture (*M*) and extrusion temperature (*T*) on specific volume (*SV*), water resistance (*WR*), floatability (*F*), residual phytase activity (*RPA*), leached phosphorus (*LP*), calcium (*LCa*), zinc (*LZn*), and iron (*LFe*) a central composite design (3^2) was used. The levels of each variable were: *T*: 160–180–200 °C and *M*: 140–160–180 g/kg. A multiple response optimization of physicochemical properties of extruded feed (*WR*, *F*, *RPA* and mineral leaching) was performed using the Derringer's desirability function. The global desirability function value was 0.8990, and the obtained optimal conditions were 183.6 °C and 158 g/kg of moisture content. Phytase extruded feed (*PEF*) was obtained at such conditions and it had the following physical properties: *WR*: $81.8 \pm 2.5\%$, *F*: $94.5 \pm 0.72\%$, *LP*: $9.40 \pm 0.61\%$, *LCa*: $2.20 \pm 0.2\%$, *LZn*: $2.00 \pm 0.15\%$, and *LFe*: $11.2 \pm 2.8\%$. Fish consuming *PEF* with *RPA* of 3934.9 ± 47.7 UP/kg had higher iron, zinc, and phosphorus retention than those fed with control extruded feed (*CEF*) obtained under the same optimal conditions ($p < 0.05$). However, no significant difference in final body weight was detected between dietary treatments ($p > 0.05$) after 38 days of feeding trial at 25 °C. Extrusion process can be optimized to obtain fish feed based on vegetable meals with high residual phytase activity and low mineral leaching, increasing *P. mesopotamicus* mineral retention.

1. Introduction

Phosphorus (P) is the most important mineral required by fish, as its requirement is higher than that of other minerals (Rocha et al., 2014). If the diet does not supply sufficient bioavailable P, fish easily show deficiency signs such as poor growth and bone deformity. However, it has also been reported that excessing amounts of dietary P inhibit zinc utilization (Satoh et al., 1996). On the other hand, P is considered to be a major factor of eutrophication in aquaculture environment (Phillips et al., 1993). Thus, it is necessary that P excretions from cultured fish were kept at minimum level. Other minerals, such as iron and zinc are essential

Abbreviations: CEF, control extruded feed; PEF, extruded feed added with phytase enzyme; *M*, blend moisture; *T*, extrusion temperature; *SV*, specific volume; *WR*, water resistance; *RPA*, residual phytase activity; *F*, floatability; *LP*, leached phosphorus; *LCa*, leached calcium; *LZn*, leached zinc; *LFe*, leached iron; *SMEC*, specific mechanical energy consumption; *TMR*, total mineral retention; *LPO*, lipid peroxidation

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elements having fundamental roles in fish cellular biochemistry and metabolism (Rocha Aride et al., 2010; Aisen et al., 2001). Moreover, fish diets are commonly supplemented with inorganic iron and zinc sources to satisfy nutritional requirement of the specie. However, clinical signs of nutritional iron and zinc deficiency might be observed if the content of these minerals in the feed is inappropriate (Carriguirborde et al., 2004; Do Carmo et al., 2005). Therefore, a loss of minerals in water decreases the nutritional quality of the food and can affect the fish growth, increasing production cost due to greater supplementation.

Fish feed can be produced by pelleting or extrusion cooking. Extrusion technology is commonly used to produce fish feeds, since physical properties, such as water stability, durability, hardness, and buoyancy control, usually are improved compared to steam pelleted diets (Sørensen et al., 2009). On the other hand, most of studies showed that partial replacement of fish meal by other protein sources such as soybean meal can be successful (Hossain and Koshio, 2017; Jirsa et al., 2015). In this regard, partial replacement of fish meal with soybean meal improved physical quality of feed in terms of breaking force and durability, reducing bulk density and increasing radial expansion (Sørensen et al., 2002). However, the direct use of the leguminous oilseed as a dietary ingredient is limited since the presence of phytic acid and other anti-nutritional factors such as tannin, glucosinolates, saponins, soluble no starch polysaccharides and gossypol, which are not destroyed or inactivated by processes involved with product manufacture or during extrusion pelleting. (Roy et al., 2014; Hardy, 2010).

Soybean meals contain around 14 g/kg phytic acid (Deak and Johnson, 2007). Up to 80% of total P content is in the form of phytate and is practically not available for monogastric or agastric aquatic animals (Hardy, 2010). Phytates present in plant meals are negatively charged. They can bind cations or positively charged functional groups of molecules. The complexes formed with minerals are not absorbed through the gastrointestinal tract and bioavailability of minerals is decreased (Albarracín et al., 2015). Therefore, it is important to find ways to improve mineral availability of minerals in extruded fish feeds based on vegetable meals. A promising alternative is using a microbial phytase. Phytase (myo-inositol hexakisphosphate phosphohydrolase) is a phosphatase enzyme that catalyzes the hydrolysis of phytate to inositol and inorganic P (Lemos and Tacon, 2017). Different studies have reported the use of phytase increases mineral bioavailability such as phosphorus, iron, magnesium, calcium, manganese, and zinc (Roy et al., 2014; Morales et al., 2014; Cao et al., 2007; Cheng and Hardy, 2003; Lemos and Tacon, 2017). However, the effects of extrusion conditions on phytase activity and mineral leaching have not been studied. Moreover, there are not reports about residual phytase activity after the extrusion process and even less about optimized extrusion conditions to obtain a fish feed with maximum residual phytase activity and minimal mineral leaching. In this context, the aims of this work were: (i) to develop a fish feed formula based on vegetable meals added with phytase enzyme, (ii) to optimize extrusion conditions in order to have a product with maximum water resistance, floatability, and residual phytase activity; but minimum mineral leaching, (iii) to assess P, Ca, Fe, and Zn retention of this feed using a juvenile *P. mesopotamicus* model.

2. Materials and methods

2.1. Production of experimental diets

Experimental diets (CEF and PEF) were formulated with commercial corn meal (613 g/kg), soybean meal (200 g/kg), bovine plasma protein concentrate (130 g/kg), corn starch (20 g/kg), vitamin-mineral mix (7 g/kg), and canola oil (30 g/kg) taking into account the nutrient requirement for *P. mesopotamicus* (Bicudo et al., 2009). Soybean meal and bovine plasma protein concentrate were donated by America Pampa Agroindustrial S.A. (America, Argentina) and Yeruvá S.A. (Esperanza, Argentina), respectively. Thermo-resistant microbial phytase (Ronozyme, NOVOZYME®) was added to PEF at 0.2 g/kg.

The ingredients were mixed using a Yelmo 2202 dough mixer and water was added in order to achieve moisture content. The blends were sealed in polyethylene bags and stored 1 h at room temperature before each run for moisture stabilization. Moisture content of blends was checked using AOAC (2000) methods.

The extrusion process was carried out with a Brabender 10 DN single-screw extruder, using a 3:1 compression ratio screw, a 3/20 mm (diameter/length) die and 175 rpm screw speed. A Central Composite Design (CCD) (3^2), with three replicates in the central point resulting in 11 runs, was used to study the simultaneous effect of blend moisture (*M*) and extrusion temperature (*T*) on specific volume (SV), water resistance (WR), floatability (F), residual phytase activity (RPA), leached phosphorus (LP), leached calcium (LCa), leached zinc (LZn), and leached iron (LFe) of PEF. The levels of each factor were: *T*: 160–180–200 °C and *M*: 140–160–180 g/kg. Experiments were randomized. While the extruder feeding section temperature was maintained by circulating water through the jacketed device, the metering and die section temperature were both kept at that corresponding to each run by using the heat control device of the extruder. The feeding rate of the extruder was at full capacity. Experimental samples were taken after stationary state was established, then torque (Brabender Units – BU) and mass output (g/min) were measured.

Extrudates were dried in an oven at 40 °C (Bioelec) until a moisture content of ~80 g/kg was reached, divided into several portions and kept in plastic bags hermetically sealed until evaluation. For chemical analysis, extruded feeds were ground with a cyclone sample mill (UDY Corp Boulder Colorado, USA) using a 1 mm sieve.

In order to evaluate the effect of PEF obtained in optimal extrusion conditions on mineral retention of juvenile *P. mesopotamicus*, an extruded feed without phytase (CEF) was obtained under the same extrusion conditions than PEF, and used as a control for *in vivo* assay.

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