Animal Feed Science and Technology 234 (2017) 54–64

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Optimization of single screw extrusion process for producing fish feeds based on vegetable meals and evaluation of nutritional effects using a juvenile *Piaractus mesopotamicus* model

Raúl E. Cian[a,*], Carla Baccheta[b], Jimena Cazenave[b], Silvina R. Drago[a]

[a] *Instituto de Tecnología de Alimentos, CONICET, FIQ - UNL, 1o de Mayo 3250, 3000, Santa Fe, Argentina*
[b] *Instituto Nacional de Limnología, CONICET-UNL, Santa Fe, Argentina*

ARTICLE INFO

Keywords:
Extrusion process
Physical properties
Fish feed
Growth performance
Pacu

ABSTRACT

Optimization of extrusion process for producing extruded feeds based on vegetable meals using a single screw extruder was study. Also growth performance of extruded feed obtained in optimal conditions using a juvenile *P mesopotamicus* model was evaluated. In order to study the simultaneous effect of blend moisture (*M*) and extrusion temperature (*T*) on expansion (E), bulk density (BD), water absorption index (WAI), water solubility index (WSI), and floatability (F) a central composite design (3^2) was used. The levels of each variable were: *T*: 160–180–200 °C and *M*: 14–16–18 g/100 g. A multiple response optimization of physical properties of experimental extruded feed (E, F, BD and WSI) was performed using the Derringer's desirability function. The global desirability function value was 0.8805, and the obtained optimal conditions were 181.5 °C and 15.8 g/100 g of moisture content. Experimental extruded feed (EF) obtained at such conditions presented the following physical properties: E: 2.23 ± 0.03, F: 99.0 ± 1.0%, BD: 270.9 ± 13.8 g/L, and WSI: 12.9 ± 1.5 g/100 g. Crude protein content of EF was higher than that of control feed (CF). However, no significant difference for crude lipid (~37.9 g/kg) and total starch content was found. Gelatinized starch for EF (430.5 ± 7.0 g/kg) was higher than that obtained for CF (378.9 ± 6.7 g/kg), corresponding to 97.0 ± 2.1 and 88.1 ± 2.2% of degree of gelatinization, respectively. Chemical score of CF was 93.3%, lysine being the limiting amino acid. However, in the case of EF no limiting amino acid was found. Both diets presented a predominance of unsaturated fatty acids of C18 series (C18:1 and C18:2 (n–6)). EF showed higher content of oleic, linolenic, *cis*-11-eicosenoic and di-homo-γ-linolenic acid than CF. Nevertheless, only for CF, eicosapentaenoic acid, docosapentaenoic, and docosahexaenoic acid were detected. Significant differences in final fish body weight, weight gain, specific growth rate, and condition factor were not detected between dietary treatments after 120 days of feeding trial. Extrusion using a single screw extruder in optimal process conditions could be used to obtain fish feed based in vegetable meals and good physical properties with proper growth performance on juvenile *P. mesopotamicus*.

Abbreviations: FM, fish meal; CM, corn meal; SM, soybean meal; BPPC, bovine plasma protein concentrate; CF, control feed; *M*, blend moisture; *T*, extrusion temperature; E, expansion; BD, bulk density; WAI, water absorption index; WSI, water solubility index; F, floatability; DG, degree of gelatinization; DC, degree of cooking

[*] Corresponding author.

E-mail address: rec_704@yahoo.com.ar (R.E. Cian).

http://dx.doi.org/10.1016/j.anifeedsci.2017.09.004
Received 16 December 2016; Received in revised form 3 September 2017; Accepted 7 September 2017

0377-8401/ © 2017 Elsevier B.V. All rights reserved.

1. Introduction

Pacu (*Piaractus mesopotamicus*) is grown from northeastern Argentina to the central-western region of Brazil. In general, it is reared in ponds under intensive system in monoculture or under semi-intensive system in polyculture together with other omnivorous species (Abimorad et al., 2009). Studies have demonstrated that minimum dietary protein requirements of Pacu fingerlings are 27% (Bicudo et al., 2009). However, especially when derived from fish meal (FM), protein is the most expensive nutrient in the preparation of diets for aquatic organisms (Abimorad et al., 2009). This explains the need for the replacement of FM by vegetable alternatives (Draganovic et al., 2011). Most of studies showed that partial replacement of FM by other protein sources can be successfully accomplished with respect to nutritional and health aspects (Hossain and Koshio, 2017; Jirsa et al., 2015). According to Hardy (2010), in the near future fishmeal will no longer be the primary protein source in aqua feeds for carnivorous fish species, but rather be a specialty ingredient added to enhance palatability, balance dietary amino acids, supply other essential nutrients and biologically active compounds or enhance product quality. In general, inclusion of plant meals led to increased hardness, rougher surface, and greater radial expansion of fish feed. However, due to potential problems associated with insufficient levels of indispensable amino acids (e.g., lysine and methionine), anti-nutritional factors, and poor palatability, the commercial use of vegetable protein is often limited (Abimorad et al., 2009; Samocha et al., 2004). A promising alternative is using a mixture of complementary ingredients to improve the nutritional and physical quality of pellets. In this sense, plasma protein from animal blood produced during slaughtering is a valuable protein source (Salgado et al., 2011) which can be used with plant proteins for formulation of fish food.

Fish feed can be produced by pelleting or extrusion cooking. During extrusion cooking, raw ingredients undergo many chemical and structural changes, such as protein denaturation, starch gelatinization and formation of complexes between amylose and lipids (González et al., 2013), which changes physical properties of products. Physical quality of fish feed is often defined as the ability of feed to be handled without creating an excessive amount of fines. All feeds used in intensive aquaculture should be resistant to mechanical stress during transport handling and in pneumatic feeding devices (Aarseth et al., 2006). At the same time, the feed should have a texture and size that facilitate high feed intake and efficient digestion by the fish (Sørensen, 2012). Physical quality of fish feed can be assessed by physical properties of products like water stability, bulk density, sinking velocity, hardness and durability (De Cruz et al., 2015). Water stability of the extrudates gives information about nutrient leaching caused by the disintegration of feed pellets (Bandyopadhyay and Rout, 2001). Bulk density is related to sinking velocity and floatability, and hardness to durability of fish feed (Sørensen, 2012). Although extrusion technology is used with the purpose of producing feed with high nutritional and physical quality, formula composition and processing conditions can affect physicochemical properties (Sørensen, 2012). In this context, the aims of this work were: (i) to develop a formulation with corn meal, soybean meal and bovine plasma protein concentrate, (ii) to optimize extrusion conditions in order to have a product with maximum expansion and floatability, but minimum bulk density and water solubility, using a single screw extruder, (iii) to assess the nutritional effects of this feed using a juvenile *P. mesopotamicus* model.

2. Materials and methods

2.1. Raw materials and production of experimental extruded feeds (EF)

Commercial corn meal (CM), soybean meal (SM), and bovine plasma protein concentrate (BPPC) were analyzed regarding their proximate composition and mixed to obtain the chemical composition showed in Table 1, according to that proposed by Bicudo et al. (2009) for juvenile pacu. Soybean meal and BPPC were donated from America Pampa Agroindustrial S.A. (America, Argentina) and Yeruvá S.A. (Esperanza, Argentina), respectively. The ingredients were mixed using a Yelmo 2202 dough mixer (Buenos Aires, Argentina) and water was added in order to achieve the 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 before extrusion using AOAC

Table 1
Chemical composition of corn meal (CM), soybean meal (SM), bovine plasma protein concentrate (BPPC), control feed (CF), and experimental extruded feed (EF).

Components[a]	CM (g/kg)	SM (g/kg)	BPPC (g/kg)	CF (g/kg)[b]	EF (g/kg)[b]
Dry matter	851.6 ± 0.4	960.4 ± 0.4	909.7 ± 0.2	899.9 ± 1.4*	914.6 ± 5.3**
Crude protein	97.5 ± 0.8	495.4 ± 10.1	769.5 ± 26.2	249.3 ± 0.6*	285.5 ± 2.2**
Crude lipid	4.0 ± 0.1	76.6 ± 2.4	2.0 ± 0.0	41.3 ± 1.9*	34.4 ± 2.6*
Total starch	N.a.	N.a.	N.a.	430.6 ± 7.2*	444.0 ± 4.3*
Ash	28.2 ± 0.9	53.6 ± 2.5	100.2 ± 2.5	81.9 ± 0.7**	23.6 ± 0.9*
Calcium	0.03 ± 0.00	1.58 ± 0.02	0.71 ± 0.01	18.7 ± 0.7**	1.61 ± 0.08*
Phosphorous	0.88 ± 0.03	5.54 ± 0.19	2.99 ± 0.01	10.5 ± 0.2**	2.16 ± 0.21*
Zinc	0.01 ± 0.00	0.06 ± 0.00	0.08 ± 0.00	0.09 ± 0.01*	0.09 ± 0.02*
Iron	0.01 ± 0.00	0.08 ± 0.00	0.15 ± 0.01	0.28 ± 0.00**	0.17 ± 0.00*
Phytic acid	N.a.	N.a.	N.a.	9.4 ± 0.5**	8.1 ± 0.2*

N.a: not analyzed

[a] Chemical composition expressed as mean ± SD (n = 3).

[b] Different symbols in a row mean significant differences between samples ($p < 0.05$).

Table 2
Central composite design responses for expansion (E), bulk density (BD), water absorption index (WAI), water solubility index (WSI), and floatability (F).

Extrusion conditions		E	BD (g/L)	WAI (g water/g)	WSI (%)	F (%)
Temperature (*T*)	Moisture (*M*)					
160 °C	14 g/100g	2.33	321.40	4.74	15.26	96.32
160 °C	16 g/100g	2.03	454.03	4.39	13.73	84.06
160 °C	18 g/100g	1.71	611.38	2.99	6.75	70.02
180 °C	14 g/100g	2.31	276.87	4.95	16.18	100.01
180 °C	16 g/100g	2.22	263.16	4.53	14.59	98.02
180 °C	16 g/100g	2.26	262.74	4.30	11.67	99.05
180 °C	16 g/100g	2.18	286.90	4.33	12.45	100.01
180 °C	18 g/100g	1.84	443.45	3.60	9.86	85.05
200 °C	14 g/100g	1.93	324.19	4.40	13.69	95.06
200 °C	16 g/100g	1.88	296.68	3.66	7.98	97.01
200 °C	18 g/100g	1.65	530.74	2.60	8.17	76.0.2

(2000) approved methods. A commercial extruded fish feed was used as control feed (CF), which was characterized as described below.

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 a screw speed of 175 rpm. 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 expansion (E), bulk density (BD), water absorption index (WAI), water solubility index (WSI) and floatability (F). The levels of each factor were: *T*: 160–180–200 °C and *M*: 14–16–18 g/100 g (Table 2). Experiments were randomized. While the extruder feeding section was maintained by circulating water through the jacketed device, the metering and die sections were both kept at the temperature 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 ~8 g/100 g was reached, divided into several portions and kept in plastic bags hermetically sealed until evaluation. For chemical analysis, extruded feeds were ground with a Cyclotec mill (UD Corp Boulder Colorado, USA) using a 1 mm sieve.

2.2. Physical properties of experimental extruded feeds

2.2.1. Expansion (E)

Expansion (E) was determined according to González et al. (2002). Diameters of extruded feeds were measured with a caliper (Vernier, 0–150 mm, Stronger Argentina) on 10 pieces of sample randomly selected. Expansion (E) was calculated as the ratio between extruded product diameter and die orifice diameter (3 mm).

2.2.2. Bulk density (BD)

A tared 1000 mL measuring cylinder was filled with extrudates and the content was weighed. Bulk density (BD) was calculated as the ratio between the weight of the extrudates and 1000 mL. BD was expressed as g extruded feed/L (Aas et al., 2011). All determinations were performed at least in triplicate.

2.2.3. Water absorption index (WAI) and water solubility index (WSI)

Water absorption index (WAI) was performed according to Honorato et al. (2010), with some modifications. Extruded feeds were dispersed at 50 g/kg in distilled water (at 25 °C), stirred during 30 min and filtered through a 50-mesh sieve (0.297 mm). The residue of the filtration (particle size > 0.297 mm), was weighed and its solids content was determined after drying (24 h at 105 °C). Water absorption index (WAI) was expressed as g water/g extruded feed.

Water solubility index (WSI) was determined according to González et al. (2002). An aliquot of 1.25 g of ground sample was dispersed in 25 mL of water, stirred for 30 min and centrifuged at 2000 × *g* for 30 min at 25 °C. The supernatant was dried at 105 °C and the soluble solids were determined by weight. The WSI was calculated as: (g soluble solids x 100)/g dry extruded feed. All determinations were performed at least in triplicate.

2.2.4. Floatability (F)

Floatability (F) was determined according to De Cruz et al. (2015), with some modifications. Ten pieces of each extrudate were poured into a 100 mL beaker filled with distilled water at room temperature. The number of floating extrudates (Nf) suspended in the beaker was observed after 30 min and F was calculated as [(Nf/10) x 100]. All determinations were performed at least in triplicate.

2.3. Optimization of extrusion process

Derringer's desirability function was used for multiple response optimizations according to Derringer and Suich (1980). The method involves transformation of each predicted response to a dimensionless partial desirability function (*di*). The global desirability function (D) is defined as the geometric mean of the different *di* values. A value of D different from zero implies that all responses are in a desirable range simultaneously and, consequently, for a value of D close to 1, the combination of the criteria is globally optimal. In this work, E and F were maximized, while BD and WSI were minimized.

The specific mechanical energy consumption (SMEC) of extruded feed obtained in optimal conditions was determined according to González et al. (2002).

2.3.1. Model validation

Experiments were carried out to validate the models at T and M given by the optimization procedure. Samples of experimental extruded feed were obtained in the same way as it was previously described. The experimental data (E, F, BD and WSI), were compared to values of these responses predicted by the models. Additionally, experimental data responses (E, F, BD and WSI) obtained in each confirmatory experiment were compared to values predicted from the developed models by a *t*-test analysis.

2.4. Chemical analysis

Total nitrogen by semi micro Kjeldahl method (*N x 6.25*), Crude lipids (petroleum ether extract), moisture, ash, phytic acid and phosphorous contents were determined using AOAC (2000) approved methods. Total starch (g/kg) and gelatinized starch were quantified according to Tovar et al. (1990) and Holm et al. (1986), respectively. Degree of gelatinization (DG) was calculated as the ratio between gelatinized starches to total starch. Urease activity was determined according to AACC (2000) approved methods. Calcium, zinc and iron content in the sample were measured by atomic absorption spectroscopy after dry mineralization. Ash was removed with 10% HCl (v/v). An atomic absorption spectrophotometer analyst 300 Perkin-Elmer (Norwalk, CT, USA) was used. All determinations were performed at least in triplicate.

2.5. Amino acid and fatty acid profile

Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), according to the method of Alaiz et al. (1992), using D,L-α-aminobutyric acid as internal standard. The HPLC system consisted in a Perkin Elmer Series 200 pump, with Perkin Elmer 785A UV/vis detector, equipped with a 300 × 3.9 mm i.d. reversed-phase column (Novapack C18, 4 m; Waters). The chemical score was calculated according to Abimorad et al. (2008).

Fatty acids were determined by gas chromatographic quantification of their methyl esters prepared according to Masson et al. (2015). All determinations were performed at least in triplicate.

2.6. SDS–PAGE

SDS–PAGE was performed according to Laemmli (1970), using a Mini-Protean IV Electrophoresis cell (Bio-Rad) equipment with a Power Pac 300 Bio-Rad source with stacking gel of 3% w/v polyacrylamide and separating gel of 10% w/v polyacrylamide in 25 mM Tris–HCl, pH 8.3, 0.18 M glycine and 0.1% w/v SDS. The separation was carried out at 180 V for 50 min. The meals used as protein source standard were: soybean meal (SM), corn meal (CM) and fish meal (FM). Gel plates were fixed and stained with 0.125% w/v Coomassie Blue R-250 solution containing 50% v/v methanol and 10% v/v acetic acid. Then, polyacrylamide gels were distained with a 25% v/v methanol and 10% v/v acetic acid solution. Polyacrylamide gel were scanned with Shimadzu Dual – Wavelength Chromatogram Scanner Model CS – 910 at 550 nm and data acquisition was performed with CSW Chromatography Station DataApex Ltd program.

2.7. Nutritional effect of experimental extruded feed (EF) obtained at optimal conditions on juvenile P. mesopotamicus model

Juvenile pacu were obtained from Pez Campero fish farm (Paraná, Argentina). The experiment was performed in the Aquaculture Laboratory at the Instituto Nacional de Limnología (CONICET, Argentina) in a recirculating water system supplied with dechlorinated city (tap) water, and equipped with an external quartz-anthracite filter (Multiválvula Vulcano Filtro VC10). Prior to the feeding trial, all fish were acclimated to the indoor rearing conditions for 2 weeks. At the start of the feeding experiment, two hundred and ten fish (initial body weight 13.43 ± 2.56 g) were stocked in six 300 L tanks with 35 fish per tank. The diets, EF and a control one (CF), were randomly assigned to triplicate tanks. Fish were fed manually with these diets to satiation for 17 weeks. The daily ration was divided into two, and given at 09:00 and 14:00 h. The fish were weighed every 3 weeks and their ration adjusted accordingly. Uneaten diet was collected to avoid water quality impairment. Water flow to the tanks was at 15.1 L/min with artificial aeration and 12 h light/12 h dark photoperiod regime provided by artificial illumination. The water was maintained at 22.0 ± 1 °C, dissolved oxygen at 6.67 ± 0.63 mg/L, pH 6.15 ± 0.32, electrical conductivity of 189.40 ± 25.93 µs/cm, and total ammonia nitrogen of 0.24 ± 0.1 mg/L. The experiment was conducted in accordance with national and institutional guidelines for the protection of animal welfare (CONICET, 2005).

Growth and morphometric parameters were calculated according to Bicudo et al. (2009) as follows: weight gain (%)

Table 3
Analysis of variance for the overall effect of the two variables on Expansion (E), Bulk density (BD), Water absorption index (WAI), Water solubility index (WSI) and Floatability (F); and predicted and experimental mean values obtained at 181.5 °C and 15.8 g/100 g of moisture content.

Source of variation			*P-values*		
	E	BD (g/L)	WAI (%)	WSI (%)	F (%)
Temperature (*T*)	**0.0228**	**0.0201**	0.2795	0.2518	**0.0180**
Moisture (*M*)	**0.0048**	**0.0026**	**0.0064**	**0.0315**	**0.0017**
T^2	**0.0123**	**0.0082**	**0.0492**	0.1636	**0.0056**
T x *M*	**0.0460**	0.0946	0.0656	0.4267	0.0728
M^2	0.0632	**0.0116**	0.3407	0.9154	**0.0096**
Lack of fit	0.3670	0.0757	0.1686	0.4086	0.0517
r^2	0.9808	0.9484	0.9189	0.8509	0.9478
Model validation	**E**	**BD (g/L)**	**WSI (%)**		**F (%)**
Predicted Value[a]	2.21	262.7	13.1		99.9
Experimental value[b]	2.23 ± 0.03	270.9 ± 13.8	12.9 ± 1.5		99.0 ± 1.0

Bold values indicates significant differences ($p < 0.05$). Degrees of freedom: n-1.

[a] Values obtained using the second-order polynomial equation and the corresponding regression coefficients.

[b] Mean ± SD ($n = 3$).

= 100 × [(final body weight (g) – initial body weight (g))/initial body weight (g)] and specific growth rate (%/day) = 100 × [(ln final weight (g) – ln initial weight (g))/days of the trial]. Condition factor (K) = [total body weight (g)/standard length^3 (cm)] was calculated according to Goede and Barton (1990).

2.8. Statistical analysis

STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, Virginia, USA) was used to perform ANOVA, to fit the second-order polynomial equations to the experimental data given in Table 2 and to obtain the coefficients of the equations. The significance of each term of the models was evaluated referred to the pure error. For verification of the model adequacy, the lack of fit, the coefficient of determination (r^2) was calculated. STATGRAPHICS Centurion XV 15.2.06 was used as well for the numerical optimization procedure through the Derringer's desirability function. The statistical differences among samples were determined using the least significant difference (LSD) test with a level of signification $\alpha = 0.05$.

3. Results

3.1. Effect of extrusion conditions on physical properties of experimental extruded feeds

Table 2 shows physical properties of experimental extruded feeds obtained at different extrusion conditions. ANOVA results (Table 3) showed the degree of significance (*p* values) corresponding to the effects of each polynomial term of the regression model. In all cases the lack of fit was not significant ($p > 0.05$), and the coefficient of determination (r^2) was acceptable, thus regression models can be considered adequate to describe the effects of *T* and *M* on each response.

For expansion (E), all terms, except M^2, were significant ($p < 0.05$). E was inversely related to *M* and was observed a maximum at intermediate temperatures (179.5 °C) and low *M* (Fig. 1**a**). The highest values corresponded to the extruded feed obtained at 160 °C and 14 g/100 g, suggesting that melt elasticity level was also inversely related to *M*. In the case of bulk density (BD), the effects of *T* and *M* were significant in both linear and the quadratic terms (Table 3). The lowest value of BD was obtained at 180 °C and 16 g/100 g (Table 2) and a minimum in BD response surface at 182.8 °C and 15.8 g/100 g was observed (Fig. 1**b**). Regarding water absorption index (WAI), only *M* and T^2 terms were significant. WAI increased with *T* up to ~180 °C and decreased with *M*, for all moisture range studied and a maximum at 183.4 °C and 14.0 g/100 g was observed (Fig. 1**c**). The values of water solubility index (WSI) ranged 6.75–16.18%. The ANOVA results showed that only the linear term of *M* was significant. As for WAI, WSI was inversely related with *M* (Fig. 1**d**), being this effect more noticeable at low *T*. For floatability (F), the effects of *T* and *M* were significant in both linear and quadratic terms. F responses surface (Fig. 1**e**) showed a maximum at intermediate values of *T* and *M* (182.8 °C and 15.7 g/100 g). In this regard, an inverse relationship between F and BD (r^2: 0.9883) was observed (Fig. 1**f**), the minimum value of BD matching with the maximum of F response surface.

3.2. Optimization of extrusion process

A multiple response optimization of physical properties of experimental extruded feeds (E, F, BD and WSI), was performed using the Derringer's desirability function. Optimization criteria were to maximize the E and F ($P = 2$ and $P = 5$, respectively), and to minimize the BD and WSI ($P = 5$ and $P = 1$, respectively). The global desirability function value was 0.8805, and the obtained optimal conditions were 181.5 °C and 15.8 g/100 g of moisture content. The specific mechanical energy consumption (SMEC) in this condition was 419.2 ± 31.0 J/g. This result are in agreement with those reported by Pastor-Cavada et al. (2011) for extruded products based on whole corn and brown rice added with wild legumes.

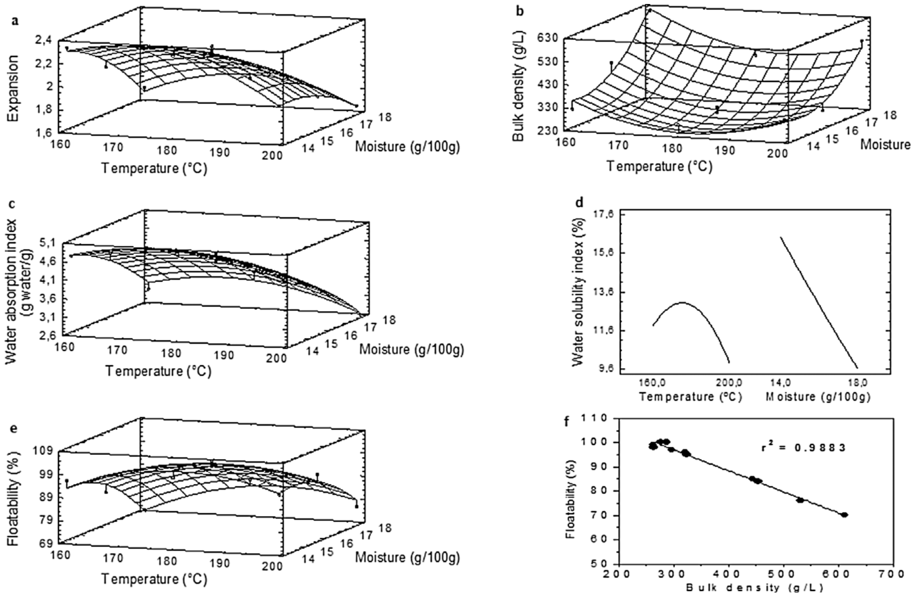

Fig. 1. Response surface plot corresponding to the effects of extrusion temperature and moisture content on Expansion (a), Bulk density (b), Water absorption index (c), Principle effects for percentage of Water solubility index (WSI), Floatability (e) and relationship between Floatability and Bulk density (f).

The suitability of the generated mathematical model to predict maximum E and F and minimum BD and WSI was experimentally validated using the conditions determined in the optimization. The experimental values as well as those predicted by the generated model are shown in Table 3. In this regard, the experimental and predicted values generated by the mathematical model showed adequate agreement ($p > 0.05$).

3.3. Chemical analysis, amino acid profile, fatty acid profile and SDS-PAGE

Table 1 shows chemical composition of control feed (CF) and experimental extruded feed (EF) obtained at optimal conditions (181.5 °C and 15.8 g/100 g). Crude protein content of EF was higher than that obtained for CF ($p < 0.05$). However, no significant differences for crude lipid and total starch content were found. Gelatinized starch for EF was higher than that obtained for CF (430.5 ± 7.0 and 378.9 ± 6.7 g/kg, respectively). In this sense, the degree of gelatinization (DG) obtained for EF and CF was 97.0 ± 2.1 and 88.1 ± 2.2%, respectively. Ash content of CF was higher than that obtained for EF. In accordance with this, calcium, phosphorous, and iron contents from CF were higher than that found for EF ($p < 0.05$). However, no significant difference for zinc content was found. The P/Ca ratio for CF and EF was 0.6 and 1.3, respectively. Phytic acid content of EF was lower than that obtained for CF. Urease activity was not detected in both diets (ΔpH ~ 0.00).

Table 4 shows the amino acid profile of CF and EF. Aspartic + glutamic acid and leucine were the most abundant amino acids in both diets. For each amino acid, the ratio of content between EF and CF was approximately 1.0. However, lysine, histidine, threonine and cysteine were higher in EF, while proline was lower. The chemical score of CF was 93.3%, lysine being the limiting amino acid. However, in the case of EF no limiting amino acids were found.

Fatty acid profile is shown in Table 5. Both diets presented a predominance of unsaturated fatty acids of C18 series (C18:1 and C18:2 (n–6)). CF showed higher content of saturated fatty acids than EF, C16:0 being the most abundant. The second most abundant saturated fatty acid in both products was C18:0, although it was in a much smaller proportion than C16:0. Among unsaturated fatty acids, EF showed higher content of oleic, linolenic, *cis*-11-eicosenoic and di-homo-γ-linolenic acid than CF. However, only for CF, eicosapentaenoic acid (EPA), docosapentaenoic (DPA), and docosahexaenoic acid (DHA) were detected. These results indicate control feed has fish meal (FM), which was confirming by SDS–PAGE of CF (Fig. 2). As can be seen in Fig. 2**a**, CF shows the characteristic peak corresponding to FM, which was used as standard (peak 4). Nevertheless, in the case of EF, this peak is not present (Fig. 2**b**).

3.4. Nutritional effect of diets on juvenile P. mesopotamicus model

Fish promptly accepted both experimental diets, and no mortality occurred during the feeding trial. Final body weight was 25.58 ± 0.92 g and 28.23 ± 0.87 g for fish fed with CF and EF, condition factor being 3.48 ± 0.06 and 3.54 ± 0.10, respectively. Weight gain was 101.00 ± 11.75 and 110.27 ± 15.08% for fish fed with CF and EF, specific growth rate being 0.58 ± 0.05 and 0.62 ± 0.06%/day respectively. No significant difference in growth performance and condition factor was detected between dietary treatments ($p > 0.05$) after 120 days of feeding trial.

Table 4

Amino acid profile of control feed (CF) and experimental extruded feed (EF) obtained at 181.5 °C and 15.8 g/100 g of moisture content.

Amino acids	Total amino acids (g/kg protein dry basis)[a]	
	CF [b]	EF [b]
Asp + Glu	256.6 ± 14.7**	207.5 ± 8.5*
Ser	71.8 ± 3.7*	80.5 ± 2.0**
His	30.6 ± 2.9*	48.8 ± 2.4**
Gly	84.9 ± 2.9**	56.6 ± 2.5*
Thr	50.0 ± 0.1*	72.8 ± 2.9**
Arg	71.8 ± 0.1*	81.5 ± 3.0**
Ala	91.1 ± 1.4**	76.7 ± 3.0*
Pro	68.0 ± 5.8**	24.1 ± 0.3*
Tyr	42.8 ± 6.7*	56.6 ± 2.5*
Val	52.3 ± 0.3*	72.1 ± 3.2**
Met	16.1 ± 2.2*	12.8 ± 0.2*
Cys	14.1 ± 0.2*	22.6 ± 1.6**
Ile	42.0 ± 0.9*	46.8 ± 1.1**
Leu	130.3 ± 0.1*	143.4 ± 5.8*
Phe	61.8 ± 1.5*	73.4 ± 2.6**
Lys	66.3 ± 2.7*	92.2 ± 3.6**

[a] Total amino acids content expressed as mean ± SD (n = 3).

[b] Different symbols in a row mean significant differences between samples ($p < 0.05$).

Table 5

Fatty acid profile of control feed (CF) and experimental extruded feed (EF) at 181.5 °C and 15.8 g/100 g of moisture content.

Fatty acids	Fatty acids (g/kg crude lipid)[a]	
	CF[b]	EF[b]
C12:0	1.3 ± 0.3	N.d.
C14:0	5.9 ± 0.1	N.d.
C16:0	175.3 ± 7.3**	60.5 ± 5.3*
C17:0 iso	2.3 ± 0.1	N.d.
C16:1 9c	21.0 ± 0.1**	0.9 ± 0.0*
C17:0	1.4 ± 0.2	N.d.
C18:0	46.1 ± 0.9**	21.0 ± 0.3*
C18:1 9t	4.0 ± 0.0	N.d.
C18:1 10t	0.3 ± 0.0	N.d.
C 18:1 11t	0.4 ± 0.3	N.d.
C18:1 9c	334.7 ± 4.3*	494.9 ± 7.3**
C18:1 11c	10.9 ± 0.9*	14.9 ± 0.5**
C18:1 12c	0.6 ± 0.3	N.d.
C18:2 9c 12c n-6 (linoleic)	362.2 ± 4.5**	319.2 ± 1.7*
C18:3 6c9c12c n-3 (γ-linolenic)	1.9 ± 0.6*	3.3 ± 0.8**
C18:3 9c12c15c n-3 (α-linolenic)	20.6 ± 2.3*	77.5 ± 1.5**
C20:1 11c	3.2 ± 0.3*	5.4 ± 0.2**
C20:2	1.5 ± 0.1	N.d.
C20:3 n-6 (di-homo-γ-linolenic)	N.d.	2.4 ± 0.3
C20:4 n-6 (arachidonic acid)	1.3 ± 0.1	N.d.
C20:5 n-3 (eicosapentaenoic acid)	3.0 ± 0.2	N.d.
C22:5 (docosapentaenoic acid)	1.1 ± 0.0	N.d.
C22:6 (docosahexaenoic acid)	4.4 ± 0.5	N.d.

N.d.: not detected.

[a] Fatty acid content expressed as mean ± SD (n = 3).

[b] Different symbols in a row mean significant differences between samples ($p < 0.05$).

4. Discussion

4.1. Effects of extrusion conditions on physical properties of experimental extruded feeds and optimization of extrusion process

The expansion is an important factor in aquafeeds as it affects the density, fragility, hardness and oil holding capacity (Rosentrater et al., 2009). It is greatly affected by the flour moisture (Draganovic et al., 2011). For experimental formulation, an increase in M caused a decrease in E, since E is positively correlated with the elastic component of the melt coming out from the die, which would decrease as M increases (González et al., 2013). This result is in agreement with those reported by González et al. (2002), Perez et al.

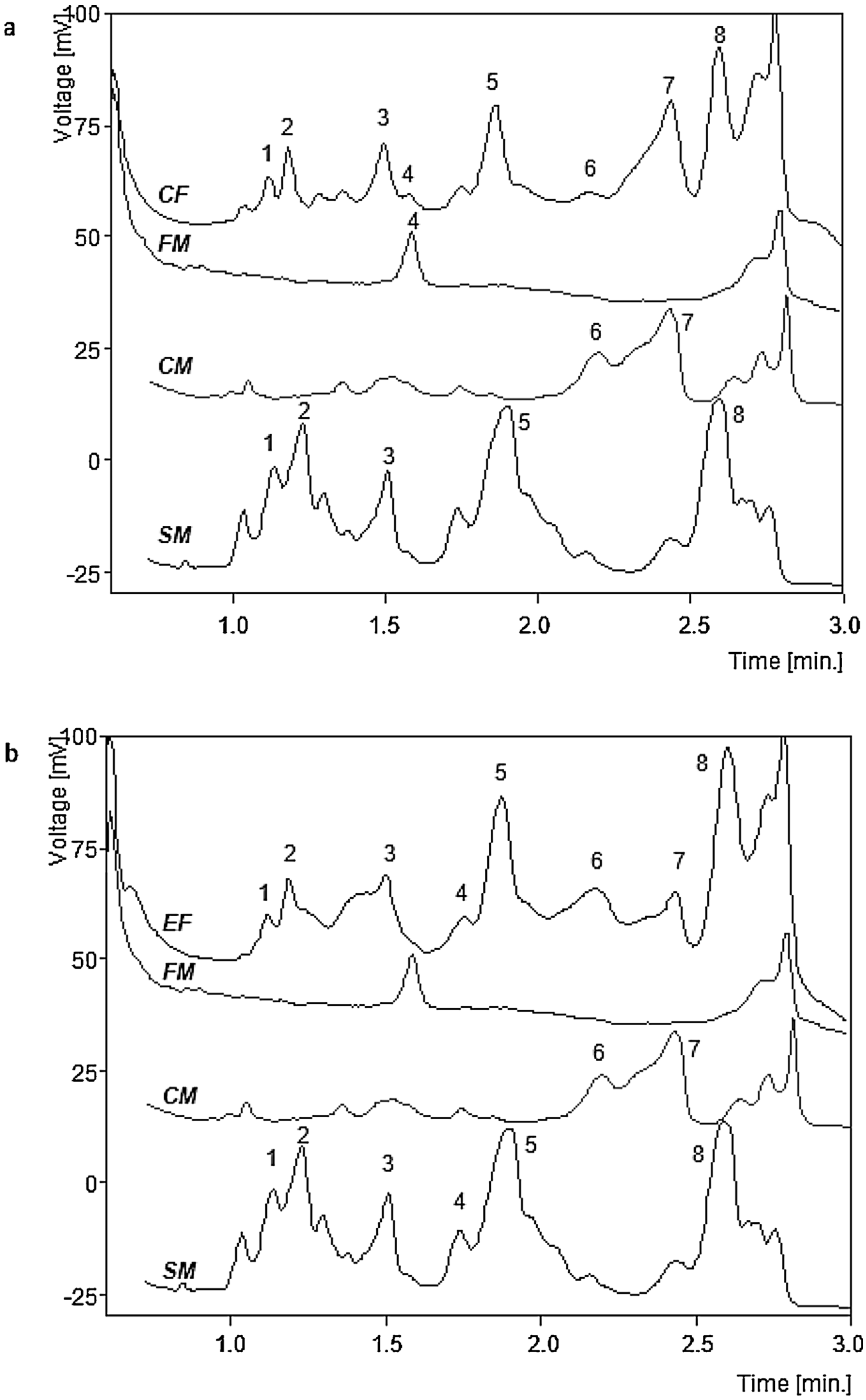

Fig. 2. SDS-PAGE profile of a) control feed (CF) and b) experimental extruded feed (EF). Soybean meal (SM), corn meal (CM) and fish meal (FM) are protein source standard.

(2008), Hagenimana et al. (2006) and Llopart et al. (2014) who worked with maize grits, maize-soy blend, rice flour and whole grain red sorghum, respectively.

Expansion and BD are the best properties to describe product porosity (Pastor-Cavada et al., 2011). Moreover, BD determines the floatability of fish feed (Chevanan et al., 2009). In this sense, an inverse relationship between BD and F was found. This result was also observed by De Cruz et al. (2015) for extruded fish pellets containing taro or broken rice starch. They observed that as die temperature increased from 125 °C to 170 °C, BD decreased. We found similar tendency until 182.8 °C. As *T* increases, degree of cooking (DC) increases and therefore the product is less dense (González et al., 2013). However, the reduction of viscosity in the last section of the screw at *T* higher than 182.8 °C reduced friction levels, decreasing DC and increasing BD of extruded feeds (González et al., 2014). In the range of 160–200 °C, BD increased with moisture level, indicating a reduction of DC. This can be due to high values of *M* produce a reduction of friction. Specific volume of extruded feed (the inverse of BD) is directly related with DC and is considered as a DC indicator (Llopart et al., 2014; González et al., 2002).

On the other hand, the ability of the pellets to float for a period of time is also related to water stability (Sørensenet al., 2002) and hydration properties (WAI and WSI). We found that WAI and WSI were inversely related to *M*. This would indicate that at low moisture level, mechanical effects (friction level) are more important, however at high *M*, thermal effects predominate (Llopart et al., 2014). In the range of 14–18 g/100 g of moisture content, WSI was directly related to *T* for values lower than 180 °C. Moreover WSI is directly related to DC (Pastor-Cavada et al., 2011). However, an increase of *T* from 180 °C to 200 °C produced a reduction of DC, probably due to the increase of *T* impacted more on the reduction of friction than on thermal effect on DC. This incomplete cooking process could be attributed to perturbation of the particles transport inside the extruder that not only could retard the cooking process of starchy particles caused by friction, but also could broaden the residence time distribution of particles inside the extruder (Haller et al., 2012). Thus, some particles would reach the die very fast, without suffering much change, obtaining a compaction product (González et al., 2013). The reduction of WSI at high *T* also was reported for extruded oat products. The high contents of lipids, proteins, and dietary fiber may influence starch cooking levels, reducing WSI with *T* (Gutkoski and El-Dash, 1999).

As previously mentioned, both BD and water stability of the extruded feed are important factors determining floatability or sinking velocity of pellets and leaching caused by the disintegration of feed (Sørensenet al., 2012). Therefore, in order to optimize floatability and water stability of extruded feed, the BD and WSI were minimized.

4.2. Chemical analysis, amino acid profile, fatty acid profile and SDS-PAGE

Crude protein and crude lipid of both feeds were similar to that reported by Abimorad and Carneiro (2007). EF had higher gelatinized starch than CF indicating higher degree of gelatinization (DG) probably due to differences in processing conditions such as temperature, moisture and screw speed used to obtain EF and CF. Although extruder systems are versatile and can produce pellets with high quality from ingredients with low pelletability index, feed constituents such as starch, protein, fiber and fat affect physical properties such as strength, durability and expansion ratio. It has been generally accepted that high starch gelatinization improves pellet quality (Sørensenet al., 2012). According to this, EF would have better pellet quality than CF.

EF had lower phytic acid content than CF which could provide better mineral availability and protein digestively. 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 in the case of protein; the digestibility is decreased (Albarracín et al., 2015). Thus, phytic acid decreases nutritional quality of feeds (Forster et al., 1999). On the other hand, it is known that soybean is a source of protease inhibitors which might affect nutrient utilization (Francis et al., 2001). An indirect measure of this anti-nutritional factor is urease activity (Osella et al., 1997). EF had no urease activity, indicating complete inactivation of trypsin inhibitor.

Regarding the amino acids content EF satisfied Bicudo et al. (2009) recommendations for juvenile *P. mesopotamicus*. Chemical score was calculated taking as reference amino acid profile of white muscle protein of juvenile *P. mesopotamicus* (Abimorad et al., 2008). Lysine was the first limiting amino acid in CF, but no limiting amino acid was found for EF. Therefore, EF would provide better quality protein than CF. It is important to note that bovine plasma protein concentrate and soybean meal are excellent lysine sources, and may contribute up to 100 and 24.8 g/kg digestible lysine (respectively) in their compositions, whereas FM may only contribute 24.8 g/kg digestible lysine (Abimorad et al., 2008).

CF has higher content of saturated fatty acids (mainly C16:0) and lower amounts of mono and polyunsaturated fatty acids than EF. The apparent digestibility coefficient of C16:0 is lower than that of monounsaturated or polyunsaturated fatty acids by *P. mesopotamicus* (Gonçalves and Cyrino, 2014). Thus EF would provide better lipid quality than CF. Corn oil and soybean oil are interesting sources of 18:3n-3 dietary fatty acids with high apparent digestibility coefficients (> 93%), EF being a good source of this essential fatty acid for *P. mesopotamicus.*

4.3. Nutritional effect of diets on juvenile P. mesopotamicus model

For obtaining a suitable production in aquaculture, it is necessary to reach the greatest and fastest fish growth with the less expenses (Baldisserotto et al., 2013). Overall growth performance was almost the same in both experimental groups. However, the results presented herein highlighted a slight enhancement of growth performance in fish fed with EF. In agreement with Fernandes et al. (2000), the results showed that FM can be substituted totally by other protein source (plant or animal protein) without the impairment of growth performance on juvenile *P. mesopotamicus*.

5. Conclusion

The present study has documented for the first time the simultaneous effect of blend moisture and extrusion temperature on physical properties (expansion, bulk density, water absorption index, water solubility index, and floatability) of experimental extruded feed, using a single screw extruder. Extrusion conditions were selected in order to obtain a product with very good physical properties for aquaculture. The optimal conditions were 181.5 °C and 15.8 g/100 g of moisture content. This product without fish meal was evaluated in a juvenile *P. mesopotamicus* model. Significant differences in growth performance and condition factor between dietary treatments were not observed. Thus, extrusion process using a single screw extruder in optimal conditions could be used to obtain fish feed based in vegetable meals and with very good physical properties.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was funded by project PICT-2013-1804 from ANPCyT.

References

AACC, 2000. Approved Methods of the American Association of Cereal Chemists. Methods 38-12 and 46-13, 10th ed. American Association of Cereal Chemists, St. Paul (MN).

AOAC., 2000. Official Methods of Analysis (17th Edn.). Vol. I and II. Gaithersburg (MD): Baldisserotto, B., 2013. Fisiologia de Peixes Aplicada à Piscicultura 3a Edição. Ed. Editoraufsm. Santa Maria, RS.

Aarseth, K.A., Sørensen, M., Storebakken, T., 2006. Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength: weibull analysis. Anim. Feed Sci. Technol. 126, 75–91.

Aas, T.S., Oehme, M., Sørensen, M., He, G., Lygren, I., Åsgård, T., 2011. Analysis of pellet degradation of extruded high energy fish feeds with different physical quality in a pneumatic feeding system. Aquacult. Eng. 44, 25–34.

Abimorad, E.G., Carneiro, D.J., 2007. Digestibility and performance of pacu (*Piaractus mesopotamicus*) juveniles-fed diets containing different protein, lipid and carbohydrate levels. Aquacult. Nutr. 13, 1–9.

Abimorad, E.G., Squassoni, G.H., Carneiro, D.J., 2008. Apparent digestibility of protein, energy, and amino acids in some selected feed ingredients for pacu *Piaractus mesopotamicus*. Aquacult. Nutr. 14, 374–380.

Abimorad, E.G., Favero, G.C., Castellani, D., Garcia, F., Carneiro, D.J., 2009. Dietary supplementation of lysine and/or methionine on performance, nitrogen retention and excretion in pacu *Piaractus mesopotamicus* reared in cages. Aquaculture 295, 266–270.

Alaiz, M., Navarro, J., Giron, J., Vioque, E., 1992. Amino acid analysis by highperformanceliquid chromatography after derivatization with diethylethoxymethylenemalonate. J. Chromatogr. 591, 181–186.

Albarracín, M., González, R.J., Drago, S.R., 2015. Soaking and extrusion effects on physicochemical parameters phytic acid, nutrient content and mineral bioaccessibility of whole rice grain. Int. J. Food Sci. Nutr. 66, 210–215.

Bandyopadhyay, S., Rout, R.K., 2001. Aquafeed extrudate flow rate and pellet characteristics from low-cost single-screw extruder. J. Aquat. Food Prod. Technol. 10, 3–15.

Bicudo, A.J.A., Sado, R.Y., Cyrino, J.E.P., 2009. Dietary lysine requirement of juvenile pacu *Piaractus mesopotamicus* (Holmberg, 1887). Aquaculture 297, 151–156.

CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), 2005. Marco Ético de Referencia para las Investigaciones Biomédicas en Animales de laboratorio, de granja y obtenidos de la naturaleza, Buenos Aires. CONICET, Argentina. http://web.conicet.gov.ar/documents/11716/0/OCR+RD+20050701-1047. pdf.

Chevanan, N., Muthukumarappan, K., Rosentrater, K.A., 2009. Extrusion studies of Aquacultulture feed using distillers dried grains with solubles and whey. Food Bioprocess Technol. 2, 177–185.

De Cruz, C.R., Kamarudina, M.S., Saada, C.R., 2015. Effects of extruder die temperature on the physical properties of extruded fish pellets containing taro and broken rice starch. Anim. Feed Sci. Technol. 199, 137–145.

Derringer, G., Suich, J., 1980. Simultaneous optimization of several response variables. J. Qual. Technol. 12, 214–219.

Draganovic, V., Van der Goot, A.J., Boom, R., Jonkers, J., 2011. Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Anim. Feed Sci. Technol. 165, 238–250.

Fernandes, J.B.K., Carneiro, D.J., Sakomura, N.K., 2000. Fontes e Níveis de Proteína Bruta em Dietas para Alevinos de Pacu (*Piaractus mesopotamicus*). Rev. Bras. Zootec. 29, 646–653.

Forster, I., Higgs, D.A., Dosanjh, B.S., Rowshandeli, M., 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 °C fresh water. Aquaculture 179, 109–125.

Francis, G., Makkar, H.P., Becker, K., 2001. Anti-nutritional factors present in plant derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.

Goede, R.W., Barton, B.A., 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. In: Am. Fish. Soc. Symp.. In: Adams, S.M. (Ed.), Biological Indicators of Stress in Fish 8. pp. 93–108.

Gonçalves, L.U., Cyrino, J.E.P., 2014. Digestibility of energy, lipids and fatty acids of vegetable oils and poultry fat by pacu *Piaractus mesopotamicus* (Holmberg 1887). Aquacult. Nutr. 20, 567–573.

González, R.J., Torres, R.L., De Greef, D.M., 2002. Extrusión cocción de cereales. Food Sci Technol. 36, 83–136.

González, R.J., Pastor Cavada, E., Vioque Peña, J., Torres, R.L., De Greef, D.M., Drago, S.R., 2013. 2013. Extrusion conditions and amylose content affect physicochemical properties of extrudates obtained from brown rice grains. Int. J. Food Sci., 584148 (8 pages).

González, R.J., Drago, S.R., Torres, R.L., De Greef, D.M., 2014. In: de Pinho Ferreira Guine, R., dos Reis Correia, P.M. (Eds.), Extrusion Cooking of Cereal Based Products. Engineering Aspects of Cereal and Cereal-Based Products, Chapter 12, pag. (Portugal). ISBN 9781439887028.

Gutkoski, L.C., El-Dash, A.A., 1999. Effect of extrusion process variables on physical and chemical properties of extruded oat products. Plant Foods Hum. Nutr. 54, 315–325.

Hagenimana, A., Ding, X., Fang, T., 2006. Evaluation of rice flour modified by extrusion cooking. J. Cereal Sci. 43, 38–46.

Haller, D., González, R.J., Drago, S.R., Torres, R.L., De Greef, D.M., 2012. Harina de maíz para tortillas: Efectos de la dureza del endospermo y de las variables de extrusión. Revista No 52 de la Facultad de Ingeniería Química. Universidad Autónoma de Yucatán, Mérida, México, pp. 3–10 (ISSN: 0188-5006).

Hardy, R., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac. Res. 41, 770–776.

Holm, J., Björck, I., Drews, A., Asp, N.G., 1986. A rapid method for the analysis of starch. Starch-Stärke 38, 224–226.

Honorato, C.A., Almeida, L.C., Da Silva Nunes, C., Carneiro, D.J., Moraes, G., 2010. Effects of diets with distinct levels of carbohydrates and lipids: the outcomes on growth of pacu (Piaractus mesopotamicus). Aquacul. Nutr. 19, 91–99.

Hossain, M.S., Koshio, S., 2017. Dietary substitution of fishmeal by alternative protein with guanosine monophosphate supplementation influences growth, digestibility, blood chemistry profile, immunity, and stress resistance of red sea bream, Pagrus major. Fish. Physiol. Biochem. http://dx.doi.org/10.1007/s10695-017-0398-4.

Jirsa, D., Barrows, F.T., Hardy, R.W., Drawbridge, M., 2015. Alternative protein blends as a replacement for fish meal in diets for white seabass, *Atractoscion nobilis*. Aquacul. Nutr. 21, 861–867.

Laemmli, U., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680.

Llopart, E.E., Drago, S.R., De Greef, D.M., Torres, R.L., González, R.J., 2014. Effects of extrusion conditions on physical and nutritional properties of extruded whole grain red sorghum (sorghum spp). Int. J. Food Sci. Nutr. 65, 34–41.

Masson, L., Alfaro, T., Camilo, C., Carvalho, A., Illesca, P., Torres, R., Tavares do Carmo, M., Mancini-Filho, J., Bernal, C., 2015. Fatty acid composition of soybean/sunflower mix oil, fish oil and butterfat applying the AOCS Ce 1j-07 method with a modified temperature program. Grasas y aceites 66 (1), e064. http://dx.doi.org/10.3989/gya.0692141. (66).

Osella, C.A., Gordo, N.A., González, R.J., Tosi, E., Ré, E., 1997. Soybean heat-treated using a fluidized bed. LWT Food Sci. Technol. 30, 676–680.

Pastor-Cavada, E., Drago, S., González, R., Juan, R., Pastor, J., Alaiz, M., Vioque, J., 2011. Effects of the addition of wild legumes (*Lathyrus annuus* and *Lathyrus clymenum*) on the physical and nutritional properties of extruded products based on whole corn and brown rice. Food Chem. 128, 961–967.

Perez, A., Drago, S.R., Carrara, C., De Greef, D.M., Torres, R.L., González, R.J., 2008. Extrusion cooking of a maize-soybean mixture: factors affecting expanded product characteristics and flour dispersion viscosity. J. Food Eng. 87, 333–340.

Rosentrater, K.A., Muthukumarappan, K., Kannadhason, S., 2009. Effects of ingredients and extrusion parameters on aquafeeds containing DDGS and potato starch. J. Aquacult. Feed Sci. Nutr. 1, 22–38.

Sørensen, M., Ljøkjel, K., Storebakken, T., Shearer, K.D., Skrede, A., 2002. Apparent digestibility of protein, amino acids and energy in rainbow trout (*Oncorhynchus mykiss*) fed a fish meal based diet extruded at different temperatures. Aquaculture 211, 215–225.

Sørensen, M., 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquacult. Nutr. 18, 233–248.

Salgado, P., Fernández, G., Drago, R.S., Mauri, A., 2011. Addition of bovine plasma hydrolysates improves the antioxidant properties of soybean and sunflower protein-based films. Food Hydrocolloids 25, 1433–1440.

Samocha, T.M., Allen Davis, D., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by coextruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 231, 197–203.

Tovar, J., Bjoerck, I.M., Asp, N.G., 1990. Starch content and aamylolysis rateinprecooked legume flours. J. Agric. Food Chem. 38, 1818–1823.