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Effects of increasing the dietary lipid levels on the growth performance, body composition and digestive enzyme activities of the teleost pejerrey (*Odontesthes bonariensis*)

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ABSTRACT

The present study aimed at determining the growth performance, body composition and digestive enzyme activities of pejerrey (*Odontesthes bonariensis*) juveniles fed with graded levels of dietary lipids: 6% (L6), 10% (L10) and 25% (L25). Following a 14-week growth trial, weight increase decreased in group L6. The protein productive value increased in groups L10 and L25, whereas group L10 showed the highest lipid productive value. Hepatosomatic and mesenteric fat indexes decreased in group L6. Body protein content decreased with the increase in dietary lipids (L6 > L10 > L25); whereas body lipid content, and nitrogen and energy retentions were the lowest in group L6. The muscle fatty acid composition was differentially regulated by the diets except EPA, DHA and n-3 PUFA contents. The total n-6 PUFA decreased in group L6. The n-3 PUFA tended to accumulate in the muscle of the fish; group L10 exhibiting the lowest accumulation rate. The total activity of neutral lipase was stimulated in group L25 whereas the specific activity of pancreatic lipase decreased in this same group. While group L6 showed the lowest total activity of alkaline proteases, group L10 showed the highest total activity of trypsin. A decrease in the specific activity of amylase in group L25 followed the decrease in dietary starch. In summary, 25% of dietary lipids did not have additional stimulatory effects on the digestive proteases activity compared to 10%, but negatively affected pancreatic lipase and trypsin activities. Moreover, the decrease of the latter in group L25 suggests that approximately 10% of dietary lipids might support the growth of pejerrey juveniles reared in captivity.

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1. Introduction

The pejerrey (*Odontesthes bonarienis*) is an atherinid eurihaline fish which during the last decades has emerged as a potential fish species for the South American aquaculture industry. The reasons for this increase are, among others, high overfishing pressure and the pollution of water bodies (Somoza et al., 2008). In addition to the excellent local market attained as a result of the high quality of its flesh, the pejerrey is also very appreciated by foreign consumers. In this sense, important advances were achieved during last years in relation to the larvae and caged culture that resulted in the current rearing of this fish species at a commercial scale (Colautti et al., 2010; Gómez-Requeni et al., 2012; Hualde et al., 2011; Somoza et al., 2008). However, the aquaculture activity of the pejerrey has not been fully developed yet due to, among others, the slow growth rates showed in captivity (Miranda et al., 2006). This impaired growth is mainly a consequence of the scarce knowledge about the nutritional requirements of larvae and juveniles of this fish species, representing one of the main limitations for its potential rearing at a commercial scale. So far, very few studies have attempted the elucidation of the physiologic and endocrine response of pejerrey larvae and juveniles to shifts in dietary formulations, as well as their influence over somatic growth (Gómez-Requeni et al., 2012; Hualde et al., 2011; Piedras et al., 2004; Toledo-Cuevas et al., 2011).

The aim of the present study was to elucidate the effects of increasing the lipid levels of the pejerrey juveniles feeds on the growth performance, body composition and digestive enzymes activities. This work complements additional studies focused on the response of the GH/IGF system and muscle growth biomarkers in fish subjected to the same dietary treatments (P. Gómez-Requeni and L.F. Canosa, unpublished data). To address these issues, we developed a small-scale experiment in which pejerrey juveniles were fed with formulated isoproteic diets (approx.

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36% crude protein) with increasing levels of dietary lipids (6%, 10% and 25%) during 14 weeks. At the end of this period, the growth performance was evaluated, the body composition of the fish was analyzed and the activities of digestive amylase, alkaline proteases, trypsin, and pancreatic and neutral lipases were assessed.

2. Materials and methods

2.1. Diets

The experimental diets (Table 1) were based on fishmeal and haddock filet as protein sources (crude protein: 36.4–38.2% of dry matter, DM). The lipid composition of the diets was established at 6% DM (diet L6), 10% DM (diet L10) and 25% DM (diet L25). Fish oil was used as lipid source and soy lecithin was added as additional source of phospholipids. The energy content of the experimental diets was 12.02 kJ/g (diet L6), 13.11 kJ/g (diet L10) and 16.85 kJ/g (diet L25).

The fatty acid (FA) composition of the experimental diets is shown in Table 1. On a general basis, when expressed as % DM, all FA increased progressively with the increase of dietary lipid content. The polyunsaturated FA (PUFA) of the n-3 series ranged from 1.91% DM (diet L6) to 6.70% DM (diet L25), whereas the n-6 PUFA ranged from 0.48% DM (diet L6) to 5.17% DM (diet L25). Similarly, the EPA plus DHA content varied from 1.57% DM (diet L6) to 4.95% DM (diet L25), whereas the DHA/EPA ratio remained relatively constant (2.13–2.27%). In relative terms, as % of total FA, the polyenes slightly increased with the increase of the dietary lipid content, with the subsequent decrease of the monoenes fraction. The n-3 and n-6 PUFA were slightly higher in diet L25, whereas the ratio DHA/EPA varied among 1.79% (diet L6), 2.12% (diet L10) and 2.17% (diet L25).

Table 1
Ingredients, proximate and fatty acid compositions of the experimental diets.

	L6	L10	L25
Ingredients (g/100 g)			
Fish meal (59% CP)[a]	38.06	38.06	38.06
Haddock filet[b]	26.17	26.17	26.17
Wheat gluten	4.28	4.28	4.28
Wheat starch	15.00	9.21	0.60
Fish oil[a]	0.10	3.08	7.37
Vitamin and mineral mix[c]	8.32	8.32	8.32
Sodium chloride	3.33	3.33	3.33
Agar–agar	1.33	1.33	1.33
Na-CMC[d]	3.41	3.41	3.41
Soy lecithin	0.00	3.08	7.13
Proximate composition (dry matter, %)			
Dry matter	87.96	89.60	91.82
Protein	36.53	36.38	38.18
Fat	6.13	10.20	24.79
Carbohydrates	21.95	18.53	6.99
Ash	23.35	24.49	21.86
Energy (kJ/g)	12.02	13.11	16.85
Fatty acid composition (dry matter, %)			
EPA + DHA	1.57	1.91	4.95
DHA/EPA	2.27	2.13	2.17
Σ Saturates[e]	1.46	2.28	4.98
Σ Monoenes[f]	2.24	3.38	7.73
Σ Polyenes[g]	2.39	4.37	11.87
Σ n-3 PUFA	1.91	2.57	6.70
Σ n-6 PUFA	0.48	1.80	5.17
Fatty acid composition (g/100 g total FAME[h])			
DHA/EPA	1.79	2.12	2.71
Σ Saturates	21.77	22.54	20.13
Σ Monoenes	34.59	33.42	31.26
Σ Polyenes	42.88	43.20	47.95
Σ n-3 PUFA	25.24	25.42	27.06
Σ n-6 PUFA	17.64	17.78	20.89

[a] Moliendas del Sur S.A., Mar del Plata, Argentina. Fish meal composition: water 8%, protein 59%, lipid 8%, ash 25%.
[b] Composition: water 75.4%, protein 15.9%, lipid 1.4%, ash 1.2%.
[c] Provided per kg of feed: retinol, 200,000 I.U.; vitamin D_3, 40,000 I.U.; tocopherol, 4150 mg; vitamin K_3, 130 mg; thiamine, 165 mg; riboflavin, 330 mg; pyridoxine-HCl, 250 mg; vitamin B_{12}, 0.5 mg; ascorbic acid, 2500 mg; niacin, 2500 mg; calcium pantothenate, 660 mg; folic acid, 100 mg; biotin. 16.5 mg; zinc, 1150 mg; iron, 1650 mg; manganese, 800 mg; copper, 83.0 mg; cobalt, 33 mg; iodine, 28.5 mg; selenium, 5 mg.
[d] Sodium carboxymethyl cellulose.
[e] Includes 14:0, 16:0, 17:0 and 18:0.
[f] Includes 16:1, 18:1, 20:1, 22:1, 24:1.
[g] Includes 18:2 n-3, 18:4 n-3, 20:4 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3, 18:2 n-6, 20:4 n-6.
[h] Fatty acid methyl esters.

2.2. Growth trial

Juveniles of pejerrey (*O. bonariensis*) reared from eggs obtained from natural spawning at the indoor facilities of IIB-INTECH were randomly distributed into experimental tanks of 300 L capacity and acclimated to experimental rearing conditions for 20 days. Water flow was 20 L/min, and oxygen content of outlet water was always higher than 85% saturation. Day length and temperature followed the natural changes at our latitude (35°34′13″S; 58°0′42″O). Water temperature ranged from 16 to 19 °C.

Each diet was distributed to triplicate groups of fish for 98 days (14 weeks) according to a random design. Feed was offered to apparent visual satiety in 3 meals per day (10:00 h, 13:00 h, 16:00 h), and feed consumption was recorded daily.

Whole body and muscle fatty acid composition were determined in pooled samples of 10 fish at the beginning and 10 fish per diet at the end of the growth trial. Additional sampling of muscle, hepatopancreas (Toledo-Cuevas et al., 2011) and gut tissue was done at the end of the growth trial from randomly selected fish killed by scission of the spinal cord. Following overnight fasting, tissue samples of 4 fish per tank (12 animals per diet) were excised and freeze-dried and enzyme activities for amylase, alkaline proteases, trypsin, and pancreatic and neutral lipases were determined as indicated below.

2.3. Food and body composition analysis

Feed and specimens for body analysis were ground, and samples were weighed for protein, fat, moisture and ash analytical determination. Additionally, muscle samples were prepared for FA determination. Analyses were made following laboratory procedures based on published official methods (AOAC, 1990). Moisture was measured gravimetrically after drying in an oven at 105 °C for 16 h, and ash by combustion in a muffle at 550 °C for 16 h. Total nitrogen (N) was determined using Kjeldahl method and crude protein estimated as 6.25 × N. Fat was measured gravimetrically following 5 h extraction of dried samples in a mixture of equal volumes of sulfuric ether and petroleum ether, using a Twisselman apparatus. Fatty acid methyl esters (FAME) from muscle samples were prepared from aliquots of total lipid extracted by the Folch method (Folch et al., 1957) and quantified by gas chromatography with FID detector. Carbohydrates were calculated by the equation $C = 100 - F - P - W - A$; where C, F, P, W and A correspond respectively to carbohydrates, fat, protein, water and ash. Energy in kJ was calculated from the following equation: $\text{kJ}/100\ \text{g} = 37 \times F + 17 \times P + 17 \times C$; where F corresponds to fat, P corresponds to protein and C corresponds to carbohydrates.

2.4. Digestive enzyme activities

Enzymatic activities were determined on digestive organs (hepatopancreas and intestine) for each individual. Single tissues were homogenized in 350–460 μl distilled water and sonicated in ice bath until total tissue disintegration (Ultrasonic Dismembrator, Fisher Scientific, USA). The homogenates were then centrifuged for 20 min at 16900 × *g* at 4 °C and the extracts used for the analysis. Fluorometric analyses were performed to determine the digestive activities except for neutral lipase,

which was assayed as shown by Iijima et al. (1998). Trypsin and pancreatic lipase were measured according to Rotllant et al. (2008), modified by Toledo-Cuevas et al. (2011). Total alkaline proteases were performed based on Twining (1984) and amylase activity was recorded by the EnzChek Ultra AmylaseAssay kit (E33651 Molecular Probes, Invitrogen). The fluorometric measurements were carried out using a Fluoroskan Ascent equipment (Thermo Fisher Scientific, Vantaa, Finland). The activities were reported as total activity [Fluorescence Units (FU) per segment (FU segment^{-1})] and specific activity (FU mg protein^{-1}). For neutral lipase, 1 U of enzyme activity was defined as 1 mmol of hydrolyzed substrate released per min. Each assay was performed in duplicate. Protein concentration was measured in homogenates as described by Bradford (1976).

2.5. Statistics

Growth parameters (tank average values) and the relative amount of FA were checked for homogeneity and homoscedasticity. Data that failed to pass homogeneity were transformed and re-tested. Data were analyzed by one-way analysis of variance, followed by the Newman–Keuls' *post-hoc* test when significant differences were found. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Growth performance

A 4- to 6-fold increase in body weight was found over the course of the 14-week growth trial with juveniles of pejerrey (Table 2). Fish grew from 0.8–0.9 g to 3.89 ± 0.24 g (L6), 5.23 ± 0.37 (L10) and 5.41 ± 0.05 g (L25; mean ± S.E.M.). The final body weight was significantly lower ($P = 0.044$) in fish fed with diet L6, and the weight increase followed the same trend ($P = 0.040$). The weight gain was slightly lower also in fish fed with a 6% of dietary lipids but the differences were not statistically significant ($P = 0.161$). Similarly, feed efficiency was slightly but not significantly higher ($P = 0.084$) in fish fed with diets L10 and L25 than in those fed with diet L6, whereas feed intake slightly decreased ($P = 0.242$). The protein productive value (PPV) was significantly higher ($P = 0.036$) in fish fed with diets L10 and L25, whereas fish fed diet L10 showed the highest lipid productive value (LPV; $P = 0.003$). The liver and mesenteric fat weight, and the hepatosomatic (HSI) and mesenteric fat indexes (MFI) were significantly lower in fish fed with diet L6 ($P < 0.001$). The survival rate was affected by the dietary treatment; fish from group L25 showing higher survival rates ($P = 0.040$) than fish from group L6.

The progressive increase in dietary lipids was reflected in the whole body composition of the fish (Table 2). Thus, a progressive and significant decrease in water and protein content (diet L6 > diet L10 > diet L25) accompanied the progressive increase in dietary lipids ($P < 0.001$). Conversely, fish fed with diet L6 showed the lowest body lipid content and lipid gain ($P < 0.001$), and nitrogen and energy retentions ($P = 0.036$ and $P = 0.012$, respectively). The lipid content and FA profile of the skeletal muscle was also affected by the dietary treatment (Table 3). Thus, the muscle lipid content was significantly higher ($P = 0.041$) in fish fed with diet L25. The monoenes 17:1, 20:1, 22:1 and 24:1 significantly decreased ($P < 0.05$) with the increase of dietary lipids; group L6 showing higher levels than L10 and L25. Conversely, polyenes 18:2 n-6, 18:3 n-3, and 18:4 n-3 were significantly higher ($P \leq 0.001$) in fish fed with diets L10 and L25; and 20:2 n-6 increased significantly ($P < 0.001$) in group L25. The content of EPA and DHA and the total n-3 PUFA were

Table 2
Data on growth performance, whole body composition, and nutrient gain and retention of fish fed the experimental diets for 14 weeks.

	L6	L10	L25	P[1]
Initial body weight (g)	0.95 ± 0.04	0.87 ± 0.02	0.90 ± 0.10	0.727
Final body weight (g)	3.89 ± 0.24[a]	5.23 ± 0.37[b]	5.41 ± 0.05[b]	0.044
Survival (%)	65.9 ± 3.50[a]	73.6 ± 1.75[ab]	83.6 ± 2.35[b]	0.040
Liver (g)	0.11 ± 0.01[a]	0.22 ± 0.02[b]	0.22 ± 0.01[b]	<0.001
Mesenteric fat (g)	0.13 ± 0.02[a]	0.35 ± 0.04[b]	0.31 ± 0.03[b]	<0.001
HSI (%)[2]	1.94 ± 0.10[a]	2.73 ± 0.18[b]	3.30 ± 0.13[c]	<0.001
MFI (%)[3]	1.98 ± 0.24[a]	3.92 ± 0.30[b]	4.48 ± 0.28[b]	<0.001
Feed intake (% ABW/day)	2.00 ± 0.10	1.79 ± 0.01	1.79 ± 0.09	0.242
Weight increase (g)	196.3 ± 23.9[a]	291.5 ± 24.2[b]	323.6 ± 1.82[b]	0.040
Weight gain (%)	312.4 ± 41.7	500.8 ± 59.6	506.1 ± 70.3	0.161
Feed efficiency[4]	0.56 ± 0.07	0.75 ± 0.03	0.77 ± 0.01	0.084
PPV[5](%)	19.2 ± 0.69[a]	29.8 ± 2.78[b]	28.8 ± 0.56[b]	0.036
LPV[6](%)	29.6 ± 1.15[a]	61.9 ± 3.81[b]	25.9 ± 0.34[a]	0.003
Whole body composition (% wet matter)				
Moisture	76.6 ± 0.15[c]	73.3 ± 0.02[b]	70.5 ± 0.01[a]	<0.001
Crude protein	17.5 ± 0.01[c]	16.7 ± 0.01[b]	15.4 ± 0.07[a]	<0.001
Crude lipid	3.55 ± 0.06[a]	7.89 ± 0.01[c]	7.39 ± 0.15[b]	<0.001
Ash	1.96 ± 0.01[b]	1.85 ± 0.02[a]	2.33 ± 0.03[c]	<0.001
Gain [mg(g)/kg ABW/day]				
Nitrogen (mg)	269.0 ± 10.3	340.1 ± 20.1	326.2 ± 10.7	0.077
Lipid (g)	0.43 ± 0.02[a]	1.24 ± 0.03[b]	1.19 ± 0.05[b]	<0.001
Retention (% intake)				
Nitrogen	19.2 ± 0.69[a]	29.8 ± 2.78[b]	28.8 ± 0.56[b]	0.036
Energy	19.6 ± 0.79[a]	37.5 ± 2.95[b]	33.4 ± 0.69[b]	0.012

Each value is the mean ± S.E.M. of triplicate groups. Data on liver and mesenteric fat indexes were calculated from 12–24 fish.
Initial body composition: water 80.3%, protein 16.5%, lipid 1.6%, ash 2.6%.
[1] *P* values result from analysis of variance. Different superscript letters in each row indicate significant differences among dietary treatments (Newman–Keuls' test, $P < 0.05$).
[2] Hepatosomatic index = (100 × liver wt.) / fish wt.
[3] Mesenteric fat index = (100 × mesenteric fat wt.) / fish wt.
[4] Feed efficiency = wet weight gain / dry feed intake.
[5] Protein productive value = [(final body N − initial body N) / N intake] × 100.
[6] Lipid productive value = [(final body lipids − initial body lipids) / lipid intake] × 100.

Table 3
Muscle lipid and fatty acids composition of fish fed with the experimental diets for 14 weeks.

	L6	L10	L25	P[1]
Lipids (% DM)	1.95 ± 0.11[a]	2.03 ± 0.12[a]	2.51 ± 0.14[b]	0.041
Fatty acids (% FAME)				
14:0	1.03 ± 0.06	0.98 ± 0.06	1.20 ± 0.07	0.109
16:0	13.85 ± 0.80	12.79 ± 0.74	11.97 ± 0.70	0.279
16:1	2.56 ± 0.15	2.95 ± 0.17	2.79 ± 0.16	0.307
17:0	0.62 ± 0.03	0.54 ± 0.03	0.52 ± 0.02	0.101
17:1	1.03 ± 0.06[b]	0.49 ± 0.03[a]	0.40 ± 0.02[a]	<0.001
18:0	4.10 ± 0.24[b]	3.44 ± 0.20[b]	2.39 ± 0.14[a]	0.002
18:1	18.47 ± 1.09	18.20 ± 1.06	14.96 ± 0.86	0.088
18:2 n-6	5.13 ± 0.30[a]	10.82 ± 0.63[b]	11.57 ± 0.67[b]	<0.001
18:3 n-6	0.67 ± 0.03	0.69 ± 0.03	0.60 ± 0.02	0.127
18:3 n-3	0.51 ± 0.03[a]	1.48 ± 0.09[b]	1.60 ± 0.09[b]	<0.001
18:4 n-3	0.51 ± 0.03[a]	0.98 ± 0.06[c]	0.80 ± 0.05[b]	0.001
20:1	3.08 ± 0.18[b]	2.46 ± 0.14[a]	1.99 ± 0.12[a]	0.006
20:2 n-6	0.51 ± 0.03[a]	0.49 ± 0.03[a]	1.99 ± 0.12[b]	<0.001
20:4 n-6	1.63 ± 0.09	1.67 ± 0.09	1.44 ± 0.07	0.168
20:4 n-3	0.51 ± 0.03	0.54 ± 0.03	0.48 ± 0.02	0.335
20:5 n-3	2.56 ± 0.15	2.95 ± 0.17	2.39 ± 0.14	0.101
22:1	1.54 ± 0.09[b]	0.98 ± 0.06[a]	0.80 ± 0.05[a]	<0.001
22:5 n-6	1.13 ± 0.06	1.13 ± 0.06	1.00 ± 0.05	0.222
22:5 n-3	2.56 ± 0.15	2.46 ± 0.14	2.79 ± 0.16	0.347
22:6 n-3	36.94 ± 2.13	33.45 ± 1.94	37.51 ± 2.19	0.390
24:1	1.03 ± 0.06[c]	0.49 ± 0.03[a]	0.80 ± 0.05[b]	<0.001
EPA + DHA	39.51 ± 2.28	36.40 ± 2.11	39.90 ± 2.33	0.519
DHA/EPA	14.40 ± 0.83[b]	11.33 ± 0.65[a]	15.67 ± 0.90[b]	0.022
Saturates	19.60 ± 1.12	17.76 ± 1.03	16.08 ± 0.93	0.131
Unsaturates	80.40 ± 4.65	82.24 ± 4.75	83.92 ± 4.84	0.874
Monoenes	27.71 ± 1.63	25.58 ± 1.49	21.75 ± 1.26	0.071
Polyenes	52.69 ± 3.02	56.66 ± 3.26	62.17 ± 3.58	0.205
n-3 PUFA	43.61 ± 2.52	41.86 ± 2.43	45.57 ± 2.65	0.610
n-6 PUFA	9.08 ± 0.50[a]	14.81 ± 0.83[b]	16.60 ± 0.93[b]	0.001

Each value represents the mean ± S.E.M. of data from triplicate groups.
[1] *P* values result from analysis of variance. Different superscript letters in each row indicate significant differences among dietary treatments (Newman–Keuls' test, $P < 0.05$).

not significantly affected by the dietary treatment ($P > 0.05$). However, the total n-6 PUFA were significantly lower ($P = 0.001$) in fish fed with diet L6.

The differences (Δ) between muscle and dietary FA concentrations are shown in Fig. 1, where negative Δ values indicate lower values in muscle compared with the diet and positive values indicate accumulation relative to the diet. The utilization of saturated FA (especially 16:0 and 18:0) and monoenes (especially 20:1, 22:1 and 24:1) increased to a higher extent parallel with the increase of dietary lipids (Fig. 1A, B and E). Actually, the saturate 18:0 and the monoene 24:1 were mostly accumulated in the muscle of fish fed diet L6, whereas in the other groups these FA were utilized. On the other hand, the monoene 16:1 and the n-6 PUFA (Fig. 1C), especially the linoleic acid (LA, 18:2 n-6), showed higher negative values (utilization) in fish fed diet L6. Polyenes, especially the n-3 PUFA (Fig. 1D and E), tended to accumulate in the muscle of the fish, and this accumulation apparently increased parallel with the increase of dietary lipids. The arachidonic acid (AA, 20:4 n-6), the docosapentaenoic acid (DPA, 22:5 n-3) and DHA were selectively accumulated in the muscle of fish fed the 3 experimental diets. The DHA content, the sum of EPA plus DHA, and the total n-3 PUFA were not apparently modified by the dietary treatment, although fish from group L10 exhibited the lowest accumulation rate. By contrast, EPA Δ value indicates that this FA was utilized at slightly higher rates in fish fed with diet L6.

3.2. Digestive enzyme activities

The increase in dietary lipid level produced a different response in the analyzed digestive enzyme activities (Figs. 2 and 3). The activity of neutral lipase was stimulated in fish fed with diet L25, but this increase ($P = 0.015$) was only detected in the total activity per segment (or segmental activity; Fig. 2A). Segmental activity is thus defined as the total activity per digestive tract (hepatopancreas and gut) or segment, and it reflects the digestive capability of an organism. In addition, a significant decrease ($P = 0.011$) was measured in the specific activity of pancreatic lipase in group L25 (Fig. 3B), although segmental activities did not change significantly ($P = 0.124$; Fig. 2B). The increase in dietary lipid levels also produced an increase in the proteolytic capability of the fish (Fig. 2C and D). Thus, whereas the total activity of alkaline proteases was not significantly different between groups L10 and L25 (Fig. 2C), the maximum activity for trypsin corresponded to group L10 ($P = 0.004$; Fig. 2D). No significant differences were detected in the specific activities for both enzymes ($P > 0.05$; Fig. 3C and D), although the specific activity of trypsin (Fig. 3D) followed a similar trend to its segmental activity (Fig. 2D). The decrease in dietary starch (to maintain the energy levels) as a consequence of the increase in dietary lipids was followed by a parallel and significant decrease ($P = 0.007$) in the specific activity of amylase in fish fed with diet L25 (Fig. 3E). This decrease was clearly related to the significant decrease in the secretion of this enzyme [55.6 ± 6.16^{b} (L6), 43.3 ± 3.20^{b} (L10), and 28.5 ± 4.22^{a} (L25); $P = 0.001$]. The secretion rates of all the other analyzed digestive enzymes were not significantly affected by the dietary treatments ($P > 0.05$; data not shown).

4. Discussion

4.1. Growth performance and body composition

The present study was developed to improve our knowledge on the effects of shifts in the dietary composition on the growth performance and physiology of pejerrey (*O. bonariensis*) juveniles, which is currently scarce. A few technical reports, preliminary studies and scientific manuscripts have been published during the last decades exploring the feasibility of pejerrey culture in semi-intensive and/or extensive conditions in lagoons, supplementing the natural feeding resources with non-specific artificial diets (Berasain et al., 2000; Colautti et al., 2010; Freyre et al., 2009; Hualde et al., 2011). However, to our knowledge, this is the first study attempting to determine the effects of shifts in the lipid content of the diets for pejerrey juveniles reared in captivity. Our results show better growth performance and survival in fish fed with diets L10 and L25. Thus, fish showed higher body weights but also slightly lower feed intake and improved feed and protein efficiencies, reflecting the need of fish fed with diet L6 to increase feeding rates to mitigate a nutrient deficiency and/or imbalance. Moreover, although the difference in the lipid content and dietary energy between diets L6 and L10 was relatively low, most of the differences found in terms of growth performance between fish fed both diets were significant. These findings suggest that fish fed with diet L6 probably exhibited a serious shortage of energy and/or essential FA, although the high inclusion of dietary fish meal and haddock filet supports the notion

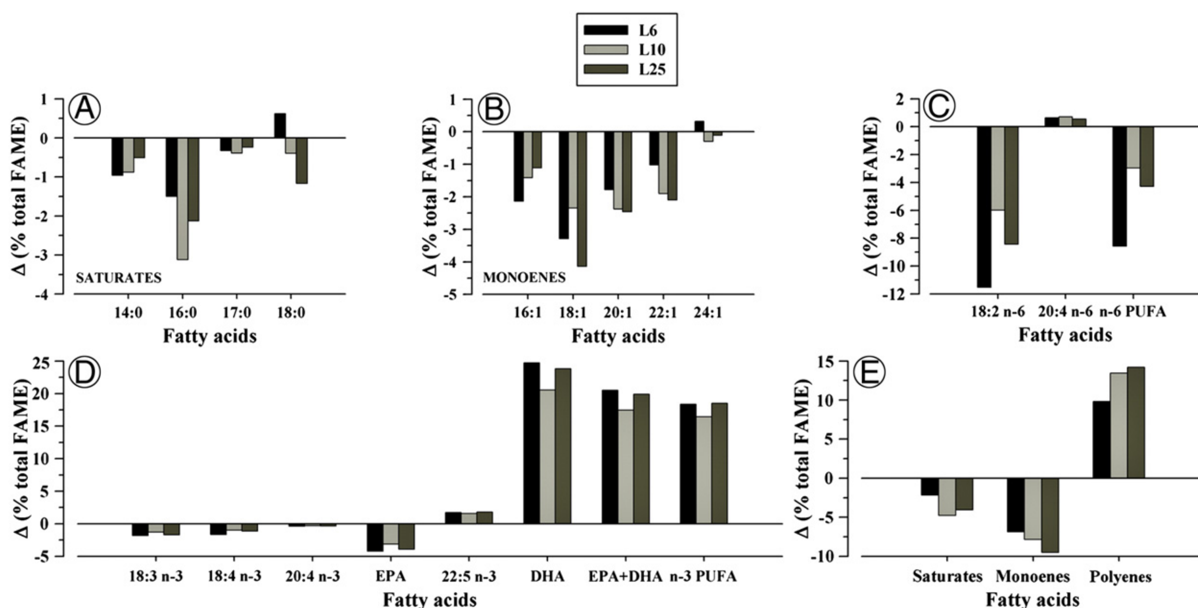

Fig. 1. Differences (Δ)* between dietary and muscle concentrations for individual (A) saturates, (B) monoenes, (C) ω-6 FA, and (D) ω-3 FA; and (E) the sum of the different FA classes (g/100 g total FAME) in fish fed the 3 experimental treatments. *Negative Δ values indicate lower values in muscle compared with the diet, whereas positive values indicate accumulation in muscle relative to the diet.

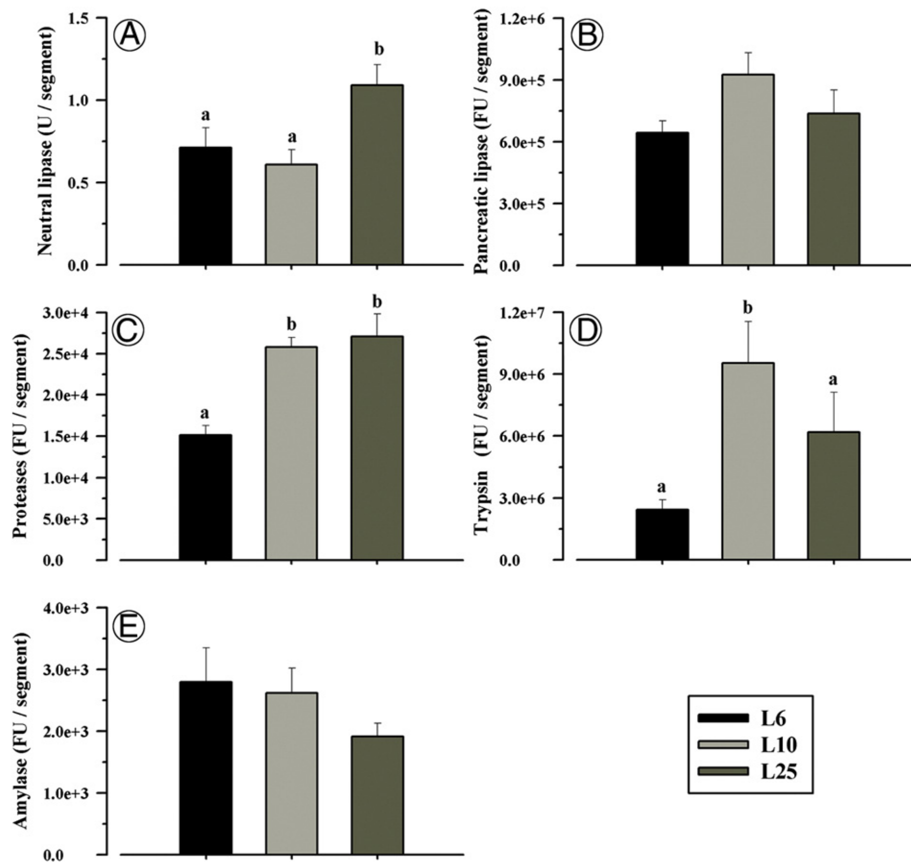

Fig. 2. Segmental activity of (A) neutral lipase, (B) pancreatic lipase, (C) alkaline proteases, (D) trypsin and (E) amylase of fish fed the 3 experimental diets. Each value is the mean ± S.E.M. of 12 animals. Values with different letters indicate significant differences among dietary treatments (Newman–Keuls' test, $P < 0.05$).

that the EPA and DHA requirements were theoretically met (Turchini et al., 2009). However, we cannot discard the possibility of a protein sparing effect event in fish fed with diet L10; allowing the slightly higher inclusion of dietary lipids in comparison to diet L6 the use of amino acids for growth instead of for energy purposes. In this sense, as weight gain also involves fat deposition and is not only the result of protein retention, it may not always be considered an accurate predictor of true growth. Therefore, the higher protein productive value (PPV) and higher nitrogen gain and retention reflected the better protein utilization for growth purposes in fish fed with diet L10. These results would agree with the common strategy of sparing dietary protein by reducing the dietary protein levels while increasing the content of dietary lipids or carbohydrates (Chatzifotis et al., 2010; Karalazos et al., 2011; Skalli et al., 2004). Moreover, our results also show that pejerrey fed with diet L6 were not capable of handling carbohydrates as effectively as lipids for energy purposes and, hence, it seems that fish needed to promote the catabolism of dietary proteins to meet their energy demands. Also, the lack of soy lecithin in diet L6 as an additional source of phospholipids might be disturbing the nutrient assimilation processes. In this sense, the rise in dietary lipids and also the incorporation of phospholipids to diets L10 and L25 had a positive effect on the growth and survival of the fish. Actually, previous studies have addressed that phospholipids show a markedly positive effect on lipid absorption and transport (Tocher et al., 2008), improving consequently growth and survival (Gisbert et al., 2005; Morais et al., 2006). The increase of dietary lipids in a higher amount than 10% did not apparently lead to better results in terms of growth performance, suggesting that dietary lipid contents around 10% are enough to provide essential FA and lipid-derived energy to pejerrey juveniles fed with a 36–38% of dietary protein at the body size assayed in this work. Actually, the low lipid productive value (LPV) as indicator of poor lipid retention showed by fish fed with diet L25 follows the same trend of another lean fish, the Senegale sole, when fed with dietary lipid levels higher than 12% (Borges et al., 2009). Furthermore, the increase of dietary energy between diets L10 and L25 did not affect the feed intake of the fish corroborating the hypothesis stating that fish seem to adjust their feed intake to protein rather than energy intake (Chatzifotis et al., 2010; Martí-Palanca et al., 1996; Peres and Oliva-Teles, 1999).

It is widely accepted that tissue total FA composition reflects the dietary FA content (Bell et al., 2002; Díaz-López et al., 2010; Figueiredo-Silva et al., 2010; Izquierdo et al., 2005; Mourente and Bell, 2006). In our study, we attempted to maintain a relatively constant proportion of the different classes of FA among diets. Thus, the increase of dietary lipid diets was achieved by increasing the amount of fish oil and soy lecithin in a 1:1 ratio. Even though, a slight decrease of total monoenes (including 16:1, 18:1 and 20:1) and a slight increase of total polyenes (including 18:2 n-6, 18:3 n-3, 22:5 n-3 and 22:6 n-3) were found with the progressive increase of dietary lipids. Also, the addition of soy lecithin (especially rich in 18:2 n-6) contributed to the progressive decrease of the n-3/n-6 ratio with the increase of dietary lipids, although this ratio was not substantially modified among diets when expressed in relative terms (as % of total FA). Thus, the increase of the dietary lipid content was reflected in the higher percentage of lipids found in the skeletal muscle of fish fed with diet L25, despite the higher whole body lipid content was attained in fish fed with diet L10. This suggests that in fish fed with diet L10 the lipid depots were probably accumulated in peripheral tissues other than the skeletal muscle, as it is reflected in the high HSI and MFI values. Actually, the muscle lipid content reported here classifies pejerrey as a low fat fish, in which the ability to deposit lipids in muscle is restricted (Chatzifotis et al., 2006, 2010; Peres and Oliva-Teles, 1999). Similarly, the whole body lipid levels of pejerrey reinforces that this is a species with very low lipid content, in contraposition to other fish species with body lipid levels ranging from 9% to 20% (Gómez-Requeni et al., 2004; Martins et al., 2007; Peres and Oliva-

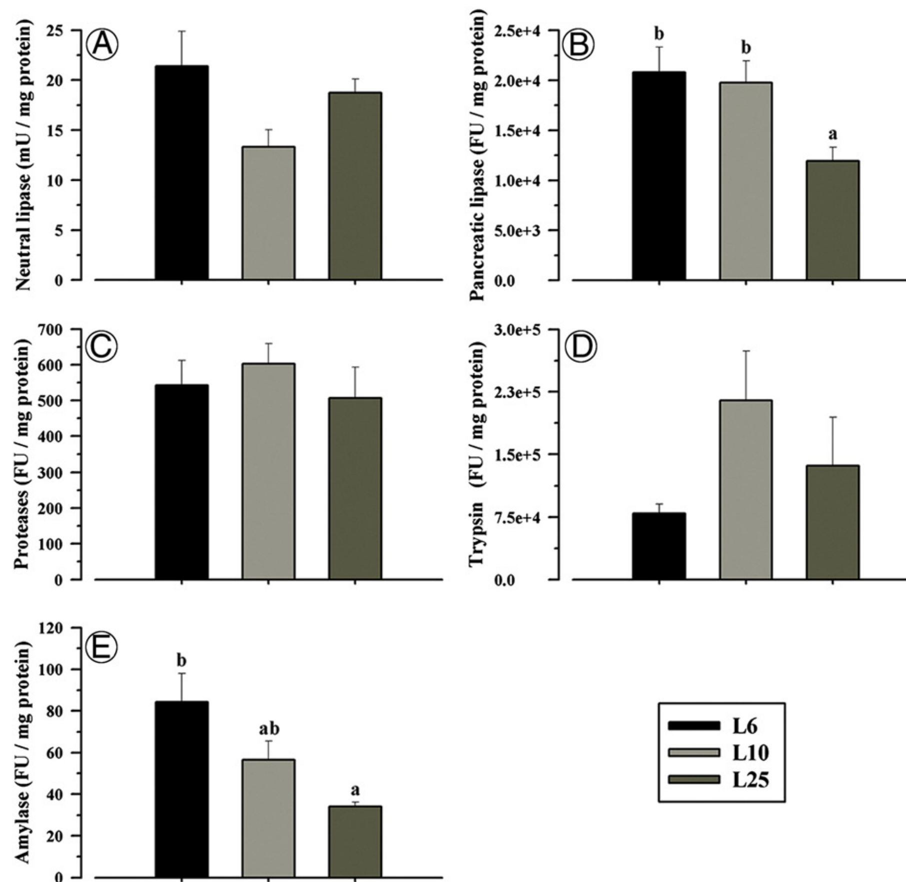

Fig. 3. Specific activity of (A) neutral lipase, (B) pancreatic lipase, (C) alkaline proteases, (D) trypsin and (E) amylase of fish fed the 3 experimental diets. Each value is the mean ± S.E.M. of 12 animals. Values with different letters indicate significant differences among dietary treatments (Newman–Keuls' test, $P < 0.05$).

Teles, 1999; Sá et al., 2006; Wang et al., 2005). In the muscle tissue of pejerrey, monoene composition reflected that of the diets, with decreasing values of 17:1, 20:1, 22:1, 24:1 and, to a lesser extent, 18:1 in diets L10 and L25. Moreover, in the skeletal muscle Δ values for 20:1, 22:1 and 24:1 were found in fish fed diets L10 and L25, suggesting that these fish selectively utilized the mentioned monoenes for energy purposes. According to Karalazos et al. (2011), FA are highly utilized in tissues when they are provided at high concentrations (Δ reflecting negative values) and, conversely, they are accumulated in the tissues when the dietary supply is reduced. This is especially relevant in the case of the essential EPA and DHA. In our study, the dietary content of both EPA and DHA progressively increased with the increase of dietary lipids, and the sum of EPA plus DHA ranged from 1.57% to 4.95% DM (diets L6 and L25, respectively). In this context, although Takeuchi and Watanabe (1979) reported poor growth and feed utilization in rainbow trout fed with EPA plus DHA levels exceeding 4 times the requirements of this fish species, the growth performance of pejerrey fed with diet L25 was apparently not affected by the high EPA plus DHA content. In any case, the relative content of both EPA and DHA within the FA pool of the experimental diets was not severely modified and the DHA/EPA ratio was kept constant between 2.13 and 2.27%. As expected, it seems that the increase of lipid levels in pejerrey diets promotes the preferable use of saturates and monoenes to meet the energy demands; and this preferential use was accompanied by a progressive selective accumulation of polyenes (especially 20:4 n-6, 22:5 n-3 and DHA) in muscle tissue. This is in accordance with previous studies that showed that specific FA such as 16:0, 18:1, 20:1, and 22:1 are readily catabolized, although 18:3 n-3, 18:2 n-6 and even EPA and DHA are also good substrates for β-oxidation when provided at high levels (Díaz-López et al., 2010; Stubhaug et al., 2005a, 2005b; Turchini et al., 2011). In our study, muscle EPA and DHA were not differentially affected by the dietary treatment although DHA was selectively accumulated in muscle tissue regardless the diet. This is in concordance with a recent report developed on *Chirostoma estor*, another teleost belonging to the Atherinopsidae family, which showed that EPA is mainly catabolized in comparison to DHA (Fonseca-Madrigal et al., 2012); implying a higher biological value of DHA during the on-growing phase of pejerrey. Interestingly, fish fed with diet L6 apparently exhibited a high utilization of 18:2 n-6, which contributed to the significant decrease of the muscle total n-6 FA pool in fish fed this diet. Conversely, fish fed with diets L10 and L25 apparently used saturates and monoenes for the same purpose. In diet L6, the content of 18:2 n-6 represented almost a 17% of the dietary FA pool (data not shown) and it seems plausible that fish fed this diet used 18:2 n-6 as a preferential substrate for β-oxidation. In this context, it is worthy to mention that we have recently cloned a partial sequence of a pejerrey Δ6 desaturase (P. Gómez-Requeni and L.F. Canosa, unpublished data) although the functionality of this enzyme and the capacity of pejerrey to undergo FA bioconversion activities (*e.g.*, desaturation of 18:2 n-6 to 18:3 n-6) have not already been established. However, in the Atherinopsidae *C. estor* this activity has recently been demonstrated (Fonseca-Madrigal et al., 2012). Similarly to diet L6, 18:2 n-6 was the most represented FA in diet L25 (20%, data not shown). Fish fed with this diet showed the lowest LPV values (a measure of the efficiency of the fish to retain dietary lipids) in concordance with a recent study in which Murray cod fed with a diet based on sunflower oil especially rich in 18:2 n-6 showed a reduced lipid efficiency during the grow-out period (Turchini et al., 2011). The positive Δ values of specific saturates and monoenes found in fish fed with diet L6 suggest a lipogenic activity that maybe reflects an attempt to generate lipid depots from the carbohydrates obtained through the diet (Dias et al., 1998). Therefore, in the present experiment it might be plausible that as a consequence of the very low dietary lipid content both lipogenesis and the β-oxidation for

energy production of selective n-6 FA might have occurred in fish fed with diet L6. However, the reasons for the preferential use of specific FA with the increase of dietary lipids and for the apparent incapacity shown by pejerrey to obtain energy from dietary carbohydrates remain unclear.

4.2. Digestive enzyme activities

Growth is a complex phenomenon that partly relies on the digestive capabilities of an organism. Several studies on fish have suggested that the activity of the main digestive enzymes and their response to different diet compositions are parameters that probably determine how effective a given diet may be in optimizing growth and food utilization (Pérez-Jiménez et al., 2009). The digestion of dietary lipids in fish seems to be accomplished by two different kinds of lipases: a pancreatic and a non-specific, also known as bile salt activated or neutral lipase. The pancreatic lipase with high substrate specificity for triacylglycerols made up of short- and low-saturated FA may not be important in fish. On the other hand, the neutral lipase shows higher activity on triacylglycerols containing PUFAs, which are abundant in the marine food chain, but also in other ester bond lipidic substrates such as phospholipids, cholesterol, vitamins, wax esters and ceramides. Therefore, the neutral lipase has been proposed as the principal lipase in most marine fish species (Iijima et al., 1998; Izquierdo et al., 2000; Kurtovic et al., 2009). In the present work, the increase in dietary lipids resulted in higher segmental activity of neutral lipase, especially in juveniles fed with diet L25, probably in response to the increase in dietary substrates, as fish oils are characterized by the high levels of PUFA. Also, it is possible that the high content of phospholipids in diet L25 may allow a better use of the dietary PUFA, as in some fish species the source of dietary lipids and not only the level significantly affected lipase activities (Morais et al., 2004a, 2007). On the other hand, a significant decrease in the specific activity of the pancreatic lipase occurred in fish fed diet L25, which might be related to the rise of neutral lipase as the major lipolytic enzyme. Alternatively, the decrease in the pancreatic lipase activity might represent an adaptive response to a highly digestible diet containing a significant amount of phospholipids (Morais et al., 2004b). However, we cannot discard the possibilities that an excess of substrate, the long-chain monoenes and saturates, or the increase in HUFA neutral lipids or both might be inhibiting the pancreatic lipase (Iijima et al., 1998; Izquierdo et al., 2000; Morais et al., 2007; Olsen et al., 1998). Nevertheless, the stimulation and decrease in neutral and pancreatic lipase activities, respectively, in fish fed with diet L25 were not a consequence of a differential secretion rate of these enzymes, as secretion was not affected by the dietary treatment.

A rise in alkaline proteases and trypsin activities was observed in fish fed with increasing dietary lipid levels although in other fish species the increase in the secretion of these enzymes is more common than the increase in the activities (Buchet et al., 2000; Mohanta et al., 2008; Zambonino-Infante and Cahu, 1999). In spite of the fact that dietary lipids represent an important stimulus for the release of cholecystokinin (CCK), a potent regulatory hormone of pancreatic secretion, the increase in the activities of alkaline proteases and trypsin were not related to a differential secretion rate due to the fact that this was maximal for all dietary treatments. Even though the dietary protein level was appropriated for a carnivorous species such as pejerrey, it is possible that the lack of a stomach (M.E. Toledo-Cuevas, unpublished data) potentially reduced the ability to digest complex dietary proteins (Rønnestad et al., 2007). In this context, stomachless fish as in the case of marine larvae lacking a functional stomach may require more highly digestible dietary proteins than those existing on live food (Carvalho et al., 2004; Tonheim et al., 2007). Then, it is plausible that both dietary lipids and the stomachless condition might have promoted a CCK-mediated stimulation of pancreatic fluid secretions, improving therefore the digestion conditions for proteases and trypsin.

The increase in the dietary lipid content was counteracted by a subsequent reduction in dietary carbohydrates, in the form of wheat starch. As a consequence, amylase activity decreased progressively and significantly with the decrease in dietary carbohydrates. The adaptation of amylase activity to the dietary starch content has been extensively reported in mammals and marine fish (Fernández et al., 2001; Hidalgo et alet al., 1999; Pérez-Jiménez et al., 2009). A previous study developed on fish larvae showed that changes in amylase activity resulted from a differential transcriptional regulation, although a hormonal-driven translational regulation of amylase synthesis was also suggested (Péres et al., 1998). Both mechanisms would potentially lead to the decrease in the secretion and activity of pancreatic amylase found in the present study.

In summary, a dietary lipid content of 25% does not have an additional stimulatory effect on the digestive protease activity of pejerrey, but does negatively affect trypsin and pancreatic lipase activities. The latter might be a response to the increase in neutral lipase substrates that are not abundant in the pampean lakes, the natural habitats of pejerrey. Moreover, as trypsin plays a key role on the activation of all other pancreatic zymogens and it is suggested to influence growth rates and feed efficiency in some fish species (Lemieux et al., 1999), the decrease in the activity of trypsin in group L25 suggests that an approximate content of 10% of dietary lipids might be enough to support the growth of pejerrey juveniles in captivity. The results obtained on growth performance and endocrine growth biomarkers (P. Gómez-Requeni and L.F. Canosa, unpublished data) strongly support this conclusion. In this scenario, current research in our group is on-going in order to improve and optimize the dietary formulations for pejerrey in addition to introducing alternative ingredients to fishmeal and fish oils, hence promoting a sustainable culture of this fish species.

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