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Dietary protein:lipid ratio modulates somatic growth and expression of genes involved in somatic growth, lipid metabolism and food intake in Pejerrey fry (*Odontesthes bonariensis*)

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ABSTRACT

Pejerrey is a freshwater fish from South America with high potential for aquaculture. This study was designed to determine the effects of different dietary protein:lipid ratio on growth rate and the expression of growth, lipid metabolism and feeding-related genes of this species during early developmental stages. Pejerrey fry were fed for 60 days with four experimental diets containing low (400 g Kg^{-1}) or high (500 g Kg^{-1}) protein (LP or HP, respectively) and low (120 g Kg^{-1}) or high (200 g Kg^{-1}) lipid (LL or HL, respectively), in the combinations: LP-LL; LP-HL; HP-LL and HP-HL. Measurements of growth, lipid and fatty acid content of fry, expression of genes from the endocrine axis (*gh, ghrs, igfs*), fatty acid metabolism (*Δ6-desaturase*), and food intake behavior (*nucb2/nesfatin-1*) were collected. Fry fed with diets LP-LL and HP-LL showed the highest growth rate and *growth hormone* (*gh*) mRNA expression levels. The gene expression of *Δ6-desaturase* was high in head of fry fed with diet LP-HL. The mRNA expression of *nucb2/nesfatin-1* and *gh* followed the same patterns in head, and the inverse pattern in body. In conclusion, diets with LL ensure a higher growth of pejerrey fry compared to those that contain HL, without altering the final lipid amount nor the fatty acid profile on fry. In LL groups, the expression of genes from the GH-IGF axis is associated with the observed promotion of somatic growth. The expression of *nucb2/nesfatin-1* indicates an effect of this peptide not related to food intake regulation, e.g., a negative regulatory role on GH expression, that would warrant future research.

1. Introduction

Fry diets (so called starter diets) usually have lower lipid levels than those used for juveniles and adults (Glencross and Turchini, 2010; Cogliati et al., 2019). Reasons for this lie in two facts. First, most fish fry need diets with a high precursor:energy ratio, i.e., high protein and low lipid content. Second, fry show a low lipid utilization efficiency, and thus diets with a high lipid content entail an unnecessary economic cost (Izquierdo et al., 2000; Ullah Khan et al., 2019). Therefore, the diet lipid: protein ratio is an important factor to ensure fry optimal growth and development. According to Morais et al. (2005), this ratio has a high impact on protein utilization because it changes the relationship between their oxidization (to obtain energy) and their synthesis (to promote grow). Furthermore, contrary to mammals, lipids and proteins are the primary dietary source of energy for fish, given that carbohydrates are poorly digested by fish (Polakof et al., 2012; Bertucci et al., 2019).

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Lipids and proteins have a relevant physiological role since they are part of most fish structures. But, in case of a negative energy balance, fish can use lipids and proteins as energy source (catabolism) instead of incorporating them into tissues (anabolism; Cho and Kaushik, 1990). The relationship between protein and dietary lipids also determines the energy storage capacity of fish. Lipids are the most energy-efficient non-protein source (Borges et al., 2013). In addition, lipids have a high digestibility value and are preferably oxidized compared to other diet components such as proteins and carbohydrates (Sargent and Tacon, 1999). Moreover, it has been shown that an increase in the content of lipid can spare the amount of protein utilized in diets from several fish (Ahmad, 2008; Chatzifotis et al., 2010, Ding et al., 2010., Xu et al., 2015). The use of diets with high lipid content has become a common practice due to their role as an energy source, which increases fish growth and protein retention, reducing nitrogen loss (Santinha et al., 1999; Ullah Khan et al., 2019). However, the increase in growth associated with the increase in protein anabolism caused by diets with high lipid content is counteracted by the negative effects that lipids can produce on fish. One of such negative effects is the high fat deposition, which reduces the commercial value of the final product (Cogliati et al., 2019). Not only the lipid content but also the fatty acid composition is an important quality of fish flesh since the presence of polyunsaturated fatty acids (PUFAs) from the omega 3 series has proven to be beneficial for human health (Dunbar et al., 2014). The ability of fish to synthesize long-chain PUFAs depends on the enzyme Δ6-desaturase. This key enzyme regulates the first step in the pathway which produces arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), converting linolenic acid (LNA) and linoleic acid (LA) obtained from diet to 18:4n − 3 and 18:3n − 6, respectively (Vagner and Santigosa, 2011), and participates in the synthesis of DHA through the process named Sprecher's shunt (Sprecher, 2000).

High fat diets have also been reported to cause a reduction in growth performance in some species, such as cobia (*Rachycentron canadum*), turbot (*Psetta maxima*), meagre (*Argyrosomus regius*), Asian seabass (*Lates calcarifer*), white seabass (*Atractoscion nobilis*), and Senegalese sole (Péres and Oliva-Teles, 1999; Chou et al., 2001; Regost et al., 2001; Espinós et al., 2003; Williams et al., 2003; Wang et al., 2005; López et al., 2006; Borges et al., 2009; Chatzifotis et al., 2010), but not in others (Vergara et al., 1996; Weatherup et al., 1997; Company et al., 1999; Ding et al., 2010., Xu et al., 2015; Vega-Aguayo et al., 2021). The imbalance between lipids and proteins can cause a negative effect on food digestion and nutrient absorption, which may be detrimental to growth and development (Chang et al., 2018; Ling et al., 2019; Zhou et al., 2020). In this regard, it has been showed that diets with a high lipid content entail low digestive capacity in *Gadus morhua* and *Scophtalmus maximus* fry (Hoehne, 1999). Also, the high lipid content of diets has been associated with the accumulation of large lipids clusters in enterocytes of fry from several species, possibly reducing lipid transport across the intestinal epithelium (Morais et al., 2005). With this background, it seems clear that the amount of lipids and proteins in a fish diet that would achieve optimal protein retention without altering the desirable characteristics of the fish flesh varies among species and must be studied individually.

An important parameter to study the effect of dietary macronutrients is the expression of genes involved in somatic growth, commonly referred to as genes from the growth endocrine axis (GH-IGF axis; Reinecke, 2010). The growth hormone (GH) is secreted from the pituitary and has been involved in different physiological functions in fish, mainly associated with somatic growth (Reinecke et al., 2005) and stress resistance (Deane and Woo, 2009; Yousefian and Shirzad, 2011), among others. This hormone exerts its action through the receptors GHR- I and GHR- II that show high expression in liver since early stages of development (Ozaki et al., 2006; Rhee et al., 2012). In response to this binding, the liver expresses and releases the insulin-like growth factor I and II (IGF- I and IGF- II), which act in an endocrine manner promoting growth on tissues (Wood et al., 2005). Also, IGF-I can act in an autocrine-paracrine manner in tissues such as brain, pancreas, kidney, gastrointestinal tract, gonads, muscle, skin and bone (Reinecke, 2010). Food intake is an important aspect to take into consideration when an environmental condition such as diet is tested, since it could modify growth and related-parameters. Nesfatin- 1 is a peptide orphan ligand with hormone-like actions, produced by the N-terminal cleavage of its precursor nucleobindin- 2 (NUCB2), encoded in the nucb2 gene (Oh-I et al., 2006). Nesfatin-1 reduces feed intake after central or peripheral administration in mammals (Gonzalez et al., 2012) and fish (Kerbel and Unniappan, 2012), which supports an anorexigenic role of this hormone. In a previous work we found that nucb2/nesfatin-1 mRNA expression is modified by fatty acids and amino acids in vitro (Bertucci et al., 2017b).

Pejerrey (*Odontesthes bonariensis*; Valenciennes, 1835) is a freshwater fish from South America, which represents an important economical resource for the region. It is highly appreciated for the quality of its flesh, which has similar characteristics to those of expensive marine species (Somoza et al., 2008). Despite the high potential of pejerrey for aquaculture production, its farming presents several difficulties related to low growth rates and high mortality during the first life stages (Grosman and González Castelain, 1995; Miranda and Somoza, 2001). Therefore, the aim of this study was to determine the dietary protein:lipid ratio that ensures the best growth and survival rates for pejerrey fry, taking into consideration possible effects on endocrine factors related to growth, lipid and fatty acid metabolism, and food intake regulation. For this, four experimental diets varying on their protein and lipid proportion were formulated and offered to fry during 8 weeks. Then, growth parameters, growth indexes, lipid and fatty acid content of fry were determined. To study the response of the growth endocrine axis to the dietary protein:lipid ratio, we quantified the mRNA abundance of the *growth hormone* (*gh*), *growth hormone receptor I* (*ghr-I*), *growth hormone receptor II* (*ghr-II*), *insulin-like growth factor I* (*igf-I*) and *insulin-like growth factor II* (*igf-II*). Finally, the mRNA expression of the enzyme Δ6-desaturase (which has a key role in lipid metabolism) and of the anorexigenic peptide nesfatin-1 (*nucb2/nesfatin-1*) involved in food intake regulation, was also measured to determine if changes in dietary lipid and protein proportions might induce changes in lipid metabolism and food intake.

2. Materials and methods

2.1. Fish management and experimental design

Pejerrey fertilized eggs were acquired from "Estación Hidrobiológica Chascomús" (Buenos Aires, Argentina). During all the experiment, fry were maintained in 140 L open flow-through water system tanks (water flow of 5 L/min), under a 12 h light: 12 h darkness photoperiod. Water salinity was 15 g/L, oxygen concentration 8 ppm, and water temperature 18 °C. After hatching, fry were fed with *Artemia* sp. nauplii 4 times per day during 30 days prior to the assay. One week before the onset of the experiment, a co-feeding schedule with *Artemia* nauplii and a commercial starter feed (crude protein, 430 g Kg^{-1}; crude fat, 30 g Kg^{-1}; crude fiber, 30 g Kg^{-1}; Shullet bebe®, Shulet, Argentina) was established. After this adaptation period, fish were divided into 12 tanks (n = 75 fish/tank). Four experimental groups were established (n = 3 tanks/group), each fed until apparent satiety 3 times per day with diets containing the same composition except for different percent of proteins and lipids, as described in Table 1. The proximal composition was determined according to AOAC (1990) methods. Crude protein was estimated as 6.25 × total nitrogen (N), determined using the Semi-micro Kjeldahl method. Crude lipid was determined gravimetrically of the sulfuric ether extract of 1 g samples. Moisture was measured gravimetrically after drying in an oven at 105 °C for 3 h, and the ash content by combustion in a muffle at 550 °C for 6 h. Fatty acid composition of each diet was determined by gas chromatography with FID detector of fatty acid methyl esters (FAME) prepared from aliquots of total lipid extracted by the Folch method (Folch et al., 1957). The fatty acid profile of diets is shown in Table 1 and Table S1. Food was weighed every day

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

	LP-LL	LP-HL	HP-LL	HP-HL
Ingredients (g kg $^{-1}$)				
CMC	11.7	116.7	42.2	146.7
Celite	50.0	50.0	50.0	50.0
Vitamin and Minerals[a]	20.0	20.0	20.0	20.0
Polyphosphates	30.0	30.0	30.0	30.0
Fish oil	62.5	142.5	54.4	133.0
Fish meal[b]	448.0	448.0	564.0	567.5
Soy Lecithin	23.8	23.8	23.8	23.8
Wheat flour	73.0	74.0	74.0	52.0
Cassava flour	326.0	140.0	187.0	22.0
Proximate composition (g kg^{-1} DM)				
Humidity	85 ± 6	80 ± 7	82 ± 5	82 ± 5
Lipid	132 ± 1	191 ± 1	126 ± 2	181 ± 2
Protein	430 ± 15	410 ± 10	525 ± 7	540 ± 10
Ash	260 ± 13	290 ± 15	212 ± 14	173 ± 4
Carbohydrate	128 ± 20	125 ± 7	119 ± 15	118 ± 9
Energy (kJ g^{-1} DM)[c]	17.61 ± 0.74	19.41 ± 0.40	19.47 ± 0.50	21.98 ± 0.47
E/P ratio	40.94 ± 0.99	47.35 ± 0.56	37.08 ± 0.55	40.70 ± 0.5
Lipid content (g Kg^{-1} DM)				
Total SFA	246 ± 3	209 ± 1	265 ± 2	230 ± 1
Total MUFA	308.5 ± 1	328.8 ± 1	295.6 ± 6	324.1 ± 0.8
Total PUFA	408 ± 2	431 ± 3	397 ± 8	408 ± 1
Total UFA	717 ± 3	760 ± 5	693 ± 14	732 ± 13
Total n-3 FA	128 ± 2	110 ± 2	159 ± 15	95.7 ± 0.8
Total n-6 FA	10.4 ± 0.3	8.6 ± 0.1	12.8 ± 0.3	7.3 ± 0.1

Experimental diets: LP, low protein (400 g Kg^{-1}); HP, high protein (500 g Kg^{-1}); LL, low lipid (120 g Kg^{-1}); HL, high lipid (200 g Kg^{-1}), combined as follow: LP-LL; LP-HL; HP-LL and HP-HL.

[a] "Vitafac Super Acqua." Provided per-kg of feed: retinol, 24,000 I.U.; vitamin D3, 4800 I.U.; tocopherol, 500 I.U.; vitamin K3, 16 mg; thiamine, 20 mg; riboflavin, 40 mg; pyrodoxine-HCl, 30 mg; vitamin B12, 0.06 mg; ascorbic acid, 300 mg; niacin, 300 mg; pantothenic acid, 80 mg; folic acid, 12 mg; biotin. 2 mg; zinc, 140 mg; iron, 200 mg; manganese, 100 mg; copper, 10 mg; cobalt, 4 mg; iodine, 3.4 mg; selenium, 6 mg.

[b] 620 g Kg^{-1} of crude protein, Moliendas del Sur S.A., Mar del Plata, Argentina. CMC, carboxymethyl cellulose; PC, proximate composition; FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. FA: fatty acid. SFA: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid. UFA: unsaturated fatty acid.

[c] Calculated based on physiological fuel values 23.7, 17.2 and 39.5 kJ g^{-1}(DM) for protein, carbohydrate, and fat, respectively (Brett and Groves, 1979).

before the first meal and after the last one to calculate the daily food consumption. This parameter was calculated as g of food consumed per day in each tank divided by the number of fry present in each tank. Total number, biomass and standard length (Ls) of fry from each tank were measured at day 0 (before the start of the assay) and at days 15, 30, 45 and 60. As we were unable to collect brain, pituitary, gut, liver, and muscle due to the fry size, samples of head (with no gills, head-kidney and heart), and trunk (from the gills to the anus) were collected, frozen in liquid nitrogen and stored at −80 °C until total RNA extraction.

2.2. RNA extraction and quantitative real-time PCR (RT-qPCR) analysis

The expression of the *gh*, *ghr-I*, *ghr-II*, *igf-I*, *igf-II*, Δ6-desaturase, and *nucb2/nesfatin-1* was measured in head and trunk from pejerrey fry after the completion of the experiment (60 days).

Tissues were disrupted using a PRO 200 homogenizer (PRO Scientific Inc., USA) and total RNA was extracted using the Ambion® TRIzol® Reagent (Life Technologies, USA) following the manufacturer protocol. Total RNA (1 μg) was reversely transcribed into cDNA using SuperScript II Reverse™ Transcriptase (Invitrogen, USA). Expression of target genes was measured by RT-qPCR with Fast Start Universal SYBR Green Supermix, (Roche Diagnostics, USA) on a Thermal Cycler StepOne Plus (Life Technologies Corporation, USA), using *β-actin* (primers pjBETA-F1 and pjBETA-R1) as reference gene. All primers sequences used for gene expression analysis are shown in Table S2. Each sample was run in duplicate and a PCR reaction, without the addition of template, was used as negative control. The RT-qPCR profiles contained an initial activation step at 95 °C for 5 min, followed by 35 cycles: 30 s at 95 °C, 30 s at 60 °C and 30 s at 73 °C. After the amplification, a melt curve of 0.5 °C increments from 65 °C to 95 °C was performed, enabling confirmation of amplification of single products. Gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ comparative threshold cycle (Ct) method (where ΔΔCt = ΔCt sample − ΔCt reference). The efficiency of amplification ranged 95–100% for all genes studied. The expression level in each group was normalized to the control and was presented as a fold of change (Livak and Schmittgen, 2001).

2.3. Fry fatty acid composition

Total lipids and fatty acids (FA) from 6 lyophilized individuals (randomly selected) were analyzed as described by Guinot et al. (2013). Identity of FA was further assessed through GC–MS after split-less injection in an Agilent 6850 Gas Chromatograph system, equipped with a Sapiens-5MS (30 m × 0.25 μm × 0.25 μm) capillary column (Teknokroma, Sant Cugat del Vallés, Barcelona, Spain) coupled to a 5975 series MSD (Agilent Technologies, Santa Clara, CA, USA).

2.4. Statistical analysis

Data were analyzed by two-way ANOVA, followed by Tukey post hoc test at a significance level of $P < 0.05$. Data that failed to pass normality and/or homoscedasticity tests were log-transformed and re-tested. All tests were performed using Infostat Version 2008 (Di Rienzo et al., 2013) software. Each tank from each treatment was taken as an experimental unit ($n = 3$).

2.5. Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journals' author guidelines page, have been adhered to and that all experimental procedures involving fish were strictly in accordance with the UFAW Handbook on the Care and Management of Laboratory Animals and the INTECH internal regulations.

3. Results

3.1. Dietary protein:lipid ratio induces differences in somatic growth

From day 30 on, fry fed with the HP-LL diet showed the highest length and weight (Fig. 1). At day 45, fry from groups fed with LP-LL and HP-LL diets showed higher values of length and weight than those fed with diets containing high lipid percent (Fig. 1). At the end of the experiment (day 60), feeding with diets containing LP-LL and HP-LL led to a higher length and weight compared to feeding on diets containing LP-HL and HP-HL. When comparing groups fed with diets containing LL, fry that were fed with the HP-LL diet showed the highest growth in both body weight and standard length (Fig. 1; Table 2). Percentage of length increase (LI%) was significantly higher in fish fed on HP-LL diet (Table 2).

Diets containing LP-LL and HP-LL caused a higher weight gain, body weight increase (BWI), and specific growth rate (SGR) than diets containing LP-HL and HP-HL (Table 2). Feed conversion ratio (FCR) was reduced, while feed efficiency ratio (FER) was increased, by feeding on LL diets (Table 2). Condition factor, daily food consumption and survival

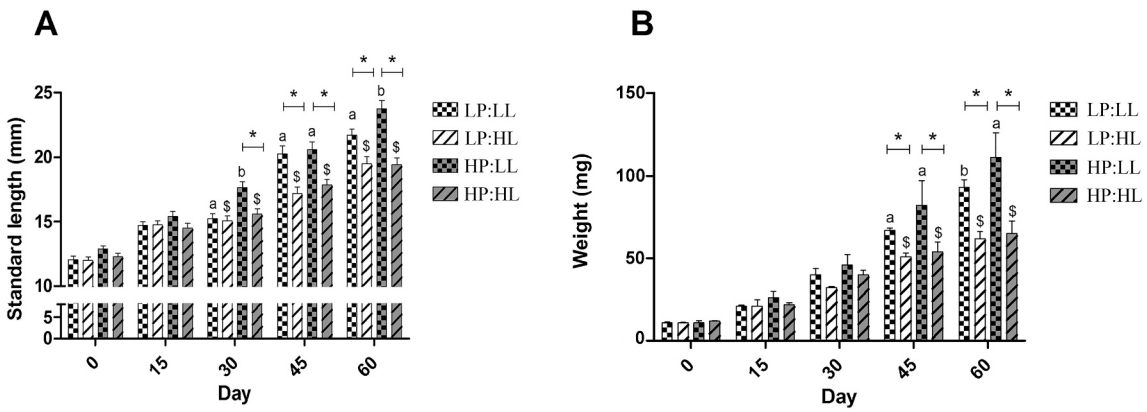

Fig. 1. (A) Standard length (mm) and (B) total weight (mg) of pejerrey fry at different times of the feeding trial with experimental diets. Data is presented as mean ± SEM (n = 3). Data were analyzed by two-way ANOVA followed by a Holm-Sidak post hoc test ($p < 0.05$). Asterisks (*) represent significant differences between diets with the same content of proteins. Different letters indicate differences between diets with 120 g Kg^{-1} of lipids, and different symbols ($, #) indicate differences between diets with 200 g Kg^{-1} of lipids ($p < 0.05$).

Table 2
Growth performance, food consumption and percent of survival of fry fed with the experimental diets for 60 days.

	LP-LL	LP-HL	HP-LL	HP-HL
Initial length (mm)	13.14 ± 0.21	13.01 ± 0.25	14.01 ± 0.44	13.45 ± 0.03
Final length (mm)	24.48 ± 0.27^{b}	21.96 ± 0.54^{a}	26.96 ± 0.69^{c}	21.83 ± 0.36^{a}
LI (%)	80.23 ± 2.42^{ab}	62.82 ± 8.69^{ab}	84.29 ± 4.96^{b}	56.35 ± 2.47^{a}
Initial weight (mg)	11.0 ± 0.2	11.0 ± 0.2	11.0 ± 0.7	12.0 ± 0.1
Final weight (mg)	93 ± 3^{b}	62 ± 2^{a}	111 ± 9^{b}	65 ± 4^{a}
Weight gain (mg)	82 ± 3^{b}	51 ± 3^{a}	100 ± 8^{b}	53 ± 5^{a}
BWI (%)	750 ± 41^{b}	457 ± 36^{a}	886 ± 28^{b}	450 ± 42^{a}
SGR	3.56 ± 0.08^{b}	2.9 ± 0.1^{a}	3.81 ± 0.05^{b}	2.8 ± 0.1^{a}
Initial K	0.48 ± 0.01	0.51 ± 0.02	0.41 ± 0.01	0.49 ± 0.01
Final K	0.64 ± 0.04	0.58 ± 0.03	0.57 ± 0.05	0.62 ± 0.02
Feed intake (% BWA/day)	2.97 ± 0.41	5.35 ± 0.57	3.48 ± 0.68	4.21 ± 0.68
DFC (g DM/ fry/ day)	2.4 ± 0.3	2.7 ± 0.3	2.5 ± 0.3	2.5 ± 0.2
FCR (g DM/g BW)	1.61 ± 0.14^{a}	2.85 ± 0.18^{b}	1.92 ± 0.33^{ab}	2.47 ± 0.31^{ab}
FER (g BW/g DM)	0.63 ± 0.05^{b}	0.35 ± 0.02^{a}	0.55 ± 0.08^{ab}	0.42 ± 0.05^{ab}
Survival (%)	65.8 ± 5.5	56.1 ± 2.9	46.4 ± 12.5	64.6 ± 2.6

Experimental diets: LP, low protein (400 g Kg^{-1}); HP, high protein (500 g Kg^{-1}); LL, low lipid (120 g Kg^{-1}); HL, high lipid (200 g Kg^{-1}). Values represent the mean ± SEM ($n = 3$). All data were analyzed with two-way ANOVA and Tukey posthoc test. Different letters indicate differences between diets ($p < 0.05$). LI: Length increase = [Final length – Initial length/ Initial length] x 100. Weight gain = Final weight - Initial weight. BWI: body weight increase = [(Final weight - Initial weight)/Initial weight] x 100. SGR: specific growth rate = [100 x (ln final fish wt. - In initial fish wt.)]/60 days. DFC: daily food consumption = food consumed per day per tank / number of fry in the tank. FCR: Food conversion ratio = feed intake (g)/ weight gain (g). FER: feed efficiency ratio = Weight gain/Food consumption. DM: dry matter. Sl: Standard length. BW: body weight. WG: weight gain.

rate did not show differences among treatments (Table 2).

The response of somatic growth to variations in feed protein content allow us to estimate the optimum protein level in aquafeed to maximize pejerrey fry growth. When SGR was plotted against feed percentage of protein content, data fit to a quadratic equation (Fig. S1) with a maximum of SGR reached at 47.7% of protein ($r^2 = 0.88$). Similar results were obtained for WG (47.8%, $r^2 = 0.87$) and BWI 47.7%, $r^2 = 0.88$) when plotted against dietary protein content (data not shown). On the other hand, no fitting was obtained when SGR was plot against dietary energy:protein ratio ($r^2 = 0.5747$; Fig. S2) or carbohydrate content ($r^2 = 0.0611$; not shown).

3.2. Dietary protein:lipid ratio modulates the expression of the gh-igf axis in pejerrey fry

Abundance of *gh* mRNAs was high in head of those groups fed with diets containing LL, compared with groups fed with HL diets (Fig. 2A). When comparing the effects caused by diets with the same lipid content, an increase in the amount of protein led to an increase in *gh* relative expression in head (Fig. 2A). HP-HL diet caused a statistically significant upregulation of *ghr-I* mRNA expression, and LP-HL diet a significant upregulation of *ghr-II* (Fig. 2B) in trunk of pejerrey fry. The expression of *igf-I* was detectable in trunk but no differences among treatments were observed (Fig. 2C). The *igf-I* mRNA expression was not detectable in head of pejerrey fry. The highest *igf-II* expression was detected in head of fry fed with HP-LL diet (Fig. 2D). The expression of *igf-II* in trunk did not show any difference among experimental groups (Fig. 2D).

3.3. Dietary protein and lipid percent regulates Δ6-desaturase gene expression

The *Δ6-desaturase* gene expression was highest in head of fry fed with diet containing LP-LL and no differences were detected among the other treatments (Fig. 2E). No differences in *Δ6-desaturase* gene expression was observed in trunk of pejerrey fry after the 60 days of trial (Fig. 2E).

3.4. Dietary protein:lipid ratio induces changes in nucb2/nesfatin-1 gene expression

Feeding on LL diets led to a significant increase in *nucb2/nesfatin-1* expression in head of pejerrey fry compared to feeding on diets with HL content (Fig. 2F). Comparing diets with the same amount of lipids, fry fed on diets with HP showed an increase in *nucb2/nesfatin-1* expression in head (Fig. 2F). Levels of *nucb2/nesfatin-1* mRNAs were high in body of pejerrey fry fed on diets with a HL.

3.5. Lipid composition and fatty acid profile of pejerrey fry

Fry of the 4 experimental groups contained approximately 200 g Kg^{-1} of total lipids (Table 3). The lipid productive value (LPV) was higher in fish fed on HP-LL diet, while lipid efficiency rate (LER) and

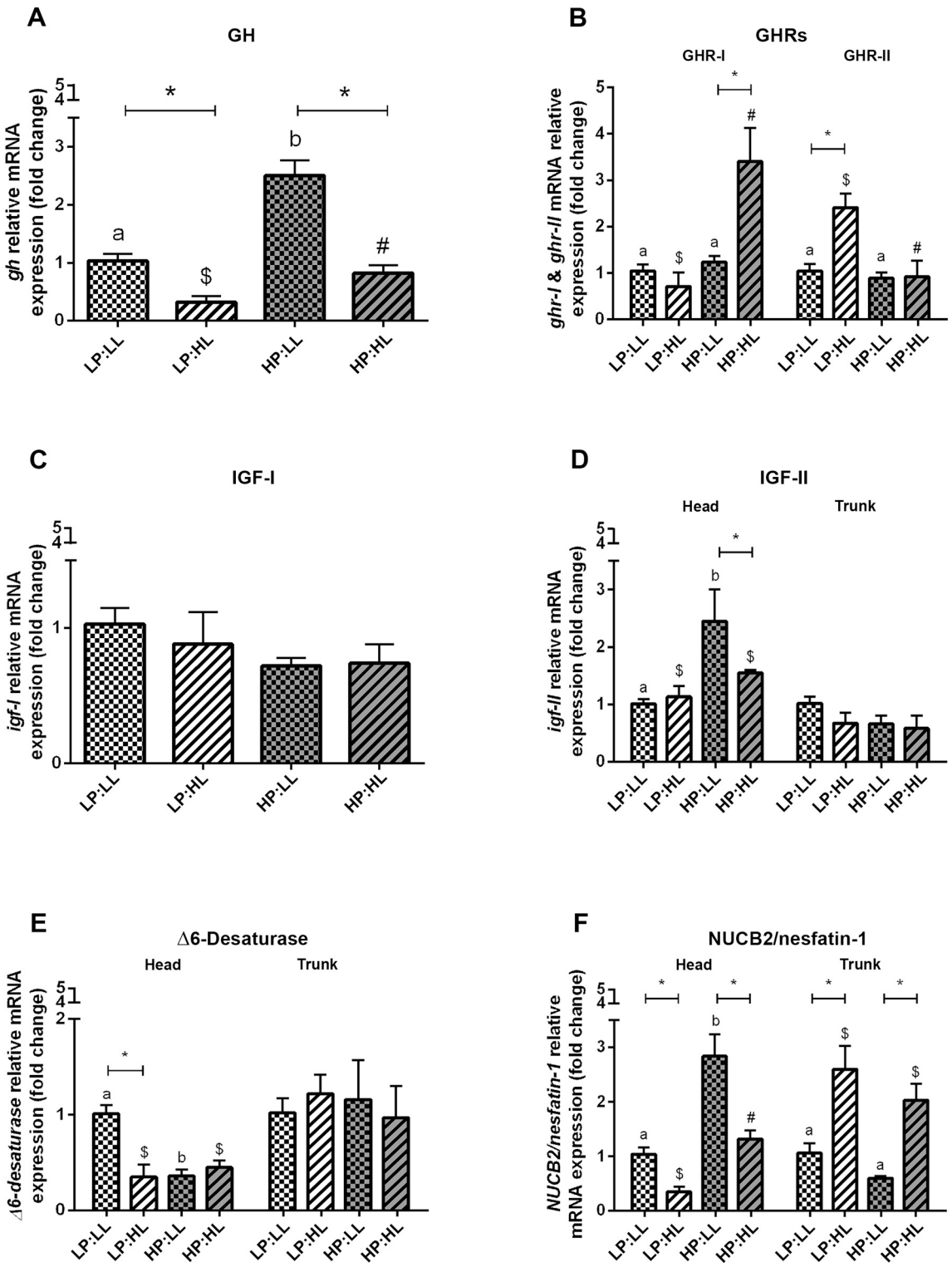

Fig. 2. (A) *gh* mRNA expression in head, (B) *ghr-I* and *ghr-II* mRNA expression in trunk, (C) *igf-I* mRNA expression in trunk, (D) *igf-II* mRNA expression in head and trunk, (E) *Δ6-desaturase* mRNA expression in head and trunk, and (F) *NUCB2/nesfatin-1* mRNA expression in head and trunk of pejerrey fry after 60 days of feeding on different experimental diets. Data is presented as mean ± SEM (n = 3). Data were analyzed by two-way ANOVA followed by a Holm-Sidak post hoc test ($p < 0.05$). Asterisks (*) represent significant differences between diets with the same content of proteins. Different letters indicate differences between diets with 120 g Kg^{-1} of lipids, and different symbols ($, #) indicate differences between diets with 200 g Kg^{-1} of lipids ($p < 0.05$).

protein efficiency rate (PER) were higher in LP-LL and HP-LL groups (Table 3). The dietary protein:lipid ratio did not change the total amount of PUFAs of the n-3 series. However, PUFAs of the n-6 series were significantly increased by feeding on a HP-HL diet (Table 3).

4. Discussion

4.1. Growth of pejerrey fry

The aim of the present study was to find a dietary protein:lipid

Table 3
Fatty acid composition, productive values of the total lipids of pejerrey fry fed with the experimental diets for 60 days.

	LP-LL	LP-HL	HP-LL	HP-HL
Lipid body content (%/WM)				
Lipids	19 ± 1	20 ± 1	20 ± 1	19 ± 1
Fatty acid composition (g Kg-1 sample)				
Total SFA	28 ± 1	27 ± 1	27 ± 1	28 ± 1
Total MUFA	28 ± 1 [a]	30 ± 1[a]	30 ± 1[a]	25 ± 2[b]
Total PUFA	23 ± 2	22 ± 1	22 ± 1	24 ± 1
Total UFA	51 ± 1	52 ± 1	52 ± 1	49 ± 1
Total n-3 FA	21 ± 1	22 ± 1	22 ± 1	22 ± 1
Total n-6 FA	20 ± 1[a]	18.1 ± 0.5[a]	18.1 ± 0.5[a]	22 ± 1[b]
Lipid and protein utilization				
LPV[1] (%)	18.08 ± 1.08[ab]	15.84 ± 0.62[ab]	20.25 ± 1.95[b]	13.31 ± 0.90[a]
LER[2] (%)	8.46 ± 0.26[a]	3.73 ± 0.24[b]	10.69 ± 1.19[a]	4.06 ± 0.45[b]
PER[3] (%)	2.60 ± 0.08[a]	1.74 ± 0.11[b]	2.57 ± 0.28[a]	1.36 ± 0.15[b]

Experimental diets: LP, low protein (400 g Kg^{-1}); HP, high protein (500 g Kg^{-1}); LL, low lipid (120 g Kg^{-1}); HL, high lipid (200 g Kg^{-1}). Values represent the mean ± SEM (n = 3). All data were analyzed with two-way ANOVA and Tukey posthoc test. Different letters indicate differences between diets ($p < 0.05$). Initial body composition: water 80.3%, protein 16.5%, lipid 1.6%, ash 2.6%. FA: fatty acid. SFA: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid. UFA: unsaturated fatty acid. [1] Lipid productive value = [(final body lipids − initial body lipids) / total lipid intake] × 100 = [(final body lipids − initial body lipids) / (feed intake (g) × % feed total lipid content)]. [2] LER: Lipid efficiency ratio = Weight gain (g) / total lipid intake (g) *100 = Weight gain (g) / [feed intake (g) × % feed total lipid content]. [3] PER: Protein efficiency ratio = Weight gain (g) / total protein intake (g) *100 = Weight gain (g) / [feed intake (g) × % CP feed].

proportion that improves the growth of pejerrey fry. To achieve this, we studied the response at the somatic and endocrine levels. Our results demonstrated that from day 30 of feeding on diets with low lipid percent and high proportion of proteins, fry begin to show significant differences in size. On day 45 of treatment, the increase in size and weight of fry fed on diets with a lower percentage of lipids were notorious. At the end of the experiment, an increase in the amount of protein within the groups fed with diets containing the lower lipid levels generates an increase in fry somatic growth. Although the HL content in diets has not affected feed intakes, the feed efficiency was significantly reduced in the LP-HL group, while in the HP-HL group this reduction was not statistical significative. Concomitantly, HL diets reduced both lipid and protein retention and utilization. Excess of dietary lipids may reduce digestive capacity and nutrient absorption in the intestinal epithelium (Morais et al., 2005; Zhang et al., 2018; Arenas et al., 2021). From our results, we conclude that a content of 200 g Kg^{-1} lipids in diets of pejerrey fry limits its growth after 30 days of treatment. It is evident that dietary lipids begin to influence the fry growth before proteins. Although at day 30 some differences in length were observed between fish feed on diets HP-LL and HP-HL, these differences were not observed in weight. Thus, the different growth patterns observed between groups fed on diets LL could suggest that protein requirements were covered by both amounts of proteins (-LP and -HP) up to day 45. However, from day 45 onwards the amount of dietary protein around to 400 g Kg^{-1} become a limiting factor. In this regard, by fitting our data to a quadratic equation we found that 477 to 478 g Kg^{-1} of dietary protein content would be required to maximize pejerrey growth at this developmental stage. A meta-analysis of data from studies available on protein requirements of fish estimated that the dietary protein requirements ranged between 24 and 70% of the diet, depending on species and the life stage (Teles and Couto, 2020). The level of dietary proteins estimated for pejerrey fry in this study is in the range found from omnivorous or carnivorous fish such as hybrid striped bass (*Morone chrysops x M. saxatilis*; 470 g Kg^{-1}, Rawles et al., 2018), *Sillago sihama* (450 g Kg^{-1}, Huang et al., 2020), and *Caranx ignobilis* (500 g Kg^{-1}, Muhammadar et al., 2021).

Amino acids play a key role in fish metabolism since they are not only used for anabolism but are also a very important source of energy (Bertucci et al., 2019; Canosa and Bertucci, 2020). However, if most of the energy requirements are covered by dietary lipids, the amino acids supplied will be used by fry to grow (Borges et al., 2013). Thus, we had anticipated a probable protein sparing effect of HL diets. However, this sparing effect was not observed; on the contrary, the protein efficiency rate was reduced by the HL content. In addition, pejerrey fry fed on diets with HL content did not show differences in their length and weight in relation to the dietary protein levels. This could suggest that a high percentage of lipids in diet generates negative effects on fry, inhibiting its normal growth and the proper nutrient utilization. Fish, in general, do not possess a very well-adapted metabolism for fat consumption, and the excess of lipids in the diet leads to negative effects on the growth and health of many species (Glencross and Turchini, 2010; Izquierdo et al., 2000). Survival rates and daily food consumption did not show significant differences between groups and fell within normal values for the developmental stage of the specie (Miranda et al., 2006).

The analysis of genes from the endocrine growth axis shows a correlation between the expression of *gh* and the fry somatic growth after 60 days of the experiment. The mRNA expression of *igf-II* was higher in the head of fry with the highest somatic growth after 60 days of the experiment (HP-LL). These results could be related to a greater expression of *gh* in the pituitary in response to diets with a favorable content of lipids and proteins, which would trigger a greater growth of pejerrey fry. The expression of *igf-II* found in head could be a signal that this factor is acting on the brain as a mediator of the GH response (Langdahl et al., 1998; Perrot et al., 1999), although more experiments should be carried out to confirm this hypothesis. The nutritional status of fish can be estimated through the gene expression pattern of GH-IGF components (Pérez-Sánchez and Le Bail, 1999; Hack et al., 2018; Canosa and Bertucci, 2020). Thus, as shown by many studies, dissociation of the action of GH on the release of IGFs in the liver of fish (presumably by desensitization of GHRs) occurs in response to fasting and malnutrition (Saera-Vila et al., 2009; Triantaphyllopoulos et al., 2019). Although we cannot consider that fry fed with HL diets in this experiment were under a malnutrition state, the retarded growth, the lower expression of *gh* in the head, and the higher expression of one of the *ghrs* in the body are clear indicators of a metabolic unbalance.

4.2. Lipid and fatty acid metabolism of pejerrey fry

The lipid content of fry was not different among groups after the completion of the experiment. This indicates that after 60 days, LL diets allow fry to accumulate lipids up to the same level as HL diets. The rate-limiting enzyme involved in the biosynthetic pathway of polyunsaturated fatty acids (PUFAs) from both n-6 and n-3 series in vertebrates is the fatty acyl Δ6-desaturase, which catalyzes the first step of the desaturation/elongation process in both pathways, converting linolenic acid (LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6) into 18:4n-3 and 18:3n-6, respectively (Vagner and Santigosa, 2011). Previous studies from our group showed that pejerrey expressed mRNA from a *Δ6-desaturase* gene (Bertucci et al., 2018; Bertucci et al., 2017a). Studies of fatty acid composition in wild pejerrey fry (Koppio et al., 2015) suggest this species could elongate and desaturate dietary PUFAs to long-chain PUFAs through a Δ6-desaturation pathway (Fonseca-Madrigal et al., 2014). To know if dietary lipid content could affect fatty acid metabolism, we studied the gene expression of the *Δ6-desaturase* in the head and trunk of fry. Gene expression levels in head and trunk mainly indicates the expression in central neural tissue and in liver, respectively. Liver and brain are the tissues in which the major expression/activity of this enzyme were found in teleost fish (Vagner and Santigosa, 2011). Liver is the major tissue for the production and storage of PUFAs, while the production in the brain is important for the

development of cell membranes vital for the normal functioning of neural systems (Ebm et al., 2021). No differences in the expression of the *Δ6-desaturase* were found in trunk, which is in concordance with the content of PUFA found in the whole fry (Table S3). The major amount of n-3 PUFAs in fry from all treatments was due to the presence of DHA (22:6 n-3), the final product from the pathway of desaturation/elongation of the n-3 series (Vagner and Santigosa, 2011). No differences among treatments were found in the amount of DHA in fry from pejerrey, nor in the total amount of PUFAs from the series n-3 (Table S3). In the case of the n-6 PUFAs, the final product of the pathway is the 22:5 n-6 and no differences were found between treatments. The major amount of fatty acids from this series found in fry from all treatments, was due to the linolenic acid (LA, 18:2n-6), which is the first precursor of the pathway (Vagner and Santigosa, 2011). This means that the pejerrey fry does not need to desaturate and elongate great amounts of fatty acids from the n-6 series to reach its requirements. Overall, considering the amount of each class of fatty acid present in the experimental diets, the final amount and composition of fatty acid present in fry, and the measured gene expression of *Δ6-desaturase* in trunk, we conclude that fry has accumulated PUFAs from diet rather than synthesizing them through the desaturation/elongation pathway. Expression of *Δ6-desaturase* show an increase only in head of pejerrey fry fed with LP-LL. PUFAs in the brain of fry are to a greater extent accumulated from the diet, especially those from the n-3 series such as EPA and DHA (Mourente, 2003). The presence of fatty acids with a high degree of unsaturation in the neural tissue is very important both for the maintenance of their structure and to obtain energy (Tocher and Harvie, 1988). The analysis of the fatty acid composition of fry did not show differences between experimental groups. But that analysis was carried out on the whole fry, and therefore, could be a different fatty acid profile in the head leading to that higher expression of desaturase. Since LP-LL is the diet with the lowest energy content, the higher expression of *Δ6-desaturase* in this tissue might respond to a particular requirement of structural precursors or energy provoked by this diet presumably in the neural tissue. Detailed experiments should be carried out in the future to elucidate this point.

4.3. Expression of nucb2/nesfatin-1 in pejerrey fry

The process for achieving somatic growth consumes available nutrient and energy obtained from the environment by feeding and transformed by metabolic cellular reactions into cell and tissue components (Canosa and Bertucci, 2020). Thus, the growth regulatory network should sense nutrient and energy levels and coordinate actions with the feed intake- and energy balance- regulation systems. The main environmental factor that regulates the GH-IGF system is the nutritional status (Moriyama et al., 2000). Therefore, there are factors involved in food intake regulation that also exert control on the expression of components from the GH-IGF axis in fish such as ghrelin, NPY, PYY (for a complete review see Bertucci et al., 2019; Canosa and Bertucci, 2020). Nesfatin-1 is the N-terminal fragment of nucleobindin-2 (NUCB2) protein, encoded by the nucb2 gene. This gene is widely distributed among mammals, reptiles, birds and fish (Mohan and Unniappan, 2013). The anorexigenic effect of nesfatin-1 in fish was initially demonstrated by Gonzalez et al. (2010) in goldfish. Nesfatin-1 is a peptide involved not only in feeding regulation but also in energy homeostasis in fish (Blanco et al., 2018). It was recently shown that nesfatin-1 is involved in the regulation of glucosensing and lipid metabolism in trout (Blanco et al., 2018), but the relation between nutrients, nesfatin-1, and the somatic growth axis was not completely elucidated. Several evidences indicate a possible interaction between the components of the somatic growth axis and nesfatin-1. For example, a similar trend of expression in response to the dietary amount of sunflower oil in diet of peripheral nesfatin-1 and central growth hormone was observed in pejerrey fry (Bertucci et al., 2018). Immunoreactive signal of nesfatin-1 was found in pituitary of the frog *Microhyla ornate* (Senejani et al., 2014) and confirmed at protein level in pituitary of rats (Stengel et al., 2009). The mRNA expression of *nucb2* was also reported in pituitary of mice (Kim et al., 2014) and goldfish (Gonzalez et al., 2010). Moreover, recent results from Blanco et al. (2021) indicate a suppressive role for nesfatin-1 on the goldfish GH/IGF axis. In our work, we found the expression levels of *nucb2/nesfatin-1* and *gh* in the head were higher in fry fed with the LL diets, while in the body the lower values of expression of *nucb2/nesfatin-1* were found in fry fed with LL-diets. Since there are no statistically significant differences in food intake, we could relate the *nucb2/nesfatin-1* peptide expression patterns found in head and body of fry with the effect that this hormone might exert (along with some other regulators of metabolism) in the expression of some components of the endocrine growth axis. According to results from Blanco et al. (2021), nesfatin-1 does not appear to act in an autocrine manner to regulate the GH secretion in the pituitary of goldfish. But, an intraperitoneal injection of nesfatin-1 significantly decreased the expression of *gh* mRNAs (approximately 90%) in pituitary at 15 min post-injection. Therefore, the inverse patter of expression found in this work between the expression of *gh* in head (assumably coming from the pituitary) and *nucb2/nesfatin-1* in trunk of fry, could be related with this endocrine negative regulation reported by Blanco et al. (2021) in goldfish.

5. Conclusion

In this study we determined that diets with 120 g Kg^{-1} of lipids improves the pejerrey fry growth compared to those that contain 200 g Kg^{-1}. Since we were unable to find a protein spearing effect of HL content, we can speculate that 120 g Kg^{-1} is close to the maximum tolerable for this species. Although more studies must be done to precisely establish the optimum for dietary lipid content, this conclusion agrees with a previous report from our group, where early juvenile pejerrey fish were fed with increasing lipid content (100, 130, 210 g Kg^{-1}) in practical diets ranging 530–560 g Kg^{-1}. Although no significant differences in growth were found, it was concluded that the diet containing 130 g Kg^{-1} was the best to ensure a proper functioning of GH-IGF axis (Gómez-Requeni et al., 2012). Similarly, when pejerrey larvae were fed for 14 weeks with diets composed by 360–380 g Kg^{-1} of protein and increasing lipids levels (60, 100, and 210 g Kg^{-1}), the results suggest that 60 g Kg^{-1} of lipids not adequate for pejerrey growth while not differences were found between the other two diets (Gómez-Requeni et al., 2013; 2019). In addition, we found that an increase from 400 g Kg^{-1} to 500 g Kg^{-1} of dietary protein content promotes fry growth after 60 days of treatment only in diets with a 120 g Kg^{-1} of lipids. We estimate that a protein content around 477–478 g Kg^{-1} should give the maximum growth rate. The study of genes from the GH-IGF axis confirm at endocrine level that diets high in protein and low in lipids promote a higher rate of growth in pejerrey fry. As changes in the expression of genes precede the somatic response, results also indicate that this growth might be sustained beyond the 60 days of the experiment. The lower content of lipids in diet that promote the maximum growth does not alter the fatty acid composition of fry neither the expression of the rate limiting enzyme for the PUFAs synthesis, which is desirable for the normal development of fish. The expression of *nucb2/nesfatin-1* seems to indicate some novel effects of this peptide may be related to energy homeostasis and growth and opens the opportunity for new research in fish.

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Declaration of Competing Interest

Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2022.111231.

References

Ahmad, M.H., 2008. Response of African catfish, *Clarias gariepinus*, to different dietary protein and lipid levels in practical diets. J. World Aquacult. Soc. 39, 541–548.

Arenas, M., Álvarez-González, C.A., Barreto, A., et al., 2021. Physiological and metabolic protein-sparing effects of dietary lipids on common Snook Centropomus undecimalis (Bloch, 1792) juveniles. Aquac. Nutr. 27, 1089–1102. https://doi.org/10.1111/anu.13250.

Bertucci, Juan Ignacio, Tovar, M.O., Blanco, A.M., Gómez-Requeni, P., Unniappan, S., Canosa, L.F., 2017a. Influence of water salinity on genes implicated in somatic growth, lipid metabolism and food intake in Pejerrey (*Odontesthes bonariensis*). Comp. Biochem. Physiol. Part B 210 (May), 29–38. https://doi.org/10.1016/j.cbpb.2017.05.005.

Bertucci, J.I., Blanco, A.M., Canosa, L.F., 2017b. Direct actions of macronutrient components on goldfish hepatopancreas in vitro to modulate the expression of ghr-I, ghr-II , igf-I and igf-II mRNAs. Gen. Comp. Endocrinol. 250, 1–8. https://doi.org/10.1016/j.ygcen.2017.05.014.

Bertucci, J.I., Tovar, M.O., Unniappan, S., Navarro, J.C., Canosa, L.F., 2018. Effects of dietary sunflower oil on growth parameters, fatty acid profiles and expression of genes regulating growth and metabolism in the pejerrey (*Odontesthes bonariensis*) fry. Aquac. Nutr. https://doi.org/10.1111/anu.12603.

Bertucci, J.I., Blanco, A.M., Sundarrajan, L., et al., 2019. Nutrient regulation of endocrine factors influencing feeding and growth in fish. Front. Endocrinol. 10, 1–17. https://doi.org/10.3389/fendo.2019.00083.

Blanco, A.M., Velasco, C., Bertucci, J.I., Soengas, J.L., Unniappan, S., 2018. Nesfatin-1 regulates feeding, glucosensing and lipid metabolism in rainbow trout. Front. Endocrinol. 9 https://doi.org/10.3389/fendo.2018.00484.

Blanco, A.M., Pemberton, J.G., Gonzalez, R., Hatef, A., Pham, V., Chang, J.P., Unniappan, S., 2021. Nesfatin-1 is an inhibitor of the growth hormone-insulin-like growth factor axis in goldfish (*Carassius auratus*). J. Neuroendocrinol. 33, e13010 https://doi.org/10.1111/jne.13010.

Borges, P., Oliveira, B., Casal, S., Días, J., Conceição, L., Valente, L.M.P., 2009. Dietary lipid level affects growth performance and nutrient utilisation of Senegalese sole (*Solea senegalensis*) juveniles. Br. J. Nutr. 102, 1007–1014.

Borges, P., Medale, F., Dias, J., Valente, L.M.P., 2013. Protein utilisation and intermediary metabolism of Senegalese sole (*Solea senegalensis*) as a function of protein:lipid ratio. Br. J. Nutr. 109 (8), 1373–1381.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, 8, pp. 280–351.

Canosa, L.F., Bertucci, J.I., 2020. Nutrient regulation of somatic growth in teleost fish. The interaction between somatic growth, feeding and metabolism. Mol. Cell. Endocrinol. 518 https://doi.org/10.1016/j.mce.2020.111029.

Chang, J., Niu, H.X., Jia, Y.D., et al., 2018. Effects of dietary lipid levels on growth, feed utilization, digestive tract enzyme activity and lipid deposition of juvenile Manchurian trout, *Brachymystax lenok* (Pallas). Aquac. Nutr. 24, 694–701. https://doi.org/10.1111/ANU.12598.

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., Mylonas, C. C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. Aquaculture 307, 65–70.

Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Rev. Nutr. Diet. 61, 132–172.

Chou, R.L., Su, M.S., Chen, H.Y., 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193, 81–89.

Cogliati, K.M., Unrein, J.R., Sealey, W.M., et al., 2019. Low-lipid diets fed at reduced ration: effects on growth, body composition, and survival of juvenile Chinook Salmon. J. Fish Wildlife Manag. 10, 500–508. https://doi.org/10.3996/062018-JFWM-059.

Company, R., Calduch-Giner, J.A., Pérez-Sánchez, J., Kaushik, S.J., 1999. Protein sparing effect of dietary lipids in common dentex (*dentex dentex*): a comparative study with sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Aquat. Living Resour. 12, 23–30. https://doi.org/10.1016/S0990-7440(99)80011-4.

Deane, E.E., Woo, N.Y.S., 2009. Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: A review. Fish Biol. Fish. 19 (1), 97–120. https://doi.org/10.1007/s11160-008-9091-0.

Rienzo, Di., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., Robledo, C., 2013. Infostat-Sofware estadístico. Universidad Nacional de Córdoba, Argentina.

Ding, L., Zhang, L., Wang, J., Ma, J., Meng, X., Duan, P., Sun, Y., 2010. Effect of dietary lipid level on the growth performance, feed utilization, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). Aquat. Res. 41 (10), 1470–1478.

Dunbar, B.S., Bosire, R.V., Deckelbaum, R.J., 2014. Omega 3 and omega 6 fatty acids in human and animal health: an African perspective. Mol. Cell. Endocrinol. 398 (1–2), 69–77. https://doi.org/10.1016/j.mce.2014.10.009.

Ebm, N., Guo, F., Kainz, M.J., Brett, M.T., Bunn, S.E., 2021. Polyunsaturated fatty acids in fish tissues more closely resemble algal than terrestrial diet sources. Hydrobiologia 848 (2), 371–383. https://doi.org/10.1007/s10750-020-04445-1.

Espinós, F.J., Tomás, A., Pérez, L.M., Balasch, S., Jover, M., 2003. Growth of dentex fingerlings (*Dentex dentex*) fed diets containing different levels of protein and lipid. Aquaculture 218, 479–490.

Folch, J., Lees, M., Sloane, S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem 226 (1), 497–509.

Fonseca-Madrigal, J., Navarro, J.C., Hontoria, F., Tocher, D.R., Martinez-Palacios, C.A., Monroig, O., 2014. Diversification of substrate specificities in teleostei Fads2: characterization of Δ4 and Δ6Δ5 desaturases of Chirostoma estor. J. Lipid Res. 55 (7), 1408–1419. https://doi.org/10.1194/jlr.M049791.

Glencross, B., Turchini, G., 2010. Fish oil replacement in starter, grow-out, and finishing feeds for farmed aquatic animals. In: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, pp. 373–404.

Gómez-Requeni, P., Kraemer, M.N., Canosa, L.F., 2012. Regulation of somatic growth and gene expression of the GH–IGF system and PRP-PACAP by dietary lipid level in early juveniles of a teleost fish, the pejerrey (*Odontesthes bonariensis*). J. Comp. Physiol. B. 182, 517–530. https://doi.org/10.1007/s00360-011-0640-9.

Gómez-Requeni, P., Bedolla-Cázares, F., Montecchia, C., Zorrilla, J., Villian, M., Toledo-Cuevas, E.M., Canosa, L.F., 2013. Effects of increasing the dietary lipid levels on the growth performance, body composition and digestive enzyme activities of the teleost pejerrey (*Odontesthes bonariensis*). Aquaculture 416-417, 15–22.

Gómez-Requeni, P., Kraemer, M.N., Canosa, L.F., 2019. The dietary lipid content affects the tissue gene expression of muscle growth biomarkers and the GH/IGF system of Pejerrey (*Odontesthes bonariensis*) juveniles. Fishes 4, 37. https://doi.org/10.3390/fishes4030037.

Gonzalez, R., Kerbel, B., Chun, A., Unniappan, S., 2010. Molecular, cellular and physiological evidences for the anorexigenic actions of nesfatin-1 in goldfish. PLoS One 5 (12), e15201. https://doi.org/10.1371/journal.pone.0015201.

Gonzalez, R., Mohan, H., Unniappan, S., 2012. Nucleobindins: bioactive precursor proteins encoding putative endocrine factors? Gen. Comp. Endocrinol. 176 (3), 341–346. https://doi.org/10.1016/j.ygcen.2011.11.021.

Grosman, F., González Castelain, J., 1995. Experiencias de alimentación y crecimiento con alevinos de pejerrey (*Odontesthes bonariensis*) dirigidas a optimizar la siembra. Revista de Ictiología Argentina. 4 (1–2), 5–9.

Guinot, D., Monroig, Ó., Hontoria, F., Amat, F., Varó, I., Navarro, J.C., 2013. Enriched on-grown Artemia metanauplii actively metabolise highly unsaturated fatty acid-rich phospholipids. Aquaculture 412, 173–178.

Hack, N.L., Strobel, J.S., Journey, M.L., et al., 2018. Response of the insulin-like growth factor-1 (Igf1) system to nutritional status and growth rate variation in olive rockfish (Sebastes serranoides). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 224, 42–52. https://doi.org/10.1016/J.CBPA.2018.05.025.

Hoehne, K., 1999. Lipid digestive enzymes in developing larva of the Atlantic cod (Gadus morhua) and turbot (Scophthalamus maximus). PhD thesis, Universität Karlsruhe.

Huang, Q.C., Qin, D.G., Tan, B.P., Du, T., Yang, Y.Z., Yang, Q.H., Dong, X.H., 2020. The optimal dietary protein level of juvenile silver sillago Sillago sihama at three dietary lipid levels. Aquacult. Res. 51 (2), 816–827.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernández-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol. Biochem. 22 (2), 97–107.

Kerbel, B., Unniappan, S., 2012. Nesfatin-1 suppresses energy intake, co-localises ghrelin in the brain and gut, and alters ghrelin, cholecystokinin and orexin mRNA expression in goldfish. J. Neuroendocrinol. 24 (2), 366–377. https://doi.org/10.1111/j.1365-2826.2011.02246.x.

Kim, J., Chung, Y., Kim, H., Im, E., Lee, H., Yang, H., 2014. The tissue distribution of Nesfatin-1/NUCB2 in mouse. Dev. Reprod 18 (4), 301–309. https://doi.org/10.12717/devrep.2014.18.4.301.

Kopprio, G.A., Graeve, M., Kattner, G., Lara, R.J., 2015. Fatty acid composition of wild Odontesthes bonariensis (Valenciennes 1835) larvae: implications on lipid metabolism and trophic relationships. J. Appl. Ichthyol. 31, 752–755. https://doi.org/10.1111/jai.12786.

Langdahl, B.L., Kassem, M., Møller, M.K., Eriksen, E.F., 1998. The effects of IGF-I and IGF-II on proliferation and differentiation of human osteoblasts and interactions with growth hormone. Eur. J. Clin. Investig. 28 (3), 176–186.

Ling, Guo J., Lang, Zhou Y., Zhao, H., et al., 2019. Effect of dietary lipid level on growth, lipid metabolism and oxidative status of largemouth bass, Micropterus salmoides. Aquaculture 506, 394–400. https://doi.org/10.1016/J.AQUACULTURE.2019.04.007.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25 (4), 402–408.

López, L.M., Torres, A.L., Durazo, E., Drawbridge, M., Bureau, D.P., 2006. Effects of lipid on growth and feed utilization of white seabass (*Atractoscion nobilis*) fingerlings. Aquaculture 253, 557–563.

Miranda, L.A., Somoza, G.M., 2001. Biología reproductiva del pejerrey *Odontesthes bonariensis*: Diferenciación sexual y endocrinología de la reproducción. Aspectos básicos y su potencial aplicación en acuicultura. In: *Fundamentos Biológicos, Económicos Y Sociales Para Una Correcta Gestión Del Recurso Pejerrey. F Grosman Ed. Editorial Astyanax,* Buenos Aires, pp. 41–45.

Miranda, L.A., Berasain, G.E., Velasco, C.A.M., Shirojo, Y., Somoza, G.M., 2006. Natural spawning and intensive culture of pejerrey *Odontesthes bonariensis* juveniles. Biocell 30 (1), 157–162.

Mohan, H., Unniappan, S., 2013. Phylogenetic aspects of nucleobindin-2/nesfatin-1. Curr. Pharm. Des. 19 (39), 6929–6934.

Morais, S., Koven, W., Rønnestad, I., Dinis, M.T., Conceição, L.E.C., 2005. Dietary protein:lipid ratio and lipid nature affect fatty acid absorption and metabolism in a teleost larva. Br. J. Nutr. 93, 813–820.

Moriyama, S., Ayson, F.G., Kawauchi, H., 2000. Growth regulation by insulin- like growth factor-I in fish. Biosci. Biotechnol. Biochem. 64, 1553–1562. https://doi.org/10.1271/bbb.64.1553.

Mourente, G., 2003. Accumulation of DHA (docosahexaenoic acid; 22:6n-3) in larval and juvenile fish brain. Big Fish Bang 239–248.

Muhammadar, A.A., Firdus, F., Muchlisin, Z.A., Samadi, S., Sarong, M.A., Boihaqi, B., Sartira, S., Sahidir, I., Batubara, A.S., 2021. Effect of Dietary Protein Level on Growth, Food Utilization, Food Conversion and Survival Rate of Giant Trevally *(Caranx ignobilis)* [Version 1; Peer Review: 2 Not Approved].

Oh-I, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., Eguchi, H., Yamamoto, M., Imaki, T., Hashimoto, K., Tsuchiya, T., Monden, T., Horiguchi, K., Yamada, M., Mori, M., 2006. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443 (7112), 709–712. https://doi.org/10.1038/nature05162.

Ozaki, Y., Fukada, H., Tanaka, H., Kagawa, H., Ohta, H., Adachi, S., Hara, A., Yamauchi, K., 2006. Expression of growth hormone family and growth hormone receptor during early development in the Japanese eel (Anguilla japonica). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 145 (1), 27–34. https://doi.org/10.1016/j.cbpb.2006.05.009.

Péres, H., Oliva-Teles, A., 1999. Effect of dietary lipid level on growth performance and feed utilization by European seabass juveniles *Dicentrarchus labrax*. Aquaculture 179, 325–334.

Pérez-Sánchez, J., Le Bail, P.-Y., 1999. Growth hormone axis as marker of nutritional status and growth performance in fish. Aquaculture 177 (1–4), 117–128.

Perrot, V., Moiseeva, E.B., Gozes, Y., Chan, S.J., Ingleton, P., Funkenstein, B., 1999. Ontogeny of the insulin-like growth factor system (IGF-I, IGF-II, and IGF-1R) in gilthead seabream (*Sparus aurata*): expression and cellular localization. Gen. Comp. Endocrinol. 116 (3), 445–460.

Polakof, S., Panserat, S., Soengas, J.L., Moon, T.W., 2012. Glucose metabolism in fish: A review. J. Comp. Physiol. B 182 (8), 1015–1045. https://doi.org/10.1007/s00360-012-0658-7.

Rawles, S.D., Green, B.W., Mcentire, M.E., Gaylord, T.G., Barrows, F.T., 2018. Reducing Dietary Protein in Pond Production of Hybrid Striped Bass (Morone chrysops x *M. saxatilis*): Effects on Fish Performance and Water Quality Dynamics, pp. 1–36.

Regost, C., Arzel, J., Cardinal, M., Robin, J., Laroche, M., Kaushik, S.J., 2001. Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (Psetta maxima). Aquaculture 193, 291–309.

Reinecke, M., 2010. Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. J. Fish Biol. 76 (6), 1233–1254. https://doi.org/10.1111/j.1095-8649.2010.02605.x.

Reinecke, M., Björnsson, B.T., Dickhoff, W.W., McCormick, S.D., Navarro, I., Power, D. M., Gutiérrez, J., 2005. Growth hormone and insulin-like growth factors in fish: where we are and where to go. Gen. Comp. Endocrinol. 142 (1–2), 20–24. https://doi.org/10.1016/j.ygcen.2005.01.016.

Rhee, J.-S., Kim, B.-M., Seo, J.S., Kim, I.-C., Lee, Y.-M., Lee, J.-S., 2012. Cloning of growth hormone, somatolactin, and their receptor mRNAs, their expression in organs, during development, and on salinity stress in the hermaphroditic fish, Kryptolebias marmoratus. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 161 (4), 436–442. https://doi.org/10.1016/j.cbpa.2012.01.004.

Saera-Vila, A., Calduch-Giner, J.A., Prunet, P., Pérez-Sánchez, J., 2009. Dynamics of liver GH/IGF axis and selected stress markers in juvenile gilthead sea bream (*Sparus aurata*) exposed to acute confinement: differential stress response of growth hormone receptors. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 154 (2), 197–203.

Santinha, Medale, Corraze, Gomes, 1999. Effects of the dietary protein:lipid ratio on growth and nutrient utilization in gilthead seabream (*Sparus aurata* L.). Aquac. Nutr. 5 (3), 147–156.

Sargent, J.R., Tacon, A.G., 1999. Development of farmed fish: a nutritionally necessary alternative to meat. Proc. Nutr. Soc. 58 (2), 377–383.

Senejani, A.G., Gaupale, T.C., Unniappan, S., Bhargava, S., 2014. Nesfatin-1/nucleobindin-2 like immunoreactivity in the olfactory system, brain and pituitary of frog, Microhyla ornata. Gen. Comp. Endocrinol. 202, 8–14. https://doi.org/10.1016/j.ygcen.2014.04.007.

Somoza, G.M., Miranda, L.A., Berasain, G.E., Colautti, D., Remes Lenicov, M., Strüssmann, C.A., 2008. Historical aspects, current status and prospects of pejerrey aquaculture in South America. Aquac. Res. 39 (7), 784–793.

Sprecher, H., 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochim. Biophys. Acta 1486 (2), 219–231.

Stengel, A., Goebel, M., Yakubov, I., Wang, L., Witcher, D., Coskun, T., Taché, Y., Sachs, G., Lambrecht, N.W.G., 2009. Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa. Endocrinology 150 (1), 232–238. https://doi.org/10.1210/en.2008-0747.

Teles, A.O., Couto, A., 2020. Dietary Protein Requirements of Fish – a Meta-Analysis, pp. 1445–1477. https://doi.org/10.1111/raq.12391.

Tocher, D.R., Harvie, D.G., 1988. Fatty acid compositions of the major phosphoglycerides from fish neural tissues; (n−3) and (n−6) polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and retinas. Fish Physiol. Biochem. 5 (4), 229–239.

Triantaphyllopoulos, K.A., Cartas, D., Miliou, H., 2019. Factors influencing *GH* and *IGF-I* gene expression on growth in teleost fish: how can aquaculture industry benefit? Rev. Aquac. https://doi.org/10.1111/raq.12402, 12:raq.12402.

Ullah Khan, K., Tellechea Rodrigues, A., Menegasso Mansano, C., et al., 2019. Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. Lat. Am. J. Aquat. Res. 47, 232–239. https://doi.org/10.3856/vol47-issue2-fulltext-3.

Vagner, M., Santigosa, E., 2011. Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review. Aquaculture 315 (1–2), 131–143. https://doi.org/10.1016/j.aquaculture.2010.11.031.

Vega-Aguayo, R., Drake, C., Guzmán, V., Mardones, A., Valdebenito, I., Ríos-Escalante, P. D.L., Encina-Montoya, F., Barile, J., 2021. Effect of protein and lipids levels in a growth diet on adult whitebait Galaxias maculatus (Jenyns 1842). Braz. J. Biol. 83, e252305 https://doi.org/10.1590/1519-6984.252305.

Vergara, J.M., Robainà, L., Izquierdo, M., Higuera, M.D.L., 1996. Protein sparing effect of lipids in diets for fingerlings of gilthead seabream. Fish. Sci. 62, 624–628. https://doi.org/10.2331/fishsci.62.624.

Wang, J.T., Liu, Y.J., Tian, L.X., Mai, K.S., Du, Z.Y., Wang, Y., Yang, H.J., 2005. Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*). Aquaculture 249, 439–447.

Weatherup, R.N., McCracken, K.J., Foy, R., Rice, D., McKendry, J., Mairs, R.J., Hoey, R., 1997. The effects of dietary fat content on performance and body composition of farmed rainbow trout *Oncorhynchus mykiss*. Aquaculture 151, 173–184. https://doi.org/10.1016/S0044-8486(96)01507-4.

Williams, K.C., Barlow, C.G., Rodgers, L., Hockings, I., Agcopra, C., Ruscoe, I., 2003. Asian seabass *Lates calcarifer* perform well when fed pelleted diets high in protein and lipid. Aquaculture 225, 191–206.

Wood, A.W., Duan, C., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. In. Int. Rev. Cytol. 243, 215–285. https://doi.org/10.1016/S0074-7696(05)43004-1.

Xu, G.F., Wang, Y.Y., Han, Y., Liu, Y., Yang, Y.H., Yu, S.L., Mou, Z.B., 2015. Growth, feed utilization and body composition of juvenile Manchurian trout, Brachymystax lenok (Pallas) fed different dietary protein and lipid levels. Aquac. Nutr. 21 (3), 332–340. https://doi.org/10.1111/anu.12165.

Yousefian, M., Shirzad, E., 2011. The review of the effect of growth hormone on immune system, metabolism and osmoregulation of fish. Aust. J. Basic Appl. Sci. 5 (5), 467–475.

Zhang, J., Zhao, N., Sharawy, Z., et al., 2018. Effects of dietary lipid and protein levels on growth and physiological metabolism of *Pelteobagrus fulvidraco* larvae under recirculating aquaculture system (RAS). Aquaculture 495, 458–464. https://doi.org/10.1016/J.AQUACULTURE.2018.06.004.

Zhou, Y.-L., Guo, J.-L., Tang, R.-J., et al., 2020. High dietary lipid level alters the growth, hepatic metabolism enzyme, and anti-oxidative capacity in juvenile largemouth bass Micropterus salmoides. Fish Physiol. Biochem. 46, 125–134. https://doi.org/10.1007/s10695-019-00705-7.