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Phenotypic flexibility in juvenile flounder *Paralichthys orbignyanus* (Valenciennes, 1839): differential modulation of digestive enzymes and energy reserves in relation to diet

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Abstract. The flounder *Paralichthys orbignyanus* is a flatfish of great ecological and economic importance that lives mainly in estuaries and coastal waters in Southwest Atlantic. Integrative studies on modulation of digestive enzymes and energy reserves along with growth upon differential proportions of key dietary substrates are lacking. We determined amylase, maltase, sucrase, trypsin, aminopeptidase-N (APN) and lipase activities in intestine and glycogen, triglycerides and proteins concentration in liver and muscle of juveniles fed with two diets (Diet 1; 35.5% carbohydrates, 41% proteins, 13.6% lipids; Diet 2; 41.8% carbohydrates, 46% proteins, 2.8% lipids). Juveniles fed with Diet 2 exhibited higher maltase, sucrase and trypsin activity (54%, 39% and 110%), lower triglycerides concentration in liver and muscle (65% and 14%), lower proteins content in liver (35%) and higher weight gain. Amylase, APN and lipase activity, glycogen and free glucose in liver and muscle and proteins in muscle were similar. The differential modulation of digestive enzymes and energy reserves suggest specific digestive and metabolic adjustments allowing growth upon diet with high carbohydrate and low lipids content. This work contributes to the knowledge of digestive and metabolic physiology of flatfishes which are of importance for the development of activities with economic impact in our region.

Key words: Flatfish, digestive flexibility, metabolic flexibility, dietary substrates.

Resumen: Flexibilidad fenotípica en juveniles del lenguado *Paralichthys orbignyanus* (Valenciennes, 1839): modulación diferencial de enzimas digestivas y reservas de energía en relación a la dieta. El lenguado *Paralichthys orbignyanus* es un pez plano de gran importancia ecológica y económica que habita principalmente en estuarios y aguas costeras del Atlántico sudoccidental. Faltan estudios integrativos sobre modulación de enzimas digestivas, reservas de energía y crecimiento en relación a diferentes dietas. Determinamos la actividad de amilasa, maltasa, sacarasa, tripsina, aminopeptidasa-N (APN) y lipasa en intestino y el contenido de glucógeno, triglicéridos y proteínas en hígado y músculo de juveniles alimentados con dos dietas comerciales (Dieta 1; carbohidratos 35.5%, proteínas 41%, lípidos 13.6%; Dieta 2; carbohidratos 41.8%, proteínas 46%, lípidos 2.8%). Juveniles alimentados con Dieta 2 exhibieron una mayor actividad de maltasa, sacarasa y tripsina (54%, 39%, 110%), menor contenido de triglicéridos en hígado y músculo (65%, 14%), menor contenido de proteínas en hígado (35%) y mayor ganancia de peso. La actividad de amilasa, APN y lipasa, glucógeno y glucosa libre en hígado y músculo y proteínas en músculo fueron similares. La modulación diferencial de enzimas digestivas y reservas de energía sugiere ajustes digestivos y metabólicos

que permitirían el crecimiento bajo dieta con alto contenido de carbohidratos y bajo de lípidos. Este trabajo contribuye al conocimiento sobre fisiología digestiva y metabólica de peces de importancia económica para la región.

Palabras Claves: Lenguado, flexibilidad digestiva, flexibilidad metabólica, sustratos dietarios

Introduction

Flatfishes constitute a sought after fishing resource in Southwest Atlantic, standing out the species of the Genera *Paralichthys* (Actinopterygii, Paralichthyidae) as one of the most commercially valuable fishes in demersal fisheries of Argentina, Brazil and Uruguay (Díaz de Astarloa 2002). The mire flounder, *Paralichthys orbignyanus*, is a marine estuarine-dependent flatfish that lives mainly in estuarine and coastal waters from Rio de Janeiro (Brazil) to the Gulf of San Matías, Argentina (Rivera-Prisco *et al.* 2001, Sampaio *et al*. 2001). This species has been described as a non-selective active predator that feeds on or near the bottom, mainly crustaceans, polychaetes and some fishes (Carneiro 1995, Rivera-Prisco *et al*. 2001). *P. orbignyanus* has great potential for aquaculture due to its wide tolerance to various environmental factors and for its meat quality (Sampaio *et al*. 2001, 2007, 2008, Radonic & Macchi 2009, Magnone *et al*. 2015). However, little is known yet about digestive characteristics and physiology at the biochemical level and the possible occurrence of digestive flexibility under different conditions (del Valle *et al*. 2016, Candiotto *et al*. 2018).

Fish require different sources of energy to maintain physical condition and fundamental processes, such as growth, metabolism and reproduction. Phenotypic flexibility implies reversible within individual variations in phenotypic traits which can increase the chances of survival for animals under different internal and external conditions. The acquisition and efficient processing of food energy are critical to survival and reproductive success (Karasov & Douglas 2013). In this context, the performance of the digestive system at different levels (*e.g*. biochemical) influences all physiological processes. Digestive enzymes have a main physiological role since they constitute a link between digestion, absorption and storage of nutrients. Modulation of digestive enzymes is an important strategy to maximize energy intake in various animals (Karasov & Douglas 2013). The occurrence and proportion of the main macromolecules in diet can influence digestive characteristics such as the existence and modulation of key digestive enzymes and the performance of an individual. Furthermore, the type of macromolecules consumed can also modify metabolic and physiological responses (Lazzari *et al*. 2010). Despite their physiological importance, this kind of studies in *P. orbignyanus* is lacking.

Most of the fish species studied show ability to use different dietary components to cope with energy and nutrient requirements (Karasov & Douglas 2013, Steimberg 2018). Intestine is the main site of digestion and absorption of nutrients. The presence and modulation of specific digestive enzymes in the intestine are indicators of the digestive capacity for the corresponding substrate (del Valle *et al*. 2016, Candiotto *et al*. 2018). Digestion of carbohydrates such as starch, glycogen and disaccharides and absorption of glucose via the intestine are main sources of circulating glucose (Bakke *et al*. 2011, Polakof *et al*. 2011, 2012, Steimberg 2018). Similar to other vertebrates, amylases in fish have a central physiological importance due to their role in the initial steps of digestion of dietary starch and glycogen and of stored glycogen in preys (Date *et al*. 2015). To establish the presence and modulation of amylase activity in the intestine of fish species of commercial importance and culture is of particular interest due to the possibility of incorporating low-cost carbohydrates such as starch in the meal. Disaccharidases activity such as maltase and sucrase would further allow the potential use of diet-specific glycogenic disaccharides (del Valle *et al*. 2016, Steimberg 2018). Maltase plays a main role in final steps of glycogenic substrates to produce glucose (Lin *et al*. 2016). In several species of teleost fish, lipids are used as sources of energy for maintenance of various physiological processes such as growth, reproduction and movement (Tocher 2003, Sandre *et al*. 2017, Steimberg 2018). Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) have a central physiological importance due to their role in digestion of dietary lipids into small molecules that can be absorbed (Casas-Godoy *et al*. 2012, Karasov & Douglas 2013, Chang & Leung 2014, Michiels *et al*. 2015b**).** Levels and modulation of lipase activity in the intestine will determine the ability for digestion and use of dietary lipids. To know the occurrence and modulation of

key proteases such as trypsin and aminopeptidase-N (APN) in the intestine it is essential to determine the proteolytic digestive capacity of an organism (Bakke *et al*. 2011, Steimberg 2018). Trypsin, an endopeptidase, is one of the most important proteases in the vertebrate digestive tract, including several species of teleost fish (Sainz 2004, Perera *et al*. 2015, Rittschoff 2017, Steimberg 2018). APN, which is a membrane-bound ectopeptidase, plays an essential role in the final steps of protein digestion (Fairweather *et al*. 2012, Michiels *et al*. 2015a). This enzyme is used as an indicator of the ability to digest proteins (Ramirez-Otarola *et al*. 2011, 2018). Modulation of specific proteases in the intestine is particularly important in farmed fish species, since proteins must be incorporated into diet at specific levels to achieve normal growth and maintenance (Rahman *et al*. 2017).

The aim of this work was to study the effect of two commercial diets with different percentages of carbohydrates, proteins and lipids on: i) amylase, maltase, sucrase, trypsin, APN and lipase activities in the anterior intestine; ii) the content of energy reserves (glycogen, triglycerides, proteins) in liver and muscle and iii) growth parameters in juveniles of *P. orbignyanus*, as index of digestive and metabolic adjustments at the biochemical level to support growth under differential proportions of key dietary macromolecules.

Materials and methods

Animal maintenance: Juvenile flounder (mean length: 8.70 ± 0.50 cm, mean body weight: 9.37± 0.18g) (Programa Maricultura y Biología Experimental, INIDEP, Mar del Plata, Argentina) were acclimated during 22 days, in six 100 L fiberglass circular tanks at 28 psu, pH 8.1 and at a temperature of 22 ± 2°C under a regime of 12 h light/12 h dark. At time 0, juveniles were randomly divided into two groups (n=60 per group) and received two different diets. Diet 1 contained 35.5% carbohydrates, 41% proteins, 13.6% lipids. Diet 2 contained 41.8% carbohydrates, 46% proteins, 2.8% of lipids . Components of both diets were fish meal, wheat flour, mineral and vitamins complexes. Food were prepared by Asociación de Cooperativas Argentina CL and by Núcleo Alimentos MDQ S.R.L. Three tanks connected to a closed recirculation system, consisting of a settler, a foam skimmer, a biological filter, and two UV lights, as described by Radonic *et al.* (2018), were used for each group. Effluent water was biofiltered for a biological conversion of ammonia to nitrate. The water was continuously aerated. Fish were fed *ad libitum* three times a day (at 9:00, 12:00 and 15:00 h) and 6 days a week throughout the experimental period which extended for 150 days. Excess food and feces were daily removed by manual siphoning the bottom of the tanks. Individual fish length and weight were recorded at the beginning and at the end of the experimental period. This study was conducted under the regulations and statements of the Ethics Committee CICUAE del Instituto Nacional de Investigación y Desarrollo Pesquero .Res- 0159 Ministerio de Agroindustria, INIDEP, Argentina.

Sampling procedures: At the end of the experimental period, individuals (n = 10 per diet) were food deprived for 24 h (del Valle *et al*. 2016, Pujante *et al*. 2016) prior being used for biochemical determinations. The sampling procedure used was that one we previously described (del Valle *et al*. 2016). Briefly, fish were weighed and cold-anaesthetized by placing them on ice for approximately 10 minutes. Small intestine, liver and epiaxial muscle were immediately excised and frozen at -80°C until be used for homogenates preparation in 50 mM Tris / HCl, pH 7.4 (4 ml of tissue g^{-1} for intestine and liver; 8 ml of tissue g^{-1} for muscle) (CAT 9 120 homogenizer, T10 tool) (del Valle *et al.* 2016)

Biochemical assays: Enzyme activities were determined with samples without previous thawing and using optimal temperature, pH and substrate concentrations as determined previously for the intestine of juveniles of *P. orbignyanus* (del Valle *et al*. 2016, Michiels *et al*. 2017, Albanesi *et al.* 2020). Freezing procedure did not alter the activity values.

Amylase activity was measured by incubating the sample for 15 min at 30 °C in the presence of starch (15 mg mL^{-1}) in 50 mM phosphate buffer (pH 7.4), 1.5 ml of dinitrosalicylic acid reagent (DNS) (Miller 1959), was added for further incubation for 10 min at 100 °C. After cooling, the released maltose was assessed with absorbance being read at 540 nm (ZL5000 PLUS, Zelte).

Maltase and sucrase activities were assayed by incubating samples for 10 min in 0.1 M maleate-NaOH buffer (pH 6.4) at 37°C plus 42 mM of substrate maltose or sucrose. Glucose released was determined by the addition of 1.5 ml of the combined enzyme color glucose reagent solution (oxidase glucose 10 kU L, peroxidase 1 kU, l, 4-aminophenazone 0.5 mmol L^{-1}, phosphates pH 7.0 100 mmol L^{-1}, hydroxybenzoate12 mmol L^{-1})

(Wiener Lab AA Kit cod. 1400101), further incubation for 5 min at 37 °C and with absorbance of the colored quinone complex formed being read at A_{505nm}

Lipase activity was determined colorimetrically adding samples to the assay mixture, which contained 0.85mM of p-nitrophenyl-palmitate substrate and buffer 50mM Tris-HCl pH 8.5. After incubation during 5 minutes at 37° C, 0.1% (w/v) TCA was added to stop the reaction and released product p-nitrophenol (pNP) was quantified by reading the absorbance at 410 nm.

Trypsin activity was determined using N-α-benzoyl-DL-arginine-4-nitroanilide (BAPNA) as substrate as described (Michiels *et al*. 2017, Albanesi *et al*. 2020). After incubation of samples during 45 minutes at 45 ° C in 50 mM Tris buffer / HCl pH 9.0/ 400 mM Cl_2Ca and in the presence of 1.23 mM BAPNA, 0.1M KOH was added and absorbance was measured at 405 nm.

APN activity was assayed by incubating the sample in the presence of L-alanine-p-nitroanilide (L-Ala pNA) (final concentration 0.33mM) as substrate in 50mM Tris-HCl buffer pH 7.4 for 15 minutes at 45° C. Cold 2 M acetic acid was added to stop the reaction and absorbance was determined at 384 nm.

Glycogen in the liver and in the epiaxial muscle was determined by hydrolysis of α-amyloglucosidase (Sigma Chemicals) (Albanesi *et al*. 2020). Samples were boiled for 4 minutes and incubated in acetate buffer (pH 4.8) in the presence and absence of 0.2 mg ml^{-1} of α-amyloglucosidase during 2.5 h at 55 °C. After incubation and centrifugation at 3000 rpm for 15 minutes, glucose was quantified in the supernatant by using a commercial kit for enzyme glycemia (Wiener Lab AA). Glucose from the hydrolysis of glycogen was determined as the difference between the tests with and without α-amyloglucosidase and free glucose from the assay in the absence of α-amyloglucosidase.

Triglycerides (TAG) in the liver and the epiaxial muscle was measured by the colorimetric method of glycerol phosphate oxidase (TAG Wiener-Lab AA code 861110001) (Albanesi *et al*. 2020). After incubation of samples for 5 minutes at 37° C with the reactant, released glycerol was determined by reading the absorbance at 505 nm of the formed colored quinone complex.

Proteins was assayed according to Bradford (1976). Bovine serum albumin (0.96 mg x ml^{-1}) was used as standard.

Statistical analysis: Statistical analysis was performed using the Sigma-Stat 4.0 program for Windows, which automatically performs a previous test of equality of variances of Levene and a normality test of Kolmogorov-Smirnov. A t-test was used to estimate the statistical significance of the differences and $P<0.05$ was considered significant. A non-parametric test (Mann-Whitney) was used when variance or normality were not equal.

Results

Digestive enzyme activities in anterior intestine: Amylase activity in intestine was similar in juveniles fed with both diets (Fig. 1a). Maltase and sucrase activities in intestine of individuals fed with Diet 2 were higher (about 54% and 39% respectively) with respect to the value of juveniles fed with Diet 1 (Fig. 1b- 1c).

Trypsin activity of individuals fed with Diet 2 was higher (around 110%) than in juveniles fed with Diet 1 (Fig. 2a). No statistical differences were observed in APN and lipase activity in individuals fed with both diets (Fig. 2b- 2c).

Energy reserves in storage tissues and growth parameters: Glycogen and free glucose concentrations in liver and muscle were similar in individuals fed with both diets (Fig. 3a- 3b- 3c- 3d). Triglycerides concentration in liver and muscle of individuals fed with Diet 2 was lower (65% and 14%, respectively) compared to the corresponding value of juveniles fed with Diet 1 (Fig. 4a- b). Proteins concentration in liver of individuals fed with Diet 2 was lower (around 35%) compared to that of juveniles fed with Diet 1 (Fig. 4c). Proteins concentration was similar in muscle of fishes fed with the different diets (Fig. 4d).

At the end of the experimental period, individuals fed with Diet 2 showed higher mean length and weight and higher weight gain (final weight – initial weight) compared to those fed with Diet 1 (Table I).

Discussion

This work shows a differential modulation of carbohydrases, proteases and lipase activities in intestine and that of energy reserves in liver and muscle in juveniles of the flounder *P. orbignyanus* in response to diet, which suggests the occurrence of coordinated and specific adjustments in key components of carbohydrates, lipids and proteins metabolism.

In flatfishes, the effect on growth of varying lipid concentration in the diet is very different

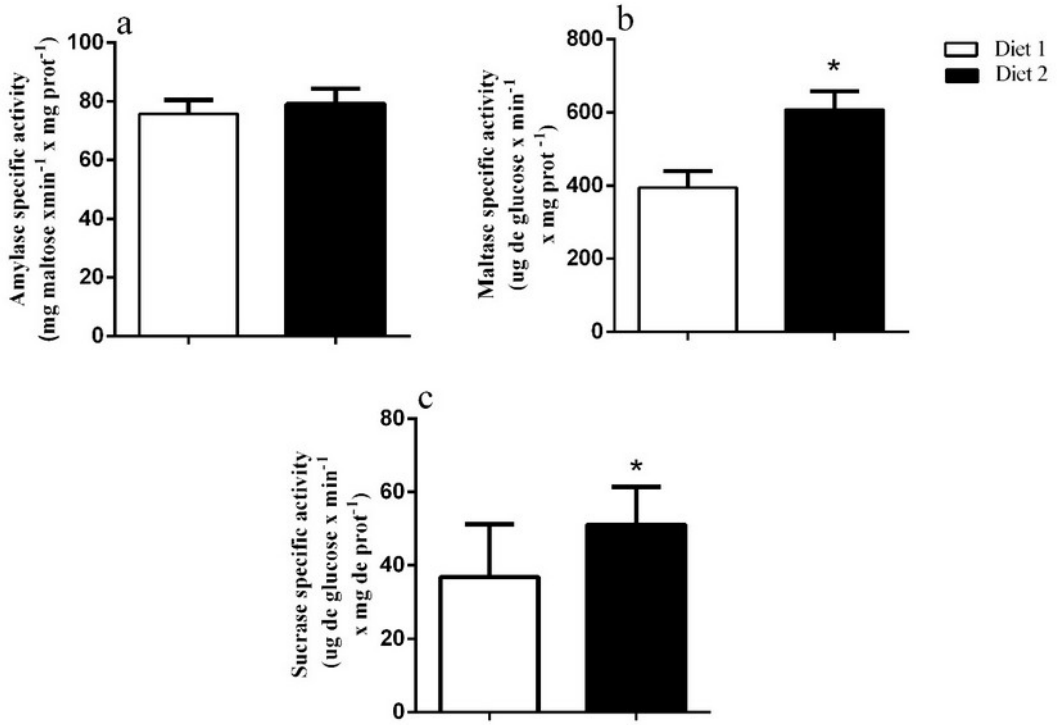

Figure 1. Amylase (a) maltase (b) and sucrase (c) specific activity in intestine of juveniles of *Paralichthys orbignyanus* fed with Diet 1 (35.5% carbohydrates, 41% proteins, 13.6% lipids) and Diet 2 (41.8% carbohydrates, 46% proteins, 2.8% lipids). * Indicate significant differences between diets ($P < 0.05$). Data are the mean ± SE for ten individuals.

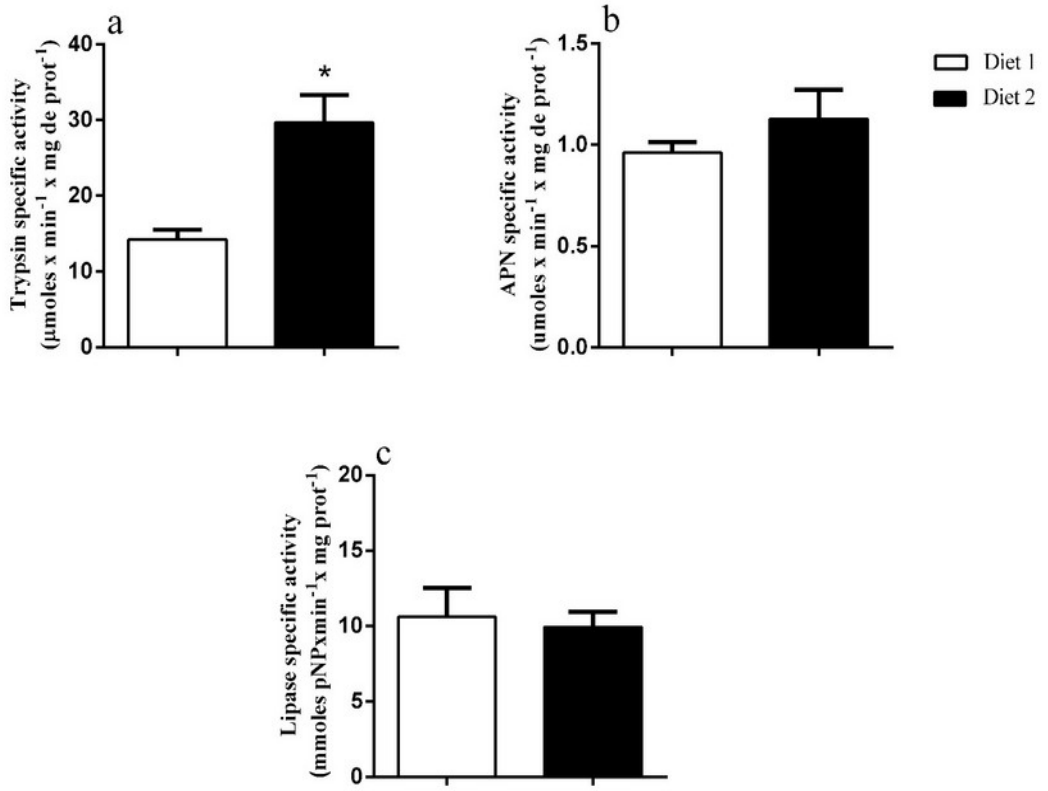

Figure 2. Trypsin (a), APN (b) and lipase (c) specific activity in intestine of juveniles of *Paralichthys orbignyanus* fed with Diet 1 (35.5% carbohydrates, 41% proteins, 13.6% lipids) and Diet 2 (41.8% carbohydrates, 46% proteins, 2.8% lipids). * Indicate significant differences between diets ($P < 0.05$). Data are the mean ± SE for ten individuals.

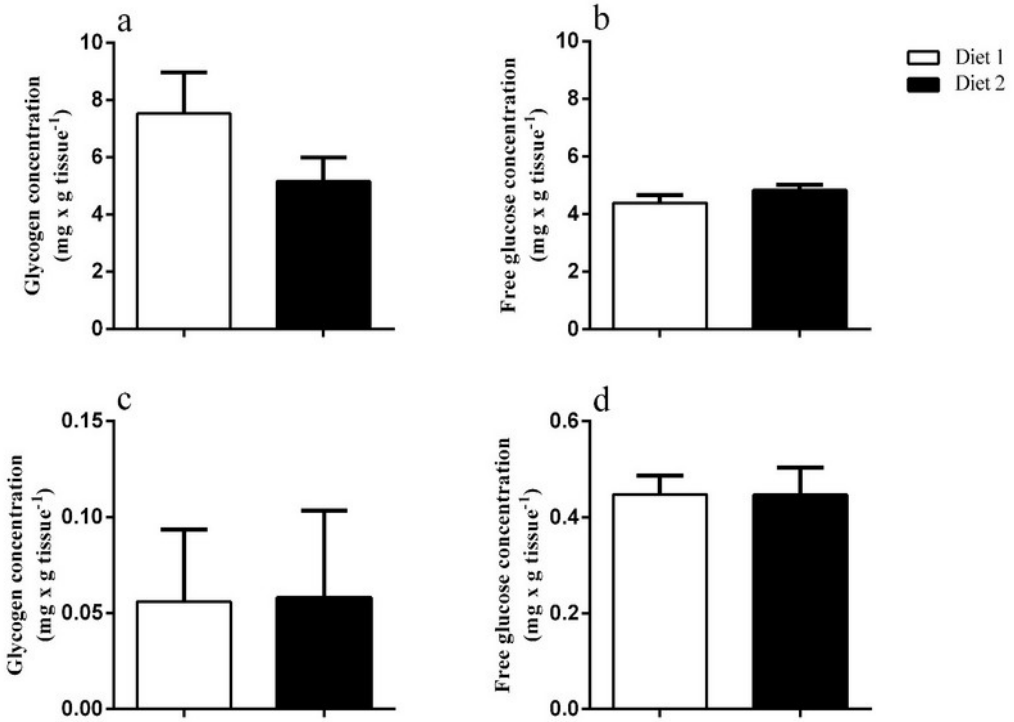

Figure 3. Glycogen and free glucose concentration in liver and muscle of juveniles of *Paralichthys orbignyanus* fed with Diet 1 (35.5% carbohydrates, 41% proteins, 13.6% lipids) and Diet 2 (41.8% carbohydrates, 46% proteins, 2.8% lipids). Glycogen (a) and free glucose (b) concentration in liver. Glycogen (c) and free glucose (d) concentration in muscle. * Indicate significant differences between diets ($P < 0.05$). Data are the mean ± SE for ten individuals.

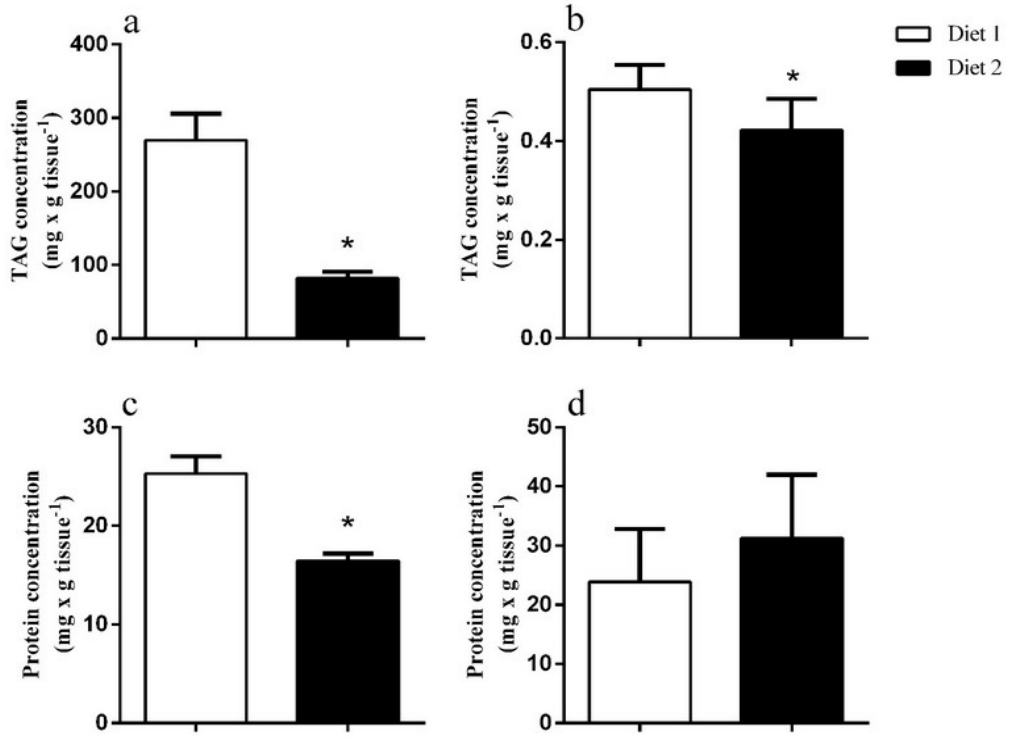

Figure 4. Triglycerides and proteins concentration in liver and muscle of juveniles of *Paralichthys orbignyanus* fed with Diet 1 (35.5% carbohydrates, 41% proteins, 13.6% lipids) and Diet 2 (41.8% carbohydrates, 46% proteins, 2.8% lipids). Triglycerides concentration in liver (a) and muscle (b). Proteins concentration in liver (c) and muscle (d) * Indicate significant differences between diets ($P < 0.05$). Data are the mean ± SE for ten individuals.

(positive, negative, no effect) depending on the species (Borges *et al*. 2009, 2013). A higher average weight and greater weight gain indicate the ability of juveniles of *P. orbignyanus* to adapt to a diet with a lower proportion of lipids (Diet 2). Similar levels of lipase activity in anterior intestine suggest that adjustments such as an increase in total lipolytic digestive capacity would not be a strategy to compensate a lower level of dietary lipids in juveniles of *P. orbignyanus*. In general, levels of digestive enzymes in the digestive tract of vertebrates correlate positively with those of the corresponding substrate in the diet (Melo *et al.* 2012, Karasov & Douglas 2013). However, some species exhibit an inverse relationship; while in others, the level is similar (Karasov & Douglas 2013, Xie *et al.* 2017). In juveniles of *P. orbignyanus*, lipase activity is only present in anterior intestine being the main site for the degradation of dietary lipids while the liver constitutes a main triglyceride reserve site (del Valle *et al*. 2016). A lower content of triglycerides in liver and muscle in juveniles of *P. orbignyanus* suggests that a mobilization or a decrease in the building of these reserves occurred in the presence of a lower dietary lipid content. This could provide energy metabolites to sustain the higher weight gain. In the Papu *Piaractus mesopotamicus* a lower content of body lipids is related to a better growth performance (Favero *et al*. 2018). Higher lipid deposits in fish are not necessarily related to greater energy availability when growth is stimulated by feeding after a period of prolonged food deprivation (Favero *et al*. 2018). Triglyceride biosynthesis pathway appears to be generally the same in fish and mammals (Tocher 2003, Karasov & Douglas 2013, Steimberg 2018). During feeding, excess fatty acids are exported from the liver in the form of lipoproteins, accumulated, and stored in the form of triglycerides in specific storage sites (Grosell *et al*. 2011, Ballantyne 2014). Body fat stores vary among fish species and depend mainly on dietary factors (Borges *et al*. 2009, 2013). Storage of significant amounts of fat within the muscle occurred in several fishes, which may explain a large proportion of the total reserves (Tocher 2003). An enhancement in dietary lipids leads to an increase in lipid deposition in various species (Borges *et al*. 2009, 2013). This could be the case for juveniles of *P. orbignyanus* since, triglycerides concentration in liver and muscle was higher when fed with a higher lipid content diet.

Glucose homeostasis is essential to support the regular functions of various organs and responses to environmental stress in fish (Polakof *et al*. 2011, 2012, La Fleur *et al*. 2014, Chen *et al*. 2018). Digestion of glycogenic carbohydrates is one of the main sources of glucose in various fish (Grosell *et al*. 2011, Ballantyne 2014, Steimberg 2018). In several species of flatfish, a different tolerance to dietary carbohydrates has been reported (Hamre *et al*. 2003, Salas-Leiton *et al*. 2018). Our results show that juveniles of *P.orbignyanus* which are carnivorous in the field (Rivera-Prisco 2001) tolerate diets with high carbohydrate content (≥ 20% carbohydrates, Xu *et al*. 2017). Coordinated action of amylase and maltase, and in some species along with sucrase activity, is essential for the total digestion of glycogen substrates in the diet (Diaz-Sotomayor *et al*. 2013, Lin *et al*. 2012, 2014, 2016, Steimberg 2018). Increased maltase and sucrase activity in anterior intestine while amylase activity was not affected suggests a differential regulation of specific carbohydrases and of the digestion of

Table I. Growth parameters for *Paralichthys orbignyanus* juveniles fed with Diet 1 and Diet 2.

Growth parameters	Diet 1	Diet 2
Initial weight (g)	5.71± 0.12	5.73 ± 0.11
Initial length (cm)	1.97 ± 0.93	1.97 ± 0.82
Final weight (g)	8.72 ± 0.22	9.92 ± 0.26*
Final length (cm)	7.28 ± 0.55	9.91 ± 0.77 *
Weight gain	6.2 ± 0.55	8.8 ± 0.77

* Denote significant statistical difference in a row ($P < 0.05$). Data of growth parameters are presented as mean ± SE

distinct glycogenic substrates. Enhanced disaccharidases activity could lead to a potential increase of final steps of carbohydrates digestion and of glucose availability. However, if such were the case for juveniles of *P. orbignyanus*, the potential greater accessibility of glucose would not lead to an increase in the glycogen stores in the liver or muscle. Several species of carnivorous fish seem to have a potential flexibility for the use of glucose under conditions of limited availability of lipids (Yang *et al*. 2019). Dietary carbohydrates can not only promote growth, but also provide metabolic intermediates for the synthesis of other biologically important compounds in some fishes (Ballantyne 2014, Sanz *et al.* 2015, Xu *et al*. 2017). Availability of substrates derived from the glucose metabolism favors the synthesis of triglycerides in liver, which are later processed and exported to extra hepatic tissues such as muscle (Tocher 2003, Sandre *et al*. 2017). Whether this be the case for juveniles *P. orbignyanus* requires further investigation.

Dietary proteins must be at appropriate levels to achieve normal growth and maintenance of fish, since an excess can be harmful (Rahman *et al*. 2017). Determination of key proteases such as trypsin and APN (involved in initial and final steps of diet protein digestion, respectively) in intestine is particularly important in the case of farmed fish species (Perera *et al*. 2015, Rittschoff 2017). Increased trypsin activity in anterior intestine while APN activity was not affected in juveniles of *P. orbignyanus* fed with higher protein content diet supports the idea of a specific modulation of proteases. Specific modulation of trypsin activity suggests a potential increase in digestive capacity for initial steps of protein digestion, while final steps appear not to be affected. However, we cannot discard the occurrence and modulation of other membrane-bound aminopeptidases involved in the final steps of protein digestion. Apart from APN, other intestinal membrane-bound aminopeptidases occur in some fish (Bakke *et al*. 2011, Steimberg 2018). A lower protein content in liver of juveniles of *P. orbignyanus* fed with a higher protein content diet, suggests the modulation of protein metabolism such as decreased synthesis or increased degradation or mobilization. This could lead to a modification in the use of protein metabolites to support the growth of other organs or tissues. Since proteins concentration was not affected, the muscle could not be involved in adjustments in protein metabolism of juveniles of *P. orbignyanus*. In several teleost fish, and under certain physiological conditions, amino acids are absorbed at a rate that exceeds that used for protein synthesis by different organs and storage sites. Amino acids that are not used for protein synthesis are deaminated and the carbon skeleton is used as energy source or in gluconeogenic and lipogenesis pathways. In addition, several amino acids that are not used in the deposition of body proteins serve as precursors for the synthesis of different nitrogen compounds (Cowey 1996, Li *et al*. 2009).

In conclusion, results of this work show that juveniles of *P. orbignyanus* exhibit digestive and metabolic flexibility, which could allow the tolerance of a diet with high carbohydrate and low lipid content. Differential and specific responses of key components of the carbohydrate, lipid and protein metabolism such as modulation of digestive enzymes in intestine and of energy reserves in liver and muscle, suggest that coordinated digestive and metabolic adjustments at the biochemical level occur in relation to diet. Whether differential adjustments of digestive enzymes activity in the intestine of juveniles of *P. orbignyanus* are due to qualitative or quantitative variations such as modulation by chemical messengers of existing enzymes, changes in expression and synthesis or degradation require further investigation. Routes of digestion and absorption of nutrients or those involved in the construction and use of energy reserves in *P. orbignyanus* are not known yet. Therefore, future works should focus on establishing the interplay among carbohydrates, lipids and proteins metabolic pathways in a specific and among different organs.

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