Received: 8 May 2019 | Revised: 1 November 2019 | Accepted: 11 December 2019

DOI: 10.1111/are.14474

ORIGINAL ARTICLE

Aquaculture Research WILEY

Protein and cellulose level in diet: Effects on enzymatic activity, metabolite and amino acid profiles in freshwater anomurans *Aegla uruguayana* (Decapoda: Anomura)

Gabriela E. Musin[1] | Débora A. Carvalho[1] | María F. Viozzi[1] | María C. Mora[1] | Pablo A. Collins[1,2] | Verónica Williner[1,3]

[1]Instituto Nacional de Limnología (CONICET – Universidad Nacional del Litoral), Santa Fe, Argentina

[2]Escuela Superior de Sanidad Dr. Ramón Carrillo, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina

[3]Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Santa Fe, Argentina

Correspondence
Verónica Williner, Instituto Nacional de Limnología (CONICET – Universidad Nacional del Litoral), 3000 Santa Fe, Argentina.
Email: vwilliner@gmail.com

Funding information
Fondo para la Investigación Científica y Tecnológica, Grant/Award Number: PICT 2014-1996 and PICT 2016-2542; Consejo Nacional de Investigaciones Científicas y Técnicas, Grant/Award Number: PIP 790

Abstract

The aims of this study were to evaluate the short-term effects of artificial diets with different protein: cellulose ratios (D1 = 60:0, D2 = 45:15 and D3 = 30:30) on the enzymatic activity and metabolite profile and to probe the suitability of the amino acid profile of the different diets in *Aegla uruguayana*. The different artificial diets affected the digestive total protease and amylase activities in a short period of time. Lipases were not affected by the diets. In the haemolymph, an effect of the diet was found in glucose and triglyceride measurements. Particularly, the glucose of the organisms fed D1 was significantly lower than that of organisms fed D2, without significant differences in D3. The concentrations of triglycerides were different among organisms subjected to the treatments, indicating a greater concentration in organisms fed D2 than in those fed D1. In muscle tissue, the concentration of glycogen followed the same trend as haemolymphatic glucose, indicating that the organisms fed D2 had a higher concentration of this metabolite compared to the rest. The amino acid profile of muscle tissue of wild and fed aeglids had a high and significant correlation in all cases, showing few changes in the short-term feeding regime. Some changes were observed mainly in aeglids fed D3, which exhibited the lowest amounts of methionine, lysine and arginine and the highest amounts of aspartic + glutamic acids. In addition, D2 has a good quality of EAA, fulfilling almost all the requirements of a wild aeglid.

KEYWORDS

aeglids, artificial diets, haemolymph, hepatopancreas, muscle

1 | INTRODUCTION

In aquaculture, in the absence of natural food, it is necessary to provide an artificial diet that meets the nutritional needs of the animal growth (Kaushik, 2000). This must also be in accordance with the physiological characteristics and the development phase corresponding to the life cycle of the species being cultivated (Lall & Dumas, 2015). Freshwater decapods are organisms widely used in aquaculture with different applications and intentions (FAO, 2006). There are several species recognized worldwide for their commercial value as a product for human consumption (Phillips, Hanson, Dasgupta, & Ohs, 2010; Pillay & Kutty, 2005), as a nutritive material to produce food for other species of commercial interest, for the extraction of astaxanthin, for the nutrition of farmed fish (Hooshmand, Shabanpour, Moosavi-Nasab, & Golmakani, 2017; Lim, Yusoff, Shariff, & Kamarudin, 2017; Ribeiro, Genofre, & McNamara, 2001),

and extraction of chitosan for various purposes (Mármol, Gutiérrez, Páez, Ferrer, & Rincón, 2004), and as species of interest for the ornamental market (Panne Huidobro, 2010).

However, on the decapods that inhabit the fluctuating freshwater environments of southern South America, these aspects have been poorly studied. Trophic studies realized on decapod crustacean species of the Paraná River floodplain, together with other sub-basins in Argentina, revealed that vegetal resource is a prominent part of their natural diet, as well as those organisms that characterize the communities of benthos (i.e., oligochaetes and insect larvae) and plankton (i.e., cladocerans and copepods) (Collins, Williner, & Giri, 2007a; Williner, 2010; Williner, Giri, & Collins, 2011). In this environmental scenario of alternation of flood and drought periods and fluctuation of the trophic supply, the Neotropical decapods have shown the ability to adjust (Collins, Carnevali, Carvalho, & Williner, 2012; Collins, Williner, & Giri, 2007b). This last ecological information can be relevant to deepen the studies on nutritional requirements and resource utilization capacity for species with potential to be cultivated.

Within decapods, the aeglids are the only family of anomurans that colonized freshwater environments (Schmitt, 1942). In 2009, the genus *Aegla* was the group with the highest participation within continental aquatic invertebrates exported from Argentina, with more than 92% of total transactions (8,000 organisms in total) (Panne Huidobro, 2010). Another fact to be noted is that they are captured entirely from natural environments, which implies possible consequences on natural populations that in many species are restricted to very limited distributions in their ranges (Tumini, Giri, Williner, & Collins, 2016). This is a reason why this group's export was forbidden (Panne Huidobro, 2014). From these data, aeglids could be a potential crop given the growing interest in ornamental use (Panne Huidobro, 2010).

Aegla uruguayana is an anomuran species that inhabits the system of La Plata basins (Bond-Buckup & Buckup, 1994). The principal components of its diet are plant debris and algae, followed by small varieties of animal items. Nevertheless, the morphology of the ossicles of the stomach suggests a macrophagic trophic habit despite the several small-sized items recorded in the stomach contents (Williner, 2010). On the way to lay the foundations to establish the aquaculture conditions of this species, Musin, Rossi, Diawol, Collins, and Williner (2017, 2018) studied physiological aspects related to the digestion in the natural environment. Taking into account the fluctuating conditions of the environment in which these organisms inhabit, it is expected that they respond to these changes by altering their behaviour, their physiology or even their development, adjusting to the perceived risk in the environment (Ricklefs, 2008).

In other latitudes and in several decapod species, the effect of different diets on the metabolite profile, digestive enzymatic activity and digestibility has been studied (López-López, Nolasco, Villarreal-Colmenares, & Civera-Cerecedo, 2005; Pavasovic, Anderson, Mather, & Richardson, 2007; Rosas, Cuzon, Gaxiola, Arena, et al., 2000a; Sacristán et al., 2016; Velurtas, Diaz, Fernandez Gimenez, & Fenucci, 2011). Furthermore, metabolic issues have been studied in another species of aeglid crustacean under laboratory culture conditions (Ferreira, Hack, Oliveira, & Bond-Buckup, 2005; Oliveira, Hack, Almerão, Bond-Buckup, & Dutra, 2011; Silva-Castiglioni, Valgas, Machado, Freitas, & Oliveira, 2015). In organisms of *A. uruguayana* from their natural environment, metabolite profile was determined in various tissues (Musin, Rossi, Diawol, Collins, & Williner, 2017), as well as the enzymatic digestive activity of lipases, total proteinase and amylase (Musin, Rossi, Diawol, Collins, & Williner, 2018). However, the effect of the diet type on the activities of digestive enzymes, the metabolite profile and the amino acid profile of muscle has not yet been studied in this species.

Needless to say the study of digestive enzymes, which involves how they respond to certain ingredients in the diet, allows us to better understand the mechanisms involved in digestion and optimal use of the resources of the cultured species (Le Moullac, Klein, Sellos, & Van Wormhoudt, 1996; López-López et al., 2005). The amount and quality of the protein source are important factors that must fulfil the nutritional requirements of a species. The amino acid profile of a feedstuff should be correlated with the amino acid composition of the muscle tissue (Cowey & Tacon, 1983), being necessary the evaluation of whether the quantitative amino acids are being satisfied through A/E ratio (Arai, 1981). Thus, the selection of nutrients and, particularly, protein sources is a key step in feed development for the ongrowing of species, to be able to create a balanced diet in terms of amino acids, necessary to generate new proteins in organisms. In turn, studies on the effects of diets on metabolism within the genus are also scarce, although their relevance in the context of aquaculture is essential (Ferreira et al., 2005).

For the above reasons, the aims of this study are, on one hand, to evaluate the short-term effects of artificial diets with different levels of protein and cellulose inclusions on the enzymatic activity and metabolite profile in *Aegla uruguayana*. On the other hand, to probe the suitability of the amino acid profile of the different diets, the essential amino acid profile index (EAAI) was applied using the muscle tissue of wild specimens of *A. uruguayana* as reference.

2 | MATERIALSANDMETHODS

2.1 | Preparation of pelleted diets

Three types of pelleting were elaborated (D1, D2 and D3) using and varying, as main components, the fishmeal in 60%, 45%, and 30%, and micro-cellulose in 0%, 15%, and 30% respectively. In addition, other ingredients such as starch, fish oil, gelatin, cholesterol, vitamins and minerals were used in the most advantageous proportions obtained in previous works (Collins, 1997; Collins & Petriella, 1996) and based on the survival and growth of other freshwater decapod species (González-Peña, Anderson, Smith, & Moreira, 2002). Values expressed as dry matter g/100 g are detailed in Table 1. The cold extrusion method described by Fenucci, Petriella, and Müller (1981) was used as the production method of pellets.

TABLE 1 Ingredients and proximate composition of the experimental diets in *Aegla uruguayana* (g/100 g)

	D1 (%)	D2 (%)	D3 (%)
Ingredients			
Fish meal[a]	60	45	30
Microcellulose[b]	0	15	30
Starch	27	27	27
Fish oil	3	3	3
Unflavoured gelatin	4	4	4
Mixture of vitamins and minerals[c]	2	2	2
Bicalcium phosphate	4	4	4
Analysed (dry matter g/100 g)			
Protein	36.45	33.78	21.76
Carbohydrate	40.34	46.71	62.07
Lipids	10.11	8.64	5.34
Humidity	4.16	3.82	4.43
Ash	19.05	16.69	11.74

[a]Coomarpes Internacional (Mar del Plata, Buenos Aires, Argentina).
[b]Sistemas Analiticos S.A.—Biopack® (Zárate, Buenos Aires, Argentina).
[c]Manufactured by Nutralia S.R.L. (Santa Fe, Santa Fe, Argentina). Maximum values of active principles in g/1,000 g: vitamin B1 ((0.550); vitamin B2 (1.925); vitamin B6 (1,238); vitamin B12 (4.125); niacin; pantothenic acid (5.978); vitamin C (27.500); biotin (5.500); vitamin A (3.385); vitamin D (0.550); vitamin E (44.000); vitamin K (11.000); iron (50.417); zinc (64,706; copper (15.714); manganese (0.917); selenium (18.750); phosphorous (0.314); and maltodextrin (excipient).

2.2 | Sample collection and laboratory maintenance

Specimens of aeglid crustaceans were collected in the Espinillo stream (31°47′09.16″S and 60°18′57.46″W), Entre Ríos (Argentina), during the month of September 2016. Males and females of adult size were used in the same proportion. The specimens were transferred to the laboratory to perform acclimation to the experimentation conditions based on the same protocols followed in previous studies (Devercelli & Williner, 2006). Around four adult specimens were separated to carry out the amino acid profile analysis of wild specimens. The organisms were kept under controlled conditions of temperature (21 ± 1°C) and photoperiod (12:12 light:darkness). To avoid stress situations and agonistic behaviour, they were provided oxygen and refuge environment (rocks and artificial refuges) in all experiment replicates.

2.3 | Experimental design of assay

Plastic aquariums with a capacity of 140 L (60 cm in diameter and 50 cm high) were filled with 80 L of dechlorinated water and used for each diet. Each of these had an individual aeration system, with the same amount of water, and the same number of animals (30 individuals). The allocation of the specimens to the aquariums and the selection of the aquariums to the diets were carried out randomly considering the interspersion of the treatments and replicates (Krebs, 1994). Artificial refuges and stones were added to each aquarium to reduce the stress of the organisms. At the beginning of the trials, specimens in the intermoult state were selected by observing the silk morphology of the uropod, according to Diawol and Collins (2012), in order to ensure the initial metabolic stability. The organisms were weighed and sexed, and afterwards placed in the aquariums. The sizes of the specimens were homogeneous for the three treatments: 20.53 (+3.41) mm. The organisms were fed twice a day (9:00 and 14:00), with an amount of food equal to 5% of the biomass present in each aquarium. The temperature (21°C) and the photoperiod (12:12 light:darkness) were kept constant. Daily levels of water temperature, conductivity, pH and O_2 (ppm and % saturation) were recorded with sensors electronic (Hanna HI 98130/9146). To maintain the optimum quality of the parameters, the water of each aquarium was filtered twice a day, every day before the alimentation period, and the remains of food, moults and dead animals were eliminated daily. The total duration of the assay was 15 days. Feeding management and animal's manipulation were conducted in accordance with the guidelines for Sneddon (2015).

2.4 | Extraction of haemolymphatic, hepatopancreatic and muscular tissues

At the end of the diet assay, the animals were anesthetized by hypothermia, weighed and measured. The haemolymph was extracted from the arthrodial membrane with a syringe previously rinsed with an anticoagulant solution of sodium citrate (10%). The hepatopancreas and muscle tissue of chelipeds were removed under a stereomicroscope. Tissues were immediately frozen and subsequently stored at −20°C (for amino acid profile) and −80°C (for enzymatic analysis and metabolite profile) according the biochemical determinations.

2.5 | Digestive enzyme analysis and determination of biochemical profile of the metabolites

For the determinations of enzymatic activities in the hepatopancreas, an enzyme preparation was carried out previously. This consisted in homogenizing the digestive gland in a buffer Tris-HCl (50 mM, pH 7.5), in a 1:4 ratio (1 g of digestive gland and 4 volumes of Tris-HCl). The homogenates were centrifuged at 20,000 *g* (4°C) for 30 min. Lipid layer was removed, and the supernatant (enzyme extract) was collected and stored at −80°C for enzyme measurement.

2.5.1 | Lipase activity determination

Lipase activity of each enzyme extract was determined according to Versaw, Cuppett, Winters, and Williams (1989). The reaction mixture

consisted of 100 µl of sodium taurocholate (100 mM), 920 µl Tris-HCl (50 mM, pH 8), 10 µl of the enzymatic extract and 10 µl β-naphthyl caprylate as substrate (100 mM dimethyl sulphoxide [DMSO]). The mixture was incubated at 25°C for 30 min. Then, 10 µl of Fast Blue BB (100 mM in DMSO) was added and the mixture was incubated at the same temperature for 5 min. The reaction was stopped with 100 µl trichloroacetic acid (TCA) (0.73 N), clarified with 1.350 ml ethyl acetate:ethanol (1:1 V/V) and the absorbance recorded at 540 nmin Metrolab 330 (made in Argentina). The reference tubes were prepared in a similar manner, but the enzyme extract was subsequently added to the TCA solution.

2.5.2 | Total protease activity determination

The proteolytic activity of the samples was determined according to García-Carreño (1992), using azocasein as substrate. The reaction mixture consisted of 20 µl of enzyme extract, 230 µl of Tris-HCl (50 mM, pH 7.2) and 500 µl of azocasein (0.5% in Tris-HCl). The mixture was incubated at room temperature for 30 min. The reaction was stopped with 500 µl of 20% TCA and clarified by centrifugation (20,000 *g*, 4°C for 30 min). The absorbance was recorded spectrophotometrically in Metrolab 330 (made in Argentina) at 440 nm.

2.5.3 | Amylase activity determination

Amylase activity of each crude extract was determined according to Vega-Villasante et al. (1999), with modifications. The reaction mixture consisted of 500 µl Tris-HCl (50 mM, pH 7.5), 5 µl enzymatic extract and 500 µl of a starch solution (1% in Tris-Cl, 50 mM, pH 7.5). It was incubated at environment temperature for 10 min. Amylase activity was determined by quantifying the production of reducing sugars resulting from the hydrolysis of starch. Immediately after incubation, 200 µl of sodium carbonate (Na_2CO_3) (2 N) and 1.5 ml of dinitrosalicylic acid reagent (DNS) were added to the reaction mixture and boiled at bain-marie for 15 min. The volume was adjusted to 10 ml with distilled water, and the coloured solution was read at 540 nm in Metrolab 330 (made in Argentina).The reference tubes were prepared in a similar manner, but the enzyme extract was subsequently added to the DNS reagent solution.

Specific enzyme activities were expressed as the number of enzyme units per milligram of protein (U/mg protein). A unit of enzyme activity was defined as the amount of enzyme required to increment per minute 0.01 units of optical density at the wavelength corresponding to the evaluated enzyme (López-López et al., 2005).

2.5.4 | Total proteins

Protein determination of enzyme extracts from the digestive gland was performed by the method of Bradford (1976), using bovine serum albumin as standard.

Haemolymph levels of total proteins, cholesterol, triglycerides and glucose were determined with enzymatic colorimetric methods, using the appropriate kits and according to the protocols of the manufacturer (Wiener Lab®). Total plasma protein concentration was measured with a reagent containing the EDTA/Cu complex in an alkaline medium, which reacts with peptide bonds to yield a purple-blue complex (Gasbarro, Bandinelli, & Tomassini, 1972). Plasma levels of total cholesterol and triglycerides were analysed by using a peroxidase-coupled method (Fossatti & Prencipe, 1982; McGowan, Artis, Strandbergh, & Zak, 1983). Plasma glucose was determined by a test based on the glucose oxidase method (Trinder, 1969). The concentrations were expressed in g/L.

Glycogen, lipid and protein contents were measured in muscle. Glycogen was estimated using 60 mg of muscle tissues treated with 1 ml KOH 30% subsequently with 0.5 ml KOH 60% at 100°C. After alkaline tissue disruption, glycogen was precipitated by ethanol, and glucose was determined using the anthrone reagent method according to Seifter, Dayton, Novic, and Muntwyler (1950). Lipid content was extracted using chloroform:methanol (2:1) by the method described by Folch, Lees, and Stanley (1957). Total protein concentration was estimated in tissue homogenates according to Lowry, Rosebrough, Far, and Randall (1951) using bovine serum albumin as standard. Specimens of the same size range were pooled when the amounts of tissue per individual crab were not sufficient. Results were expressed as µmol/g of wet tissue for glycogen and lipids, and as mg/g of wet tissue (gwt) for proteins.

2.6 | Determination of the proximal composition of pelleted diets and the amino acid profile

Analysis of the proximal content of the diets used (Table 1) was carried out according to the standard methods (AOAC, 1990). The moisture content was determined by drying the samples in an oven at 110°C for 24 hr, while the ash content was obtained by calcination of the samples (approximately 2 g) in a muffle, at 550°C for 2 hr. The total nitrogen content was determined by the Kjeldahl method; the crude protein was calculated as %N × 6.25. Lipid content was extracted using chloroform:methanol (2:1) by the method described by Folch et al., (1957).

The content of individual amino acid (AA) was determined according to Alaiz, Navarro, Giron, and Vioque (1992) after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), using α-aminobutyric acid as internal standard. The HPLC system consisted in a Shimadzu Series LC-20AT pump, with Shimadzu SPD-M20A diode array detector, equipped with a 300 × 3.9 mm i.d. reversed-phase column (Novapack C18, 4 µm; Waters). A binary gradient was used for elution with a flow of 0.9 ml/min. The solvents used were sodium acetate (25 mmol/L) containing sodium azide (0.02 g/100 ml) pH 6.0 and acetonitrile. Eluted AA was detected at 280 nm and expressed as mg/100 g d.b using a concentration–response curve of 0–200 nmol/mL.

The nine selected essential amino acids (EAAs) used in the following analysis were chosen according to D'Abramo and New (2010)

and Karasov and Martinez del Río (2007), and were expressed as grams amino acids per 100 g protein. The amino acids that showed values below the levels of detection (not detectable—ND) were assigned with the value of 0.01 in order to perform the calculations described below. Each essential amino acid ratio (A/E) (Arai, 1981) was calculated as a percentage from the total EEA. The essential amino acid index (EAAI) of the three diets was calculated using the muscle tissue of wild *A. uruguayana* (WAU) as the reference profile with the following formula:

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \ldots \times \frac{aa_n}{AA_n}}$$

where aa_1=*A*/*E* ratio in the diet; AA_1 = *A*/*E* in the anomuran; and *n* = number of essential amino acids. A feedstuff considered of good quality should have an EAAI of more than 0.9, those within 0.7 and 0.9 are useful and with less than 0.7, inadequate (Peñaflorida, 1989).

2.7 | Analysis of data

To evaluate the effect of the different diets on the digestive enzymes and on the metabolite profile, one-way ANOVA was carried out, taking as a factor the diet and, later, the Tukey post-test. Furthermore, the amylase/protease ratio was calculated to determine the digestive capacity of organisms for each treatment. A *t* test was performed for each aquarium, in order to know whether the values of the parameters of the aquariums were kept within a similar range. For the comparison among the three aquariums, an ANOVA test of one way was conducted. To verify the correlation between the amino acid profile of the three diets and the muscle tissue of wild specimens and those fed D1, D2 and D3, the Spearman's rank correlation coefficient was applied. Differences were considered statistically significant at $p < .05$. All the statistical analyses were carried out using R software (R Development Core Team, 2008).

3 | RESULTS

3.1 | Enzymatic activity of hepatopancreas

The enzymatic activities of lipases were not significantly different on the different treatments ($p > .05$) (Figure 1a). However, enzymatic activity of total protease ($F = 7.0113$, $p = .006019$) and amylases ($F = 9.2769$, $p = .001297$) was significantly different among the treatments. In the case of total protease activity, it was significantly higher for the diet D2 (8.74 ± 2.84 U/mg protein) compared to diet D3 (3.82 ± 1.53 U/mg protein), but not for diet D1 (5.91 ± 2.28 U/mg protein) (Figure 1b). Amylases reported lower activity in organisms fed with diet D3 (0.39 ± 0.21 U/mg protein) with respect to the other diets (Figure 1c). The calculated values of the amylase/protease ratio between the diets were similar in the animals fed the

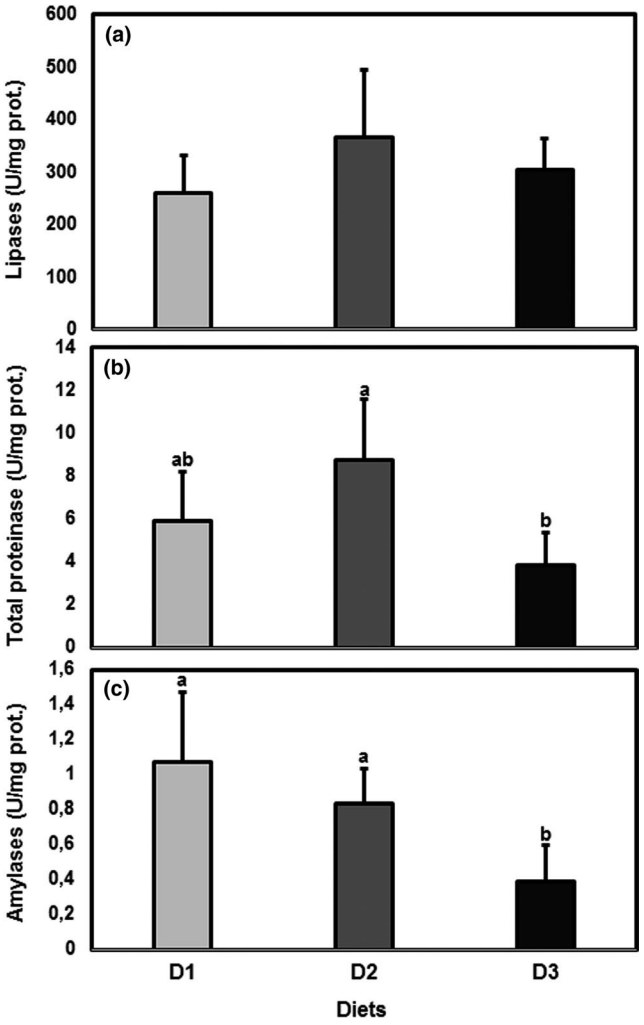

FIGURE 1 Enzymatic activities of lipases (a) (D1 *n* = 9, D2 *n* = 7, D3 *n* = 7), total proteinase (b) (D1 *n* = 8, D2 *n* = 6, D3 *n* = 6) and amylases (c) (D1 *n* = 8, D2 *n* = 6, D3 *n* = 8) in hepatopancreas of *Aegla uruguayana* after being fed with different diets (D1, D2 and D3). Bars representmean +*SD*. Different letters on the bars represent significant differences between the diets

D2 (0.10 ± 0.03) and D3 (0.10 ± 0.04) diets and higher in those fed the D1 diet (0.19 ± 0.03).

3.2 | Biochemical metabolite profile

The amounts of proteins measured in the hepatopancreas of the organisms of each diet group were not significantly different from each other ($p > .05$) (D1: 6.66 ± 1.37; D2: 6.23 ± 1.75; and D3: 7.72 ± 1.91 mg/L).

In the haemolymph, the glucose was mostly concentrated in organisms that were fed with diet D2 (0.09 ± 0.04 g/L) ($F = 4.532$, $p = .02092$), with respect to organisms fed with diet D1 (absence of cellulose) (0.04 ± 0.03 g/L) (Figure 2a).

Regarding lipids, the concentrations of triglycerides were different among organisms subjected to the treatments ($F = 4.0821$, $p = .02872$), indicating that there was greater concentration in organisms fed with diet D2 (0.23 ± 0.07 g/L) than in those that ate the

diet D1 (0.15 + 0.07 g/L) (Figure 2b). In turn, the concentration of cholesterol did not show significant differences among treatments ($p > .05$) (Figure 2c).Total protein content was similar between treatments ($p > .05$) (Figure 2d).

In muscle tissue, the concentration of glycogen followed the same trend as haemolymphatic glucose, indicating that the organisms fed diet D2 had a higher concentration of this metabolite compared to the rest (15.48 ± 5.33 µmol/g wt) ($F = 9.4921$, $p = .0008571$) (Figure 3a). In this same tissue, there were no significant differences in the concentration of lipids or proteins (Figure 3b-c).

3.3 | Amino acid profile

A total of 16 AA (15 plus aspartic and glutamic acids) were identified in the samples analysed (Table 2). The total AA and EAA profile of muscle tissue of WAU was significantly correlated with all diets. However, with regard to the total AA, diet D1 had the lowest *rho* value (Table 3) showing lower amount of proline than WAU (Table 2). The muscle tissues of both wild and fed aeglids were also significantly correlated in all cases with high *rho* values ($\rho > 0.8$) (Table 3). In aeglids fed diet D3 (with the lowest *rho* values) (Table 3), the muscle tissue had proportionally fewer EAA amounts of arginine, methionine and lysine, while the non-EAA proline and aspartic + glutamic

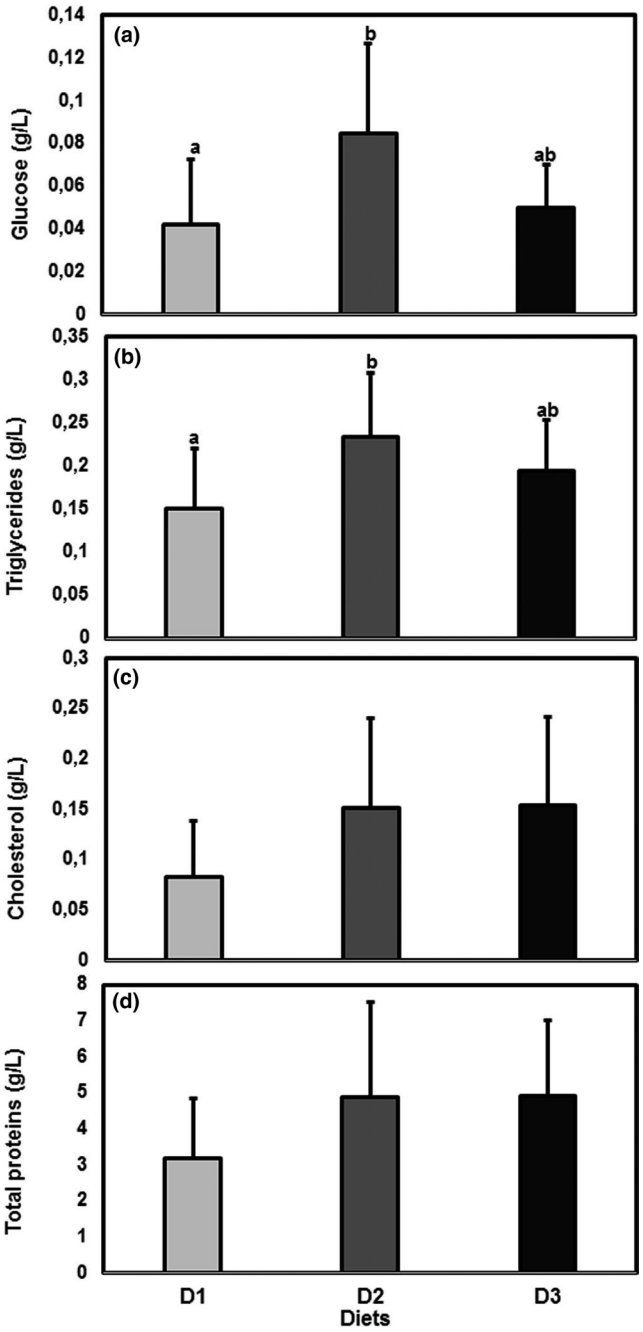

FIGURE 2 Concentrations of glucose (a) (D1 $n = 10$, D2 $n = 10$, D3 $n = 8$), triglycerides (b) (D1 $n = 9$, D2 $n = 10$, D3 $n = 10$), cholesterol (c) (D1 $n = 10$, D2 $n = 8$, D3 $n = 10$) and total proteins (d) (D1 $n = 10$, D2 $n = 8$, D3 $n = 10$) in the haemolymph of *Aegla uruguayana* after being fed with different diets (D1, D2 and D3). Bars representmean +*SD*. Different letters on the bars represent significant differences between the diets

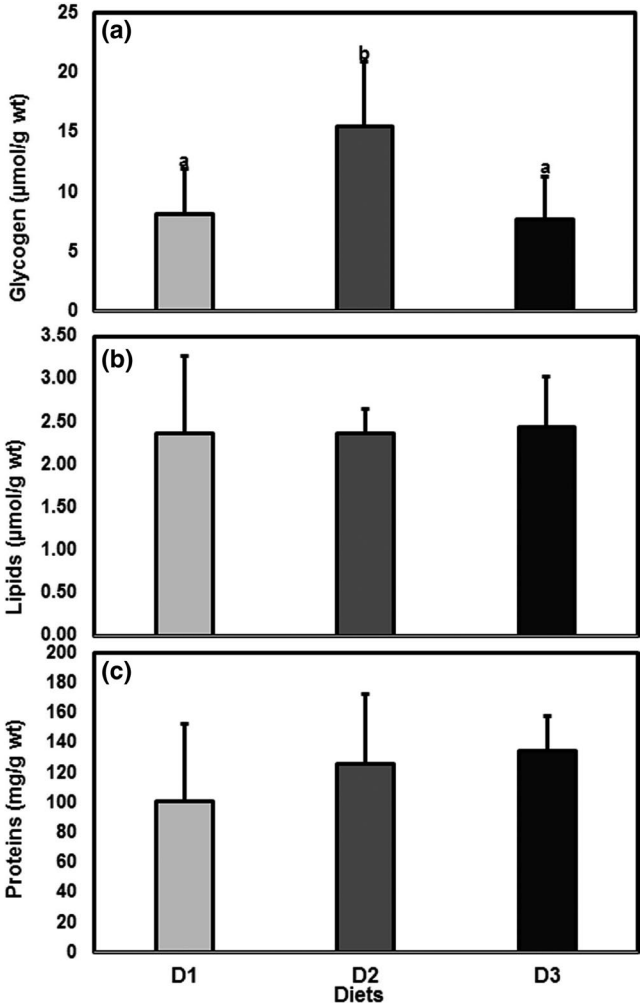

FIGURE 3 Concentrations of glycogen (a) (D1 $n = 11$, D2 $n = 9$, D3 $n = 8$), lipids (b) (D1 $n = 8$, D2 $n = 7$, D3 $n = 11$) and proteins (c) (D1 $n = 10$, D2 $n = 7$, D3 $n = 10$) in the muscle of *Aegla uruguayana* after being fed with different diets (D1, D2 and D3). Bars representmean +*SD*. The letters on the bars represent significant differences between the diets

TABLE 2 Amino acid composition (g AA per 100 g protein) (mean of duplicates ± *SD*) of the three diets proposed and the muscle tissue of *Aegla uruguayana* that were fed these diets

	WAU	AUD1	AUD2	AUD3	D1	D2	D3
Aspartic + glutamic acid	18.4 ± 0.24	23,3 ± 1.29	24.6 ± 3.57	29.4 ± 0.16	22.7 ± 2.25	19.3 ± 2.35	18.7 ± 1.01
Serine	5,6 ± 0.05	5.2 ± 0.05	4.6 ± 0.12	3.41 ± 0.18	6.9 ± 0.05	5.9 ± 0.06	7.4 ± 0.18
Histidine	3,8 ± 0.05	4.5 ± 0.66	4.5 ± 0.20	4.7 ± 1.96	8.7 ± 0.64	7.5 ± 0.28	2.6 ± 0.06
Glycine	8.3 ± 0,09	9.5 ± 0.36	10.9 ± 0.69	10.31 ± 1.49	16.4 ± 0.46	15.8 ± 0.51	15.9 ± 1.47
Threonine	4.9 ± 0.03	5.0 ± 0.10	4.9 ± 0.04	4.65 ± 0.11	6.1 ± 0.07	5.2 ± 0.04	4.8 ± 0.46
Arginine	11.7 ± 0.05	11.0 ± 1.70	9.1 ± 0.31	8.0 ± 0.70	8.9 ± 0.74	9.1 ± 0.07	8.5 ± 0.63
Alanine	8.3 ± 0.02	8.3 ± 0.10	6.8 ± 0.76	7.2 ± 0.04	8.6 ± 0.16	6.8 ± 0.76	9.3 ± 0.73
Proline	9.6 ± 0.75	7.6 ± 0.23	6.4 ± 3.65	5.1 ± 1.26	1.5 ± 0.31	9.2 ± 4.6	15.9 ± 4.36
Tyrosine	4.5 ± 0.16	4.4 ± 0.87	4.3 ± 0.51	3.6 ± 0.11	4.5 ± 0.70	4.5 ± 0.34	3.2 ± 0.11
Valine	3.8 ± 0.37	4.0 ± 0.26	4.1 ± 0.41	5.0 ± 0.11	ND	5.3 ± 0.48	4.9 ± 0.34
Methionine	3.2 ± 0.15	2.7 ± 0.73	1.2 ± 0.09	0.7 ± 0.30	2.1 ± 0.95	2.1 ± 0.17	ND
Cysteine	0.96 ± 0.12	0.8 ± 0.16	0.6 ± 0.07	0.6 ± 0.10	0.6 ± 0.27	0.5 ± 0.07	ND
Isoleucine	4.9 ± 0,01	5.2 ± 0.06	4.4 ± 0.19	4.1 ± 0.38	5.2 ± 0.06	4.6 ± 0.12	4.1 ± 0.08
Leucine	10.1 ± 0.11	9.6 ± 0.34	8.8 ± 0.39	8.4 ± 0.63	9.8 ± 0.34	8.5 ± 0.44	8.7 ± 0.18
Phenylalanine	5.5 ± 0.17	5.6 ± 0.7	5.3 ± 0.26	5.1 ± 0.35	5.9 ± 0.60	5.9 ± 0.34	4.7 ± 0.11
Lysine	10.9 ± 0.62	8.6 ± 0.59	7.9 ± 1.37	5.9 ± 0.13	8.1 ± 0.59	6.0 ± 1.3	8.3 ± 0.19
%NEAA	55.8 ± 0.25	58.9 ± 0.46	58.1 ± 1.57	59.5 ± 0.61	61.15 ± 0.75	62.0 ± 1.67	70.4 ± 1.52
%EAA	58.9 ± 0.20	56.13 ± 0.49	50.1 ± 0.49	46.5 ± 0.58	54.6 ± 0.30	54.2 ± 0.38	46.5 ± 0.20

Abbreviations: AU1, *Aegla uruguayana* fed diet 1; AU2, *Aegla uruguayana* fed diet 2; AU3, *Aegla uruguayana* fed diet 3; D1, diet 1; D2, diet 2; D3, diet 3; ND, not detectable; WAU, wild *Aegla uruguayana*.

TABLE 3 Spearman's rank correlation coefficient of total and essential amino acid profile of *Aegla uruguayana* and each type of diet consumed

	Total amino acids		Essential amino acids	
	Spearman's rho	*p* (uncorr)	Spearman's rho	*p* (uncorr)
D1 and AU1	.68	3.6×10^{-3}	.72	3.1×10^{-2}
D2 and AU2	.82	9.8×10^{-5}	.72	3.1×10^{-2}
D3 and AU3	.86	1.7×10^{-5}	.77	2.1×10^{-2}
AU1 and WAU	.96	2.9×10^{-9}	.97	1.7×10^{-4}
AU2 and WAU	.94	3.9×10^{-8}	.97	1.7×10^{-4}
AU3 and WAU	.82	8.8×10^{-5}	.80	1.1×10^{-2}

Abbreviations: AU1, *Aegla uruguayana* fed diet 1; AU2, *Aegla uruguayana* fed diet 2; AU3, *Aegla uruguayana* fed diet 3; D1, diet 1; D2, diet 2; D3, diet 3; WAU, wild *Aegla uruguayana*.

acids were higher (Table 2). Moreover, the proportion of EAA in these organisms was proportionally lower than in the others fed and wild organisms (Table 2).

Among the 16 AAs, the most abundant EAA in the muscle tissue of aeglids was arginine, leucine and lysine according the E/A ratio, while the non-EAA (NEAA) was aspartic + glutamic acids, glycine, alanine and proline (Table 4). These AAs were also proportionally the most abundant in the three diets, while threonine increased in amount with the decrease of fishmeal inclusion (Table 2). Methionine was the limiting amino acid in all cases, mainly in the muscle tissue of aeglids fed with D3 (Table 4). Figure 4 shows the E/A ratios of each diet (available for nutrition) and that required for the animal (data from the muscle tissue of wild specimens). The diagonal line represents the case in which the feed fulfil ideally the AA requirements of animals. Values above the diagonal value indicate shortage of some AA, while values below, excess. In general, lysine, arginine and methionine were lacking in variable degrees in the three diets (Figure 4). EAAI values of diets D1, D2 and D3 were 0.97, 1.00 and 0.57 respectively.

4 | DISCUSSION

4.1 | Effects on enzymatic activities

The different protein and cellulose levels of the artificial diets affected the digestive total protease and amylase activities of *A. uruguayana*, in

TABLE 4 Essential amino acid (A/E) ratio of muscle tissue of *Aegla uruguayana* and D1, D2 and D3

	WAU	AUD1	AUD2	AUD3	D1	D2	D3
Histidine	8.15	9.87	10.88	12.10	17.03	16.62	6.51
Threonine	10.51	11.04	11.86	12.07	12.01	11.58	11.84
Arginine	24.69	24.29	22.23	20.74	14.94	20.11	21.10
Valine	8.06	8.92	9.97	12.99	10.72	11.74	12.86
Methionine	6.82	6.07	2.82	1.79	4.05	4.71	0.03
Isoleucine	10.43	11.48	10.73	10.60	10,20	10.19	10.32
Leucine	21.44	21.15	21.54	21.85	22.84	18.97	21.6
Phenylalanine	11.53	13.38	13.02	13.23	11.57	12.98	11.64
Lysine	23.06	19.9	19.18	15.36	15.9	13.21	20.67

Note: The ratio values of wild anomurans are included for comparison.

Abbreviations: AU1, *Aegla uruguayana* fed diet 1; AU2, *Aegla uruguayana* fed diet 2; AU3, *Aegla uruguayana* fed diet 3; D1, diet 1; D2, diet 2; D3, diet 3; WAU, wild *Aegla uruguayana*.

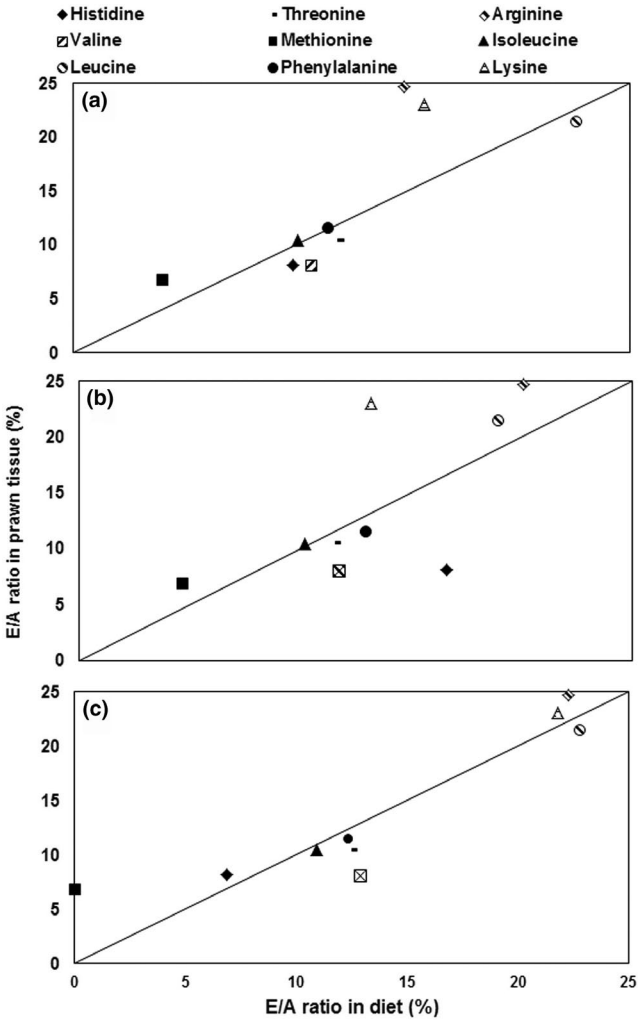

FIGURE 4 Relationship between the EAA profile of muscle tissue of wild *Aegla uruguayana* and diet D1 (a) (n = 3), diet D2 (b) (n = 3) and diet D3 (c) (n = 3) through E/A ratio. The diagonal line represents the perfect balance of amino acids between each diet and the aeglid's tissue

a short period of time. This did not happen with lipases. The lipase activities were similar among treatments. In the organisms under test with diet D2, the enzymatic activities reached the maximum average value of lipases, although no significant differences were recorded. This same trend was registered for *Cherax quadricarinatus* when it was subjected to different supplemental ingredients in practical diets (López-López et al., 2005). This would be an indicator that lipid macromolecules, probably from the ingested diet or adipose reserve tissue, are cleaving or mobilizing to another area of the body in order to meet energy demands (Miled et al., 2000; Rivera-Pérez & García-Carreño, 2011). The amount of triglycerides recorded in the haemolymph would be supporting this assumption (Figure 2b).

In our study, it is possible to think that diet D1 (with higher protein content) caused a effect of inhibition in enzymatic activity in *A. uruguayana*, which would explain the total protease activity values recorded for this diet (Figure 1). Le Moullac et al. (1996)reported that by increasing the level of protein in the diet (casein), the enzymatic activity of trypsin also increased to a certain level, after which the activity stabilized and was inhibited. The authors argue that the inhibition of the synthesis for the high level of casein may be related to an excess of amino acids in the haemolymph, which in turn is highly dependent on the protein source and its digestibility. Similarly, Muhlia-Almazán, García-Carreño, Sánchez-Paz, Yepiz-Plascencia, and Peregrino-Uriarte (2003) showed that in *L. vannamei* fed with a diet containing 30% of proteins exhibited higher trypsin and chymotrypsin activities than those fed 15% or 50% protein.

As mentioned above, the activity of amylases was affected by diet D3 (with lower protein content and higher proportion of cellulose), with less activity in the organisms fed with this diet compared to the rest. On the contrary, the highest activities of these enzymes were recorded in diets D1 and D2, both with higher protein content (Figure 1c). In juveniles of *Litopenaeus stylirostris*, an induction of α-amylase and α-glycosidase enzymes was found related to an increase in carbohydrate levels of the diet, indicating an adaptation of these enzymes to the level of the wheat meal diet (Rosas, Cuzon, Gaxiola, Arena, et al., 2000a).

The data of enzymatic activities recorded indicate that the presence of cellulose together with the decrease of proteins in the diet has significant effects on the proteases and amylases of *A. uruguayana*. In *Macrobrachium rosenbergii*, the increase in cellulose levels in

the diet was associated with a decrease in the enzymatic activity of amylases in hepatopancreas (González-Peña et al., 2002). It was not yet possible to determine the enzymatic activity of the cellulase enzyme in *A. uruguayana* (Musin et al., 2018), so it is expected that this enzyme is present as previously observed in other decapod species (Linton & Greenaway, 2007).

Regarding the amylases/protease, the proportions calculated for each diet indicate that protease enzymatic activity was greater than amylase activity, which would account for a greater use of proteins with respect to metabolized carbohydrates for the amylases.

4.2 | Effects on metabolite profile

When analysing the metabolite profile of haemolymph, an effect of the diet was found in glucose and triglyceride measurements. Particularly, the glucose of the organisms fed with diet D1 was significantly lower than that for organisms fed with diet D2, without significant differences in the measurements corresponding to diet D3. According to the previous studies, it is known those 4 hr after ingestion there is an increase in haemolymphatic glucose and glycogen in the digestive gland (Rosas et al., 1995). Several authors showed that dietary carbohydrate levels affect carbohydrate metabolism in decapods (González-Peña et al., 2002; Rosas, Cuzon, Gaxiola, Pascual, et al., 2000b). In this sense, González-Peña et al. (2002) concluded that glucose levels in the haemolymph of *M. rosenbergii* were higher with increased dietary cellulose. Similar results were found for the species *Aegla platensis* when it was subjected to experimentation with diets rich in carbohydrates and proteins (Ferreira et al., 2005).

For the case of triglycerides, the level of protein and cellulose in the diet had an effect on their concentrations (differences between organisms fed diets D1 and D2). However, for the cholesterol and total proteins there were no effects, reaching the latter the maximum levels in organisms fed with diets D2 and D3. In this case, the increase in proteins in the circulation is possibly a response to the demand of proteins in various tissues, which is necessary to satisfy glycogen synthesis, taking into account the low assimilation of carbohydrates in the diet (Rosas, Cuzon, Gaxiola, Pascual, et al., 2000b). In addition, the increase in the level of proteins circulating in haemolymph in organisms fed diets D2 and D3 may also be due to the presence of cellulose in the diet, which increases the time of gastric retention by improving protein absorption, as González-Peña et al. (2002) concluded.

Considering the metabolite profile in the muscle tissue, a modification was found in the concentration of glycogen and not so for those of lipids and proteins, which were kept in similar conditions for each case. The muscle glycogen measured in organisms fed with low dietary cellulose was significantly lower than that measured in organisms that were fed with the intermediate amount of this nutrient. This would also be an evidence of the effect of the dietary content of cellulose on the storage of glycogen, as was also recorded for *A. platensis* (Ferreira et al., 2005). It is necessary to bear in mind that the mobilization of metabolites in muscle tissue, for the short time of the trial, is not a direct reflection of the composition of the diet (De Vries, del Rio, Tunstall, & Dawson, 2015). In this case, it may be that in the muscle are being mobilized elements necessary for a normal metabolism that are not provided by the diets tested.

4.3 | Effects on amino acid profile of muscle tissue

The amino acid profile of muscle tissue of wild and fed aeglids had high and significant correlation in all cases showing, in general, few changes in the short-term feeding regime. However, some changes were observed mainly in aeglids fed diet D3, which exhibited lowest amounts of methionine, lysine and arginine and highest of aspartic + glutamic acids. On the one hand, diet D3 had the lower inclusion of fishmeal and, consequently, the lower total proteins available to animals and proportionally lower EAA. Indeed, methionine was the most limiting EAA of diet D3. The deficiency of this EAA in the diet could limit its participation in protein synthesis and reduce the pool of this AA in muscle (Mai, Wan, et al., 2006a; Wilson, 2002). The deficiency of arginine and lysine could affect the growth performance once arginine is the most versatile AA, participating in many metabolic pathways (i.e. growth and development regulation, energy utilization), and lysine is involved in the transport of long-chain fatty acids for oxidation (Li, Kangsen, Trushenski, & Wu, 2009). On the other hand, aspartic + glutamic acids are ubiquitous in animal tissue (van Waarde, 1988) and the excess of these AA in aeglids fed diet D3 could be indicating higher transamination reactions of amino groups from most amino acids (Hill, Wyse, & Anderson, 2012).

According to the E/A ratio, not only diet D3 had AA deficiency, at least three AAs were in shortage in all diets. Among them, methionine and lysine are often the most limiting AA in ingredients of feedstuffs (Mai, Wan, et al., 2006a; Mai, Zhang, et al., 2006b). The requirements of methionine can be partially spared by cysteine because it can be synthetized from methionine, reducing the amount of this EAA in protein synthesis (Wilson, 2002). Although cysteine is a NEAA, its amounts in the diets proved in this study were low in comparison with the muscle tissue of aeglids, indicating a nutritional deficiency of the formulated diets. The requirement of lysine and arginine by *A. uruguayana* is high if compared with other crustaceans. While in this aeglid both AA are around 11% of the total AA, in other species, such as *Portunus tuberculatus* and *Penaeus monodon*, the requirements of these AA are minor (Jin et al., 2015; Millamena, Bautista-Teruela, Reyes, & Kanazawab, 1998). Even though methionine and lysine are commonly limiting amino acids in feedstuffs, in *A. uruguayana*, the deficiency may be greater. These results will be very important to identify the limiting amino acids in this species, enabling the formulation of a diet that meets the amino acid requirements of this anomuran species in a culture system. In this context, the EAAI is a useful tool to evaluate the adequacy of a determined diet. In the present study, this index indicated that diets D1 and D2 had good quality of EAA, while D3 can be considered inadequate (Peñaflorida, 1989), emphasizing what has been previously stated.

5 | CONCLUSIONS

It could be said that *A. uruguayana* shows a differential response to different proportions of dietary cellulose and protein and that has the capacity so both the activities of its digestive enzymes and its metabolite profile are modified according to the components of the diet. Nonetheless, given certain proportions of these ingredients (protein–cellulose) this species does not seem to have the ability to make better use of these nutrients. In this sense, we concluded that *A. uruguayana* exhibited a better use of the nutrients formulated for diet D2. In addition, this feedstuff had a good quality of EAA, fulfilling almost all requirements of a wild aeglid. However, some EAA appeared in high proportion in the muscle of these animals, emphasizing the need to look for sources of protein or other supplements in order to meet the species requirements.

ACKNOWLEDGMENTS

This work was supported by Pip 790 CONICET, PICT 2014-1996 and PICT 2016-2542.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study. All relevant data are within the paper.

ORCID

Débora A. Carvalho https://orcid.org/0000-0001-8278-4416
Verónica Williner https://orcid.org/0000-0001-7950-6968

REFERENCES

Alaiz, M., Navarro, J., Giron, J., & Vioque, E. (1992). Amino acid analysis by high performance liquid chromatography after derivatization with diethylethoxymethylene-malonate. *Journal of Chromatography*, *591*, 181–186. https://doi.org/10.1016/0021-9673(92)80236-N

AOAC (1990). *Official methods of analysis*, 15th ed. Washington, DC: Association of Official Analytical Chemist.

Arai, S. (1981). A purified diet for coho salmon, *Oncorhynchus kisutch*. *Fry Bulletin of the Japanese Society of Scientific Fisheries*, *47*(4), 547–550. https://doi.org/10.2331/suisan.47.547

Bond-Buckup, G., & Buckup, L. (1994). A família Aeglidae (Crustacea, Decapoda, Anomura). *Arquivos De Zoologia*, *32*(4), 159–346.https://doi.org/10.11606/issn.2176-7793.v32i4p159-346

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Collins, P. A. (1997). Cultivo del camarón *Macrobrachium borellii* (Crustacea: Decapoda: Palaemonidae), con dietas artificiales. *Natura Neotropicalis*, *28*(1), 39–45. https://doi.org/10.14409/natura.v1i28.3695

Collins, P. A., Carnevali, R., Carvalho, D., & Williner, V. (2012).Dynamics of decapod crustaceans in a trophic web of continental aquatic environments in Southern South America.In J. A. Daniels (Ed.), *Advances in environmental research* (Vol. *21*, pp. 159–185). Hauppauge, NY: Nova Publishers.

Collins, P. A., & Petriella, A. M. (1996). Crecimiento y supervivencia del camarón *Macrobrachium borellii* (Decapoda: Palaemonidae) alimentado con dietas artificiales. *Neotropica*, *42*(107–108), 3–7.

Collins, P., Williner, V., & Giri, F. (2007a). Trophic relationships in crustacean decapods of a river with a floodplain. In A. M. T. Elewa (Ed.), *Predation in organisms. A distinct phenomenon* (pp. 59–85). Berlin Heidelberg: Springer – Verlag.

Collins, P., Williner, V., & Giri, F. (2007b). Littoral communities. Macrocrustaceans. In M. H. Iriondo, J. C. Paggi, & M. J. Parma (Eds.), *The Middle Paraná River: Limnology of a subtropical wetland* (pp. 277–302). Berlin Heidelberg: Springer – Verlag.

Cowey, C. B., & Tacon, A. G. J. (1983). Fish nutrition-relevance to marine invertebrates. In G. D. Pruder, C. J. Langdon, & D. E. Conklin (Eds.), *Proceedings of the second international conference on aquaculture nutrition: biochemical and physiological approaches to shellfish nutrition*, (p. 13). Baton Rouge, LA: Louisiana State University, Division of Continuing Education.

D'Abramo, L. R., & New, M. B. (2010). Nutrition, feeds and feeding. In M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, & M. N. Kutty (Eds.), *Freshwater prawns: Biology and farming*. Chichester, UK: Wiley-Blackwell.

De Vries, M. S., del Rio, C. M., Tunstall, T. S., & Dawson, T. E. (2015). Isotopic incorporation rates and discrimination factors in mantis shrimp crustaceans. *PLoS ONE*, *10*(4), e0122334. https://doi.org/10.1371/journal.pone.0122334

Devercelli, M., & Williner, V. (2006). Diatom grazing by *Aegla uruguayana* (Decapoda: Anomura: Aeglidae): Digestibility and cell viability after gut passage. *Annales De Limnologie - International Journal of Limnology*, *42*(2), 73–77. https://doi.org/10.1051/limn/2006011

Diawol, V. P., & Collins, P. A. (2012). Caracterización de los estadíos del ciclo de muda del pseudocangrejo dulceacuícola Aegla uruguayana Schmitt, 1942 (Decapoda, Anomura). *Natura Neotropicalis*, *43*(1–2), 21–28. https://doi.org/10.14409/natura.v1i43.4280

FAO (2006). *Programa de información de especies acuáticas. Penaeus vannamei*. Departamento de Pesca y Acuicultura de la FAO. Retrieved from http://www.fao.org/fishery/culturedspecies/Penaeus_vannamei/es

Fenucci, J. L., Petriella, A. M., & Müller, M. I. (1981). Efectos de alimentación natural y artificial en el crecimiento del camarón *Artemesia longinaris*. *Revista Latinoamericana De Acuicultura*, *10*, 10–17.

Ferreira, B. D. P., Hack, C., deOliveira, G. T., & Bond-Buckup, G. (2005). Perfil metabólico de *Aegla platensis* Schmitt, (Crustacea, Aeglidae, Anomura) submetida a dietas ricas em carboidratos ou proteínas. *Revista Brasileira De Zoologia*, *22*(1), 161–168. https://doi.org/10.1590/S0101-81752005000100018

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Biological Chemistry*, *226*, 497–509.

Fossatti, P., & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, *28*, 2077–2080.

García-Carreño, F. L. (1992). The digestive proteases of langostilla (*Pleuroncodes planipes*, Decapoda): Their partial characterization, and the effect of feed on their com-position. *Comparative Biochemistry and Physiology B*, *103*(3), 575–578. https://doi.org/10.1016/0305-0491(92)90373-Y

Gasbarro, L., Bandinelli, R., & Tomassini, G. (1972). A new unified biuret reagent stabilized with EDTA. *Clinica Chimica Acta*, *36*, 275.

González-Peña, M. C., Anderson, A. J., Smith, D. M., & Moreira, G. S. (2002). Effect of dietary cellulose on digestion in the prawn *Macrobrachium rosenbergii*. *Aquaculture*, *211*, 291–303. https://doi.org/10.1016/S0044-8486(02)00129-1

Hill, R. W., Wyse, G. A., & Anderson, M. (2012). *Animal physiology*, 3rd ed. Sunderland, MA: Sinauer Associates.

Hooshmand, H., Shabanpour, B., Moosavi-Nasab, M., & Golmakani, M. T. (2017). Optimization of carotenoids extraction from blue crab (*Portunus pelagicus*) and shrimp (*Penaeus semisulcatus*) wastes using organic solvents and vegetable oils. *Journal of Food Processing and Preservation*, *41*(5), e13171. https://doi.org/10.1111/jfpp.13171

Jin, M., Wang, M.-Q., Huo, Y.-W., Huang, W.-W., Mai, K.-S., & Zhou, Q.-C. (2015). Dietary lysine requirement of juvenile swimming crab, *Portunus trituberculatus*. *Aquaculture*, *448*, 1–7. https://doi.org/10.1016/j.aquaculture.2015.05.021

Karasov, W. H., & Martínez del Río, C. (2007). *Physiological ecology*. Princeton, NJ: Princeton University Press.

Kaushik, S. J. (2000). *Feed formulation, diet development and feed technology. Recent advances in Mediterranean aquaculture finfish species diversification* (Vol. *47*, pp. 43–51). Zaragoza, Spain: CIHEAM (Cahiers Options Méditerranéennes).

Krebs, C. J. (1994). *Ecology: The experimental analysis of distribution and abundance*. New York, NY: Harper Collins College Publisher.

Lall, S. P., & Dumas, A. (2015).Nutritional requirements of cultured fish: Formulating nutritionally adequate feeds. In D. A. Davis (Ed.), *Feed and feeding practices in aquaculture*, (pp. 53–109). Oxford, UK: Woodhead Publishing Series in Food Science, Technology and Nutrition.

Le Moullac, G., Klein, B., Sellos, D., & Van Wormhoudt, A. (1996). Adaptation of trypsin, chymotrypsin and α-amylase to casein level and protein source in *Penaeus vannamei* (Crustacea Decapoda). *Journal of Experimental Marine Biology and Ecology*, *208*, 107–125. https://doi.org/10.1016/S0022-0981(96)02671-8

Li, P., Kangsen, M., Trushenski, J., & Wu, G. (2009). New developments in fish amino acids nutrition: Towards functional and environmentally aquafeeds. *Amino Acids*, *37*, 43–53. https://doi.org/10.1007/s00726-008-0171-1

Lim, K. C., Yusoff, F. M., Shariff, M., & Kamarudin, M. S. (2017). Astaxanthin as feed supplement in aquatic animals. *Reviews in Aquaculture*, *10*(3), 738–773. https://doi.org/10.1111/raq.12200

Linton, S. M., & Greenaway, P. (2007). A review of feeding and nutrition of herbivorous land crabs: Adaptations to low quality plant diets. *Journal of Comparative Physiology B*, *177*, 269–286. https://doi.org/10.1007/s00360-006-0138-z

López-López, S., Nolasco, H., Villarreal-Colmenares, H., & Civera-Cerecedo, R. (2005). Digestive enzyme response to supplemental ingredients in practical diets for juvenile freshwater crayfish *Cherax quadricarinatus*. *Aquaculture Nutrition*, *11*(2), 79–85. https://doi.org/10.1111/j.1365-2095.2004.00305.x

Lowry, O. H., Rosebrough, M. J., Far, A. L., & Randall, R. L. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Mai, K., Wan, J., Ai, Q., Xu, W., Liufu, Z., Zhang, L., …Li, H. (2006a). Dietary methionine requirement of juvenile yellow croaker *Pseudosciaena crocea* R. *Aquaculture*, *251*, 564–572. https://doi.org/10.1016/j.aquaculture.2005.08.010

Mai, K., Zhang, L., Ai, Q., Duan, Q., Zhang, C., Li, H., …Liufu, Z. (2006b). Dietary lysine requirement of juvenile seabass (*Lateolabrax japonicus*). *Aquaculture*, *258*, 535–542. https://doi.org/10.1016/j.aquaculture.2006.04.043

Mármol, Z., Gutiérrez, E., Páez, G., Ferrer, J., & Rincón, M. (2004). Desacetilación termoalcalina de quitina de conchas de camarón. *Multiciencias*, *4*(2), 1–10.

McGowan, M., Artis, J., Strandbergh, D., & Zak, B. (1983). A peroxidase coupled method for the colorimetric determination of serum triglycerides. *Clinical Chemistry*, *29*, 538–542.

Miled, N., Canaan, S., Dupuis, L., Roussel, A., Rivière, M., Carrière, F., …Verger, R. (2000). Digestive lipases: From three-dimensional structure to physiology. *Biochimie*, *82*, 973–986. https://doi.org/10.1016/S0300-9084(00)01179-2

Millamena, O. M. M., Bautista-Teruela, M. M., Reyes, O. S., & Kanazawab, A. (1998). Requirements of juvenile marine shrimp, *Penaeus monodon* (Fabricius) for lysine and arginine. *Aquaculture*, *164*, 95–104. https://doi.org/10.1016/S0044-8486(98)00179-3

Muhlia-Almazan, A., García-Carreño, F. L., Sánchez-Paz, J. A., Yepiz-Plascencia, G., & Peregrino-Uriarte, A. B. (2003). Effects of dietary protein on the activity and mRNA level of trypsin in the midgut gland of the white shrimp *Penaeus vannamei*. *Comparative Biochemistry and Physiology B*, *135*, 373–383. https://doi.org/10.1016/S0044-8486(98)00179-3

Musin, G. E., Rossi, A., Diawol, V., Collins, P. A., & Williner, V. (2017). Dynamic metabolic pattern of *Aegla uruguayana* (Schmitt, 1942) (Decapoda: Anomura: Aeglidae): Responses to seasonality and ontogeny in a temperate freshwater environment. *Journal of Crustacean Biology*, *37*(4), 436–444. https://doi.org/10.1093/jcbiol/rux048

Musin, G. E., Rossi, A., Diawol, V., Collins, P. A., & Williner, V. (2018). Development of enzymes during ontogeny of two freshwater Decapoda: *Aegla uruguayana* (Aeglidae) and *Macrobrachium borellii* (Palaemonidae). *Aquaculture Research*, *49*, 3889–3897. https://doi.org/10.1111/are.13858

Oliveira, G. T., Hack, C., Almerão, M., Bond-Buckup, G., & Dutra, B. K. (2011). Tissue composition and haemolymphatic metabolites during gonadal development in *Aegla platensis* (Crustacea, Decapoda) maintained in experimental culture. *Revista Brasileira De Biociências*, *9*(1), 64–71.

Panne Huidobro, S. (2010). *Organismos acuáticos ornamentales: su importación y exportación en el 2009*. Informe de la Dirección de Acuicultura Subsecretaría de Pesca y Acuicultura – Ministerio de Agroindustria de la Presidencia de la Nación Argentina.

Panne Huidobro, S. (2014). *Estadísticas de importación y exportación de organismos acuáticos ornamentales durante el año 2014*. Informe de la Dirección de Acuicultura Subsecretaría de Pesca y Acuicultura – Ministerio de Agroindustria de la Presidencia de la Nación Argentina.

Pavasovic, A., Anderson, A. J., Mather, P. B., & Richardson, N. A. (2007). Effect of a variety of animal, plant and single cell-based feed ingredients on diet digestibility and digestive enzyme activity in redclaw crayfish, *Cherax quadricarinatus* (Von Martens 1868). *Aquaculture*, *272*(1–4), 564–572. https://doi.org/10.1016/j.aquaculture.2007.08.027

Peñaflorida, V. (1989). An evaluation of indigenous protein source as potential component in the diet formulation for tiger prawn, *Penaeus monodon*, using essential amino acid index (EAAI). *Aquaculture*, *83*, 319–330. https://doi.org/10.1016/0044-8486(89)90043-4

Phillips, H., Hanson, T. R., Dasgupta, S., & Ohs, C. (2010). Marketing and preparation for consumption. In M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, & M. N. Kutty (Eds.), *Freshwater prawns. Biology and farming* (pp. 420–447). West Sussex, UK: Wiley-Blackwell.

Pillay, T. V. R., & Kutty, M. N. (2005). *Aquaculture. Principles and practices*, 2nd ed. Ames, IA: Blackwell Publishing Ltd.

R Development Core Team (2008). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Ribeiro, E. A., Genofre, G. C., & McNamara, J. C. (2001). Identification and quantification of carotenoid pigments during the embryonic development of the freshwater shrimp *Macrobrachium olfersii* (Crustacea, Decapoda). *Marine and Freshwater Behaviour and Physiology*, *34*(2), 105–116. https://doi.org/10.1080/10236240109379063

Ricklefs, R. E. (2008). *The economy of nature*, 6th ed. New York, NY: W.H. Freeman and Company.

Rivera-Pérez, C., & García-Carreño, F. (2011). Effects of fasting on digestive gland lipase transcripts expression in *Penaeus vannamei*. *Marine Genomics*, *4*, 273–278. https://doi.org/10.1016/j.margen.2011.07.002

Rosas, C., Bolongaro-Crevenna, A., Sanchez, A., Gaxiola, G., Soto, L., & Escobar, E. (1995). Role of digestive gland in the energetic metabolism of *Penaeus setiferus*. *The Biological Bulletin*, *189*(2), 168–174.

Rosas, C., Cuzon, G., Gaxiola, G., Arena, L., Lemaire, P., Soyez, C., & Van Wormhoudt, A. (2000a). Influence of dietary carbohydrate on the

metabolism of juvenile *Litopenaeus stylirostris*. *Journal of Experimental Marine Biology and Ecology*, *249*(2), 181–198. https://doi.org/10.1016/S0022-0981(00)00184-2

Rosas, C., Cuzon, G., Gaxiola, G., Pascual, C., Brito, R., Chimal, M. E., & Van Worhoudt, A. (2000b). El Metabolismo de los carbohidratos de *Litopenaeus setiferus*, *L. vannamei* y *L. stylirostris*. In L. E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M. A. Olvera-Novoa, & R. Civera-Cerecedo (Eds.), *Avances en nutrición acuícola V*. Memorias del V Simposium Inter-nacional de Nutrición Acuícola, Mérida, Yucatán.

Sacristán, H. J., Fernández Gimenez, A. V., Chaulet,A., Franco Tadic, L. M., Fenucci, J., & López Greco, L. S. (2016). Effect of different diets on digestive enzyme activities, in vitro digestibility, and midgut gland structure in juvenile crayfish, Cherax quadricarinatus. *Acta Zoologica*, *97*(4), 407–418. https://doi.org/10.1111/azo.12134

Seifter, S., Dayton, S., Novic, B., & Muntwyler, E. (1950). The estimation of glycogen with the anthrone reagent. *Archives of Biochemistry and Biophysics*, *25*, 191–200.

Silva-Castiglioni, D., Valgas, A. A. N., Machado, I. D., Freitas, B. S., & Oliveira, G. T. (2015). Effect of different starvation and refeeding periods on macromolecules in the haemolymph, digestive parameters, and reproductive state in Aegla platensis (Crustacea, Decapoda, Aeglidae). *Marine and Freshwater Behaviour and Physiology*, *49*(1), 27–45. https://doi.org/10.1080/10236244.2015.1099205

Sneddon, L. U. (2015). Pain in aquatic animals. *Journal of Experimental Biology*, *218*, 967–976. https://doi.org/10.1242/jeb.088823

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry*, *6*, 24–27.

Tumini, G., Giri, F., Williner, V., & Collins, P. A. (2016). The importance of biogeographical history and extant environmental conditions as drivers of freshwater decapod distribution in southern South America. *Freshwater Biology*, *61*(5), 715–728. https://doi.org/10.1111/fwb.12742

van Waarde, A. (1988). Biochemistry of non-protein nitrogenous compounds in fish including the use of amino acids for anaerobic energy production. *Comparative Biochemistry and Physiology B*, *91*, 207–228. https://doi.org/10.1016/0305-0491(88)90136-8

Vega-Villasante, F., Fernández, I., Preciado, R. M., Oliva, M., Tovar, D., & Nolasco, H. (1999). The activity of digestive enzymes during the molting stages of the arched swimming *Callinectes arcuatus* Ordway, 1863 (Crustacea: Decapoda: Portunidae). *Bulletin of Marine Science*, *65*(1), 1–9.

Velurtas, S. M., Diaz, A. C., Fernandez Gimenez, A. V., & Fenucci, L. J. (2011). Influence of dietary starch and cellulose levels on the metabolic profile and apparent digestibility in penaeoid shrimp. *Latin American Journal of Aquatic Research*, *39*(2), 214–224. https://doi.org/10.4067/S0718-560X2011000200003

Versaw, W. K., Cuppett, S. L., Winters, D. D., & Williams, L. E. (1989). An improved colorimetric assay for bacterial lipase in nonfat dry milk. *Journal of Food Science*, *54*(6), 1557–1558. https://doi.org/10.1111/j.1365-2621.1989.tb05159.x

Williner, V. (2010). Foregut ossicles morphology and feeding of the freshwater anomuran crab *Aegla uruguayana* (Decapoda, Aeglidae). *Acta Zoologica*, *91*(4), 408–415. https://doi.org/10.1111/j.1463-6395.2009.00416.x

Williner, V., Giri, F., & Collins, P. A. (2011). Metapopulations of decapods in the floodplain of Paraná River, South America. In M. A. Álvarez (Ed.), *Floodplains: Physical geography, ecology and societal interactions* (pp. 179–199). New York, NY: Nova Publishers.

Wilson, R. P. (2002). Amino acids and proteins.In J. E. Halver, & R. W. Hardy (Eds.), *Fish nutrition* (pp. 143–179). New York, NY: Academic Press.

How to cite this article: Musin GE, Carvalho DA, Viozzi MF, Mora MC, Collins PA, Williner V. Protein and cellulose level in diet: Effects on enzymatic activity, metabolite and amino acid profiles in freshwater anomurans *Aegla uruguayana* (Decapoda: Anomura). *Aquac Res*. 2019;00:1–12. https://doi.org/10.1111/are.14474