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Research Article

Seasonal Variation in Fatty Acid and Amino Acid Composition of the Patagonian Marine Polychaete *Abarenicola pusilla* and Its By-Products

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This study aims to provide a quantitative analysis of the amino acids (AAs) and fatty acids (FAs) found in by-products of the Patagonian marine polychaete *Abarenicola pusilla*. Samples of polychaetes were taken in different seasons and processed to generate three by-products: eviscerated whole body, press cake, and press liquid. The results showed a clear seasonal difference, with essential nutrients decreasing significantly in winter. For example, from eviscerated whole body specimens, essential fatty acids, eicosapentaenoic acid (EPA), and arachidonic acid (ARA) maintained a relationship of 1 in summer, 1.24 in winter, and 1.5 in spring and autumn. In comparison, docosahexaenoic acid (DHA) was undetected in some extracts in winter. However, the fatty acid content in press liquid resulted in high variation, with the lowest content during the autumn. In contrast, the press liquid and press cake responded similarly. DHA decreased from spring to winter (from 0.1 to 0.08), whereas EPA resulted in high variation along the seasons (from 0.57 to 0.03) from summer to autumn. The highest protein values were observed in the spring and summer. In contrast, lipid values remained under 10% constant throughout all seasons, unless in the eviscerated carcass, where 5 to 3% higher lipids were found, indicating that *A. pusilla* uses protein more efficiently than lipids as an energy source. The most abundant EAAs were lysine (LYS) and leucine (LEU), while glycine (GLY), glutamic acid (GLU), and alanine (ALA) were the most abundant NEAAs. Finally, *A. pusilla* harvest conditions and the type of extract recommended to produce a valuable ingredient for marine aquafeeds are discussed.

1. Introduction

With the increased global aquaculture production and the high competition for feed ingredients for marine aquaculture production, new local ingredients and additives are required to fulfill the nutrient requirement[1]. Therefore, research on new species of microalgae, yeast, and insects, among others, is needed to identify other potential ingredient sources [2–5]. Among these novel ingredients, the by-products obtained from marine polychaetes could be of interest if some species can be successfully cultured [6]. In nature, deposit-feeding polychaetes play an essential role in degrading mudflats, so a controlled culture is needed to produce sufficient biomass and avoid harvesting from natural populations. Furthermore, due to their biochemical composition and detritivorous capabilities, they have been used as bait [7, 8], for sludge treatment of marine recirculation systems [6, 9], as feed for *Panulirus ornatus* juveniles [10], and as an ovarian maturation stimulant for shrimp [11, 12]. According to Gómez et al. [13], *A. pusilla* is

an efficient remediating species for reducing organic waste in RAS aquaculture sludge with a density of 200 organisms m^{-2}, representing a conversion efficiency of 4.22 g of total organic matter per g *A. pusilla*. This result shows that *A. pusilla* efficiently removes higher organic matter than other works reported [14]. However, since the chemical composition of polychaetes is subject to spatial and temporal variation, their nutritional properties could be related to seasonal characteristics, sediment features, hydrodynamic stress [38], or proximity to aquaculture farms [38]. Therefore, this study aims to compare and analyze the amino acid and fatty acid contents of *Abarenicola pusilla*by-products throughout the year. The species A. *pusilla* is a marine polychaete distributed from 29°S to Southern Patagonia (41°S) along the Chilean coast [17] that has reproduced under controlled conditions in the laboratory and its possible aquaculture applications (Valenzuela et al.). The knowledge obtained from this research will be an essential contribution to the best practices for rearing *A. pusilla* as an accessible ingredient of high nutritional value in the aquaculture feed sector in Chile. However, even if Polychaeta are not produced massively under laboratory conditions, they could also be grown together with feces mixed with mud as coculture, which is a fact that could improve the water quality of aquaculture effluents.

2. Materials and Methods

Two hundred adult, *A. pusilla* individuals, were randomly sampled in April (autumn), August (winter), November (spring), and January (summer) in Quetalmahue close to Ancud in Chile (41°51′S-41°85′S, 73°49′W-73°96′W). The bristle worms were transported to the facilities of the Marine Invertebrates Hatchery (HIM) of the Universidad Austral de Chile, located nearby Puerto Montt, and maintained in flat-bottom tanks with an open seawater flow of 100 $L{\cdot}h^{-1}$, with dissolved oxygen over 8 $mg{\cdot}L^{-1}$, and at natural temperature and photoperiod for 24 h. Next, they were euthanized by hypothermia, eviscerated, and immediately processed. Three random samples of the whole body, eviscerated specimens, were pooled and stored frozen (−20°C) for further biochemical analysis. In each sampled period, the remaining *A. pusilla* samples were pressed to extract the liquid (press liquid) and solid portions (press cake). The samples of each extract were maintained at −20°C until analysis.

2.1. Fatty Acid Analysis. The total lipid content and the fatty acid quantification profile were performed in duplicate on 10 mg freeze-dried samples by following the method described by Farías et al. [18]. Total lipids were extracted according to Folch et al. [19] using methanol and chloroform, and further methylation followed the method described by Gordon Bell et al. [20]. Fatty acid methyl esters (FAMEs) were analyzed using gas chromatography equipped with a flame ionization detector (Thermo Fisher Scientific Inc., Waltham, MA, USA) and an autosampler. Separation was performed using hydrogen as a carrier gas in a 100 m RESTEC RT-2560 capillary column (0.25 mm internal diameter, 0.2 m split thick film). An initial temperature of 140°C was maintained for five minutes before starting a ramp up to 240°C for 20 minutes (5°C min^{-1}). Then, the temperature was maintained at 240°C for the last 20 min, whereas the detector was set at 260°C. Nonadecanoic acid (19 : 0) was used as an internal standard. FAMEs were identified by comparing retention times with those found in the Supelco 37 FAME Mix standard (Sigma-Aldrich ChemieGmbH, Buchs, Switzerland). The amount of each FAME was calculated and reported as milligrams of FAME per Gram of dry matter.

2.2. Protein and Amino Acid Analysis. Samples from each season and product type were analyzed for crude protein. In summary, carbon, hydrogen, and nitrogen of samples were analyzed using a CHN analyzer (LECO CHN-900) with a 1 mg freeze-dried sample weighed in a microbalance (±0.001 mg; METTLER-TOLEDO XP2U), and the crude protein content was calculated by nitrogen conversion (% $N \times 6.25$).

For the amino acid analysis, freeze-dried 50 mg samples were hydrolyzed using 6N HCl containing 0.06% phenol in a closed vial and heated to 110°C for 24 h. The samples were chromatographed through a reverse-phase column (3.9 × 150 mm), 4μm AccQ·Tag™ C-18 (Waters, Milford, MA, USA), using the water-acetonitrile gradient recommended by the Waters AccQ·Tag™ system. A fluorescence detector was set up for an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Analyses were run at a constant temperature of 39°C. Calibration and standard curves were obtained using an amino acid standard solution at three concentrations ranging from 18.75 to 150 pmol of each amino acid. The amount of each AA was calculated and reported as a percentage of each AA in the dry matter.

2.3. Statistical Analysis. Data for proximate and fatty acid composition were tested for normal distribution and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. In addition, the one-way analysis of variance (ANOVA) was applied when the data proved normality. At the same time, means were compared with Tukey's multiple range test, while nonparametric data were analyzed using the Kruskal-Wallis test. Differences were reported as statistically significant when $p < 0.05$. All the statistical analyses were performed according to the method by Sokal and Rohlf [21]. The amino acid composition corresponded to a pooled sample per season; statistical analyses were not run, and qualitative results are presented. Therefore, the graphs of AA considered the annual average among the four seasonal samples.

2.4. Ethical Statement. Protocols involving animal work were strictly conducted according to the ethics statement from the Universidad Austral de Chile (UACh) approved by Decree 95 of 2020 (https://adminvidca.uach.cl/wp-content/uploads/2022/05/Decreto-95-de-2020.pdf) and the ANID 2022 guidelines on the protection of animals for scientific

purposes (https://adminvidca.uach.cl/wp-content/uploads/2022/05/Lineamientos-Bioeticos-para-la-Investigacio%CC%81n-con-Animales-ANID.pdf). All procedures were approved and supervised by the Bioethical Committee of the Universidad Austral de Chile (VIDCA, UACh).

3. Results

Lipids from the eviscerated whole body *(A. pusilla)* showed a highly significant difference among seasons ($F3, 6 = 14.40$, $p = 0.004$). Higher values were observed during autumn ($12.3 \pm 0.6\%$ dry weight) than those in the rest of the seasons (Figure 1). The liquid and press cake showed similar seasonal values of crude fat with averages of 7.8 ± 0.05 and $7.6 \pm 0.9\%$, respectively (Figure 1).

Regarding the fatty acid content classes in different samples, the FAs $16:0$, $16:1n-7$, $18:0$, $18:1n-9$, and $20:1n-9$ explained a maximum of 65% and 63% of total FA in winter in the eviscerated whole body and press cake samples, respectively (Tables 1and2), and 60% in spring for press liquid (Table 3). However, in the eviscerated whole body, total highly unsaturated fatty acids (HUFA), also called long-chain PUFA (LC-PUFA), varied from 1.6% in autumn to 13.9% in summer ($F_{3,4} = 8.50$; $p = 0.03$). Alternatively, for press liquid, total saturated fatty acids (SFAs) varied from 40.1% in summer to 51.4% in winter ($F_{3,4} = 7.10$; $p = 0.04$), while total PUFAs varied from 11.6% in winter to 21.3% in summer ($F_{3,4} = 6.53$; $p = 0.05$). On the contrary, no seasonal differences were observed in the press cake in total SFA, MUFA, PUFA, or LC-PUFA.

The sum of the three essential fatty acids (EFAs) for marine organisms (ARA, EPA, and DHA) represented only 2–5% of the total FA identified in winter for the whole body and both by-products (Tables 1–3). These EFAs increased to the highest value of 21% in spring for eviscerated whole body samples (Table 1). However, for press cake and press liquid, EFAs reached only a maximum of 14 and 15% in summer, respectively (Tables 2 and 3).

The crude protein content resulted in significant differences (Figure 1) ($H(3, N = 32) = 25.25$, $p < 0.00001$), with the highest values in the eviscerated whole body in spring ($74.1\% \pm 1.5$). The press liquid had the lowest protein value of $42.0\% \pm 0.7$ ($H(3, N = 36) = 30.03$, $p < 0.00001$) in spring, whereas the press cake resulted in a higher value for the same season with $52.7\% \pm 0.9$ ($H(3, N = 27) = 22.80$, $p < 0.001$).

In the eviscerated-whole body, press cake, and press liquid, lysine (LYS) and leucine (LEU) explained 40.1, 48.9, and 49.9% of protein-bound EAAs, respectively (Figure 2). On the other hand, the most abundant nonessential amino acids were alanine (ALA), asparagine (ASP), glycine (GLY), and glutamic acid (GLU), representing 84.4, 76.1, and 82.5% of protein-bound NEAAs, respectively (Figure 2), without differences between by-products.

LYS and LEU explained 40.8, 38.9, and 35.2% of EAA of the free AAs in whole-body, press cake, and press liquid, respectively (Figure 3). On other hand, ALA, ASP, GLY, and GLU represented 86.0, 63.0, and 80.8% of free NEAAs in the three by-products, respectively. The free EAA and NEAAs were most abundant in press liquid than in the whole body and press cake (Figure 3). The exceptions was arginine (ARG) (EAA) that exhibited a similar value of $0.47\ \mu g \cdot mg^{-1}$ (± 0.12) for the three by-products, and taurine (TAU) was the only free NEAA with similar values in the three by-products (Figure 3).

4. Discussion

All products derived from *A. pusilla* showed substantial seasonal variation, with the most considerable reduction in essential fatty acids and protein-bound amino acids observed in winter. During the winter, DHA was only detectable in press liquid. Similarly, HIS and MET were only detectable as free AA during the same season in all polychaete by-products analyzed.

Regarding fatty acids, palmitic acid ($16:0$) was the highest of the saturated fatty acids, similar to the result found in the polychaete *Nereis* spp. [22], and $16:1n-7$ and $18:1n-9$ were primary monosaturated fatty acids (MUFAs). Among the polyunsaturated fatty acids (PUFAs), the polychaete extracts tended to be more abundant in ARA and EPA, with a low amount of DHA, which contradicts the early observation of marine polychaetes with a high amount of DHA observed by Nguyen et al. [12] but resembles the trend reported for *Nereis* sp. [22]. The DHA shortage in *A. pusilla* explains the low DHA/EPA ratio of around 0.2. However, the $n-3/n-6$ ratio was around 1.0 ± 0.1; below this value, Nguyen et al. [12] observed in marine polychaete extracts. EPA dominance observed in polychaete by-products up to 70% of $n-3$ PUFAs is quite similar to the composition of particulate organic matter (POM) documented in the waters of the southern fjords of Chile [23]. The nondetection of DHAs in winter could be because of their scarce food, and the diet was mainly composed of detritus, which could increase the relative importance of other fatty acids.

A similar effect was observed in bivalves, where the lack of DHA could indicate the absence of zooplankton in the diet [24]. Furthermore, high SFA values of $15:0$ and $17:0$ would denote the presence of bacteria in the diet or a high contribution of fatty acids from the intestinal flora [24, 25]. Oleic and arachidonic fatty acids could be obtained by ingesting either foraminifera or red algae [25]. Additionally, according to Sahu et al. [22], high levels of $20:4n-6$ could be associated with low oxygen environments, which could correspond to data observed during the spring. Therefore, the fatty acid characteristics indicate that *A. pusilla* feeds on organic matter derived from bacteria, algae, and foraminifers and could be considered an omnivore that grows under a low oxygen environment.

Concerning EAAs, LYS was among the most abundant in the three products, like previous observations by Nguyen et al. [12] of marine polychaete extract and by Moussa Dorgham et al. [26] in the species *Perinereis cultrifera.* Since LYS is a limiting EAA found in low quantities in vegetable proteins [27], it could be one of the main advantages of using eviscerated whole polychaetes as an additional ingredient in fish-free aquafeeds. Furthermore, it has been documented that LYS above the minimum required dietary level is

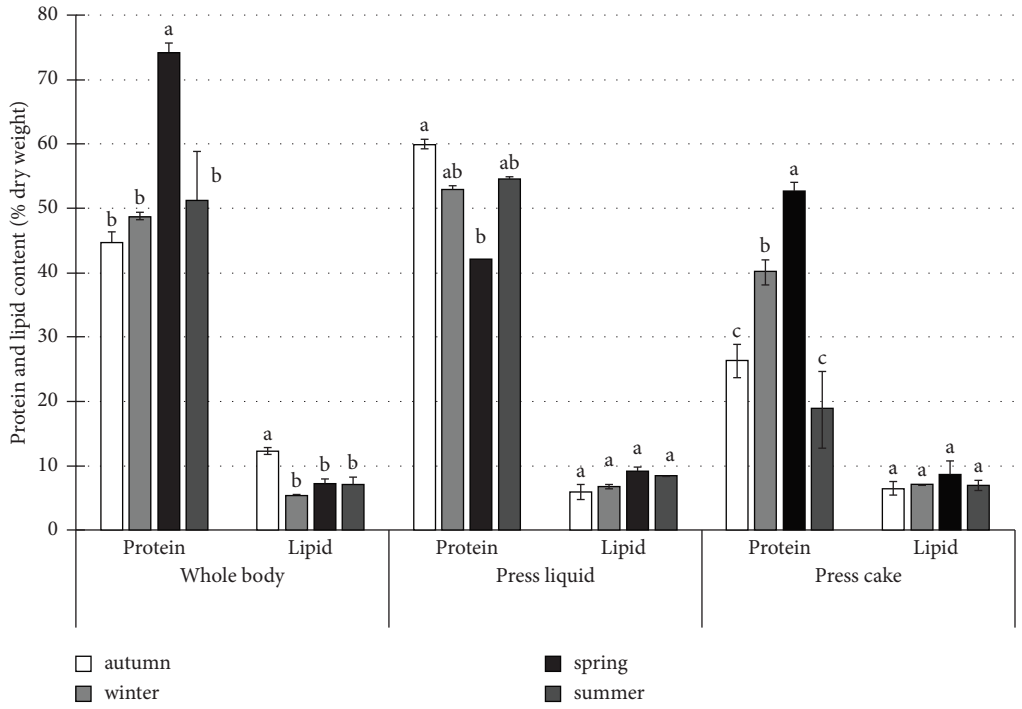

Figure 1: Seasonal variations in protein and lipid of *A. pusilla* eviscerated whole body, press liquid, and press cake. Bars correspond to the standard error of three replicates. Lower case subindices indicate a significant difference between seasons per product ($p < 0.05$).

Table 1: Fatty acid content (mg g^{-1} sample) from eviscerated whole body specimens. Each value represents the average value ($n = 3$) and the standard error.

FAME	Spring	Summer	Autumn	Winter
		Fatty acid content (mg FAME g^{-1})		
15 : 0, pentadecanoic acid	0.18 ± 0.03	0.25 ± 0.02	0.17 ± 0.01	0.15 ± 0.03
16 : 0, palmitic acid	1.14 ± 0.03^{a}	2.24 ± 0.01^{b}	0.99 ± 0.13^{a}	1.19 ± 0.12^{a}
16 : 1$n-7$, palmitoleic acid	0.56 ± 0.01^{a}	0.99 ± 0.01^{b}	0.47 ± 0.09^{a}	0.45 ± 0.08^{a}
17 : 0, heptadecanoic acid	0.17 ± 0.02^{a}	0.24 ± 0.0^{b}	0.16 ± 0.01^{a}	0.16 ± 0.01^{a}
17 : 1$n-7$, heptadecenoic acid	0.14 ± 0.02	0.15 ± 0.01	0.10 ± 0.01	0.08 ± 0.03
18 : 0, stearic acid	0.29 ± 0.03^{ab}	0.89 ± 0.08^{c}	0.18 ± 0.02^{a}	0.43 ± 0.07^{b}
18 : 1$n-9$, oleic acid	0.51 ± 0.01^{a}	1.77 ± 0.19^{b}	0.36 ± 0.11^{a}	0.68 ± 0.23^{a}
20 : 1$n-9$, eicosaenoic acid	0.46 ± 0.05	1.08 ± 0.38	0.25 ± 0.02	0.27 ± 0.04
18 : 3$n-3$, α-linolenic acid	0.15 ± 0.02^{b}	0.15 ± 0.02^{b}	0.08 ± 0.02^{a}	0.07 ± 0.01^{a}
18 : 2$n-6$, linoleic acid	0.22 ± 0.04	0.22 ± 0.03	0.17 ± 0.06	0.07 ± 0.01
20 : 4$n-6$, arachidonic acid (ARA)	0.46 ± 0.01^{c}	0.61 ± 0.05^{d}	0.19 ± 0.02^{b}	0.05 ± 0.02^{a}
20 : 5$n-3$, eicosapentaenoic acid (EPA)	0.69 ± 0.05^{c}	0.55 ± 0.04^{bc}	0.30 ± 0.06^{ab}	0.06 ± 0.02^{a}
22 : 6$n-3$, docosahexaenoic acid (DHA)	0.15 ± 0.01	0.20 ± 0.11	0.07 ± 0.07	0.00 ± 0.00
Other SFA	0.49 ± 0.09	0.84 ± 0.12	0.21 ± 0.03	0.64 ± 0.06
Other MUFA	0.56 ± 0.01	1.17 ± 0.46	0.37 ± 0.02	0.28 ± 0.03
Other PUFA	0.18 ± 0.00	2.01 ± 0.27	0.09 ± 0.03	0.05 ± 0.02
FAME ratios				
EPA/ARA	1.52 ± 0.28	0.91 ± 0.14	1.54 ± 0.13	1.24 ± 0.10
DHA/EPA	0.22 ± 0.02	0.34 ± 0.17	0.20 ± 0.20	0.00 ± 0.00
$n-3/n-6$	1.21 ± 0.12	0.96 ± 0.130	1.10 ± 0.10	0.90 ± 0.03

essential for the nonspecific immune system of the rainbow trout, *Oncorhynchus mykiss* [28], and favors the gastrointestinal development of agastric fish [29].

GLY and GLU were the most abundant NEAAs found in the three polychaete by-products, supporting the results reported by Nguyen et al. [12] and Moussa Dorgham et al. [26] in marine polychaetes. The authors found these AAs especially abundant in the eviscerated whole body samples. GLY has been reported as a feed ingestion stimulant to facilitate nutrient absorption, apart from playing an essential role in osmoregulation and creatine synthesis [29]. GLU in plasma and muscle plays a critical regulatory role in energy metabolism and oxidative stress. In herbivores, it can act as a feed stimulant [30]. Furthermore, it works as an energy

Table 2: Fatty acid content (mg·g^{-1} sample) in the press cake. Each value represents the average value ($n = 3$) and the standard error.

FAME	Spring	Summer	Autumn	Winter
		Press cake (mg FAME g^{-1})		
15 : 0, pentadecaenoic acid	0.28 ± 0.07	0.14 ± 0.07	0.03 ± 0.01	0.09 ± 0.02
16 : 0, palmitic acid	2.04 ± 0.24^{c}	1.23 ± 0.34^{bc}	0.16 ± 0.05^{a}	0.67 ± 0.09^{ab}
16 : 1n − 7, palmitoleic acid	0.94 ± 0.15	0.76 ± 0.33	0.07 ± 0.02	0.25 ± 0.05
17 : 0, heptadecanoic acid	0.35 ± 0.07^{b}	0.19 ± 0.09^{ab}	0.04 ± 0.02^{a}	0.09 ± 0.01^{a}
17 : 1n − 7, heptadecenoic acid	0.20 ± 0.06	0.09 ± 0.05	0.02 ± 0.01	0.04 ± 0.01
18 : 0, stearic acid	0.67 ± 0.04^{a}	0.44 ± 0.03^{c}	0.05 ± 0.02^{bc}	0.24 ± 0.04^{ab}
18 : 1n − 9, oleic acid	0.96 ± 0.06^{c}	0.76 ± 0.00^{c}	0.05 ± 0.02^{a}	0.38 ± 0.14^{b}
20 : 1n − 9, eicosaenoic acid	0.76 ± 0.27	0.35 ± 0.23	0.07 ± 0.03	0.15 ± 0.03
18 : 3n − 3, α-linolenic acid	0.29 ± 0.17	0.07 ± 0.04	0.01 ± 0.00	0.04 ± 0.01
18 : 2n − 6, linoleic acid	0.26 ± 0.07	0.17 ± 0.10	0.02 ± 0.01	0.04 ± 0.01
20 : 4n − 6, arachidonic acid (ARA)	0.55 ± 0.15	0.34 ± 0.24	0.04 ± 0.01	0.03 ± 0.01
20 : 5n − 3, eicosapentaenoic acid (EPA)	0.39 ± 0.09	0.39 ± 0.28	0.04 ± 0.01	0.04 ± 0.01
22 : 6n − 3, docosahexaenoic acid (DHA)	0.11 ± 0.04	0.09 ± 0.07	0.01 ± 0.00	0.00 ± 0.00
Other SFA	1.51 ± 1.19	0.60 ± 0.10	0.45 ± 0.02	0.46 ± 0.04
Other MUFA	0.49 ± 0.18	0.33 ± 0.21	0.04 ± 0.01	0.16 ± 0.03
Other PUFA	0.16 ± 0.06	0.07 ± 0.06	0.05 ± 0.02	0.03 ± 0.02
FAME ratios				
EPA/ARA	0.71 ± 0.16	1.12 ± 0.02	1.00 ± 0.00	1.25 ± 0.25
DHA/EPA	0.27 ± 0.04^{b}	0.20 ± 0.02^{b}	0.27 ± 0.07^{b}	0.00 ± 0.00^{b}
n − 3/n − 6	0.79 ± 0.09	0.92 ± 0.03	1.25 ± 0.15	0.94 ± 0.06

Table 3: Fatty acid content (mg·g^{-1} sample) in the press liquid. Each value represents the average value ($n = 3$) and the standard error.

FAME	Spring	Summer	Autumn	Winter
		Press liquid (mg FAME g^{-1})		
15 : 0, pentadecanoic acid	0.29 ± 0.06	0.25 ± 0.12	0.14 ± 0.00	0.15 ± 0.02
16 : 0, palmitic acid	2.23 ± 0.31	1.63 ± 0.84	0.67 ± 0.01	1.37 ± 0.39
16 : 1n − 7, palmitoleic acid	1.33 ± 0.19	1.05 ± 0.53	0.35 ± 0.02	0.66 ± 0.18
17 : 0, heptadecanoic acid	0.30 ± 0.05	0.30 ± 0.05	0.11 ± 0.0q	0.11 ± 0.14
17 : 1n − 7, heptadecenoic acid	0.18 ± 0.03	0.15 ± 0.07	0.08 ± 0.01	0.07 ± 0.02
18 : 0, stearic acid	0.40 ± 0.04	0.42 ± 0.26	0.13 ± 0.00	0.25 ± 0.04
18 : 1n − 9, oleic acid	0.77 ± 0.11	0.77 ± 0.52	0.23 ± 0.02	0.81 ± 0.13
20 : 1n − 9, eicosaenoic acid	0.71 ± 0.12	0.53 ± 0.22	0.22 ± 0.01	0.21 ± 0.06
18 : 3n − 3, α-linolenic acid	0.26 ± 0.04	0.11 ± 0.05	0.06 ± 0.01	0.12 ± 0.07
18 : 2n − 6, linoleic acid	0.34 ± 0.00	0.22 ± 0.10	0.09 ± 0.01	0.23 ± 0.16
20 : 4n − 6, arachidonic acid (ARA)	0.60 ± 0.08	0.49 ± 0.24	0.11 ± 0.02	0.06 ± 0.06
20 : 5n − 3, eicosapentaenoic acid (EPA)	0.45 ± 0.04	0.57 ± 0.27	0.03 ± 0.01	0.17 ± 0.17
22 : 6n − 3, docosahexaenoic acid (DHA)	0.10 ± 0.03	0.10 ± 0.05	0.01 ± 0.01	0.08 ± 0.08
Other SFA	0.53 ± 0.05	0.15 ± 0.05	0.50 ± 0.06	1.64 ± 1.30
Other MUFA	0.54 ± 0.02	0.48 ± 0.25	0.26 ± 0.02	0.21 ± 0.12
Other PUFA	0.07 ± 0.05	0.09 ± 0.04	0.06 ± 0.01	0.07 ± 0.07
FAME ratios				
EPA/ARA	0.76 ± 0.16^{b}	1.18 ± 0.02^{b}	0.26 ± 0.04^{b}	3.09 ± 0.00^{c}
DHA/EPA	0.21 ± 0.04	0.17 ± 0.01	0.25 ± 0.25	0.44 ± 0.000
n − 3/n − 6	0.79 ± 0.04	0.98 ± 0.00	0.59 ± 0.02	0.92 ± 0.21

substrate for leukocytes, playing a relevant role in the immune response of fish and stimulating the synthesis of muscle protein, purines, and pyrimidines in cells [29]. Although GLU is produced from glutamate, considered a conditionally essential amino acid, supplementation increases enterocyte growth and differentiation and protects them from oxidative damage [31].

Nonprotein amino acid TAU is a conditionally essential AA derived from MET and CYS that can be easily concentrated in blood and tissues; the press cake shows a nonsignificant trend to high TAU quantity (7.8% of total free AAs) that could be investigated because it has been declared essential to several fish species due to its scarcity or low synthesis capacity [32–34]. Furthermore,

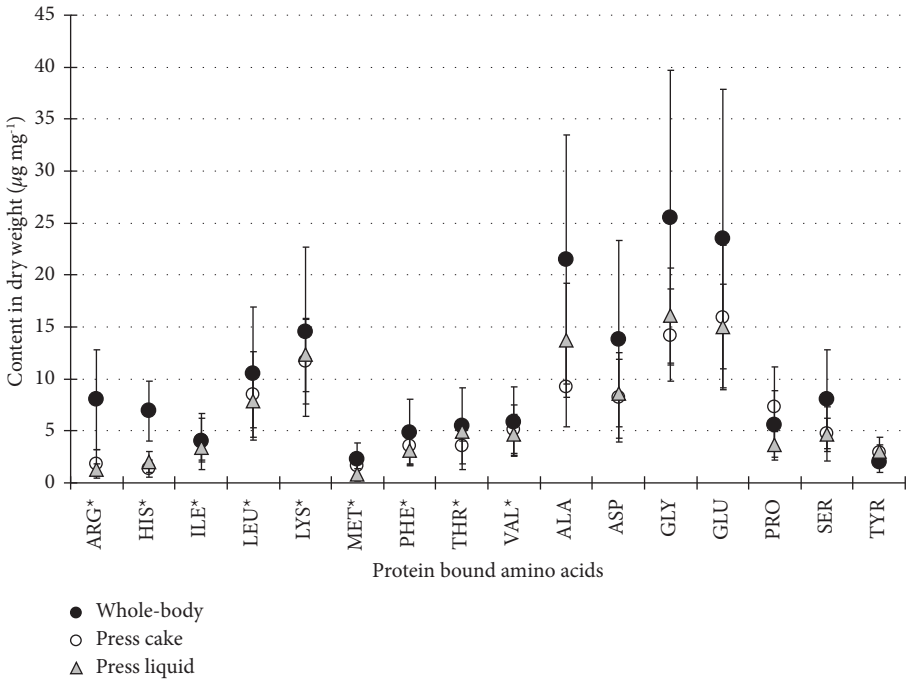

Figure 2: Protein-bound amino acids of *A. pusilla* in the eviscerated whole body, press liquid, and press cake. Bars correspond to the standard error of 4 seasonal samples (spring, summer, autumn, and winter) per amino acid ($p < 0.05$). Lower case subindices indicate a significant difference between products per amino acid ($p < 0.05$). The asterisk denotes essential amino acid (EAA).

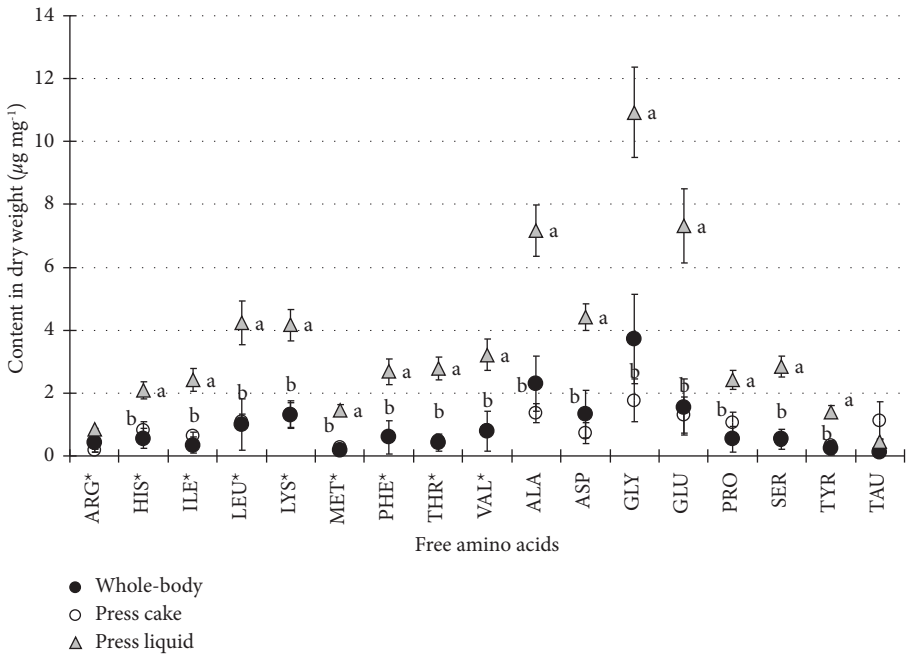

Figure 3: Free amino acids of *A. pusilla* in the eviscerated whole body, press liquid, and press cake. Bars correspond to the standard error of 4 seasonal samples (spring, summer, autumn, and winter) per amino acid ($p < 0.05$). Lower case subindices indicate a significant difference between products per amino acid ($p < 0.05$). The asterisk denotes essential amino acid (EAA).

TAU plays several roles in fish metabolism, such as fat digestibility through bile salt conformation, membrane stabilization, osmoregulation, antioxidation, immunomodulation, calcium-signaling, neuroprotection, decreasing gene expression associated with inflammation and lipogenesis, and increasing lipolytic gene expression [31, 35].

According to the data, harvesting *A. pusilla* will produce high values of LYS and LEU as EAAs and high NEAAs such as ALA, GLY, and GLU. Thus, culturing *A. pusilla* to obtain valuable aquafeed additives is possible, just as was previously reported for other species by Binh et al. [11, 12] and Yang et al. [36].

The higher protein content of the eviscerated whole body and press cake samples was observed in spring. At the same time, lower values were detected in press liquid, indicating that AAs were mainly retained for growth. On the contrary, the reduction in total protein observed in winter would indicate high AA metabolization for energy in a season with an impoverished feed offer. Furthermore, free AAs are likely used as metabolic regulators in the different functional processes of metabolic pathways, which are readily exhausted during the growing season when temperatures are higher or maintain high concentrations during nongrowing seasons under challenging environmental conditions. The three by-products compared contained an excellent source of functional amino acids, namely, LYS, LEU, GLU, GLY, ALA, and ASP as protein-bound AAs, but as free AAs, they are most concentrated in press liquid. Therefore, press liquid requires more investigation as a product focused on aquafeed formulas.

Contrary to protein, the relative average crude lipid value of polychaete by-products did not show seasonal variations, except in autumn for the eviscerated whole body. Therefore, environmental conditions affect the fatty acid composition but do not significantly influence the total lipid content of the by-products, which have an average of 7.8% (±0.9) crude fat. Therefore, lipid constancy added to protein changes could indicate that *A. pusilla* mainly mobilizes AA for energy production in harsh seasonal periods such as winter.

The eviscerated whole body was the product with the highest protein and lipid content. Also, the eviscerated whole body would be a good source of EFA, such as EPA and ARA. However, these nutrients will only be available if the polychaete is cultivated under Patagonian summer sea conditions such as feed, light, photoperiod, and temperatures.

While this polychaete could be straightforwardly harvested in natural environments, this practice is not sustainable. Large-scale harvesting could present a high ecological risk to coastal communities [7]. It is also a potential pathogen carrier that could affect aquaculture species [37]. However, polychaete species can be cultured within integrated multitrophic aquaculture systems (IMTAs) due to their ability to digest the organic matter that composes sludge [6]. IMTAs have been proved ecologically efficient by combining different extractive species and simplifying the energy and nutrients used in their production [38]. Polychaete aquaculture development has recently aroused interest. According to Mandario et al. [6], polychaetes can be cultured using muddy wastes from other culture species, even with the biofloc sediments discharged from shrimp or salmon ponds. The efficiency of *A. pusilla* has been highlighted by Gómez et al. [9, 13] as a candidate for nutrient recycling from marine aquaculture sludge.

In conclusion, the polychaete *A. pusilla* is a promising, nutrient-rich candidate that could be used as an ingredient in aquaculture feed. However, its production must be achieved with a cost-effective cultivation system. For example, it could be harvested from IMTAs, or its culture could promote using sludge from other aquaculture activities such as abalone and salmon cultivation. Nevertheless, the high amount of valuable nutrients in *A. pusilla* during the warm seasons highlights the environmental conditions for best practices.

Data Availability

The data used to generate the results in this manuscript are available from the corresponding author upon request.

Disclosure

The funders played no role in the design of the study, analysis, and interpretation of the data, writing of the manuscript, or the decision to publish the results.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Ana Farías was involved in designing, analysis of the findings, and manuscript drafting. Guillermo Valenzuela was responsible for the conceptualization of the study. Jorge Hernández was responsible for revising the manuscript. Iker Uriarte was responsible for the methodology and collection of samples. María Teresa Viana contributed to the design, methodology, and revising the manuscript. All the authors have read and agreed to the published version of the manuscript.

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