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*Original*

The use of seaweed as sustainable feed ingredient for the house cricket (*Acheta domesticus*): investigating cricket performance and nutritional composition / Ajdini, B.; Biancarosa, I.; Cardinaletti, G.; Illuminati, S.; Annibaldi, A.; Girolametti, F.; Fanelli, M.; Pascon, G.; Martinoli, M.; Tulli, F.; Pinto, T.; Truzzi, C.. - In: JOURNAL OF INSECTS AS FOOD AND FEED. - ISSN 2352-4588. - ELETTRONICO. - 10:8(2024), pp. 1313-1330. [10.1163/23524588-20230176]

*Availability:*

This version is available at: 11566/327132 since: 2024-02-28T14:18:15Z

*Publisher:*

*Published*

DOI:10.1163/23524588-20230176

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(Article begins on next page)

1 **The use of seaweed as sustainable feed ingredient for the house cricket (*Acheta***  
2 ***domesticus*): investigating cricket performance and nutritional composition**

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13 **Abstract**

14 The house cricket (*Acheta domesticus*) is considered one of the most promising farmed insect  
15 species for the novel food sector, thanks to its attractive nutritional profile and its great taste.  
16 To the best of our knowledge, crickets in Europe are reared on soybean meal-rich feeds which  
17 will not be sustainable in the long run. Insect producers in Europe have shown interest in using  
18 more environmentally friendly substrates for cricket's rearing. Among these, seaweed has been  
19 investigated as feed ingredients for insect rearing. All the existing studies on this topic are,  
20 however, focused on the black soldier fly and no such studies exist for the house crickets. In  
21 the present study we aimed to evaluate the potential use of the red alga *Palmaria palmata* in  
22 the diet of house crickets (5, 10 and 20 % of the diet), in terms of insect performances and  
23 potential changes in their nutritional composition (protein, lipid, amino acids and fatty acids).  
24 Crickets fed seaweed-enriched diets showed good performance parameters (individual weight,  
25 cricket yield and survival) compared to crickets fed the control diet without presenting  
26 statistically significant differences ( $P>0.05$ ), while their nutritional composition changed  
27 significantly for some components. Protein content of the crickets increased when more  
28 seaweed was added to their diets ( $p=0.0115$ ), while the fat content decreased ( $p=0.0451$ ). Also,  
29 the amino acid composition of the crickets remained stable between dietary groups, except for  
30 histidine, methionine and lysine which increased in crickets fed more seaweed in the diet  
31 ( $p=0.0431$ ,  $p=0.0342$ ,  $p=0.0302$ , respectively). Finally, the presence of seaweed in the diet led  
32 to a transfer of the omega-3 eicosapentaenoic acid from the seaweed to the crickets ( $p<0.0001$ ),  
33 which are naturally lacking this health-promoting fatty acid. Based on this study, we conclude  
34 that the red alga *Palmaria Palmata* is a suitable substrate for cricket mass rearing up to 20 g/100  
35 g of the diet. However, a higher inclusion of this seaweed and a longer administration time of  
36 seaweed-enriched diets could affect the nutritional composition of the house cricket differently,  
37 therefore they should be investigated.

38 **Keywords:** house cricket, novel food, nutritional composition, fatty acids, seaweed

39 **1. Introduction**

40 By 2050, the world population is expected to reach over 9 billion (WHO, 2019) and the demand  
41 for food is also expected to increase up to 70% above today's levels. The production of enough  
42 food for the growing population represents, as such, a serious challenge for the future.  
43 Nowadays, traditional animal farming of e.g., cattle, swine, poultry, fish, is considered no  
44 longer sustainable due to its massive pressure on limited resources such as land, water and feed,  
45 and severe impacts on the environment in terms of greenhouse gas emissions, significant loss  
46 of biodiversity, alteration of ecosystems, among other factors (Guiné et al., 2021). In this  
47 context, there is a need for finding fair, healthy, and environment-friendly food systems, in line  
48 with the European Green Deal priorities and the EU's climate ambition for 2030 and 2050.  
49 In the last ten years, insects have been identified as promising alternative sources of protein for  
50 the food sector, being highly nutritious for humans, and at the same time, more sustainable than  
51 traditional animal farming when it comes to resource use and their overall ecological footprint  
52 (van Huis and Oonincx, 2017). Among the insect species authorised as novel food in Europe,  
53 the house cricket *Acheta domesticus* (AD, Orthoptera: Gryllidae) is considered one of the most  
54 nutritious, being rich in protein (> 70 g/100 g of the dry matter, DM) and essential amino acids,  
55 lipid (13-18 g/100 g DM), and both macro- and micronutrients (Gasco et al., 2020; Koutsos et  
56 al., 2019). As recognized by the Food and Agriculture Organisation (FAO), one of the main  
57 advantages of using insects consists in their ability to efficiently convert a wide range of  
58 substrates into valuable products such as high-quality protein and lipids. Today, soybeans have  
59 been commonly used as a major protein source in feeds for the insect industry (Cohen AC,  
60 2004). However, the use of soybeans has a high environmental impact and compete for human  
61 and livestock consumptions (da Silva et al., 2010). According to the International Platform of  
62 Insects for Food and Feed (IPIFF), insect producers in Europe have shown interest in using  
63 environmentally sustainable materials for insect rearing to reduce footprint of the insect sector  
64 and enhance its circularity (IPIFF, 2022).  
65 Among potential substrates for insect rearing, seaweed biomass has been investigated  
66 (Biancarosa et al. 2018; Liland et al. 2017; Osimani et al. 2021). Seaweeds are known to be rich  
67 in valuable nutrients such as omega-3 polyunsaturated fatty acids (PUFAs), essential amino  
68 acids (EAAs), vitamins, minerals, among others (Gupta and Abu-Ghannam, 2011; Mæhre et  
69 al., 2014; Skjermo et al., 2014). Also, in Europe, large volumes of seaweed naturally grow  
70 along the coastlines, of which only a small percentage (<2.9 %) is harvested from wild sources  
71 (FAO, 2020). As such, seaweed represents an under-exploited resource that can be further used  
72 for several applications. Despite wild-harvesting, seaweed farming is an emerging sector, and  
73 it has been promoted for climate and environmentally friendly bioeconomy development (FAO,  
74 2020). Seaweed production in fact does not need fertilizers or pesticides but instead, extracts  
75 nutrients from the water, purifying surrounding water and maintaining ecosystems' health  
76 (Ahmed et al. 2022). Also, seaweed biomass can be obtained from side-streams and by-products  
77 from seaweed cultivation and alginate production, which improves the sustainability of the  
78 seaweed sector (Stévant et al., 2017).  
79 The existing studies on the use of seaweed as feed ingredient for insects are limited to the black  
80 soldier fly (*Hermetia illucens*, HI) (Biancarosa et al., 2018; Liland et al., 2017; Osimani et al.,  
81 2021). To the best of our knowledge, no such studies exist for AD, thus cricket's acceptance of  
82 seaweed-enriched diets is uncertain. In the present study, we aimed to investigate the possible  
83 use of seaweed biomass from the red alga *Palmaria palmata* (L.) Weber and Mohr (i.e., dulse)  
84 as sustainable and novel feed ingredient for house cricket mass rearing. To reach this aim, a 7  
85 days supplementation (before harvesting) of the cricket's diets with seaweed was carried out  
86 and cricket's response was evaluated in terms of insect performance (individual weight, yield  
87 and survival). Moreover, there is clear evidence that "insects are what they eat", i.e., their  
88 nutritional composition is highly affected by changes in their feeding substrates (Liland et al.,

2017; Diener et al. 2009; Oonincx et al. 2015 and 2020). This has also been proved for AD (Sorjonen et al. 2019; Jucker et al. 2022; Lundy and Parrella 2015). In this study, a second aim was to evaluate how feeding seaweed to AD would affect its nutritional composition with specific focus on fatty acid profile, protein content and amino acid composition.

93

## 94 2. Materials and Methods

### 95 *Experimental diets*

96 A feeding trial for the rearing of AD was set up to evaluate the effect of including graded levels  
 97 of dried biomass of the red alga *P. palmata* (PP) on insect performance and macronutrient  
 98 composition of AD. The ingredients ratio used in the crickets' experimental diets are reported  
 99 in Table 1. Briefly, the control diet (Ctrl) consisted entirely of a standard substrate made of 100  
 100 % plant-based ingredients, while three experimental diets (PP5, PP10 and PP20) were prepared  
 101 by mixing 5, 10 and 20% (w/w), respectively, of PP with the standard substrate. The dried PP  
 102 biomass was purchased from the company Ocean Harvest (Ireland), while the standard  
 103 substrate, specifically formulated for industrial rearing of AD, was provided by the insect  
 104 rearing company Nutrinsect s.r.l (Montecassiano, Italy). The standard substrate contained  
 105 mainly cereals such as soybean meal, wheat meal, corn, and beet, among others. However, the  
 106 company did not provide detailed information on the ingredients used for the basal reference  
 107 diet, as it was considered confidential.

108 All diets were manufactured as follows: all the dried ground ingredients (standard substrate and  
 109 dried PP; particle size 1000-2000 µm) were thoroughly blended (GastroNorm 30C1PN,  
 110 ItaliaGroup Corporate Srl) for 20 min and water was then added to the mixture to attain  
 111 appropriate consistency for pelleting. Pellets were obtained by using a 3-mm-die meat grinder,  
 112 dried at 37 °C for 48 hours in a ventilated heater and then ground, sieved (2 mm diameter) and  
 113 stored in vacuum bags until use.

114

115 **Table 1** Ingredients (g/kg) used to prepare the experimental diets for house cricket feeding trial.

116

	Ctrl	PP5	PP10	PP20
<b>Ingredients (g/kg)</b>				
Standard substrate	1000	950	900	800
<i>Palmaria palmata</i>	-	50	100	200

117 Ctrl, control diet; PP5, PP10, and PP20, diets enriched with 5%, 10% and 20% of PP, respectively. PP: *Palmaria*  
 118 *palmata*.

119

### 120 *Rearing of the house cricket*

121 The experiment was carried out at the insect rearing company Nutrinsect s.r.l, using an  
 122 established colony of AD. AD were divided into four groups: (i) Ctrl (AD fed with the control  
 123 diet); ii) PP5, iii) PP10 and iv) PP20 (AD fed with diets enriched with 5, 10 and 20% of PP,  
 124 respectively). Three replicates for each group were set, each hosting 4 g of cricket nymphs  
 125 which corresponded to ~6820 nymphs at hatching day, for a total of 12 crates. During the whole  
 126 rearing period, crickets were reared following standardised procedures established at  
 127 Nutrinsect, as follows. Crickets were kept in food grade plastic rearing containers of  
 128 682x480x385 cm (100 L) covered with a mesh lid, at 30±2 °C with 50±2% relative humidity  
 129 and a 12:12 light cycle. In each rearing crate, egg cartons were used to increase crawling space  
 130 for the crickets and provide them a shelter. From hatching and up to the start of the experimental  
 131 feeding trial with seaweed-enriched diets, the crickets were fed the sole standard substrate used  
 132 at Nutrinsect for optimal growth. The feeding experiment with the experimental diets enriched  
 133 with PP started at day 20° and finished at day 27° post hatching. During the feeding trial, total

134 feed added to the rearing crates was recorded each given time, while water was provided *at*  
135 *libitum*. At the end of the experiment, crickets were harvested and weighed to record both  
136 individual weight and net crate weight from each rearing crate, then sacrificed at -40 °C in an  
137 industrial blast chiller. Samples of crickets were taken and stored at -80 °C for further analysis.

### 138 *Proximate analyses and amino acid composition*

139 The dried red alga PP, the experimental diets and the freeze-dried house crickets were analysed  
140 for proximate composition following AOAC (2016) procedures. Moisture content was  
141 measured by weight loss after drying samples in an oven at 105 °C until a constant weight was  
142 achieved. Ash content was evaluated by incineration in a muffle furnace by combustion at  
143 550 °C for 4 h (HD 230, AMSE s.r.l.; Torino, Italy). Crude protein was determined as total  
144 nitrogen (N) through the Kjeldahl method. The Nitrogen-to-Protein (N-Prot) conversion factor  
145 of 4.10 was used for protein content estimations of the seaweed biomass (Biancarosa et al.  
146 2016), while the standard factor of 6.25 was used for the experimental diets (Galland-Irmouli  
147 et al., 1999); the value of 5.09 was used as conversion factor to estimate the house cricket  
148 protein content as suggested by Ritvanen et al. (2020). Total lipid content was determined  
149 according to the Bligh and Dyer method (Bligh and Dyer, 1959) modified by Burja et al.  
150 (2007). Crude fiber was evaluated by acid digestion followed by alkaline digestion according  
151 to the Weende Method and the analysis of acid detergent fiber (ADF) and acid detergent lignin  
152 (ADL) was performed sequentially using the Ankom Technology method (ANKOM, 2016) as  
153 described by Mertens et al. (2002). Gross energy was measured by a calorimetric bomb  
154 (Adiabatic Calorimetric Bomb Parr 1261; PARR Instru-ment, IL, U.S.A). Finally, the house  
155 cricket chitin content was determined following the methods described by Hahn et al. (2020).  
156 The amino acids determinations in the red alga, the diets and crickets were performed as  
157 described by Mohanty et al. (2014). Briefly, samples were digested for 14 h at 110°C in 6N  
158 HCl, derivatized according to the procedures of the AccQ-Fluor reagent kit from Waters  
159 (Milford, MA, U.S.A.) and determined in HPLC using Water AccQ-Tag column (3.9 x 150  
160 mm) at a flow rate of 1 ml/min and Waters 2475 fluorometer detector set on 250 nm excitation  
161 and 395 nm emission. Individual peaks were identified using the amino acid standard provided  
162 by the kit.

### 163 *Lipid extraction and quantification*

164 Seaweed biomass, experimental diets and insects' samples were first homogenized with mortar  
165 and pestle, then freeze-dried at -20°C in vacuum (0.2–0.01 mBar) (BUCHI Lyovapor L-200),  
166 until constant weight ( $\pm 0.2$  mg) (analytical balance Mettler Toledo AT261 DeltaRange). For  
167 lipid extraction, the Folch method (Folch, 1957) was used for seaweed biomass and diets, while  
168 the microwave assisted extraction (MAE) was applied for insects' samples. For the lipid  
169 extraction with the Folch method (1957) samples of seaweed and experimental diets (0.5g) were  
170 extracted overnight with 6 mL of chloroform and 3 mL of methanol (2:1 v/v) and 100  $\mu$ L of IS  
171 by vortexing (1 min). The extract, filtered through Whatman GF/C filter papers ( $\varnothing$  90 mm, GE  
172 Healthcare Life Sciences, Buckinghamshire, UK) was filled with anhydrous sodium sulphate  
173 (Carlo Erba, Milano, Italy) and rinsed twice with further 2 mL of chloroform methanol mixture  
174 (Truzzi et al., 2020). The MAE method, validated from Ramalhosa et al (2012) for organisms,  
175 is considered a valid method in sample preparation because of the minor time required for the  
176 process and the low consumption of solvents compared with the conventional methods. It has  
177 been widely used in a variety of samples, including insects and other biological matrices  
178 (Girolametti et al., 2022; Hao et al. 2021; Melgar-Lalanne et al., 2019; Truzzi et al., 2018b;  
179 Zarantoniello et al., 2021). Briefly, homogenized insect samples (0.5g) were transferred into a  
180 PTFE extraction liner and added with 15 mL of a mixed solution of petroleum ether and acetone

181 (2:1 v/v, RS Carlo Erba) and anhydrous sodium sulphate. The internal standard used (IS) was  
182 methyl nonadecanoate (100 µL of 0.2 µg/L solution, Merck). Liners were then introduced into  
183 a MARS-6 (CEM Corporation, Matthews, NC, USA) system with a dedicated program for lipid  
184 extraction (Truzzi et al., 2020). For all samples, lipid extracts were evaporated in an oven (80°)  
185 for 1h until constant weight and re-suspended in 0.5 mL of n-heptane. After drying, the mass  
186 of extracted lipids was determined gravimetrically (as g/kg DM).

#### 187 *Fatty acid determination*

188 Fatty acid determination was performed by GC-MS after their derivatization to Fatty acid  
189 methyl esters (FAMES) using sodium methylate, according to Canonico et al. (2016). Analysis  
190 was carried out on three aliquots per replicate. FAMES were determined using an Agilent-6890  
191 GC equipped with a split-splitless injector and coupled to an Agilent-5973 N quadrupole Mass  
192 Selective Detector. A CPS ANALITICA DB-WAX-UI (30 m x 0.250 mm ID, 0.25 µm film  
193 thickness) glass capillary column coated with polyethylene glycol was used. Instrumental  
194 conditions were as reported by Truzzi et al. (2018a and 2018b). Sample injections of 1 µL were  
195 made manually in a split mode ratio 1:50 using a glass cup liner (Agilent Liner, splitless, double  
196 taper 5583-4705). The inlet temperature was set at 250 °C. Helium (99.9999%, Air Liquide,  
197 Italy) (8.0 psi) was used as the carrier gas with a column flow rate of 1 mL/min. The oven  
198 temperature started at 100 °C for 1 min, and it was subsequently increased to 150 °C at the rate  
199 of 25 °C/min, to 200 °C at the rate of 5 °C/min and to 230 °C at the rate of 1 °C/min, for a total  
200 run time of 43 min. The ion source and the quadrupole temperatures were set at 230 °C and 280  
201 °C, respectively. The electron energy was 70 eV. A mass range from 50 to 400 m/z was scanned  
202 at a rate of 3.15 scan/s. Data collection, identification, and quantification of FAs were done as  
203 reported in Truzzi et al. (2017, 2023). Retention times and mass spectra of 37-component  
204 FAME (Fatty acid methyl esters Mix standard (≥ 99%, Supelco, Bellefonte, PA, USA) were  
205 used to confirm the NIST (National Institute of Standard and Technology, Gaithersburg)  
206 identification of FAs in the sample. The estimated limit of detection (LOD) and limit of  
207 quantification (LOQ) were calculated according to Truzzi et al. (2014a and 2014b) and ranged  
208 for each FAME from ~4 µg/mL to 22 µg/mL and from 13 µg/mL to 66 µg/mL, respectively.

#### 209 *Statistical analysis*

210 Data of the proximate, amino acid and fatty acid composition of the dried seaweed,  
211 experimental diets and the house crickets are reported as mean ± standard deviation (SD). The  
212 normality and homogeneity of variances were tested by using Shapiro-Wilk test for the  
213 proximate composition data, and Levene's test for amino acid and fatty acid composition data.  
214 One-way ANOVA, followed by the Tukey post hoc test to detect significant differences among  
215 groups was used (significant level  $p < 0.05$ ). Statistical analyses were performed by using the  
216 SPSS package (SPSS Inc., Chicago, IL, USA), and the STATGRAPHICS software  
217 (STATGRAPHIS Centurion 2018, Manugistics Inc., Rockville, Maryland, USA). Generally,  
218 findings are reported on DM basis, unless otherwise stated.

### 219 **3. Results**

#### 220 *Proximate and amino acid composition of seaweed and diets*

221 The analysed proximate composition of the dried PP and the diets are presented in Table 2. The  
222 DM in PP accounted for 89 g/100 g of the fresh biomass. Crude protein was 14.8 g/100 g DM,  
223 total lipids 1 g/100 g and carbohydrates made up to 61.1 g/100 g of DM. Increasing levels of  
224 seaweed in the diets affected the proximate composition of the diets themselves. In general,

225 adding seaweed to the diets led to a decrease in DM, total protein, total lipid and fiber content  
 226 of the diets, with significance ( $p<0.05$ ) at the highest seaweed inclusion (PP20), while  
 227 carbohydrates and ashes increased the more seaweed was added to the diets. In particular, the  
 228 DM contents in all diets were significantly different ( $p<0.05$ ) between groups with the lowest  
 229 value in the PP20 diet, while an opposite pattern was observed in the ash content that resulted  
 230 highest ( $p<0.05$ ) in diet PP20 followed by the diets PP10 ( $p<0.05$ ), PP5 and Ctrl. Adding the  
 231 highest level of PP in the diets (PP20) led to a significant decrease ( $p<0.05$ ) of protein content  
 232 compared to the Ctrl diet ( $27.23\pm 0.04$  g/100 g DM in PP20, compared to  $28.68\pm 0.23$  g/100 g  
 233 DM in the Ctrl). A similar trend was observed for total lipid content, with the highest values in  
 234 the Ctrl diet ( $4.32\pm 0.03$  g/100 g of DM) and the lowest in PP20 ( $3.91\pm 0.01$  g/100 g DM). The  
 235 fiber content of the test diets resulted lowest in PP20 ( $5.66\pm 0.19$  g/100 g DM). On the contrary,  
 236 the highest value of carbohydrates content was recorded in the PP20 ( $51.1\pm 0.2$  g/100 g DM)  
 237 but there wasn't statistically significant difference between groups. Finally, gross energy  
 238 content was affected by seaweed inclusion only in PP20 ( $16.68\pm 0.02$  MJ/kg) (Table 2).  
 239

240 **Table 2** Proximate composition (g/100 g DM), gross energy and amino acid composition  
 241 (g/100 g DM) of the dried seaweed (*Palmaria palmata*) and the diets.

	<i>P. palmata</i>	Ctrl	PP5	PP10	PP20
<b>Proximate composition</b>					
Dry matter	88.61±0.21	92.10±0.03 <sup>a</sup>	91.73±0.02 <sup>b</sup>	91.54±0.01 <sup>c</sup>	91.12±0.02 <sup>d</sup>
Crude protein	14.76±0.12	28.68±0.23 <sup>a</sup>	28.66±0.02 <sup>a</sup>	28.44±0.05 <sup>a</sup>	27.23±0.04 <sup>b</sup>
Total lipids	1.05±0.15	4.32±0.03 <sup>a</sup>	4.24±0.01 <sup>b</sup>	4.20±0.01 <sup>b</sup>	3.91±0.01 <sup>c</sup>
Crude fiber	3.48±0.03	6.66±0.14 <sup>a</sup>	6.48±0.30 <sup>a</sup>	6.09±0.29 <sup>ab</sup>	5.66±0.19 <sup>b</sup>
Ash	19.57±0.20	9.72 ±0.22 <sup>c</sup>	9.97±0.08 <sup>c</sup>	10.68±0.04 <sup>b</sup>	12.06±0.06 <sup>a</sup>
Carbohydrate*	61.1±0.4	50.6±0.3	50.6±0.3	50.6±0.3	51.1±0.2
Gross Energy (MJ/kg)	14.71±0.18	17.09±0.07 <sup>a</sup>	17.06±0.03 <sup>a</sup>	16.98±0.05 <sup>a</sup>	16.68±0.02 <sup>b</sup>
<b>Amino acid composition<sup>#</sup></b>					
Aspartic acid	1.52±0.17	1.97±0.10	1.98±0.09	1.96±0.09	1.92±0.05
Serine	0.73±0.08	1.12±0.05	1.11±0.05	1.10±0.06	1.06±0.09
Glutamic acid	2.98±0.3	6.15±0.3	6.02±0.3	5.87±0.3	5.51±0.1
Glycine	1.93±0.19	2.13±0.10	2.15±0.09	2.16±0.10	2.15±0.05
<b>Histidine</b>	0.19±0.02	1.16±0.06 <sup>a</sup>	1.11±0.05 <sup>ab</sup>	1.06±0.05 <sup>ab</sup>	0.94±0.07 <sup>b</sup>
Arginine	1.13±0.12	2.53±0.12	2.44±0.11	2.40±0.14	2.24±0.06
Threonine	1.01±0.11	1.88±0.09	1.85±0.08	1.81±0.08	1.71±0.04
<b>Alanine</b>	1.19±0.13	0.67±0.03 <sup>c</sup>	0.71±0.09 <sup>bc</sup>	0.76±0.04 <sup>b</sup>	0.83±0.02 <sup>a</sup>
Proline	1.04±0.11	1.27±0.06	1.27±0.06	1.26±0.08	1.25±0.03
Cysteine	0.13±0.01	0.19±0.11	0.20±0.10	0.21±0.13	0.21±0.09
Tyrosine	0.29±0.03	0.55±0.03	0.54±0.02	0.53±0.03	0.50±0.01
<b>Valine</b>	1.60±0.17	0.71±0.03 <sup>d</sup>	0.78±0.03 <sup>c</sup>	0.85±0.04 <sup>b</sup>	0.97±0.02 <sup>a</sup>
<b>Methionine</b>	0.54±0.06	1.10±0.05 <sup>a</sup>	1.08±0.05 <sup>a</sup>	1.02±0.05 <sup>a</sup>	0.93±0.02 <sup>b</sup>
Lysine	1.65±0.18	1.43±0.09	1.46±0.08	1.51±0.10	1.58±0.06
Isoleucine	1.08±0.12	1.15±0.06	1.16±0.05	1.17±0.05	1.17±0.03
Leucine	2.00±0.22	2.28±0.11	2.28±0.10	2.26±0.11	2.26±0.06
Phenylalanine	1.19±0.13	1.31±0.06	1.31±0.06	1.30±0.06	1.29±0.03

242 Ctrl, control diet; PP5, PP10, and PP20, diets enriched with 5%, 10% and 20% of PP, respectively.

243 \* Estimated as follows: Carbohydrate = 100 – (crude protein + total lipid + crude fiber + ash).

244 <sup>#</sup>Asparagine, Glutamine and Tryptophan were not determined. With the exception for the dried seaweed, in each  
 245 raw different letters denote significant differences among dietary treatments ( $p<0.05$ ). Values are presented as  
 246 mean±SD (n=3).

247

248 As showed in Table 2, the seaweed dietary inclusion slightly affected the amino acid profile of  
 249 the experimental diets. Significant differences ( $p<0.05$ ) were observed for histidine, alanine,

250 valine and methionine. Histidine content was lowest in the diet with highest amount of seaweed  
 251 (PP20), while intermediate values were observed for PP5 and PP10 diets. An opposite trend  
 252 was noted for alanine, that showed the highest level in PP20. The dietary valine content was  
 253 lowest in the Ctrl and increased significantly with increasing levels of seaweed in the diet.  
 254 Finally, a significant decrease of methionine in the diets was observed only with the highest  
 255 seaweed inclusion (PP20).

256

257 *Fatty acid composition of the red seaweed and the experimental diets*

258 In general, PP biomass was mainly composed of saturated fatty acids (SFAs) (82±1 g/100 g  
 259 FAs), followed by monounsaturated fatty acids (MUFAs) (13±1 g/100 g FAs) and PUFAs  
 260 (5.7±0.4 g/100 g FAs). The most represented SFAs was 16:0 (51±1 g/100 g FAs), followed by  
 261 14:0 (25±1 g/100 g FAs) and 18:0 (4.3±0.6 g/100 g FAs). Among MUFAs, 16:1n-7 (4.9±0.4  
 262 g/100 g FAs) and 18:1n-9 (7.5±0.1 g/100 g FAs) were the most dominant FAs. The  
 263 eicosapentaenoic acid (EPA) was the most abundant PUFA (4.6±0.5 g/100 g FAs), whereas the  
 264 docosahexaenoic acid (DHA) was not detected.

265 Concerning the diets, the most represented FA class was PUFAs (average value 17.6±0.2 g/100  
 266 g FAs), followed by MUFAs (average value 24.5±0.4 g/100 g FAs) and SFAs (average value  
 267 57.9±0.4 g/100 g FAs), and no statistically significant differences were detected between the  
 268 Ctrl diet and the experimental seaweed-enriched diets (Table 3), for these FA classes and for n-  
 269 6 and n-9 FAs.

270 **Table 3** Fatty acid composition (g/100 g FAs) of *P. palmata* and the diets.

FAs	<i>P. palmata</i>	Ctrl	PP5	PP10	PP20
10:0	nd	0.0113±0.0003	0.0108±0.0003	0.0123±0.0002	0.0128±0.0003
11:0	nd	0.00071±0.00002	0.0008±0.0001	0.0009±0.0001	0.00109±0.00002
12:0	0.27±0.02	0.0111±0.0001	0.0108±0.0004	0.0123±0.0006	0.0122±0.0007
13:0	0.07±0.01	nd	nd	nd	nd
<b>14:0</b>	25±1	0.134±0.004 <sup>d</sup>	0.153±0.003 <sup>c</sup>	0.191±0.007 <sup>b</sup>	0.25±0.01 <sup>a</sup>
14:1n-5	0.13±0.04	nd	nd	nd	nd
<b>15:0</b>	0.75±0.02	0.0446±0.0003 <sup>b</sup>	0.0452±0.0006 <sup>b</sup>	0.0468±0.0008 <sup>a</sup>	0.0466±0.0008 <sup>a</sup>
16:0	51±1	12.3±0.3	12.4±0.3	12.6±0.4	12.6±0.4
16:1n-7	4.9±0.4	0.173±0.004	0.170±0.002	0.1745±0.0006	0.1832±0.0009
17:0	0.27±0.02	0.108±0.001	0.107±0.002	0.1066±0.0002	0.107±0.002
18:0	4.3±0.6	3.9±0.2	3.7±0.2	3.7±0.1	3.8±0.2
18:1n-9	7.5±0.1	24.5±1	24.0±1.0	23.6±1.0	23.7±1.0
18:2n-6	0.7±0.2	50.0±1.0	51.0±2.0	51.0±1.0	51.0±2.0
18:3n-3	0.36±0.04	7.0±0.6	6.7±0.4	6.4±0.5	6.0±0.6
20:0	0.24±0.02	0.46±0.02	0.443±0.004	0.43±0.01	0.441±0.003
20:1n-9	nd	0.41±0.03	0.382±0.005	0.37±0.01	0.36±0.01
20:2n-6	nd	0.063±0.006	0.0573±0.0004	0.058±0.002	0.057±0.002
21:0	nd	0.03±0.003	0.0276±0.0004	0.026±0.002	0.0277±0.0002
20:4n-6	nd	0.018±0.001	0.0148±0.0005	0.017±0.002	0.0152±0.0008
<b>20:3n-3</b>	nd	0.0155±0.0009 <sup>c</sup>	0.0307±0.0004 <sup>b</sup>	0.036±0.004 <sup>b</sup>	0.063±0.005 <sup>a</sup>
<b>20:5n-3(EPA)</b>	4.6±0.5	<LOD	0.021±0.003 <sup>c</sup>	0.027±0.003 <sup>b</sup>	0.048±0.004 <sup>a</sup>
22:0	0.4±0.1	0.32±0.02	0.311±0.007	0.30±0.02	0.3078±0.0006
22:2n-6	nd	0.142±0.004	0.087±0.004	0.0651±0.0006	0.067±0.008
24:0	nd	0.22±0.01	0.212±0.004	0.20±0.02	0.207±0.005
22:6n-3(DHA)	nd	0.27±0.05	0.20±0.05	0.20±0.05	0.28±0.05
<i>SFAs</i>	82±1	17.5±0.1	17.4±0.2	17.6±0.2	17.9±0.2
<i>MUFAs</i>	13±1	25±1	24.5±0.2	24.2±0.3	24.2±0.2
<i>PUFAs</i>	5.7±0.4	57±1	58±0.5	58.2±0.5	57.9±0.4



<b>n-3</b>	4.96±0.3	7.3±0.2 <sup>a</sup>	6.9±0.1 <sup>b</sup>	6.7±0.1 <sup>b</sup>	6.4±0.1 <sup>b</sup>
n-6	0.8±0.2	50±1	51.1±0.5	51.5±0.5	51.5±0.4
n-9	7.5±0.5	25±1	24.4±0.2	24.0±0.3	24.0±0.4
<b>n-6/n-3</b>	0.15±0.04	6.9±0.2 <sup>c</sup>	7.4±0.1 <sup>b</sup>	7.8±0.2 <sup>a</sup>	8.0±0.1 <sup>a</sup>

271 PP, *Palmaria palmata*. Ctrl, control diet; PP5, PP10, and PP20, diets enriched with 5%, 10% and 20% of PP,  
 272 respectively. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids, PUFAs, polyunsaturated fatty  
 273 acids; n-3, omega-3 polyunsaturated fatty acids; n-6, omega-6 polyunsaturated fatty acids; n-9, omega-9  
 274 polyunsaturated fatty acids, n-6/n-3, omega-6/omega-3 ratio. Values are presented as mean±SD (n=9). Means  
 275 within rows of experimental diets bearing different letters are significantly different ( $p<0.05$ ). FAs content lower  
 276 than  $<0.02$  g/100 g FAs were excluded from any statistical analysis because their concentrations were close to the  
 277 limit of detection (LOD). nd, not detected.

278 The increasing levels of PP in the diets led to a significantly decrease of the n-3 content in all  
 279 seaweed-enriched diets compared to the Ctrl diet, ranging from  $7.3\pm 0.2$  g/100 g FAs in the Ctrl  
 280 to  $6.4\pm 0.1$  g/100 g FAs in PP20 (Table 3). Consequently, the n-6/n-3 ratio increased  
 281 significantly ( $p<0.05$ ) with increasing inclusion levels of PP in the diets. The most represented  
 282 FAs in all the diets was 18:2n-6 (average value  $51.0\pm 3.2$  g/100 g FAs), followed by 18:1n-9  
 283 (average value  $24\pm 2$  g/100 g FAs), 16:0 (average value  $12.5\pm 0.7$  g/100 g FAs), 18:3n-3  
 284 (average value  $6.5\pm 1.1$  g/100 g FAs) and 18:0 (average value  $3.8\pm 0.4$  g/100 g FAs). For these  
 285 FAs, no statistically significant differences were observed among diets. On the contrary, the  
 286 FAs 14:0 and 15:0 showed a statistically significant ( $p<0.05$ ) increase in seaweed-enriched diets  
 287 compared to the Ctrl. The increasing inclusion of seaweed in the diets also determined a  
 288 statistically significant increase ( $p<0.05$ ) of the n-3 FAs 20:3n-3 and EPA. The health  
 289 promoting FA EPA, in fact, was not detected in the Ctrl diet, but it increased ( $p<0.05$ ) with  
 290 increasing inclusion of PP in the diets, up to  $0.048\pm 0.004$  g/100 g FAs in PP20. The DHA did  
 291 not show significant differences among the experimental diets (average value  $0.24$  g/100 g  
 292 FAs).

### 293 *Insect performance*

294 In all dietary groups, crickets consumed all the feed given at the end of the trial (no feed residue  
 295 was observed in the rearing crates). The inclusion of PP in the crickets' diet did not affect  
 296 crickets' final weight, cricket yield or survival (Figure 1 and Table S1). In particular, individual  
 297 cricket weight at the end of the experiment was not significantly ( $p>0.05$ ) different between  
 298 feeding groups, with an average value of  $0.30\pm 0.02$  g (wet weight basis) (Fig. 1A). Also, there  
 299 were no statistically differences in the cricket yield (total mass of crickets per crate) by the end  
 300 of the trial between experimental groups and the Ctrl group (Figure 1B) (average value  $1041\pm 17$   
 301 g). The FCR (total feed added/weight increase of crickets) did not present statistically  
 302 significant differences among groups (Figure 1C) (average value  $1.11\pm 0.02$ ). Of the ~7000  
 303 nymphs added to each create, around half survived through the whole trial (average value 51  
 304 %) with not statistically difference between experimental groups ( $P>0.05$ ) (Table S1) (  
 305

306 **Figure 1.** (A): final individual weight of crickets (wet weight); (B) cricket yield and (C)  
 307 feed conversion ratio (FCR). Ctrl, AD reared on the control diet; PP5, PP10, and PP20, AD  
 308 reared on diets enriched with 5%, 10% and 20% of PP, respectively.

### 309 310 *Proximate composition and amino acid composition of crickets*

311 The proximate and the amino acid composition of the house crickets are presented in Table 4.  
 312 The DM content of crickets fed the Ctrl diet was significantly ( $p<0.05$ ) higher than the DM of  
 313 crickets fed the seaweed-enriched diets. The Ctrl and PP5 groups showed a significant lower

314 protein content than PP10 and PP20 (Table 4) (Ctrl: 51.3±1.1 g/100 g DM; PP5: 50.0±1.3 g/100  
 315 g DM; PP10: 53.9±0.5 g/100 g DM; and PP20: 54.2±0.8 g/100 g DM). Crickets' total lipids  
 316 resulted the lowest ( $p<0.05$ ) in PP20, while ash, fiber and carbohydrate contents were not  
 317 significantly affected by dietary treatment ( $p>0.05$ ). Also, gross energy content was similar in  
 318 all groups. Chitin content of the crickets increased the more seaweed was included in the diets,  
 319 reaching the highest value in PP20 (10.01±1.5 g/100g DM) which was significantly higher than  
 320 the other groups ( $p<0.05$ ).

321 The amino acid profile of the house crickets fed different diets are also reported in Table 4.  
 322 Significant differences among groups were observed for aspartic acid, histidine, arginine,  
 323 methionine, and lysine. Crickets fed the Ctrl and PP20 diets showed a significant decrease in  
 324 the aspartic acid content when compared to PP5 and PP10. Histidine resulted higher ( $p<0.05$ )  
 325 in PP20 compared to the Ctrl, PP5 and PP10 crickets that did not result differ among each  
 326 other's ( $p>0.05$ ). Arginine resulted significantly higher in the Ctrl and PP20 crickets with  
 327 respect to PP5 and PP10. The Ctrl also resulted in the lowest value of both methionine and  
 328 lysine when compared to the other groups.

329 **Table 4** Proximate composition (g/100 g DM) and amino acid composition (g/100 g DM) of  
 330 house cricket (AD) fed the test diets.

	Ctrl	PP5	PP10	PP20
<b>Proximate composition</b>				
Dry matter	93.45±0.21 <sup>a</sup>	92.00±0.76 <sup>b</sup>	91.89±0.36 <sup>b</sup>	92.33±0.04 <sup>b</sup>
Crude protein	51.3±1.1 <sup>b</sup>	50.0±1.3 <sup>b</sup>	53.9±0.5 <sup>a</sup>	54.2±0.8 <sup>a</sup>
Total lipids	16.4±2.6 <sup>a</sup>	15.8±1.8 <sup>a</sup>	16.6±0.3 <sup>a</sup>	13.0±1.0 <sup>b</sup>
Ash	5.9±0.7	6.1±0.8	6.5±0.2	6.7±0.4
Crude fiber	8.87±0.05	8.60±0.60	8.85±0.17	9.25±0.41
Carbohydrate*	17.5±2.8	19.5±3.0	14.1±0.6	16.9±1.4
Chitin	8.4±0.2 <sup>b</sup>	8.6±1.3 <sup>b</sup>	9.6±1.0 <sup>b</sup>	10.1±1.5 <sup>a</sup>
Gross Energy (MJ/kg)	21.3±0.5	22.1±1.2	21.7±0.3	20.6±0.3
<b>Aminoacid composition<sup>#</sup></b>				
<b>Aspartic acid</b>	2.04±0.04 <sup>c</sup>	2.82±0.09 <sup>a</sup>	2.44±0.05 <sup>b</sup>	2.12±0.05 <sup>c</sup>
Serine	1.91±0.24	1.85±0.16	1.84±0.04	1.97±0.14
Glutamic acid	5.96±0.21	6.48±0.24	6.84±0.24	6.30±0.34
Glycine	6.74±0.13	4.93±0.16	6.20±0.13	6.55±0.14
<b>Histidine</b>	1.91±0.04 <sup>b</sup>	1.82±0.15 <sup>b</sup>	1.97±0.04 <sup>b</sup>	2.10±0.05 <sup>a</sup>
<b>Arginine</b>	5.46±0.10 <sup>a</sup>	4.01±0.13 <sup>c</sup>	5.06±0.10 <sup>b</sup>	5.43±0.12 <sup>a</sup>
Threonine	4.57±0.19	3.40±0.11	4.34±0.19	4.52±0.10
Alanine	2.06±0.24	2.17±0.27	2.19±0.14	2.12±0.05
Proline	2.82±0.15	2.40±0.18	3.52±0.17	3.22±0.07
Cysteine	0.31±0.12	0.45±0.31	0.29±0.11	0.22±0.04
Tyrosine	2.93±0.16	3.12±0.10	2.98±0.14	3.52±0.18
Valine	2.65±0.15	2.63±0.18	2.84±0.06	2.90±0.16
<b>Methionine</b>	0.79±0.01 <sup>c</sup>	1.33±0.04 <sup>b</sup>	1.45±0.03 <sup>a</sup>	1.42±0.03 <sup>a</sup>
<b>Lysine</b>	1.98±0.14 <sup>b</sup>	3.14±0.10 <sup>a</sup>	3.01±0.16 <sup>a</sup>	3.13±0.15 <sup>a</sup>
Isoleucine	2.08±0.14	2.01±0.16	2.10±0.14	2.16±0.15
Leucine	3.41±0.16	3.86±0.12	4.04±0.08	4.19±0.09
Phenylalanine	3.08±0.16	1.83±0.16	1.97±0.14	2.52±0.15

331 Ctrl, AD reared on the control diet; PP5, PP10, and PP20, AD reared on diets enriched with 5%, 10% and 20% of  
 332 PP, respectively. \* Estimated as follows: Carbohydrate = 100 – (crude protein + total lipid + crude fiber + ash).

333 <sup>#</sup>Asparagine, Glutamine and Tryptophan were not determined. Within each row, different letters denote significant  
 334 differences among dietary treatments ( $p<0.05$ ). Values are presented as mean±SD (n=3). Means within rows  
 335 bearing different letters are significantly different ( $p<0.05$ ).

336  
 337 *Fatty acid composition of crickets*

338  
 339 Figure 2 reports the FA classes percentages of AD fed the test diets, while Figure 3 shows an  
 340 example of chromatogram of the FAs in AD fed diet containing 20% of PP. In general, the

341 inclusion of PP in the diets influenced the FA composition of AD only to a minor extent.  
 342 Regarding SFAs percentage, there was an increasing trend with increasing inclusion of PP in  
 343 the diet, but only PP20 (41.4±1.3 g/100 g FAs) showed statistically significant ( $p<0.05$ ) higher  
 344 values compared to the Ctrl (38.3±1.1 g/100 g FAs). The increasing inclusion of PP in the diet  
 345 determined a statistically significant decrease ( $p<0.05$ ) of: i) MUFAs (lowest value was  
 346 18.5±0.5 g/100 g FAs in PP20); ii) n-3 (lowest value was 2.30±0.04 g/100 g FAs in PP20); iii)  
 347 n-6 (lowest value was 38±1 g/100 g FAs in PP20); iv) n-9 (lowest value was 18.3±0.5 g/100 g  
 348 FAs in PP20). PUFAs did not show statistically significant differences ( $p>0.05$ ) among all the  
 349 experimental groups (average value was 40.4±0.6 g/100 g FAs). In terms of n-6/n-3 ratio, PP10,  
 350 and PP20 (16±1 and 17±1, respectively) showed a statistically significant increase with respect  
 351 to PP5 and Ctrl (14±1 and 15±1, respectively).

352 **Figure 2.** Fatty acid classes (g/100 g FAs) of *A. domesticus* fed the test diets.

353 **Figure 3.** Example of GC-MS chromatogram (Single Ion Monitoring Mode) of the FAs  
 354 composition of AD fed with diet containing 20% *P. palmata*.

355 **Table 5** Fatty acid composition (g/100 g FAs) of house cricket (AD) fed the test diets.

FAs	Ctrl	PP5	PP10	PP20
11:0	0.0021±0.0004	0.0015±0.0005	0.00229±0.00001	0.0026±0.0001
12:0	0.05±0.01	0.049±0.003	0.052±0.002	0.059±0.005
<b>14:0</b>	0.54±0.03 <sup>c</sup>	0.57±0.03 <sup>c</sup>	0.61±0.04 <sup>b</sup>	0.74±0.05 <sup>a</sup>
14:1n-5	0.031±0.004	0.04±0.01	0.031±0.002	0.039±0.003
<b>15:0</b>	0.15±0.01 <sup>c</sup>	0.162±0.004 <sup>c</sup>	0.18±0.01 <sup>b</sup>	0.21±0.02 <sup>a</sup>
16:0	26±2	25±2	26±2	28±2
16:1n-7	0.19±0.01	0.18±0.01	0.19±0.01	0.20±0.02
<b>17:0</b>	0.26±0.01 <sup>d</sup>	0.28±0.01 <sup>c</sup>	0.30±0.01 <sup>b</sup>	0.33±0.02 <sup>a</sup>
18:0	11.2±0.5	12±1	12±1	12±1
18:1n-9	20±1	20±1	19±1	18±2
18:2n-6	38±1	37±2	39±1	38±1
18:3n-3	2.3±0.1	2.4±0.2	2.2±0.1	2.0±0.3
18:3n-6	<LOD	0±0.2	<LOD	<LOD
20:0	0.24±0.01	0.24±0.01	0.26±0.01	0.23±0.02
20:1n-9	0.06±0.01	0.07±0.01	0.05±0.01	0.05±0.01
<b>20:2n-6</b>	0.050±0.002 <sup>a</sup>	0.043±0.003 <sup>b</sup>	0.0433±0.0001 <sup>b</sup>	0.044±0.002 <sup>b</sup>
21:0	0.0048±0.0002	0.0039±0.0003	0.0054±0.0002	0.005±0.001
20:4n-6	0.016±0.001	0.015±0.001	0.0188±0.0004	0.018±0.001
<b>20:3n-3</b>	0.019±0.004 <sup>c</sup>	0.029±0.009 <sup>b</sup>	0.046±0.001 <sup>a</sup>	0.045±0.003 <sup>a</sup>
<b>20:5n-3(EPA)</b>	<LOD	0.017±0.003 <sup>c</sup>	0.033±0.002 <sup>b</sup>	0.055±0.003 <sup>a</sup>
22:0	0.013±0.001	0.012±0.002	0.014±0.001	0.012±0.002
<b>22:1n-9</b>	0.20±0.02 <sup>b</sup>	0.14±0.02 <sup>c</sup>	0.24±0.02 <sup>a</sup>	0.16±0.02 <sup>c</sup>
24:0	0.006±0.001	0.005±0.001	0.007±0.001	0.005±0.002
22:6n-3(DHA)	0.16±0.03	0.13±0.03	0.16±0.03	0.18±0.03

356 Ctrl, AD reared on the control diet; PP5, PP10, and PP20, AD reared on diets enriched with 5%, 10% and 20% of  
 357 PP, respectively. Mean within rows bearing different letters are significantly different ( $p<0.05$ ). FAs content lower  
 358 than <0.02 g/100 g FAs were excluded from any statistical analysis because their concentration was close to the  
 359 limit of detection (LOD). Values are reported as mean±SD (n=9).

360 The FAs composition AD fed the experimental diets (Table 5) was mainly characterized by  
361 high percentages of 16:0 (average value  $26\pm 1$  g/100 g FAs), 18:0 (average value  $12.0\pm 0.5$  g/100  
362 g FAs), 18:1n-9 (average value  $19\pm 1$  g/100 g FAs), 18:2n-6 (average value  $38\pm 1$  g/100 g FAs)  
363 and 18:3n-3 (average value  $2.2\pm 0.2$  g/100 g FAs). For these FAs, no statistically significant  
364 differences were observed among groups. The FAs 14:0, 15:0, and 17:0 showed a statistically  
365 significant increase ( $p<0.05$ ) with increasing level of PP in the diets (Table 5). The 20:2n-6 FA  
366 decreased significantly in AD fed PP-enriched diets with respect to AD fed Ctrl diet. The  
367 increasing inclusion level of seaweed in the diets determined a statistically significant increase  
368 ( $p<0.05$ ) of the n-3 FAs 20:3n-3 and EPA. The health promoting FA, EPA, in fact, was not  
369 detected in the AD fed the Ctrl diet, but it increased significantly ( $p<0.05$ ) in AD with increasing  
370 the level of PP in the diets, up to  $0.055\pm 0.003$  g/100 g FAs in AD fed PP20 diet. The DHA did  
371 not show significant differences between the diets. Finally, the 22:1n-9 FA showed a  
372 statistically significant difference between groups, with the highest value in AD fed PP10 and  
373 the lowest value in AD fed PP5.

#### 374 4. Discussion

375 In the present study, 20-day old AD were exposed to diets supplemented with seaweed biomass  
376 for 7 days before being harvested. To the best of our knowledge, there are no existing studies  
377 on the use of seaweed for house cricket rearing; as such, comparison of results from this study  
378 and existing evidence could be difficult. Results show that AD keep thriving even when they  
379 switch to diets supplemented with seaweed biomass (the red alga PP). At the end of the trial,  
380 crickets consumed all the feed added (there was no feed residue) in all the experimental groups,  
381 which indicates that the palatability of the diets supplemented with seaweed was high and  
382 comparable to the Ctrl diet, despite increasing the inclusion level of seaweed in the diets.  
383 Adding seaweed to the diet affected neither the individual size of the crickets nor the cricket  
384 yield (total crickets produces per crate) and the FCR (which indicates the efficiency to convert  
385 feed into body mass).

386 In a previous study, using seaweed-enriched diets for larvae of HI caused a decrease in growth  
387 parameters (i.e., individual size of the larvae, yield and feed intake) with increasing seaweed  
388 inclusion (Liland et al. 2017). The authors discussed that the poor performance of the insect  
389 larvae was due to the large size of the seaweed particles and substrate inhomogeneity and  
390 suggested that finer particle size of the seaweed biomass could improve the growth of the  
391 insects when fed seaweed-enriched diets. In the present study, the seaweed biomass was ground  
392 to powder before being added to the standard substrate for final pelleting. We believe this  
393 method to manufacture the insect diets may have improved the quality of the diets themselves,  
394 in terms of homogeneity of the ingredients and particle sizes, thus this could have enhanced the  
395 performance of the crickets fed on such diets compared to previous findings (Liland et al. 2017).  
396 Feeding seaweed-enriched diets to AD did not affect their survival which was  $55\pm 4$  % in the  
397 Ctrl group and  $53\pm 4$  % in the group fed the highest amount of seaweed (PP20). The survival  
398 rates observed in this study are lower than what reported by Sorjonen et al., (2019) (64-94 %) and  
399 Morales-Ramos et al. (2020) (~80 %) in crickets fed on by-products from the food industry  
400 but comparable to those reported by Oonincx et al., 2015 (55 %) in crickets fed plant-based  
401 diets. Cricket's survival is strongly influenced by other factors, rather than the diet only, such  
402 as density of the cricket's population, access to feed and water, available space, presence of  
403 shelters, male to female ratio, among others (pers. comm. Nutrinsect srl; Floater 1997,  
404 McCluney and Date 2008).

405 The proximate composition of seaweed is highly dependent on season, and area of harvesting  
406 (Connan et al., 2004; Marinho-Soriano et al., 2006; Zubia et al., 2008). However, the nutritional  
407 profile of the red alga used in this trial is overall comparable to previously published data on

408 the same species. The protein content of PP found in the current study was calculated using the  
409 specific N-Prot conversion factor of 4.10 for PP as suggested by Biancarosa et al. (2016). The  
410 reason to use this factor instead of the default N-Prot conversion factor of 6.25 (Kjeldahl 1883)  
411 is the presence of a large amount of non-protein nitrogen in seaweed which could lead to an  
412 overestimation of the crude protein content in seaweed samples with the Kjeldahl method  
413 (Biancarosa et al., 2016). The protein content of PP found in this study is lower than protein  
414 values for PP in previous studies which are up to 35 g/100 g on DM *basis* (Gressler et al., 2011;  
415 Naseri et al., 2020; Stévant et al., 2020; Tibbetts et al., 2016). However, we believe that previous  
416 studies mostly likely overestimated protein values in PP by using the default N-Prot conversion  
417 factor of 6.25.

418 The inclusion of PP as ingredient in the cricket's diets led to a slight decrease of the protein  
419 content of the diets. At the highest seaweed inclusion (PP20), the protein content of the diet  
420 reached 27 g/100 g on DM *basis*. This value is comparable to protein contents in substrates that  
421 are commonly used for cricket mass rearing such as chicken feed (21 g/100 g DM), hen feed  
422 (16 g/100 g DM) or maize distiller (26 g/100 g DM) (Jucker et al., 2022). Patton (1978)  
423 suggested that the "optimum" substrate for rearing house crickets should contain around 30  
424 g/100 g (DM) protein (Patton, 1978). There is evidence that protein is a key factor for cricket's  
425 development, in fact AD show an optimal growth when fed a diet rich in protein (20-30 g/100  
426 g DM) as reviewed in Van Peer et al. (2021). On the contrary, low-protein diets (<20 g/100 g  
427 DM) delays development time and slows the individual biomass gain of crickets (Bawa et al.,  
428 2020; Sorjonen et al., 2019). In the present study, the nutritional composition of the diets likely  
429 met the protein demand of crickets; also, our experiment started with 20 days old nymphs which  
430 are usually more resistant than younger nymphs. Regarding total lipid content, the value found  
431 in PP in this study (1 g/100 g DM) is consistent with literature data on this algal species which  
432 range from 0.2 to 3.8 g/100 g DM (Birkeland and Chemistry, 2019; Chandini et al., 2008;  
433 Fleurence et al., 1999; Morgan et al., 1980b). Similarly, to the protein, adding seaweed to the  
434 cricket's diets slightly decreased the level of total lipid in the diets which ranged from 4.32  
435 g/100 g (DM) in the Ctrl to 3.91 g/100 g (DM) in the diet with the highest seaweed inclusion  
436 (PP20). This was expected and likely due to the little amount of lipid in the algae compared to  
437 the other ingredients present in the standard substrate. Patton defined an optimal dietary level  
438 of lipid for cricket's growth and development in the range of 3.2-5.2 g/100 g DM (Patton 1978).  
439 The lipid content of our diets is in accordance with this range.

440 Diet is considered an important factor in insect rearing, which can influence both quantity and  
441 quality of the insects' nutrients (Barroso et al., 2017; Oonincx and Van Der Poel, 2011; Oonincx  
442 et al., 2015). Feeding seaweed-enriched diets to AD influenced their nutritional composition in  
443 terms of both total protein and lipid contents but only to a minor extent. In AD fed the highest  
444 seaweed inclusion (PP20), protein increased, despite the protein content in PP20 diet decreased,  
445 compared to the other treatments. This result could be explained by the increase of chitin  
446 content in AD fed more seaweed in the diet (10.1 g/100 g in PP20 compared to 8.4 g/100 g in  
447 the Ctrl group). Chitin is a nitrogen-containing compound found in the exoskeleton of crickets,  
448 legs, and wings; its content in AD is estimated to be between 6 and 8 g/100 g DM (EFSA 2021).  
449 When estimating crude protein via the Kjeldahl method, total nitrogen (including the nitrogen  
450 quota from chitin) is used as proxy for protein determination. The increase of chitin in AD fed  
451 PP20 might therefore explain the higher protein values found in this dietary group (PP20).

452 The lipid content in AD in the present study is comparable with the data reported in Harsányi  
453 et al. (2020). Adding seaweed to the diet led to a decrease in total lipid, the more seaweed was  
454 added to the diet, reflecting the trend of lipid in the diets. This has been shown before in HI fed  
455 seaweed-enriched diets (Liland et al., 2017). A similar trend was observed for the ash content  
456 in AD which reflected the ash content of the diets. Seaweed is high in mineral content (Rupérez  
457 et al., 2002) that can be transferred to the crickets.

458 Regarding the amino acid composition, the most abundant amino acids in the PP biomass were  
459 glutamic acid (2.98 g/100 g DM), leucine (2 g/100 g DM), glycine (1.93 g/100 g DM) and  
460 aspartic acid (1.52 g/100 g DM) in consistency with previous findings on the same species  
461 (Biancarosa et al., 2016; Galland-Irmouli et al., 1999; Mahre et al., 2014; Mouritsen et al.,  
462 2013). A balanced essential amino acids (EAAs) profile defines (in part) the quality of a protein.  
463 The PP biomass contained all the EAAs, in comparable levels to the ones found in common  
464 protein sources such as corn meal, rice meal, soymeal and wheat meal (Mahre et al., 2014).  
465 These are also the main ingredients of the standard substrate used at the insect company  
466 Nutrinsect for cricket's optimal growth. For this reason, adding seaweed in the cricket's diet  
467 affected the amino acid profile of the diets only slightly. For example, histidine in the cricket's  
468 diets decreased when more seaweed was added to the diets. The levels of histidine found in PP  
469 in this study was 3.5 times lower than histidine levels in soymeal reported by Mahre et al. (2014)  
470 which might explain the trend of histidine when the standard substrate (soymeal rich) was  
471 replaced by seaweed.

472 About the amino acid composition of AD, glutamic acid, glycine, and arginine were the most  
473 abundant amino acids found in AD, in all dietary groups. This is consistent with previous data  
474 on AD (Ghosh et al., 2017; Rumpold and Schlüter, 2013a). Also, AD in this study, had generally  
475 lower amounts of methionine and cysteine compared to other amino acids, as also reported in  
476 other edible insects (Udomsil et al., 2019; Köhler et al., 2019; Sánchez-Muros et al., 2014; Yi  
477 et al., 2013). Feeding seaweed-enriched diets to AD, slightly changed its amino acid  
478 composition. First, a significant increase of histidine levels in AD fed the highest seaweed  
479 inclusion (PP20) was observed (2.10 g/100 g DM). Histidine is an indispensable amino acid for  
480 humans because of the detrimental effects on haemoglobin concentrations that have been  
481 observed when individuals are fed histidine-free diets (FAO/WHO 2007). Also, another  
482 interesting result is that AD fed the highest seaweed inclusion (PP20) increased its methionine  
483 content (1.42 g/100 g DM in PP20 compared to 0.79 g/100 g DM in the Ctrl). This indicates  
484 that the methionine content of crickets could be modified by short term dietary supplementation,  
485 however this should be investigated further as the reason behind this remains unclear. The  
486 higher histidine and methionine content of AD fed seaweed-enriched diets found in this study  
487 improves the quality of the AD protein for human nutrition. Malla et al (2022) identified  
488 methionine as the first limiting amino acid in AD for human and animal nutrition.

489 The FAO/WHO provide guidelines and recommendations on the protein and EAAs  
490 requirements in human nutrition (FAO/WHO 2007). Based on the results from this study, we  
491 can confirm that AD is a very good source of EAAs for humans even when fed seaweed-  
492 enriched diets, as levels of EAAs in AD in all groups largely meet the daily requirements for an  
493 adult for all EAAs as defined by FAO/WHO (2007).

494 The analysis on fatty acid profile of PP in the present study showed that this species has a higher  
495 percentage of n-3 compared to n-6 fatty acids, as reported in previous studies on the same  
496 species (Mouritsen et al., 2013; Van Ginneken et al., 2011; Schmid et al., 2016). Among n-3,  
497 the EPA presented the highest percentage of FAs, as reported in previous studies on PP (Van  
498 Ginneken et al., 2011; Sánchez-Machado e al., 2004; Schmid et al., 2016; Mæhre et al., 2014);  
499 however, such studies found higher EPA percentages in PP (from 24 to 59 g/100 g FAs)  
500 compared to our finding (4.6 g/100 g FAs). It is known that the nutritional composition of  
501 seaweed highly depends on time of harvesting and location (Fleurence, 1999; Pereira et al.,  
502 2020). DHA was not detected in PP in agreement to previous reports (Biancarosa et al., 2018;  
503 Foseid et al., 2020; Mæhre et al., 2014).

504 Adding PP to the diet did not lead to statistically significant differences in FAs classes between  
505 the Ctrl diet and seaweed-enriched diets, except for the n-3 fatty acids which decreased in  
506 seaweed-enriched diets compared to the Ctrl diet. This is because in seaweed-enriched diets,  
507 compared to the Ctrl diet, the increase of 22:6n-3, 20:5n-3 and 20:3n-3 is relatively low

508 compared to the decrease of 18:3n-3, leading to a higher percentage of total n-3 in the Ctrl diet  
509 compared to seaweed-enriched diets. The health promoting fatty acid EPA was not present in  
510 the Ctrl diet and was introduced in the seaweed-enriched diets; its percentages increased when  
511 more seaweed was added in the diet (up to 0.048 g/100 g FAs in PP20). A similar result was  
512 found by Liland et al (2017) with the use of brown seaweed in the diet for HI.

513 In the present study, the FAs composition of crickets reflected the FAs composition of the diets;  
514 in both crickets and the diets the major fatty acids were (in order of magnitude) 18:2n-6, 16:0,  
515 18:1n-9, 18:0 and 18:3n-3, in all dietary treatments. This is in line with previous reports on AD  
516 using different diets (Collavo et al., 2005; Finke, 2002; Oonincx et al., 2015a, b). The  
517 unsaturated FAs oleic (18:1n-9), linoleic (18:2n-6) and alpha linolenic (18:3n-3) are considered  
518 essential FAs because they are not synthesised by humans; the consumption of food containing  
519 such FAs could reduce inflammation, risk of heart attacks, and promote many other beneficial  
520 effects in the human body (Akoh and Min, 2008).

521 Adding seaweed to the diets affected the FAs classes only slightly, with significant differences  
522 ( $p < 0.05$ ) only at the highest seaweed inclusion (PP20), compared to the Ctrl diet. When adding  
523 more seaweed in the diet, SFAs increased while MUFAs decreased; PUFAs was not affected  
524 by dietary treatment. We believe that the seaweed supplementation period (7 days) was not long  
525 enough to generate a fast response of cricket in FAs composition.

526 By growing AD on seaweed-enriched diets, the health promoting fatty acid EPA was introduced  
527 in the crickets, and increased linearly the more seaweed was added to the diet. A similar result  
528 was found by Liland et al. (2017) when using seaweed in the diet of HI. AD is a terrestrial  
529 insect, as such, it does not contain EPA naturally. The possibility to introduce this FA into the  
530 cricket's body via the diet, could therefore improve the quality of cricket-based food products  
531 destined to human consumption.

532

## 533 **5. Conclusions**

534 In the present study, we tested seaweed biomass from the red alga PP as potential feed  
535 ingredient in the rearing of AD. Seaweed-enriched diets were overall palatable for crickets and  
536 presented a high protein content, comparable to Ctrl diets, which led to an insect performance  
537 (in terms of individual weight, yield and survival) and welfare of the reared crickets on these  
538 diets comparable to the ones of crickets fed the control diet without seaweed. The PP inclusion  
539 in the diet slightly affected the nutritional composition of AD, increasing its protein content  
540 while lowering its fat content the more seaweed was added. Regarding the amino acid and fatty  
541 acid compositions, these remained quiet stable between dietary treatments. An increase of  
542 methionine levels in AD (a limiting amino acid) could be an interesting outcome of the use of  
543 seaweed for cricket rearing; however, more research in this topic is suggested as the reason for  
544 this increase is unclear. Also, the addition of PP as ingredient in the cricket's diet led to the  
545 introduction of the health promoting PUFA EPA in AD when seaweed-enriched diets were  
546 used. This confirms the possibility to tailor the fatty acid profile of insects via the diet. However,  
547 since the amount of EPA in AD was overall very low, different seaweed species (containing  
548 more EPA) or longer period of seaweed-enriched diets administration should be considered for  
549 further research. Based on the results from this study, we conclude that PP is a suitable substrate  
550 for cricket mass rearing up to 20 g/100 g of the diet. More research is suggested to test higher  
551 inclusion of PP and a longer administration time of seaweed-enriched diets, to investigate  
552 whether more significant changes of the nutritional composition of AD would occur.

## 553 **6. Conflict of interest**

554 All authors disclose any potential sources of conflict of interest.

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