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

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Abstract

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

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

1. Introduction

By 2050, the world population is expected to reach over 9 billion (WHO, 2019) and the demand for food is also expected to increase up to 70% above today's levels. The production of enough food for the growing population represents, as such, a serious challenge for the future. Nowadays, traditional animal farming of e.g., cattle, swine, poultry, fish, is considered no longer sustainable due to its massive pressure on limited resources such as land, water and feed, and severe impacts on the environment in terms of greenhouse gas emissions, significant loss of biodiversity, alteration of ecosystems, among other factors (Guiné et al., 2021). In this context, there is a need for finding fair, healthy, and environment-friendly food systems, in line with the European Green Deal priorities and the EU's climate ambition for 2030 and 2050.

In the last ten years, insects have been identified as promising alternative sources of protein for the food sector, being highly nutritious for humans, and at the same time, more sustainable than traditional animal farming when it comes to resource use and their overall ecological footprint (van Huis and Oonincx, 2017). Among the insect species authorised as novel food in Europe, the house cricket *Acheta domesticus* (AD, Orthoptera: Gryllidae) is considered one of the most nutritious, being rich in protein (> 70 g/100 g of the dry matter, DM) and essential amino acids, lipid (13-18 g/100 g DM), and both macro- and micronutrients (Gasco et al., 2020; Koutsos et al., 2019). As recognized by the Food and Agriculture Organisation (FAO), one of the main advantages of using insects consists in their ability to efficiently convert a wide range of substrates into valuable products such as high-quality protein and lipids. Today, soybeans have been commonly used as a major protein source in feeds for the insect industry (Cohen AC, 2004). However, the use of soybeans has a high environmental impact and compete for human and livestock consumptions (da Silva et al., 2010). According to the International Platform of Insects for Food and Feed (IPIFF), insect producers in Europe have shown interest in using environmentally sustainable materials for insect rearing to reduce footprint of the insect sector and enhance its circularity (IPIFF, 2022).

Among potential substrates for insect rearing, seaweed biomass has been investigated (Biancarosa et al. 2018; Liland et al. 2017; Osimani et al. 2021). Seaweeds are known to be rich in valuable nutrients such as omega-3 polyunsaturated fatty acids (PUFAs), essential amino acids (EAAs), vitamins, minerals, among others (Gupta and Abu-Ghannam, 2011; Mæhre et al., 2014; Skjermo et al., 2014). Also, in Europe, large volumes of seaweed naturally grow along the coastlines, of which only a small percentage (<2.9 %) is harvested from wild sources (FAO, 2020). As such, seaweed represents an under-exploited resource that can be further used for several applications. Despite wild-harvesting, seaweed farming is an emerging sector, and it has been promoted for climate and environmentally friendly bioeconomy development (FAO, 2020). Seaweed production in fact does not need fertilizers or pesticides but instead, extracts nutrients from the water, purifying surrounding water and maintaining ecosystems' health (Ahmed et al. 2022). Also, seaweed biomass can be obtained from side-streams and by-products from seaweed cultivation and alginate production, which improves the sustainability of the seaweed sector (Stévant et al., 2017).

The existing studies on the use of seaweed as feed ingredient for insects are limited to the black soldier fly (*Hermetia illucens*, HI) (Biancarosa et al., 2018; Liland et al., 2017; Osimani et al., 2021). To the best of our knowledge, no such studies exist for AD, thus cricket's acceptance of seaweed-enriched diets is uncertain. In the present study, we aimed to investigate the possible use of seaweed biomass from the red alga *Palmaria palmata* (L.) Weber and Mohr (i.e., dulse) as sustainable and novel feed ingredient for house cricket mass rearing. To reach this aim, a 7 days supplementation (before harvesting) of the cricket's diets with seaweed was carried out and cricket's response was evaluated in terms of insect performance (individual weight, yield and survival). Moreover, there is clear evidence that "insects are what they eat", i.e., their 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.

2. Materials and Methods

Experimental diets

A feeding trial for the rearing of AD was set up to evaluate the effect of including graded levels of dried biomass of the red alga *P. palmata* (PP) on insect performance and macronutrient composition of AD. The ingredients ratio used in the crickets' experimental diets are reported in Table 1. Briefly, the control diet (Ctrl) consisted entirely of a standard substrate made of 100 % plant-based ingredients, while three experimental diets (PP5, PP10 and PP20) were prepared by mixing 5, 10 and 20% (w/w), respectively, of PP with the standard substrate. The dried PP biomass was purchased from the company Ocean Harvest (Ireland), while the standard substrate, specifically formulated for industrial rearing of AD, was provided by the insect rearing company Nutrinsect s.r.l (Montecassiano, Italy). The standard substrate contained mainly cereals such as soybean meal, wheat meal, corn, and beet, among others. However, the company did not provide detailed information on the ingredients used for the basal reference diet, as it was considered confidential.
All diets were manufactured as follows: all the dried ground ingredients (standard substrate and dried PP; particle size 1000-2000 μm) were thoroughly blended (GastroNorm 30C1PN, ItaliaGroup Corporate Srl) for 20 min and water was then added to the mixture to attain appropriate consistency for pelleting. Pellets were obtained by using a 3-mm-die meat grinder, dried at 37 °C for 48 hours in a ventilated heater and then ground, sieved (2 mm diameter) and stored in vacuum bags until use.

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

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

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

Rearing of the house cricket

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

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

Proximate analyses and amino acid composition

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

Lipid extraction and quantification

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

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

Fatty acid determination

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

Statistical analysis

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

3. Results

Proximate and amino acid composition of seaweed and diets

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

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

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

	P. palmata	**Ctrl**	**PP5**	**PP10**	**PP20**
Proximate composition					
Dry matter	88.61±0.21	92.10±0.03^{a}	91.73±0.02^{b}	91.54±0.01^{c}	91.12±0.02^{d}
Crude protein	14.76±0.12	28.68±0.23^{a}	28.66±0.02^{a}	28.44±0.05^{a}	27.23±0.04^{b}
Total lipids	1.05±0.15	4.32±0.03^{a}	4.24±0.01^{b}	4.20±0.01^{b}	3.91±0.01^{c}
Crude fiber	3.48±0.03	6.66±0.14^{a}	6.48±0.30^{a}	6.09±0.29^{ab}	5.66±0.19^{b}
Ash	19.57±0.20	9.72 ±0.22^{c}	9.97±0.08^{c}	10.68±0.04^{b}	12.06±0.06^{a}
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^{a}	17.06±0.03^{a}	16.98±0.05^{a}	16.68±0.02^{b}
***Amino acid composition*$^{\#}$**					
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
Histidine	0.19±0.02	1.16±0.06^{a}	1.11±0.05^{ab}	1.06±0.05^{ab}	0.94±0.07^{b}
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
Alanine	1.19±0.13	0.67±0.03^{c}	0.71±0.09^{bc}	0.76±0.04^{b}	0.83±0.02^{a}
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
Valine	1.60±0.17	0.71±0.03^{d}	0.78±0.03^{c}	0.85±0.04^{b}	0.97±0.02^{a}
Methionine	0.54±0.06	1.10±0.05^{a}	1.08±0.05^{a}	1.02±0.05^{a}	0.93±0.02^{b}
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

Ctrl, control diet; PP5, PP10, and PP20, diets enriched with 5%, 10% and 20% of PP, respectively.
* Estimated as follows: Carbohydrate = 100 – (crude protein + total lipid + crude fiber + ash).
$^{\#}$Asparagine, Glutamine and Tryptophan were not determined. With the exception for the dried seaweed, in each raw different letters denote significant differences among dietary treatments (p<0.05). Values are presented as mean±SD (n=3).

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

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

Fatty acid composition of the red seaweed and the experimental diets

In general, PP biomass was mainly composed of saturated fatty acids (SFAs) (82±1 g/100 g FAs), followed by monounsaturated fatty acids (MUFAs) (13±1 g/100 g FAs) and PUFAs (5.7±0.4 g/100 g FAs). The most represented SFAs was 16:0 (51±1 g/100 g FAs), followed by 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 g/100 g FAs) and 18:1n-9 (7.5±0.1 g/100 g FAs) were the most dominant FAs. The eicosapentaenoic acid (EPA) was the most abundant PUFA (4.6±0.5 g/100 g FAs), whereas the docosahexaenoic acid (DHA) was not detected.
Concerning the diets, the most represented FA class was PUFAs (average value 17.6±0.2 g/100 g FAs), followed by MUFAs (average value 24.5±0.4 g/100 g FAs) and SFAs (average value 57.9±0.4 g/100 g FAs), and no statistically significant differences were detected between the Ctrl diet and the experimental seaweed-enriched diets (Table 3), for these FA classes and for n-6 and n-9 FAs.

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

FAs	*P. palmata*	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
14:0	25±1	0.134±0.004^{d}	0.153±0.003^{c}	0.191±0.007^{b}	0.25±0.01^{a}
14:1n-5	0.13±0.04	nd	nd	nd	nd
15:0	0.75±0.02	0.0446±0.0003^{b}	0.0452±0.0006^{b}	0.0468±0.0008^{a}	0.0466±0.0008^{a}
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
20:3n-3	nd	0.0155±0.0009^{c}	0.0307±0.0004^{b}	0.036±0.004^{b}	0.063±0.005^{a}
20:5n-3(EPA)	4.6±0.5	<LOD	0.021±0.003^{c}	0.027±0.003^{b}	0.048±0.004^{a}
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
SFAs	82±1	17.5±0.1	17.4±0.2	17.6±0.2	17.9±0.2
MUFAs	13±1	25±1	24.5±0.2	24.2±0.3	24.2±0.2
PUFAs	5.7±0.4	57±1	58±0.5	58.2±0.5	57.9±0.4

n-3	4.96±0.3	7.3±0.2[a]	6.9±0.1[b]	6.7±0.1[b]	6.4±0.1[b]
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
n-6/n-3	0.15±0.04	6.9±0.2[c]	7.4±0.1[b]	7.8±0.2[a]	8.0±0.1[a]

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

The increasing levels of PP in the diets led to a significantly decrease of the n-3 content in all seaweed-enriched diets compared to the Ctrl diet, ranging from 7.3±0.2 g/100 g FAs in the Ctrl to 6.4±0.1 g/100 g FAs in PP20 (Table 3). Consequently, the n-6/n-3 ratio increased significantly (p<0.05) with increasing inclusion levels of PP in the diets. The most represented FAs in all the diets was18:2n-6 (average value 51.0±3.2 g/100 g FAs), followed by 18:1n-9 (average value 24±2 g/100 g FAs), 16:0 (average value 12.5±0.7 g/100 g FAs), 18:3n-3 (average value 6.5±1.1 g/100 g FAs) and 18:0 (average value 3.8±0.4 g/100 g FAs). For these FAs, no statistically significant differences were observed among diets. On the contrary, the FAs 14:0 and 15:0 showed a statistically significant (p<0.05) increase in seaweed-enriched diets compared to the Ctrl. The increasing inclusion of seaweed in the diets also determined a statistically significant increase (p<0.05) of the n-3 FAs 20:3n-3 and EPA. The health promoting FA EPA, in fact, was not detected in the Ctrl diet, but it increased (p<0.05) with increasing inclusion of PP in the diets, up to 0.048±0.004 g/100 g FAs in PP20. The DHA did not show significant differences among the experimental diets (average value 0.24 g/100 g FAs).

Insect performance

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

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

Proximate composition and amino acid composition of crickets

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

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

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

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

	Ctrl	**PP5**	**PP10**	**PP20**
Proximate composition				
Dry matter	93.45±0.21^{a}	92.00±0.76^{b}	91.89±0.36^{b}	92.33±0.04^{b}
Crude protein	51.3±1.1^{b}	50.0±1.3^{b}	53.9±0.5^{a}	54.2±0.8^{a}
Total lipids	16.4±2.6^{a}	15.8±1.8^{a}	16.6±0.3^{a}	13.0±1.0^{b}
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^{b}	8.6±1.3^{b}	9.6±1.0^{b}	10.1±1.5^{a}
Gross Energy (MJ/kg)	21.3±0.5	22.1±1.2	21.7±0.3	20.6±0.3
Aminoacid composition$^{\#}$				
Aspartic acid	2.04±0.04^{c}	2.82±0.09^{a}	2.44±0.05^{b}	2.12±0.05^{c}
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
Histidine	1.91±0.04^{b}	1.82±0.15^{b}	1.97±0.04^{b}	2.10±0.05^{a}
Arginine	5.46±0.10^{a}	4.01±0.13^{c}	5.06±0.10^{b}	5.43±0.12^{a}
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
Methionine	0.79±0.01^{c}	1.33±0.04^{b}	1.45±0.03^{a}	1.42±0.03^{a}
Lysine	1.98±0.14^{b}	3.14±0.10^{a}	3.01±0.16^{a}	3.13±0.15^{a}
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

Ctrl, AD reared on the control diet; PP5, PP10, and PP20, AD reared on diets enriched with 5%, 10% and 20% of PP, respectively. * Estimated as follows: Carbohydrate = 100 – (crude protein + total lipid + crude fiber + ash). $^{\#}$Asparagine, Glutamine and Tryptophan were not determined. Within each raw, different letters denote significant differences among dietary treatments (p<0.05). Values are presented as mean±SD (n=3). Means within rows bearing different letters are significantly different (p<0.05).

Fatty acid composition of crickets

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

inclusion of PP in the diets influenced the FA composition of AD only to a minor extent. Regarding SFAs percentage, there was an increasing trend with increasing inclusion of PP in the diet, but only PP20 (41.4±1.3 g/100 g FAs) showed statistically significant (p<0.05) higher values compared to the Ctrl (38.3±1.1 g/100 g FAs). The increasing inclusion of PP in the diet determined a statistically significant decrease (p<0.05) of: i) MUFAs (lowest value was 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) 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 FAs in PP20). PUFAs did not show statistically significant differences (p>0.05) among all the experimental groups (average value was 40.4±0.6 g/100 g FAs). In terms of n-6/n-3 ratio, PP10, and PP20 (16±1 and 17±1, respectively) showed a statistically significant increase with respect to PP5 and Ctrl (14±1 and 15±1, respectively).

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

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

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
14:0	0.54±0.03^{c}	0.57±0.03^{c}	0.61±0.04^{b}	0.74±0.05^{a}
14:1n-5	0.031±0.004	0.04±0.01	0.031±0.002	0.039±0.003
15:0	0.15±0.01^{c}	0.162±0.004^{c}	0.18±0.01^{b}	0.21±0.02^{a}
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
17:0	0.26±0.01^{d}	0.28±0.01^{c}	0.30±0.01^{b}	0.33±0.02^{a}
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
20:2n-6	0.050±0.002^{a}	0.043±0.003^{b}	0.0433±0.0001^{b}	0.044±0.002^{b}
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
20:3n-3	0.019±0.004^{c}	0.029±0.009^{b}	0.046±0.001^{a}	0.045±0.003^{a}
20:5n-3(EPA)	<LOD	0.017±0.003^{c}	0.033±0.002^{b}	0.055±0.003^{a}
22:0	0.013±0.001	0.012±0.002	0.014±0.001	0.012±0.002
22:1n-9	0.20±0.02^{b}	0.14±0.02^{c}	0.24±0.02^{a}	0.16±0.02^{c}
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

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

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

4. Discussion

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

In a previous study, using seaweed-enriched diets for larvae of HI caused a decrease in growth parameters (i.e., individual size of the larvae, yield and feed intake) with increasing seaweed inclusion (Liland et al. 2017). The authors discussed that the poor performance of the insect larvae was due to the large size of the seaweed particles and substrate inhomogeneity and suggested that finer particle size of the seaweed biomass could improve the growth of the insects when fed seaweed-enriched diets. In the present study, the seaweed biomass was ground to powder before being added to the standard substrate for final pelleting. We believe this method to manufacture the insect diets may have improved the quality of the diets themselves, in terms of homogeneity of the ingredients and particle sizes, thus this could have enhanced the performance of the crickets fed on such diets compared to previous findings (Liland et al. 2017).

Feeding seaweed-enriched diets to AD did not affect their survival which was 55±4 % in the Ctrl group and 53±4 % in the group fed the highest amount of seaweed (PP20). The survival rates observed in this study are lower than what reported by Sorjonen et al., (2019) (64-94 %) and Morales-Ramos et al. (2020) (~80 %) in crickets fed on by-products from the food industry but comparable to those reported by Oonincx et al., 2015 (55 %) in crickets fed plant-based diets. Cricket's survival is strongly influenced by other factors, rather than the diet only, such as density of the cricket's population, access to feed and water, available space, presence of shelters, male to female ratio, among others (pers. comm. Nutrinsect srl; Floater 1997, McCluney and Date 2008).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5. Conclusions

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

6. Conflict of interest

All authors disclose any potential sources of conflict of interest.

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