Dipartimento
di Scienze
della Vita
e dell'Ambiente
DISVA

PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII cycle, PON)

Name of PhD student: Behixhe Ajdini

Title of PhD research: Omega-3 enriched insect (*Acheta domesticus*) as a novel and eco-sustainable food in Europe.

Name of PhD supervisor: Cristina Truzzi

Research lab name: Analytical Chemistry for Environment and Food lab

Mesi in IMPRESA (Italia): Nutrinsect 3 months

Cycle:
[ ] XXXVI
[X] XXXVII

PhD Curriculum:
[ ] Marine biology and ecology
[ ] Biomolecular Sciences
[X] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[X] Other. Please, indicate: Clean Room

ABSTRACT (1000 characters, including spaces):
Acheta domesticus (AD, Orthoptera: Gryllidae), was recently authorized by the European Commission as edible insect for human consumption in Europe (EU 2022/188). My PhD project aims to study new cost-effective, eco-sustainable rearing substrates for the production of AD powder enriched in omega-3 and omega-6 fatty acids (FA), that could be integrated among the so-called "functional food". During the first year of this PhD, I selected different species of seaweed (macroalgae), based on their FA composition, determinated by Gas Chromatography-Mass Spectrometry. The red algae *Palmaria palmata* added as feed supplement lead in AD to a statistically significant (but still poor) increase in the essential FA eicosapentaenoic acid (EPA). The rearing of AD fed diets enriched with the brown algae *Ascophyllum nodosum* is in progress. The chemical risk associated to the presence of potentially toxic elements (PTEs) of the macroalgae, and AD was carried out by Atomic Absorption Spectrophotometry. The results were comparable with literature data and demonstrated low levels of PTEs, compatible with a safe use of AD as food ingredient.

Part 1. Scientific case of the PhD Research

Background
Nowadays, traditional animal farming (e.g., cattle, swine, poultry) is considered no longer eco-sustainable due to the greenhouse gas emissions, inappropriate land use causing deforestation, significant loss of biodiversity,

alteration of ecosystems and excessive consumption of water resources [1]. In terms of sustainability, insects are a good source representing a low environmental impact compared with the livestock due to the less resources needed such as land, water and feed, and a low ecological impact (greenhouse gas emissions) [2]. *Acheta domesticus* is considered one of the most nutritious one, being rich in both macro- and micronutrients. However, like other terrestrial insects, AD has a lipid profile poor in unsaturated fatty acids (FAs), such as omega 3 FAs. The lipid profile of insects can be influenced by the type of the rearing substrate [3-4]. Existing studies on this topic have been conducted on the black soldier fly (*Hermetia illucens*) while very few studies exist for the house cricket.

Scientific aim

The aim of the first year of this PhD was to investigate the use of seaweed-enriched feeding substrates for the rearing of AD, assessing the potential transfer of omega 3 FAs from the feed to the insects. Seaweed are known to contain high-quality nutrients with health-promoting properties, such as essential amino-acids, high-quality lipids including omega-3 fatty acids, vitamins, and minerals, among others [5]. In particular, we investigated the possible inclusion of different macroalgae as feed supplement to produce omega-3 enriched AD. Moreover, we evaluated the chemical risk associated to the use of macroalgae, in terms of presence of potentially toxic elements.

<u>Workplan 1.</u>

Objective. The identification of the species of macroalgae that could enrich in omega 3 the fatty acid profile of *Acheta domesticus*. The determination of the fatty acids composition of different species of macroalgae (*Palmaria palmata, Gracilaria, Ulva lactuca, Ascophyllum nodosum* and *Porphyra dioica*) was carried out. Moreover, the determination of fatty acids composition of *Acheta domesticus* reared at the Nutrinsect SRL company and its feed, was performed to assess the quantity of seaweed to add as feed supplement.

Methods. Samples of feed, AD (frozen, dried and its derivative, AD powder) were analysed to assess their FAs composition. The Lipid extraction was performed on lyophilized samples using two methods: i) Folch [6] for seaweed and feed; ii) microwave-assisted extraction for insect samples [7]. Fatty acids were determined using a gas chromatograph (Agilent-6890) coupled to an Agilent-5973N quadrupole Mass Selective Detector, after their derivatization to Fatty Acids Methyl Esters (FAMEs).

Obtained results

Feed. Feed showed a high percentage of polyunsaturated FAs (PUFAs ~62%), with essential FAs such as 18:2n6 (~44%) and 18:3n3 (~18%) being the most abundant PUFAs. Monounsaturated FAs (MUFAs) were present at ~23% and were represented almost totally by 18:1n9 (~23%). Among the saturated FAs, SFA (~14%), 16:0 (~10 %) and 18:0 (~3%) were the predominant ones. This FAs profile, together with the high PUFAs/SFAs ratio of 4.32, and a low n6/n3 ratio of 2.5, demonstrated that the feed administered to crickets has a very good nutritional profile. However, the essential omega-3 EPA (20:5n3, eicosapentanoic acid) and DHA (22:6n3, docosaesaenoic acid), were not detected in the feed or were present at a very low percentage.

Acheta domesticus and its powder. In AD sample, both frozen and dried, PUFAs was the main class (~42%), followed by SFAs (~34%) and MUFAs (~22%) (Fig 2). The most represented PUFAs were 18:2n6 (35%), and 18:3n3 (~7%), whereas DHA was present only for ~0.03%. Among SFAs, 16:0 (~22%) and 18:0 (~11%) were the predominant acids, whereas MUFAs were dominated by 18:1n9 (~22%). The FAs composition of AD powder was similar to that of the AD.

Seaweed. Seaweed species selected showed a different FA composition. In terms of FA classes, SFAs were the main class (28–90% of total FAs), followed by MUFAs (5–37%). PUFAs were present in a low percentage (3-5% of total FAs) in *P. palmata, Gracilaria, U. lactuca* and *P. dioica*, while in *A. nodosum* it was present at about 35%. These results provided valuable information regarding the selection of the optimal seaweed species. Based on the data obtained, we chose two species of seaweed to be used as feed supplement for our project experiments, *P. palmata* and *A. nodosum*, as they showed a higher percentage of EPA (about 5%) compared to the other species (less than 3%).

<u>Workplan 2.</u>

Objective. Evaluation of the chemical risk associated to the use of seaweed as food supplement for AD. The determination of potentially toxic elements in different species of seaweed, as well as of *Acheta domesticus* reared at Nutrinsect SRL company and its feed, was carried out.

Methods. For the determination of PTEs such as Cd, Pb, Ni, As and Al, the lyophilized samples were digested in a Microwave Accelerated Reaction System, MARS-X, 1500 W (CEM, Mathews, NC, USA). Quantitative determinations of Cd, Pb, Ni, As and Al were carried out with an Agilent DUO 240FS atomic absorption spectrometer (Agilent, Santa Clara, CA 95051, USA) equipped with graphite furnace (GTA120 Graphite Tube Atomizer) and with Zeeman-effect background corrector. The total mercury content was quantified by a Direct Mercury Analyzer (FKV, Milestone, Italy).

Obtained results

Results for Hg, Cd, As, Pb, Ni, Cr and Al content are reported in Table 1 and 2.

Table 1. PTEs concentrations (mg kg^{-1} wet weight) in feed, AD sample and its derivative (AD powder). Results are reported as mean ± standard deviation.

	Feed	AD_F	AD_D	AD_P
Hg	0.00237±0.00002	0.00554±0.00014	0.0023±0.0002	0.00230±0.00008
Cd	0.3±0.1	0.02±0.01	0.09±0.03	0.12±0.02
As	0.06±0.01	0.08±0.01	0.031±0.002	0.032±0.007
Pb	0.17±0.02	0.009±0.005	0.030±0.004	0.039±0.001
Ni	5.27±0.04	0.27±0.02	0.7±0.1	0.6±0.0
Cr	6.0±0.4	0.8±0.1	0.5±0.1	0.42±0.05
Al	81.7±10.3	12.0±0.4	13.8±0.5	25.0±0.0

AD_F, AD frozen; AD_D, AD dried; AD_P, AD powder

Table 2. PTEs concentrations (mg kg^{-1} wet weight) in seaweed. Results are reported as mean ± standard deviation.

	P. palmata	*Gracilaria*	*U. lactuca*	*A. nodosum*	*P. dioica*
Hg	0.0051±0.0001	0.021±0.002	0.0032±0.0001	0.027±0.001	0.0050±0.0001
Cd	0.26±0.06	0.2±0.01	1.0±0.1	1.0±0.1	0.4±0.0
As	0.5±0.1	1.0±0.1	0.6±0.1	23.3±1.5	10.4±0.3
Pb	11.9±0.4	33.6±0.4	12.1±1.1	8.8±0.5	8.6±0.2
Ni	2.95±0.41	4.8±0.5	2.0±0.3	0.7±0.1	0.66±0.03
Cr	0.97±0.38	10.4±1.2	1.8±0.4	0.2±0.3	-0.2±0.1
Al	414.2±58.7	1343.5±114.9	483.4±20.9	133.0±23.4	140.5±19.2

P. palmata, Palmaria palmata; U. lactuca, Ulva lactuca; A. nodosum, Ascophyllum nodosum; P. dioica, Porphyra dioica.

For feed, AD, and seaweed, recorded concentrations were comparable with literature data and were below the legal limit according to the EC/1881/2006 Commission Regulation and amending regulations. Taking the precautionary principle into consideration, the results reported above are generally suitable for our project experiments.

Workplan 3.

Objective. First experiment with the inclusion of the red algae *P. palmata* in AD feed. The determination of the fatty acids composition of *Acheta domesticus* reared with feed enriched with the seaweed, was carried out.

Methods. For the first experiment, *P. palmata* was added to the traditional feed used at the insect company for the optimal cricket growth, in three percenteges (5%, 10% and 20% of total feed) using three rearing crates for each feed. The experiment started at day 20 post hatching and ended at day 27 post hatching.

Obtained results

Fatty acid composition of feed. About the lipid profile of feed enriched in seaweed, there were no statistically significant differences between experimental diets in terms of SFA, ~17.5%, MUFA, ~24.5%, and PUFA, ~58% of total FAs of which n6 (~51%) and n3 (~7%). EPA was not present in the control diet, and it increased with increasing the inclusion of PP in the diet, while DHA did not show any statistically significant differences between control diet and the enriched ones.

Fatty composition of Acheta domesticus. In insects fed feed enriched in *P. palmata*, PUFAs were the predominant class (39-41% of total FAs), followed by SFAs (38-41%) and MUFAs (19-21%) (Fig. 1). With respect to AD fed control diet, insect reared with feed enriched with 10 and 20% of *P. palmata* showed a statistically significant increase in SFAs, and a statistically significant decrease in MUFAs. DHA counts only for ~0.15 % and there are no significant differences between AD fed control diet and AD fed diets enriched with *P. palmata*. The addition of *P. palmata* to the feed lead in AD to a poor (in absolute terms) but statistically significant increase in EPA (Fig. 1).

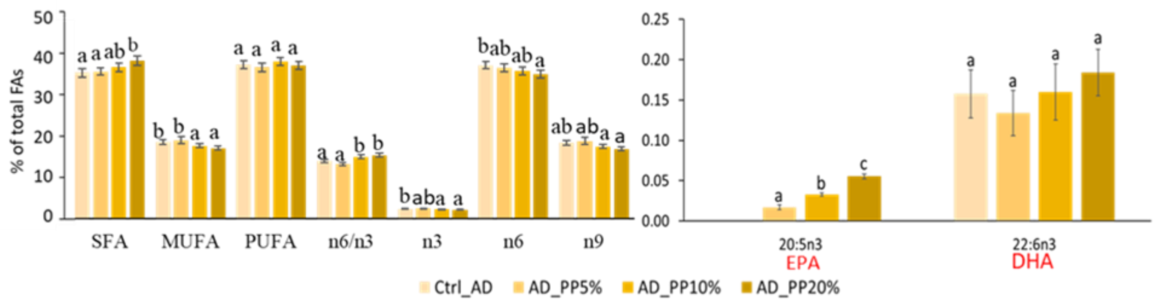

Figure 1. Percentage of SFA, MUFA, PUFA, n6/n3, n3, n6, n9, EPA and DHA in *A. domesticus* enriched with *P.palmata*. Ctrl_AD, control *Acheta domesticus*; AD_ PP5%, *Acheta domesticus* enriched with 5% of *P.palmata*; AD_PP10%, *Acheta domesticus* enriched with 10% of *P. palmata*; AD_PP20%, *Acheta domesticus* enriched with 20% of *P.palmata*; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n6/n3, omega 6/omega 3 ratio; n3, omega-3 polyunsaturated fatty acids; n6, omega-6 polyunsaturated fatty acids; n9, omega-9 polyunsaturated fatty acids. Values are expressed as mean ± SD. Different letters for each FAs class indicate statistically significant differences among samples ($p < 0.05$).

Results are encouraging, but the enrichment of AD in omega 3 is poor. We decided to perform a second experiment with another seaweed richer in omega 3 than the *P. Palmata* (the brown algae *Ascophyllum nodosum*) as feed supplement, extending the time of administration.

<u>Workplan 4.</u>

Objective. Second experiment: inclusion of the brown algae *Ascophyllum nodosum* in AD feed. The determination of the fatty acids composition of *Acheta domesticus* reared with feed enriched with the seaweed was carried out. The rearing of AD fed with feed enriched in *A. nodosum* is in progress.

Methods. For the second experiment, *A. nodosum* was added to the traditional feed used at the insect company for the optimal cricket growth, in two percentages (20%, and 40% of total feed) using three crates for each feed. The experiment started at day 15 post hatching and ended at day 33 post hatching.

Expected results

We expect to obtain an *A. domesticus* with a fatty acid composition richer in omega 3 FAs (and EPA) with respect to AD fed with the control feed.

Future perspectives

- Determination of the lipid profile of *A. domesticus* enriched with *A.nodosum.*
- Assessment of the chemical risk of *A. domesticus* enriched with both *P. palmata* and *A. nodosum.*
- To use other seaweed as feed supplement to produce omega 3 enriched feeds.
- To find other sources of PUFA to be used as feed supplement to obtain an AD rich in omega 3. In this regard, we plan on using by-products coming out from the process of extraction of alginates from seaweed, which are generally discarded. This extraction process does not affect the protein and lipid quality, as well as the presence of PUFA, but, on the contrary, promotes a significant increase in the percentage of such molecules, as well as the content of bioactive compounds [8, 9].

References

1. *Guiné, R. P., Correia, P., Coelho, C., & Costa, C. A. (2021). The role of edible insects to mitigate challenges for sustainability. Open Agriculture, 6(1), 24-36.*
2. *Oonincx, D. G., & De Boer, I. J. (2012). Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. PloS one, 7(12), e51145.*
3. *Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., ... & Olivotto, I. (2020). Fatty acids profile of black soldier fly (Hermetia illucens): Influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Animal Feed Science and Technology, 259, 114309.*
4. *Biancarosa, I., Espe, M., Bruckner, C. G., Heesch, S., Liland, N., Waagbø, R., ... & Lock, E. J. (2017). Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. Journal of Applied Phycology, 29(2), 1001-1009.*
5. *Leandro, A., Pacheco, D., Cotas, J., Marques, J. C., Pereira, L., & Gonçalves, A. M. (2020). Seaweed's bioactive candidate compounds to food industry and global food security. Life, 10(8), 140.*
6. *Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem, 226(1), 497-509.*
7. *Truzzi, C., Illuminati, S., Annibaldi, A., Antonucci, M., & Scarponi, G. (2017). Quantification of fatty acids in the muscle of Antarctic fish Trematomus bernacchii by gas chromatography-mass spectrometry: Optimization of the analytical methodology. Chemosphere, 173, 116-123.*
8. *Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. Journal of applied phycology, 23(3), 543-597.*
9. *Cebrián-Lloret, V., Metz, M., Martínez-Abad, A., Knutsen, S. H., Ballance, S., López-Rubio, A., & Martínez-Sanz, M. (2022). Valorization of alginate-extracted seaweed biomass for the development of cellulose-based packaging films. Algal Research, 61, 102576.*

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Design of research: European Projects

2. Technology Transfer and Innovation

3. Public Speaking: comunicazione efficace per la scienza_Introduzione alla comunicazione e al Public Speaking.

4. A shot of science, wastewater and marine quality: are they connected?

List of conferences/workshops attended and of contributions eventually presented

1. 7 MS Food Day, Florence 5-7 October 2022. Omega-3 enriched house cricket (*Acheta domesticus*) as novel and eco-sustainable food product in Europe. <u>Behixhe Ajdini</u>*, Irene Biancarosa, Silvia Illuminati, Anna Annibaldi, Federico Girolametti, Matteo Fanelli, Francesca Tulli, Gloriana Cardinaletti, Cristina Truzzi.
2. X Convegno Nazionale sul Particolato Atmosferico, PM2022- Bologna, 18-20 May-2022. Seasonal evolution of atmospheric aerosol in the city of Ancona. Annamaria Falgiani, Flavio Vagnoni, Sébastien Bau, Matteo Fanelli, Federico Girolametti, Giada Giglione, <u>Behixhe Ajdini</u>, Cristina Truzzi, Anna Annibaldi, Silvia Illuminati.
3. IX International Symposium – 14-16 June Livorno 2022. Il monitoraggio costiero Mediterraneo: problematiche e tecniche di misura. Water column phosphatase activity assessment in a marine coastal environment and its relationship with rain events. M. Fanelli*, F. Girolametti, <u>B. Ajdini</u>, C. Truzzi, S. Illuminati, A. Annibaldi, C. Totti, S. Accoroni.
4. ABC – XIX Congresso Nazionale della Divisione di Chimica dell'Ambiente e dei Beni Culturali – Torino, 20-23 June 2022. Vertical distribution of Hg in sediment cores of the western-central and southern adriatic sea. M. Fanelli*, A. Annibaldi, C. Cerotti, F. Girolametti, <u>B. Ajdini</u>, S. Illuminati, E. Prezioso, C. Truzzi, R. De Marco, E. Frapiccini, A. Gallerani, M. Tramontana, G. Baldelli, F. Spagnoli.
5. XXIX Congresso della Divisione di Chimica Analitica della Società Chimica Italiana (SCI), Milazzo 11-15 September 2022. Influence of the Antarctic Convergence on the distribution of fatty acids associated with surface marine suspended particulate matter. F. Girolametti, S. Illuminati, A.

Annibaldi, F. Ardini, M. Fanelli, B. Ajdini, C. Truzzi. XXIX Congresso della Divisione di Chimica Analitica della Società Chimica Italiana (SCI).

6. World health day, 7 April 2022 Ancona_ Poster presentation “Salviamo l’ambiente mangiando insetti”.
7. Sharper Ancona 2022 “Insetti come fonte di cibo sostenibile”.

Part 3. PhD student information on publications

None

List of publications on international journals

1. Girolametti, F et al 2022. Dissolved Potentially Toxic Elements (PTEs) in Relation to Depuration Plant Outflows in Adriatic Coastal Waters: A Two-Year Monitoring Survey. Water 2022, 14(4):569. DOI: 10.3390/w14040569.
2. Fanelli, M et al 2022. Impact of Depuration Plants on Nutrient Levels in the North Adriatic Sea. Water 2022, 14(12):1930. DOI: 103390/w14121930.

List of publications on conference proceedings

None

List of other publications (books, book chapters, patents)

None

[DATE] 14/11/2022

Student signature

Behixhe Ajdini

Supervisor signature