Deliverable 26.1
Title Analysis of access provided by UNITO-DISAFA Infrastructures

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Deliverable 26.1
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Executive Summary

Objectives

The objectives of the **UNITO-DISAFA** infrastructures were to provide access to 2 installations:

1. **DISAFA – AQUA**: this infrastructure is an aquaculture facility (Fish nutrition farm), mainly dedicated to rainbow trout nutritional trials (whole life cycle). DISAFA-AQUA offered for TNA 3 independent growing systems and one digestibility system.
2. **DISAFA-INSECTS**: this unique infrastructure enabled to conduct insect trials. TNA users were able to work with 2 insect species: *Hermetia illucens* and *Tenebrio molitor* which are two of the most promising species for aquaculture feeds.

Main Results:

For DISAFA-AQUA, a total of 5 applications were submitted. Three were approved, for a total of 720 units of access.

For DISAFA-INSECTS 3 applications were submitted and granted. Unfortunately, for the last proposal, all the TNA available access slots were already used by the first two projects. Moreover, the review process was lengthy and it was accepted late, with timing that was not compatible with the trials already planned in the infrastructure under other projects. A total of 90 units of access were used.

TNA provided important benefits for both users and the host installations. In fact, it allowed users to use infrastructure, equipment, and expertise at no cost (including travel and accommodation) and carry out a trial that they would otherwise not have been able to perform. Users received guidance and collaboration from experienced staff to maximize the quality and impact of their research. As far as installation is concerned, hosting international researchers increased visibility and raised the profile of the installation within the scientific community. TNA fostered collaborations across countries and disciplines, that could lead to co-authored publications, new partnerships and project opportunities (i.e. joint project proposals).

Therefore, a total of 5 approved project were performed: 3 for DISAFA-Aqua, and 2 for DISAFA-INSECTS.

Authors/Teams involved:
For UNITO-DISAFA, scientific supervision was done by Laura Gasco, Ilaria Biasato, and Sara bellezza Oddon. Farm technicians were also involved to support TNA users during their stay at the infrastructure
Laura Gasco (UNITO-DISAFA)
Ilaria Biasato (UNITO-DISAFA)
Sara Bellezza Oddon (UNITO-DISAFA)
Christian Caimi (UNITO-DISAFA)
Dario Sola (UNITO-DISAFA)

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Contents

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1. Overview of TNA users projects realized in TNA UNITO-DISAFA

1.1.1. Installations

UNITO- DISAFA infrastructures offered 2 installations for TNA:

1. DISAFA –AQUA (Fish nutrition farm)
2. DISAFA-INSECTS (Insect rearing farm).

Both infrastructures were located at the UNITO-DISAFA experimental facility, in Carmagnola (Italy).

DISAFA-AQUA was the experimental farm for aquaculture trials. The infrastructure is mainly dedicate to rainbow trout nutritional trials.
For TNA users, 3 independent growing systems and one digestibility system were available.

Growing tanks system: included 12 (3000L) indoor (Figure 1) and 24 (100L) + 24 (400L) outdoor (Figure 2) tanks. For all tanks, the water was provided by an artesian well water (constant temperature of 14 ± 1 °C all over the year) supplied in flow-through open system.

Figure 1. Twelve (3000L) indoor tanks

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Figure 2. Outdoor tanks (24 * 400 L + 24 * 100L)

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Digestibility system: 2 series of 6 cylindro-conical tanks (240L - flow-through open system) with automatic faeces collector (Choubert et al. 1982) (Figure 3), recognised by EIFAC as the most valid method for in vivo evaluation of apparent digestibility coefficients (ADC) of diets and ingredients, and the estimation of suspended matter loss of dietary origin.

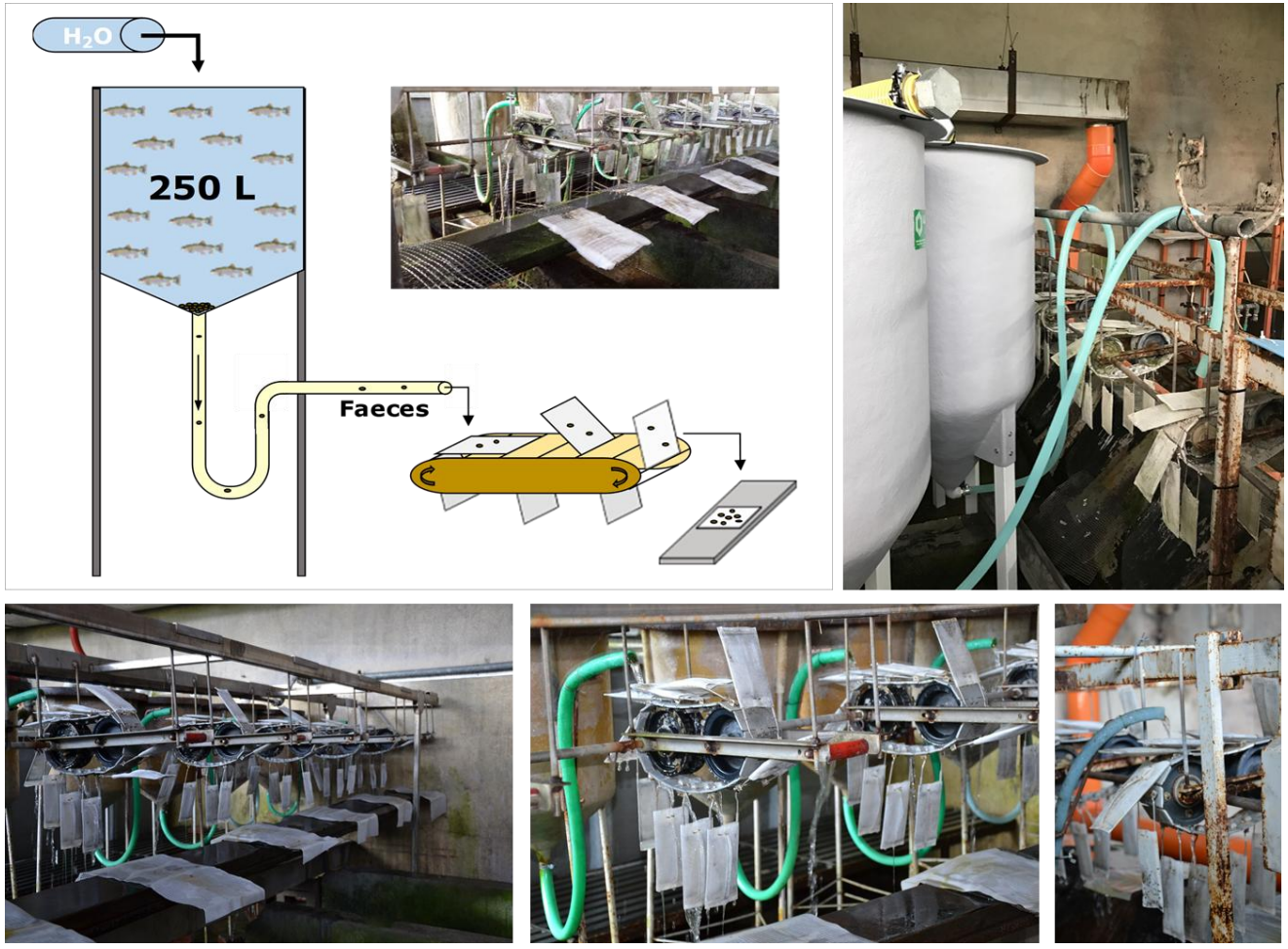

Figure 3. Digestibility system

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DISAFA-INSECTS was the facility devoted to insect trials.
Two insect species were available for TNA studies: *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) (the 2 main insect species for aquafeed production). TNA access were required only for *Hermetia illucens* trials.

The Insect sector is divided into: (i) HI Reproduction and hatching room with controlled environmental conditions (Light, T°, RH) where the adult colony is maintained and eggs production performed, together with the hatching of 1-day old larvae. (ii) HI larvae growing climatic chamber (3*3*2.5 m): where 1 to 6 days old larvae, and 6 days-old larvae are grown till the prepupae (o pupae) stage are reared. (iii) TM reproduction and larval growing chamber (3*3*2.5 m) with controlled T and RH (iiii) The climatic chamber for trial, which has controlled environmental conditions (T° and RH) that can be changed according to the experimental protocols.

Figure 4. Hermetia illucens and Tenebrio molitor facility

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1.1.2. User projects

Min. quantity of access units to be provided according ther DoA:

- DISAFA-AQUA: 720 access
- DISAFA-INSECTS: 90 access

Total number of access units (sum of access units in the table): 720 + 90

Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator	Already used installation (Yes/No)	Nature of the access unit*	Number of used access units during the project	(Potential) paper	How many people was trained by this procedure ?
22-1	DISAFA-AQUA	Dietary chitin effects on rainbow trout (Oncorhynchus mykiss) gut microbiota modulation and health condition	CHITHEALTH	Rainbow trout juveniles were fed diets containing chitin from different origin. Performances and impact on gut microbiota modulation, and fish health (gut morphology and humoral immune and antioxidant responses) were assessed.	P. Enes – F. Rangel (CIIMAR - Portugal)	yes	tank. week	168	yes	1 TNA user + 1 master degree
22-1	DISAFA-AQUA	Insect meals mix in fish	MIXIM	Rainbow trout were fed diets containing insect meals from *Hermetia illucens* (HI) or *Tenebrio molitor* (TM) or increasing levels of a mix of HI + TM. Performances, product quality and fish health were assessed.	C. Trespeuch (MUTATEC, France) – S. Crépieux (Invers, France)	yes	tank. week	336 (252 for growth + 84 for digestibility)	yes	2 insect farmers + 1 master degree + 1 PhD
22-1	DISAFA-AQUA	Dietary Medium Chain Fatty Acids (MCFA) on Rainbow trout (*Oncorhynchus mykiss*) performance, lipid digestion and	SMART	Rainbow trout were fed diets added with MCFA-rich fat by-products. The impact on nutritional value, gut health and productive performance, including the effect on fillet quality in terms of lipid	R. Sala Pallares & A.C. Barroeta (Univ. Autonim	yes	tank. week	216	yes	2 TNA + 1 PhD + 4 master degree

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		metabolism, health and product quality		composition and other quality parameters was investigated.	a de Barcelona, Spain)					
22-2	DISAFA-INSECTS	Determination of the optimal feeding rate for black soldier fly larvae	FEEDBSFL	This research aimed to verify the use of the different levels of feeding rates to identify the optimal one in terms of growth performance, bioconversion efficiency and biomass quality.	M. Kulma (Czech University of Life Sciences Prague, CZ)	yes	container.substrate	32	yes	1 TNA + 1 PHD
22-2	DISAFA-INSECTS	Black soldier fly production: Unlocking it's Potential via Genetic Research and Dietary Enhancement	BSF-UPGRADE	The project aimed to assess 2 different BSF populations and a hybrid to assess the economic impact and potential improvement	D. Deruytter & L. De Praetere (Inagro, Belgium)	yes	container.substrate	58	yes	2 TNA + 1 master degree

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2. TNA projects

2.1.1. TNA projects description

DISAFA-AQUA

PID 19393 - Dietary chitin effects on rainbow trout (Oncorhynchus mykiss) gut microbiota modulation and health condition (CHITHEALTH).
Applicants: Paula Enes - Fábio Rangel
Organisation name: CIIMAR: Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal (NUTRIMU: Nutrition and Immunobiology Group).
Status: completed
The project aimed to study the impact of dietary chitin of different sources on rainbow trout gut microbiota modulation, and thus on fish health, through assessing its effect on gut morphology and humoral immune and antioxidant responses.
A total of 7 fish meal based diets were formulated: one control, 4 diets containing insects-derived chitin, one diet with crustacean-derived chitin, and one diet containing pure chitin from shrimps. The trial lasted 60 days. At the end, performance parameters were calculated, and samples collected for gut microbiota, histology and immune system analysis.
The TNA user (Fábio Rangel) was able to come only at the end of the trial. However, all operations were agreed between the NUTRIMU and the DISAFA-AQUA teams.
Possibility of publication: yes (peer review journal)
Nature and number of persons from the user groups who were trained: (i) a PhD student followed the protocol preparation, the fish maintenance, and sampling procedures, a (iii) a Master degree student was trained regarding the fish maintenance and feeding, sampling procedures, and chemical analyses protocols conducted on fish.

Name of user: Fábio Rangel
Affiliation institution of the user: CIIMAR: Interdisciplinary Centre of Marine and Environmental Research, University of Porto
Country where the institution is based: Portugal
When the user accessed the RI: 24/07/2023 – 27/07/2023
Number of units allocated to the user: 168

PID 23116 - Interest of using mix of insect meals to support the aquaculture sustainability (MIXIM)
Applicant: Christophe Trespeuch - Organisation name: MUTATEC
Applicant: Sébastien Crépieux – Organisation name: INVERS
Status: completed

The aim of the project was to evaluate the mixture between *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) meals as individual ingredients in the diets of rainbow trout (*Oncorhynchus mykiss*). The meals derived from the two insect species was always tested as single ingredient and not as combination.
Seven experimental diets were formulated to be iso-nitrogenous, -lipidic, and -energetic, as follows: a low-fishmeal control diet; a diet with 100% replacement of fishmeal by HI meal; a diet with 100% replacement of fishmeal by TM meal; and 4 diets including a 1:1 mixture of HI and TM meals at increasing levels of fishmeal replacement: 25%, 50%, 75%, and 100%. A total of 378 fish (weight: 126 g ± 1.71) were weighed and randomly assigned to experimental tanks (3 replicates/treatment and 18 fish/replicate)This project investigated the impact of the dietary use of an insect meal mix in rainbow trout feeds on: performance parameters, digestibility, fish fillets physical and chemical quality, gut

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microbiota modulation, and fish health. Data were analysed by One-Way ANOVA. The results showed no significant differences in growth performance, carcass yield, Fulton and somatic indexes, or chemical composition among the experimental diets.

In the case of this TNA project, unfortunately, the two applicants were unable to visit the installation in person due to work commitments. However, there was strong interaction throughout the entire process—both during the preparation of the project and its implementation into an experimental protocol. Continuous contact was maintained between the users and the hosting team, ensuring close collaboration and effective progress of the research activities despite the remote format.
Possibility of publication: yes, abstract accepted as oral presentation to the Insect-Plus Conference (May 2025). The publication is currently being written)
Nature and number of persons from the user groups who were trained: (i) Two insect farmers were involved and trained in the experimental protocol preparation, a (ii) PhD student followed the protocol preparation, the fish maintenance, and sampling procedures, a (iii) a Master degree student was trained regarding the chemical and physical analyses protocols conducted on fish fillets.

Name of user: Christophe Trespeuch and Sébastien Crépieux
Affiliation institution of the user: MUTATEC and INVERS
Country where the institution is based: France
When the user accessed the RI: the two applicants were unable to visit the installation in person due to work commitments.
Number of units allocated to the user: 336

PID 35208 – Dietary Medium Chain Fatty Acids (MCFA) on Rainbow trout (Oncorhynchus mykiss) performance, lipid digestion and metabolism, health and product quality (SMART)
Applicant: Roser Sala Pallarés - Ana Cristina Barroeta Lajusticia
Organisation name: Universitat Autònoma de Barcelona (UAB)
Status: ongoing

Medium-chain fatty acids (MCFAs) are considered to be a fast-absorbing source of energy with an impact on fat deposition and food quality, with a possible beneficial effect in gut health and function. MCFA-rich fats sources available on the market are coconut and palm kernel oils and insect oils (rich in triacylglycerols, TAGs) and coconut and palm kernel fatty acid distillates (FADs; by-products of edible oil refining, rich in free fatty acids). Knowledge of the effects of these alternative ingredients on fish performance, health and final food product quality is scarce and rather confusing, but these aspects are of paramount importance in assessing the suitability of these ingredients for animal feed, including farmed fish. The aim of the project was to investigate the potential use of MCFA-rich fat by-products in rainbow trout diets and their impact on nutritional value, gut health and productive performance, including the effect on fillet quality in terms of lipid composition and other quality parameters. The proposal aimed to upcycle and valorise these by-products and generate applied information to establish guidelines for new feed formulations for trout.

The two TNA users were able to come during the trial (beginning of April). During this period, end of trial and sampling to be done were discussed and organised. One of the TNA user is also expected to come at the end of the trial to participate in fish sampling.
Possibility of publication: yes (peer review journal).
Nature and number of persons from the user groups who were trained: (i) one post-doc fellow followed the protocol preparation and is taking care of the fish maintenance and feeding, (ii) one PhD

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student will follow the sampling procedures, and (iii) four Master degree student will be trained regarding the fish maintenance and feeding, the growth performance calculation, the sampling procedures, and the chemical and physical analyses protocols that will be conducted on fish faeces and fillets.

Name of user: Roser Sala Pallarés - Ana Cristina Barroeta Lajusticia
Affiliation institution of the user: Universitat Autònoma de Barcelona (UAB)
Country where the institution is based: Spain
When the user accessed the RI: 30/03/2025 - 03/04/2025.
Number of units allocated to the user: 216

PID 23227 – Determination of the optimal feeding rate for black soldier fly larvae (FEEDBSFL).
Applicants: Martin Kulma - Michal Kurečka
Organisation name: Czech University of Life Sciences Prague (CZ)
Status: completed

This research aimed to verify the use of the different levels of feeding rates to identify the optimal one and determine the effect between feeding rate and different experimental diets. For this purpose, 2 waste-based diets composed of Italian and Czech ingredients, along with Gainesville diet (ITA, REP and GA, respectively), were evaluated with four feeding rates: 0.4, 0.6, 0.8 and 1 g/larva. 6-days-old larvae were estimated and inoculated in varying number corresponding to the feeding rate into 10 kg of diet. The growth performance and bioconversion efficiency were monitored. Furthermore, the nutritional composition of both substrates and BSFL were determined to evaluate the effect of feeding rates on biomass quality. Data were analysed by GLM model. Throughout the trial, weight was influenced by diets, feeding rate and time. The GA treatment displayed heaviest larvae, followed by ITA and REP. Improved larval weights were also obtained with increasing feeding rate. The bioconversion capacity decreased with increasing feeding rates. Based on the results, it is evident that increasing the feeding rate and, as consequence, reducing the larval density, the bioconversion capacity of the larvae is deceased, even though larval weight appears to increase. Considering the interaction between diet and feeding rate, the optimal amount of diet is related to its nutrient availability.

Possibility of publication: a scientific paper is ready for the submission and an abstract was accepted as oral presentation at the Conference Insect to Feed the World 2024.
Nature and number of persons from the user groups who were trained: one researcher and one PhD from Czech Republic were trained in the protocol design and sampling operations, the PhD was also trained for the chemical analyses protocols.

Name of user: Martin Kulma
Affiliation institution of the user: Czech University of Life Sciences Prague (CZ)
Country where the institution is based: Czech Republic
When the user accessed the RI: 23/11/2022 – 30/11/2022
Number of units allocated to the user: 32

PID 34105 – Black soldier fly production: Unlocking it's Potential via Genetic Research and Dietary Enhancement (BSF-UPGRADE)
Applicants: David Deruytter - Lukas De Praetere

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Organisation name: Inagro vzw (Belgium)
Status: completed

The aquaculture sector is growing fast and with that need for quality aquaculture feed. *Hermetia illucens* (HI) protein meal could be part of this feed as, partial, substitution for the currently commonly used but scarce fishmeal. Yet, the economic viability of HI farming is still difficult. The HI industry is currently not utilizing the vast genetic differences that exist, and any genetic improvement may rapidly improve the economic situation of the sector. The project aimed to assess 2 different HI populations and a hybrid to assess the economic impact and potential improvements. The 2 populations have known differences in the pupal stage, yet assessed in a common environment. The project wanted to test the production in a common environment and assess potential hybrid vigour by crossing. Besides the known pupal differences, the project assessed growth, survival, nutritional quality and reproduction to fully understand their potential. Preliminary data are not yet available, as the trial has just concluded.

Possibility of publication: yes (peer review journal).
Nature and number of persons from the user groups who were trained: One researcher from Belgium collaborated with the UNITO team on protocol preparation and trial execution. Additionally, one technician from Belgium received training in sampling operations and colony maintenance. A Master student was also trained in maintaining the HI rearing cycle, preparing experimental protocols, conducting trial sampling, and performing chemical analyses.

Name of user: David Deruytter and Lukas De Praetere
Affiliation institution of the user: Insect Research Centre, Inagro
Country where the institution is based: Belgium
When the user accessed the RI: 3/03/2025 – 12/03/2025 (David Deruytter) 11/03/2025 – 20/03/2025 (Lukas De Praetere)
Number of units allocated to the user: 58

2.1.2. Selection of One exemplary project

PID 23116 - Interest of using mix of insect meals to support the aquaculture sustainability (MIXIM)

This study focused on the effects of including a mixture of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) meals on the physical quality of the test feed pellets and the influence of this substitution on the growth performance, carcass yield, somatic indexes, nutrient digestibility, intestinal microbiota, histopathology and fillet quality of rainbow trout. Six experimental iso-nitrogenous, iso-lipidic, and iso-energetic diets were formulated compared with a control diet with 15% FM: 100% HI meal; 100% TM meal; and four diets including a 1:1 mixture of HI and TM meals at increasing levels of fishmeal replacement: 25%, 50%, 75%, and 100%. The physical quality of the pellets from the seven diets was evaluated. A total of 378 fish (average weight: 126g ± 1.71) were randomly distributed in 21 experimental tanks (3 replicates/treatment and 18 fish/replicate). Fish were hand-fed twice daily and the feeding ratio was adjusted during the experience. A digestibility trial was also conducted to assess the nutrient apparent digestibility coefficients of the diets. After 12 weeks, fish were slaughtered, eviscerated and organs were weighed. Fillets, feaces, and internal organs were collected and stocked for analyses.
Results showed significant differences on the physical quality of the pellet while miner difference for the physical quality of fillet. No significant differences were found in growth performance, carcass

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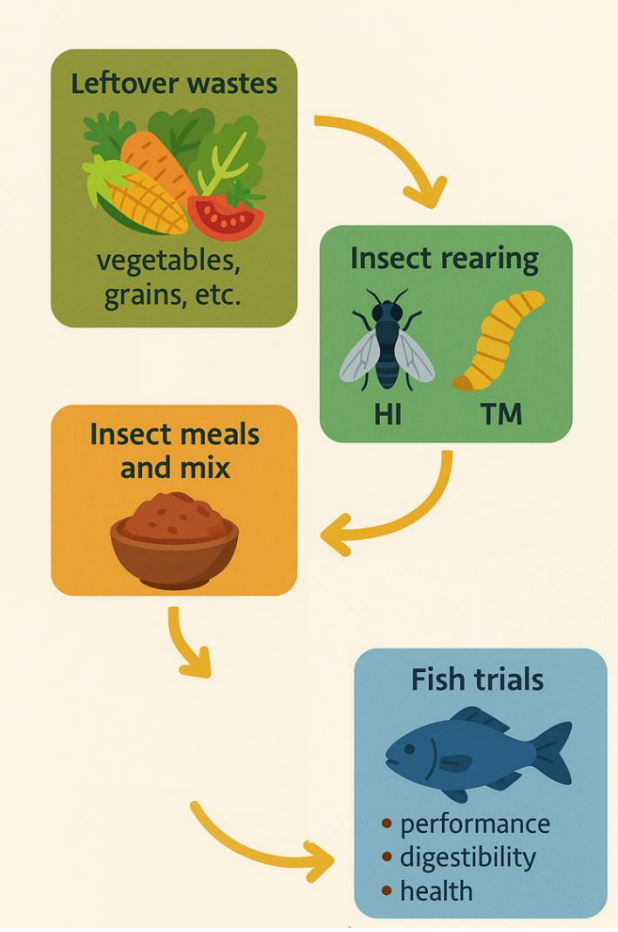
yield, somatic indexes, chemical composition, nutrient digestibility, intestinal microbiota and the histopathology of internal organs among the experimental diets ($P > 0.05$). These findings suggest the potential of using HI and TM meals, either individually or in combination, as viable protein alternatives in rainbow trout diets.

This study confirms that *Hermetia illucens* and *Tenebrio molitor* meals, alone or combined, are viable alternatives in low fish meal diets for rainbow trout. Despite variations in the physical properties of the feed pellets, growth, digestibility, gut health, and fillet texture remained unchanged, with only slight modifications in color, pH, and WHC.



3. Reflection on results of the TNA programme

The TNA installation successfully enabled hands-on access to advanced aquaculture and insect-rearing infrastructure. TNA users had access to infrastructures not present in their countries and gained valuable technical skills in experimental setup, data collection, and biological analysis.

DISAFA AQUA provided insights into sustainable fish farming practices and the use of new ingredients for a more resilient aquaculture.

DISAFA-INSECTS highlighted the potential of insect farming for aquafeed production, emphasizing circular bioeconomy models.

TNA users learned best practices in facility operation, biosecurity, and species-specific handling.

TNA programme enable intensive collaboration between DISAFA and other researchers fostering knowledge exchange and network-building among researchers. These collaborations will surely facilitate joint research projects submission.

Participants noted the high-quality infrastructure and support.

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A key challenge was the overly long evaluation and selection process, which delayed trial planning and implementation. We recommend shortening the evaluation timeline in future calls to avoid logistical constraints.

4. References

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Work Package Leader	Laura Gasco			
Work Participants	Ilaria Biasato, Christian Caimi, Dario Sola, Sara Bellezza Oddon, Zaira Loiotine			

Lead Beneficiary	UNITO (DISAFA-AQUA and DISAFA-INSECTS), 22
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