

Brussels, 17 May 2024

COST 040/24

DECISION

Subject: Memorandum of Understanding for the implementation of the COST Action “Group on Insect Nutrition: To Open Nutritional Innovative Challenges” (GIN-TONIC) CA23127

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action Group on Insect Nutrition: To Open Nutritional Innovative Challenges approved by the Committee of Senior Officials through written procedure on 17 May 2024.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA23127

GROUP ON INSECT NUTRITION: TO OPEN NUTRITIONAL INNOVATIVE CHALLENGES (GIN-TONIC)

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to foster connections, facilitate knowledge sharing, and inspire forthcoming research collaborations among prominent and upcoming researchers, and various stakeholders within the edible insect farming industry. The Action primary aim is to facilitate knowledge transfer on insect nutrition.. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.

OVERVIEW

Summary

The current global food system faces several challenges. These include producing sufficient high quality protein, decreasing environmental impact, and managing waste and by-products. Certain insect species are able to upcycle waste streams and valorize underutilized industrial by-products. As approximately 70% of production costs, and about 50% of the environmental costs are associated with feed production optimizing this element is an essential step in maturing the insect industry.

If nutritional and dietary requirements of insects are understood, this decreases financial and environmental costs, and increases yields. In this way, high quality protein is produced, associated environmental impact decreases and waste cannot only be managed, it can be valorized. Hence, a thorough understanding of insect nutritional physiology is needed to further develop insect production systems. In the last 15 years the number of studies on insect nutrition has grown exponentially. To a large extent these studies tested a small set of ingredients to determine optimal inclusion percentages. Although this has furthered our understanding, the required fundamental knowledge on nutritional requirements is largely lacking, and more importantly, it is fragmented.

This project creates a platform on which this fragmented knowledge is gathered from academic and industrial partners, synthesized and made publicly available. Besides creating an overview of the state of the art, it identifies gaps in knowledge fueling the creation of new projects to fill these gaps by partners within this open network. It functions as a linking pin between applied and fundamental research, furthering our collective knowledge and fostering business opportunities.

Areas of Expertise Relevant for the Action • Animal and dairy science: Agriculture related to animal husbandry, dairying, livestock raising, animal welfare • Other agricultural sciences: Sustainable production • Biological sciences: Zoology, including animal behaviour • Agricultural biotechnology: Other bioproducts (products manufactured using biological material as feedstock)	Keywords • Circular economy • Animal nutrition • Nutritional requirements • Entomology
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Specific Objectives

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination

- RC1: Knowledge mapping
- RC2: Digestive systems
- RC3: Effects on requirements
- RC4: Methodologies & best practices

Capacity Building

- Fostering communication and collaboration among researchers to expedite advancements in edible insect nutrition, emphasising on enhanced efficiency and developing better feeds.
- Facilitating the transfer and exchange of knowledge between well-established research areas like

Drosophila research and conventional livestock.

- Creating a bridge between academics and industry to enhance information exchange, while equipping young scientists to facilitate sector growth via knowledge on insect nutrition.
- Building partnerships for research collaborations, particularly among young researchers and participants from Inclusiveness Target Countries.
- Enabling conference participation by participants targeted by the COST Excellence and Inclusiveness policy.
- Enabling and financially supporting Short-Term Scientific Missions to develop skills and increase the knowledge level of participants.
- Transfer knowledge into practice by organising workshops tailored to industry partners.
- Hosting collaborative workshops that draw contributions from diverse research fields, including insect farming, entomology, aquaculture, and conventional livestock.
- Disseminating knowledge concerning insect rearing across Europe to enhance the resilience of European food and feed supply chains.
- Engaging in communication with policymakers to make informed decisions on regulating feeds for insect production.

TECHNICAL ANNEX

1. S&T EXCELLENCE

1.1. SOUNDNESS OF THE CHALLENGE

1.1.1. DESCRIPTION OF THE STATE OF THE ART

Food production is associated with 20-40% of global greenhouse gas emissions (Tubiello et al., 2021), but is generally overlooked compared to energy production. Population growth and increased wealth in populous countries, particularly in the Global South, will increase the demand for protein-rich animal-based foods. It is forecasted that food demand will increase by 30-62% between 2010 and 2050 (Van Dijk et al., 2021). Present animal production systems are currently at, or above, their sustainable limits in many EU countries. Further intensification would further impair natural resources such as water availability, lead to soil and air pollution, and aggravate climate change. Hence, sustainably meeting the increased food demand requires both increased production and a decreased environmental impact.

A large part of the environmental impact is due to feed production for livestock animals. Moreover, this feed production can be in competition with food, which means that an increase in feed production hampers the increase in food production, unless more land is devoted to agricultural crops. This alternative, however, would mean changes in land use, with concurrent increased greenhouse gas emissions, biodiversity loss and an impact on water resources. At the same time, consumer perceptions of livestock production systems regarding environmental impact and animal welfare are changing, demanding new approaches to provide safe, nutritious, sustainable and affordable animal-based foods (Liu et al., 2023). One of these approaches is insect farming.

Insect farming addresses several of these challenges by directly replacing conventional livestock species as food, or by using insects to form a protein-rich feed ingredient. Compared to traditional livestock, several insect species are better in utilising low-value, or contaminated (e.g., microbes, mycotoxins) agri-food byproducts and wastes such as animal manures and food wastes. Hence, insect farming can valorise such products, while preventing feed-food competition. Moreover, these systems contribute to managing organic byproducts/wastes, which form a public and environmental health issue in the food system. Insect farming can thereby ensure that nutrients and energy in agri-food by-products are recycled within the food system, limiting environmental impact such as greenhouse gas and ammonia emissions. A key aspect in the sustainable use of insect production systems is matching low-value feedstocks to the palette of insect species, and to proper post-harvest processing of insects, to create safe feed and food products with a low environmental impact (Oonincx, 2021).

Whereas over 2000 species of edible insects are described globally for their use as food or feed, scalable rearing systems and protocols exist for only a limited set of species. The current palette of commercially produced insect species, includes the following: the black soldier fly (BSF; *Hermetia illucens*), the common housefly (*Musca domestica*), the yellow mealworm (*Tenebrio molitor*), the lesser mealworm (*Alphitobius diaperinus*), the house cricket (*Acheta domesticus*), the banded cricket (*Grylloides sigillatus*) and the Jamaican field cricket (*Gryllus assimilis*). These species are economically relevant and authorised as food or feed within the European Union.

These edible insects are rich sources of proteins and lipids, providing ample amounts of amino acids, energy, certain minerals (for instance iron and zinc), and various vitamins (Finke & Oonincx, 2023). Hence, they know various applications, either as a whole or as ingredients. In addition, insect frass (a mixture of residual feed, faeces and exuviae) is a potent plant fertiliser, a biostimulant that increases plant yields and health, and a means to improve the soil microbiome and overall quality (Barragán-Fonseca et al., 2022). As healthy soils form the basis of our food production system, insect production systems naturally fill the voids by improving circularity. Consequently, the rearing of edible insects can be a cornerstone of European agriculture providing an alternative to currently imported protein sources such as soybean and fish meals. Moreover, providing insects as feed or enrichment for conventional livestock has health and welfare benefits compared to currently used proteinaceous ingredients (e.g. Ipema et al., 2022).

Animal nutrition for production animals

In poultry and livestock farming feed makes up approximately 60-70% of total costs (Lawrence et al., 2008). Similar to conventional livestock farming, also in insect farming, feedstock utilisation is a crucial determinant for yields, financial viability, and environmental impact. Due to decades of research and development of conventional livestock farming systems, production yields have seen a large increase. This progress was in part due to the characterisation of feed ingredients via standardised protocols, with much of this information finding its way to publicly available databases. Perhaps more importantly, our understanding of animal physiology, their microbiome, digestive system, and metabolism has grown immensely. This included detailed feed digestibility metrics per species, and nutrient requirements per breed and development phase. By matching this knowledge with feed composition, the efficiency of livestock farming has been greatly enhanced, with - for example - feed conversion efficiency doubling in broiler production (Van Emous, 2015).

The field of insect nutrition: the current state of the art

Similar to conventional production animals, insect nutrition can be divided into several aspects. A first, quite basic aspect of insect nutrition is feeding behaviour. What is considered feed; that is, a potential source of nutrients, and how is it acquired? Several aspects influence feeding behaviour. For instance, feeding behaviour is affected by environmental conditions such as temperature, light, and moisture (e.g. Holmes, 2010). Ambient temperature influences body temperature, which greatly affects insects as they are ectothermic organisms. Furthermore, high densities can cause thermal stress and reduce feeding. Light is also known to influence feeding behaviour, for instance, it is known that BSF adults avoid feeding in areas with a blue background and prefer a white background. Another condition that influences feeding behaviour is the moisture content of a substrate. For instance, BSF larvae can abandon substrates with excessive moisture. Besides environmental conditions, feeding behaviour is influenced by factors such as larval age, substrate choice, the presence of pathogens, and competition. In addition, female feeding behaviour can be influenced by seminal fluid proteins transferred during mating. Also, the presence of foreign gut bacteria can distort the feeding behaviour of insects by changing their sense of smell. Various other elements of feeding behaviour, such as continuous vs. discontinuous, occurrence of cannibalism and morphology of mouthparts are relevant factors that could affect it.

When a feed is consumed, it needs to be digested and subsequently absorbed. Hence, it is important to gain insight into the processes of digesting macromolecules, as well as the absorption of the digested compounds. In order to understand these two processes, knowledge of digestive physiology and on the functional morphology of the digestive tract is needed. Unfortunately, relative to the interest in edible insects for practical applications such as food and feed production, our understanding of their physiology remains limited (Bonelli et al., 2020). The most obvious is an understanding of the digestive system, which is the linchpin of the natural bioconversion process that we aim to harness. The gut plays a pivotal role in digesting and absorbing nutrients, boasting an impressive array of digestive enzymes that facilitate the absorption of nutrients to be used for ATP production or as building blocks for the biosynthesis of macromolecules. Digestion and absorption are both essential elements for efficiently utilising feeds. Interestingly, some insect species exhibit remarkable abilities to finely regulate digestive processes and nutrient absorption through post-ingestion mechanisms. This ensures their nutritional needs are met, even when the rearing substrate has low nutritional content, which might reduce larval growth. Moreover, microorganisms have clear nutritional and metabolic effects on their insect hosts. The level of knowledge greatly differs over species for the aforementioned aspects. While very little is known for the lesser mealworm, much more is known for the common housefly. For instance, for the latter species, it is known that it is specialised in consuming bacteria, the role of several enzymes in digesting these microbiota, and the functionality and morphology of different parts of the gut. This combined knowledge on feeding behaviour, microbiota, and digestive physiology, provides valuable information for improving nutrient utilisation. Within this project, expertise available on certain species will be used to gain insight into potential mechanisms in other species, to formulate hypotheses and prepare steps to test these hypotheses (see section 1.2).

Moreover, a large portion of existing research consists of feeding studies that explore the suitability of a limited amount of feedstocks and mixtures thereof, focusing on optimal inclusion levels. While informative, these have limited value for understanding underlying mechanisms. Some feeding trials have aimed to disentangle nutrient requirements using artificial diets. These have mainly focused on protein and carbohydrate levels, without considering differences in quality (Barragan-Fonseca, 2019). While the crude protein content of a feed is relevant, protein quality, as indicated by the amount and

ratios of amino acids, is a more important factor in fulfilling nutritional requirements. Currently, little is known about the amino acid requirements of insects, nor how well feeds provide digestible amino acids to the various insect species.

Besides proteins and amino acids, dietary intake of lipids and carbohydrates is also an important aspect of fulfilling the nutrient requirements of animals, including insects. These components fulfil various functions, including the production of cell walls, the synthesis of sex pheromones and cuticular waxes, and for the production of various defensive secretions. Moreover, they are needed as a source of energy to be used during moult, flight, migration, diapause, starvation, and for developing embryos. Despite the fact that these basic concepts are known, there are still knowledge gaps regarding how genes, and major cell types are involved and how lipids and carbohydrates are enzymatically metabolised. Such knowledge is needed to understand digestibility and absorption and therewith the required quantities of different lipids, and carbohydrates in specific feed ingredients.

While these macronutrients have received some attention from researchers, this is less so for vitamins and micronutrients. These components, however, play a key role in insect growth and development. Vitamins can function as cofactors in various biochemical pathways and contribute to defensive reactions (e.g., serving as antioxidants). Similarly, minerals are involved in various metabolic processes and have multiple functions (e.g., role in muscle function and energy utilisation, insect immunological responses). Despite these compounds having an important function in insect physiology, knowledge about their nutritional availability and species-specific requirements is largely absent.

Current methodologies used in insect feeding studies are highly diverse. This prevents harmonisation and reproducibility among studies. In recent years some attempts have been made to fill this gap, for instance, to standardise the approach to quantify conversion efficiency in the BSF (Bosch et al., 2020). Within such studies relevant elements for describing the basic experiment design, fly colony origin, rearing procedures, reference and experimental feeding substrates, and sampling preparations including microbiota and chemical analyses are described. Such studies form a foundation for creating standardised protocols, which are instrumental to allow conversion efficiencies to be calculated, and hence for feeds to be evaluated. More recently, the next step was made to progress the field of insect nutrition further, with the development of a standardised protocol (Deruytter et al., 2023). Even for the BSF, the insect species that has received most attention in the last decade, methodological challenges still persist. A very basic example is the collection of faeces. As this species lives within its feeding substrate, it also defecates within that substrate, making the collection of pure faeces challenging. A noteworthy attempt to fill this void is the publication by Gold et al., (2020), in which a larval diaper was first developed and tested. While for the first species, rudimentary steps have now been taken, for others these are still required.

Fortunately, the field of insects as food and feed is developing, and with that collaborations between researchers from different sub-disciplines are also developing. At the current moment, these collaborations are often initiated at the individual level, with networks being created for specific projects, or as part of larger initiatives (See section 2.1). Within the sub-discipline of insect nutrition, collaborations are yet to be coordinated.

In summary, insect farming has great potential to provide valuable nutrients to meet European and global demand for animal-based foods, while reducing environmental impacts, improving animal welfare, increasing protein and fertiliser sovereignty and managing agri-food byproducts and wastes. However, in comparison to conventional livestock systems, the provision and utilisation of feedstock nutrients by insects is poorly understood. Understanding the nutrient requirements of edible insects will improve the viability of valorizing underutilised by-products and waste, and increasing the circularity of our food system. This contributes to food security, and reduces the environmental footprint of our most basic needs. As such, insects as food and feed contribute to sustainability of modern farming systems via insect farming, supporting EU and global targets such as Farm-to-Fork, the Paris Agreement, and several of the Sustainable Development Goals defined by the United Nations

1.1.2. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

This Action seeks to foster connections, facilitate knowledge sharing, and inspire forthcoming research collaborations among prominent and upcoming researchers, and various stakeholders within the edible insect farming industry. The Action primary aim is to facilitate knowledge transfer on insect nutrition. It does so by gathering knowledge from researchers and producers of edible insects, with input from other economically important insect species, and by repurposing established knowledge from conventional livestock. As such, this Group on Insect Nutrition, aims To Open Nutritional Innovative Challenges (GIN-TONIC).

The main research questions addressed by this COST Action are formulated and described below. These questions are interconnected and the work on addressing them is to be seen as an iterative process. Progress made on one aspect informs the next, and so on.

What is known on insect digestive systems and feeding behaviour?

A detailed understanding of edible insect physiology, digestive systems and feeding behaviour is pivotal to understand their nutritional needs. **The Action will characterise the relevant digestive systems across commercially produced insect species (section 1.1.1) and their feeding behaviours.** Moreover, it will identify what is known regarding the role of microorganisms in modifying the nutritional conversions and metabolism of their host insects, including their impact on the host's immune system. For instance, this Action explores the multifaceted roles of the peritrophic matrix (PM) in digestive physiology and its function in defence mechanisms against pathogens. It also examines the interplay between fungal and bacterial microorganisms within the digestive system of insects. The metabolism of different nutrients will be considered, such as the determination and differentiation of the major cell types involved in lipid and carbohydrate metabolism and the determination of key genes involved in lipogenesis and glycogenesis. It aims to uncover the determinants of feeding behaviour, and the distinct adaptations of mouthparts and their influence on feeding patterns. Furthermore, it explores potential trade-offs between feeding behaviour and other behavioural aspects.

Genetic and genomic tools can be used to select and produce strains with traits that enhance bioconversion efficiency, performance on specific substrates, and the quality of insect biomass and derived products. Hence, these tools can be used to understand insect physiology, digestive systems, and feeding behaviours. Within this Action, the specific use of these tools will be identified, and the required methodologies will be described in order to facilitate their use by this Action's participants and the scientific community.

Another tool is the use of *in vitro* digestion models, which mimic the digestive system of edible insect species. Similar to other livestock, these can prove to be powerful research tools, able to predict outcomes from *in vivo* insect feeding. These can mitigate unforeseen variation in insect growth performance on diverse by-products, an aspect that is essential for industrial-scale bioconversion. Hence, by establishing morphological, physiological, and molecular parameters linked to insect digestion processes, lean methods can be created and applied to improve our understanding of which feed items are suitable and why. This will also lead to reliable diagnostic markers for monitoring larval health and development during mass rearing, and identifying when edible insects are unhealthy. Nutritional immunology approaches can be used to modulate systemic immunity through interventions in the diet, increasing insect resistance to pathogens, and optimising health status during mass rearing.

Which methods are currently the most suitable for insect nutritional research?

This research question addresses the currently available and most suitable methodologies in nutritional research methods for determining the nutrient requirements of edible insects. The Action will **identify and compare the currently used methods in academia and industry.** Furthermore, it **taps into the robust and established approaches from conventional livestock species** (e.g., poultry, pigs, fish) and well-studied insects (e.g., *Drosophila*). These approaches are then synthesised and tailored towards the aforementioned insect species (Section 1.1.1.).

For instance, focus will be on negating challenges such as determining the digestibility of feeds for species that live, moult and defecate in their feeding substrate. Moreover, attention will be paid to identify best practices in experimental design and data analysis, and how these might differ over the species of

edible insects. Various other challenges such as the scalability of research results from laboratory and bench-scale settings towards industrial-scale applications, and how to deal with effects of different genetic strains on the extrapolability of results, need to be identified and mitigation measures need to be created. These elements are to be embedded in the foundation of any future studies on nutrient requirements of edible insects. By mitigating these challenges, the large amount of research that is produced in the field is reproducible and comparable, allowing the drawing of overarching conclusions and streamlining the development of the scientific discipline.

Which nutrients are required in which ratios and in which form?

Analogous to conventional livestock species, **this Action aims to lay the foundation for feeding tables for the economically most relevant edible insects produced in Europe.** Information that can be drawn from insights from the digestive system, physiology, feeding behaviour, and the role of microorganisms will be the basis for the development of feeding tables, as well as typical nutrient contents and digestibilities in edible insect farming feedstocks, including organic waste and agri-food byproducts. This will be done by extracting information from nutrient tables for conventional livestock systems. A key part will be summarising potentially limiting nutrients (e.g., macronutrients, amino acids, vitamins, and minerals) and quantifying the optimal range of macronutrients. Moreover, compensatory mechanisms for the utilisation of nutrients (for instance, carbohydrates and lipids) will be identified. These elements are influenced by environmental factors and dietary characteristics, such as water holding capacity and structural texture, which will also be taken into account. Specific attention will be paid to the different roles (competitor, pre-digester or nutritional source) the microbial community has in meeting the nutrient requirements of the aforementioned insect species.

Timeliness of this COST Action

In the fifties, sixties, and early seventies of the last century, the foundation of insect nutritional research was laid. During that period, basic insights into qualitative nutritional requirements were explored for a small array of model species. It was shown that these requirements were fairly similar to those of so-called higher organisms, with certain noticeable exceptions, such as sterol requirements. Then, for some decades, this academic niche laid largely dormant.

At the onset of the new millennium, an interest in the use of insects as food or feed started to emerge. Supported by the Food and Agricultural Organisation, the impact of gathered insects on the livelihoods of people in Central Africa came into focus. Subsequently, interest in the use of edible insects in other geographical locations, noticeably South America and Southeast Asia, developed and the scope of interest broadened to include produced insects. In 2013, this led to the iconic publication “Edible insects: Future prospects for food and feed security” (Van Huis et al., 2013). At that time, a handful of researchers focused on the topic, and the first large-scale producers had started their facilities. Now, after a decade of pioneering the field of insect nutrition, researchers in academia and industry have gathered enough tacit and explicit knowledge, to take the next step.

The consortium underlying the GIN-TONIC COST initiative is committed to sharing their individual progress so that they can jointly move this scientific discipline forward. By addressing the aforementioned key research questions as a group and in concert, the state of the art is comprehensively synthesised, and a foundation for future collaborations is established.

1.2. PROGRESS BEYOND THE STATE-OF-THE-ART

1.2.1. APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE OF THE ART

There are numerous researchers within academia, research institutes, and insect producing companies, interested in understanding nutrient utilisation by edible insects. They are driven to harness the power of improved nutrient utilisation, either from a fundamental or an applied perspective, as it can be a lever for economic improvement needed for edible insect farming to become a viable alternative to established plant and animal-based ingredients. Moreover, both perspectives are supported by the notion that society requires sustainable and circular systems in order to persist in the long run.

With the market for edible insects expected to grow by 29% per year, reaching \$16.39 billion by 2032 (Meticulous research., 2023), feed requirements would be expected to grow at a similar rate, unless feed can be used more efficiently. Compared to conventional livestock, the knowledge level needed for efficient feed formulation and utilisation of edible insects is low. Moreover, the available knowledge is fragmented, sometimes inconsistent, and has varying levels of detail among the different insect species. Consequently, the nutrient utilisation rate of current edible insect farming systems is **likely to have much room for improvement**. Research in this field has recently seen tremendous growth alongside the expansion of the edible insect industry. This research is often directed at one target species and/or a single aspect. **The next step, therefore, is to break through discipline and species boundaries**. This can be done by bringing experts working on physiology, digestive systems, nutrient requirements, and interactions with microbiota together in topically divided working groups. These groups will gather the currently available information related to insect nutrition, synthesise it, agree on standardised methodologies and procedures and disseminate it through group interactions. In order to do so, within this COST Action the available expertise on certain species, will be used to gain insight into potential mechanisms in other species, to formulate hypotheses and prepare the next steps to test these hypotheses. As such it will fuel further interactions, aiming to fill knowledge gaps by initiating specific projects.

1.2.2. OBJECTIVES

1.2.2.1. *Research Coordination Objectives*

RC1: Knowledge mapping

This Action presents a unique opportunity for sharing knowledge, identifying best practices, and pitfalls. It will use these to make recommendations, and to coordinate a joined research agenda. This will require a multidisciplinary set of researchers from various academic fields (e.g., entomology, livestock nutrition, agricultural science, environmental engineering, microbiology, zoology, etc.) and industry (e.g., feed, technology, breeding and rearing companies), which will be connected in order to identify synergies and achieve mutual goals. In this respect, this Action will:

- **Map current areas of activity, priorities, and long-term strategies**
- **Map the strengths and capacities of partners across the Action**
- **Facilitate information sharing between academia and industry and *vice versa*, integrating the theoretical framework with practical and observational experience**
- **Transfer knowledge from other disciplines, such as well-studied insect species and conventional livestock to edible insects**
- **Come to a common understanding of insect nutrition and coordinate research required to close knowledge gaps**

RC2: Digestive systems

An in-depth understanding of the digestive system, physiology, and feeding behaviour is indispensable for research on edible insect nutrition and the generating tangible outputs to improve insect rearing efficiency. In this respect, this Action will:

- **Collect state of the art information on the digestive system, physiology, and feeding behaviour for the selected insect species**
- **Transfer knowledge from other well-studied insects and conventional livestock systems to fill gaps**
- **Coordinate future research regarding insect nutritional “hardware” and behaviour**

RC3: Effects on requirements

Edible insects have adaptation mechanisms to fulfil their nutritional requirements, which makes determining their nutrient requirements more complex. Quantifying these therefore requires disentangling the interconnected factors or identifying approaches to incorporate those complexities:

- **Identify the most relevant factors influencing nutrient requirements**
- **Map effects of substrate physical properties on nutrient requirements**
- **Map effects of the microbiome on nutrient requirements**

- **Identify similarities and differences between the selected insect species, using available information from better studied insect species, and conventional livestock**

RC4: Methodologies & best practices

A current limitation of research on edible insect nutrition is the lack of harmonised analyses, methods, and experimental setups. This Action will:

- **Gather information on currently used analysis methods and experimental setups**
- **Provide a platform to discuss these best practices and pitfalls**
- **Reach consensus on best practices, and formulate protocols for feeding experiments and analyses to facilitate data extrapolation**
- **Develop new and creative experimental setups to determine digestibility metrics and determine nutrient requirements**

1.2.2.2. Capacity-building Objectives

Building capacity in all Action countries is a central goal of this COST Action. While focused on young researchers, this includes more senior researchers new to this research field. The Action focuses on providing access to state of the art knowledge on edible insect nutrition across Action members. Specific capacity-building objectives of the GIN-TONIC Action include:

Fostering communication and collaboration among researchers from diverse backgrounds, or focusing on various edible species, to expedite advancements in edible insect nutrition, emphasising on enhanced efficiency and developing better feeds.

Facilitating the **transfer and exchange of knowledge** between well-established research areas like *Drosophila* research and conventional livestock.

Creating a bridge between academics and industry to enhance information exchange, while **equipping young scientists** to facilitate sector growth via knowledge **on insect nutrition**.

Building partnerships for research collaborations, particularly among young researchers and participants from Inclusiveness Target Countries.

Enabling and financially supporting Short-Term Scientific Missions to develop skills and increase the knowledge level of participants.

Transfer knowledge into practice by organising **workshops tailored to industry partners**.

Hosting **collaborative workshops** that draw **contributions from diverse research fields**, including insect farming, entomology, aquaculture, and conventional livestock.

Disseminating knowledge concerning **insect rearing** across Europe to enhance the resilience of European food and feed supply chains.

Engaging in **communication with policymakers** to make informed decisions on regulating feeds for insect production.

Enabling conference participation by participants targeted by the COST Excellence and Inclusiveness policy.

2. NETWORKING EXCELLENCE

2.1. ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Even though edible insect production has seen exponential growth in the last decade, scientific progress has been limited. This was due to non-harmonized or insufficiently described experimental setups, improper statistical considerations, limited analytical methods and a lack of coordination of research efforts. To counter this, in 2019 an initiative of 40 researchers within the Insect Commission of the European Federation of Animal Science (EAAP) embarked on standardising insect research. This initiative, while fruitful (Deruytter et al., 2023), required further strengthening, coordination and funding. The coordination of research efforts within the discipline of insect genetics has been initiated via the recently granted COST-Action, INSECT-IMP. However, as seen with conventional livestock and aquaculture, adequate insect genetics and reproduction programs require detailed insights in insect nutrition to unleash its full potential. In concert, nutrition and genetics fuel the efficient use of resources within insect production systems, hence the proposed GIN-TONIC Action connects to, and compliments

the INSECT-IMP. This Action will actively connect to this running action to facilitate synergies between the programs.

This Action also builds further on three recent European initiatives: 1) Insect Doctors, focussing on disease resistance in farmed insects, 2) SUSINCHAIN, which focused on increasing the economic viability of the insect value chain, and 3) INSECTFEED, focussing on insect health and welfare, and benefits for insect-fed poultry. To these initiatives, this Action adds a focus on knowledge sharing and standardisation. This aligns with a workshop funded by the Organization for Economic Cooperation and Development (OECD) during the last (2022) Insects to Feed the World Conference in Quebec, Canada, that aimed to harmonise methodologies and research setups. Via several presentations, the workshop identified the challenges of measurements in insect feeding studies, showcased a ring-test among nine research centres which highlighted a lack of research reproducibility and harmonised research methods, and made the comparison with the evolution and standardisation of bee research. This Action follows up on that workshop, further developing appropriate feeding systems, based on species-specific nutritional requirements, while learning from more-studied species.

The next Insects to Feed the World Conference In Singapore (2024) will be a perfect platform to introduce the GIN-TONIC COST Action to the global community working on insect production and continue the expansion of this network within and beyond Europe. Moreover, this Action will make use of the EAAP annual meeting to connect further to the European community of insect researchers. This platform has seen rapid growth, from 2 presentations on insect-related topics in 2016, to 65 in 2023. This COST Action aligns closely with the activities of the EAAP Insect Commission, focussing on the transfer of knowledge from well-studied insect species and other livestock species to advance the field of insect nutrition as is needed for further maturation of insect production. This Action is however additive as it provides a more inclusive umbrella, involving early-stage and senior researchers, coming from both industry and academia. Moreover, it facilitates interactions, between geographically distant research groups, with a diverse demography as is exemplified by the network of proposers.

2.2. ADDED VALUE OF NETWORKING IN IMPACT

2.2.1. SECURING THE CRITICAL MASS, EXPERTISE AND GEOGRAPHICAL BALANCE WITHIN THE COST MEMBERS AND BEYOND

To achieve its primary goal of promoting reproducible knowledge and advancing the field of edible insect nutrition, this Action starts out with a diverse and inclusive community of researchers, industry and policy representatives with varying backgrounds and expertises. The GIN-TONIC Network of Proposers comprises researchers working on the full spectrum of aforementioned insect species, but also conventional livestock, such as fish, poultry, and pigs. The network of Participants is expected to expand further, by openly presenting themselves at events, as mentioned in the previous section. Given the relative novelty of edible insect species, expertise on certain target species is underrepresented. It is anticipated that these gaps will gradually close as the community-building aspect of this Action progresses, fostering stronger connections among Participants and building further on their networks. The Action will facilitate the mobilisation of these networks to address any underrepresented topics and involve stakeholder groups beyond the existing network. Researchers and producers of Inclusiveness Target Countries (ITC) are well-represented in the Network of Proposers.

The Action proposers consist of a diverse group of professionals, including engineers, agricultural scientists, insect farmers, entomologists, animal scientists, zoologists, and veterinarians. As such, the expertise within the Action encompasses a range of fields, with particular emphasis on edible insect physiology, microbiology and insect nutrition. However, to further bridge the gap between scientific research and practical applications, input from industry stakeholders is crucial. Hence, a key objective is to provide a neutral platform for insect farmers to participate in research initiatives, enabling direct contributions from and to the industry. Historically, insect farming businesses were hesitant to collaborate due to industry immaturity, competition for funding, and limited supply. However, several small and large companies have committed to this Action, while ensuring the security of privileged data. It seems likely that during this Action more producers will be inclined to join, following the example of these colleagues.

2.2.2. INVOLVEMENT OF STAKEHOLDERS

This Action includes a range of stakeholders associated within the insects as food and feed value chain. Further involvement of stakeholders benefiting from efficient feedstock utilisation, such as insect feed producers, insect processors and policy makers will be sought when the COST Action is granted. As this Action aims to draw knowledge from stakeholders in well-established livestock nutrition and well-studied insects, further integration of value chain partners is likely. Several proposers have strong bonds with existing networks such as IPIFF (International Platform of Insects for Food and Feed), CEIF (Center for Environmental Sustainability Through Insect Farming), Asian Food and Feed Insect Association (AFFIA), and the North American Coalition for Insect Agriculture (NACIA), the different commissions of the EAAP (e.g. Animal Nutrition, Animal Physiology, etc.). Through these platforms, stakeholders will be actively invited to participate in and support this Action where needed. Similarly, presence of Proposers at key sector events, including INSECTA in Berlin (May 2024), the global conference Insects to Feed the World (June 2024) in Singapore, the EAAP meeting in Florence (August 2024), and the International Congress of Entomology in Kyoto (August 2024), will facilitate further stakeholder involvement. Moreover, targeting these established events will foster a dialogue between current COST Action participants and newcomers in the field.

3. IMPACT

3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAK-THROUGHS

3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

This Action addresses aspects such as substrate texture, feeding behaviour, nutrient digestibility requirements, all of which are essential in responsible farming. By combining the expertise of different research groups working on related topics defining the nutritional recommendations per species will speed up. It will facilitate the viewpoint that waste is simply resources out of place, which can, and should be valorized.

While the insect rearing sector is growing worldwide, currently the required information is lacking or is fragmented. By synthesising available knowledge, identifying knowledge gaps, and addressing these with a joined research agenda, insect producers are enabled to better valorise waste and by-products. This will decrease environmental impact on the one side and increase the production of valuable biomass (mainly protein and fat) on the other side. This improved economic and environmental sustainability will facilitate further growth and development of this sector, supporting the current global leadership of European producers.

Finally, the standardisation of scientific methodologies to be used in insect nutritional studies will allow further progress in understanding this field, and extrapolating information from individual studies. It fosters a robust foundation of knowledge and thereby scientific progress, which in turn can be applied for exploiting bioconversion capabilities.

3.2. MEASURES TO MAXIMISE IMPACT

3.2.1. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

This Action brings together researchers, and industry stakeholders. All proposers of this Action have committed to share their relevant data. Hence, this COST Action is unique as it will have access to published, unpublished and industry research data, currently not available to most stakeholders. By creating a safe platform, anonymization of data, and the establishment of data use agreements, maximal knowledge creation is facilitated.

Further explorations of potential partnerships will be explored to create more knowledge. This knowledge will be disseminated via the website, social media and open access publications. Moreover, the Action will lay the foundation for enduring collaborations amongst and between academics and industry. It is foreseen that these will translate into new projects, filling gaps in knowledge while training junior (PhD) and medior (postdoctoral) students. As such, the Action's impact will reach beyond the COST Action period. Moreover, this Action will create a cohort of young researchers with up to par knowledge on insect nutrition. The foundation for this is laid by the demography of the current proposers, consisting of the most prominent senior researchers in the field of insect nutrition within Europe, combined with a range of junior and mid-level researchers. The young researchers will be given priority

access to Workshops, Short Term Scientific Missions, and Training Schools, with active promotion through channels such as social media.

Considering the growth of insect production during the last decade, and taking into account the growth projections (Meticulous Research, 2021), these well-trained young researchers will have good opportunities to contribute to industry growth within and beyond the European borders. As the Network of proposers already has strong networks to Africa and Southeast Asia, where particularly high growth of insect farming is expected, due to its potential in ensuring food safety, it can be expected that young researchers trained within this Action, will find their place there as well. Exemplary partners/points of contacts include here the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, and the Center for Environmental Sustainability through Insect Farming (CEIF) in the USA, the National University of Singapore and the University of Colombia in Bogota.

3.2.2. PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

The intended audience for the outcomes of this Action comprises individuals engaged in insect nutrition, edible insect farming, researchers, policy makers, and the general public. The Action's initiatives will be conveyed through a dedicated website featuring a Knowledge Hub section and various social media platforms such as LinkedIn. These online channels will be consistently updated with information about the Action's progress to ensure broad outreach to academic, industrial, and public audiences. All participants in the Action will be encouraged to actively share information through social media and the Knowledge Hub, serving as a shared repository for publications and other documents of common interest. A messaging and collaboration platform among Action members and interested parties is foreseen to be utilised for effective internal communication within and over working groups.

Participants in Workshops and Training Schools will regularly give presentations at conferences focused on edible insects, insect nutrition, animal nutrition or entomology. These presentations will serve to raise awareness about the Action and promote its scientific achievements. Given the diverse backgrounds of researchers within the Action network, these presentations will offer a comprehensive representation across various fields. To enhance the dissemination of results, white papers and scientific papers containing unified knowledge regarding insect nutrition will be generated and made available open access. Additionally, these findings will be expanded into publications in popular science magazines where possible (e.g. Feed Navigator, All About Feed), featuring less technical language to ensure accessibility to a broader audience, including the industry, and the general public.

The Action will actively facilitate and support participants in connecting with their respective national and international communities, with a special emphasis on providing assistance to Early Career Investigators as they build their networks. Moreover, we will strongly encourage individuals from underrepresented countries, sectors, genders, and diverse linguistic backgrounds to take part.

Recognizing that prevailing regulatory limitations on the use of certain feedstocks (e.g. food wastes, agri-food byproducts including meat/fish) represent a significant barrier to realising the European insect industry's potential, a key aspect of the Action's efforts will involve engaging with regulatory authorities and related associations. This engagement will be sought by collaboration with IPIFF and by actively seeking opportunities to interact with legislators at regional, national, and EU levels.

4. IMPLEMENTATION

4.1. COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

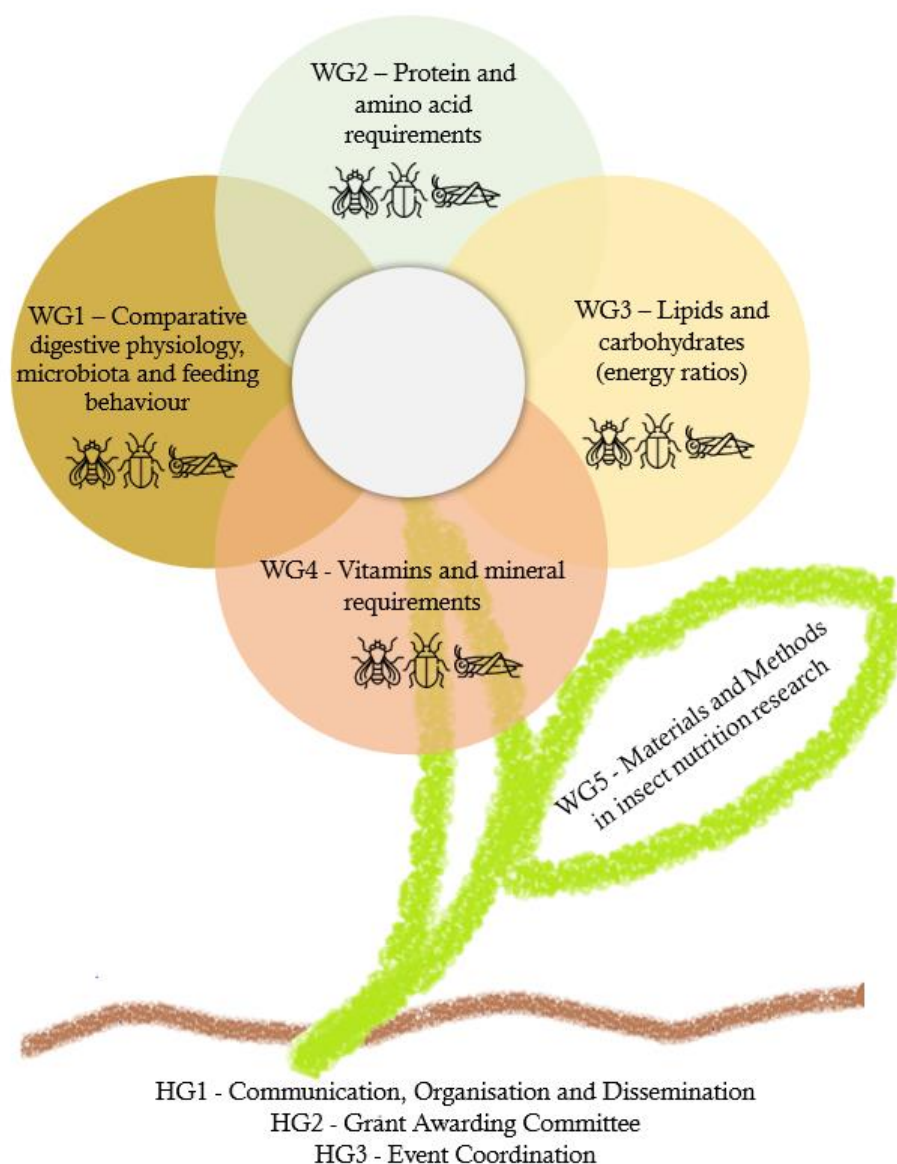
This Action consists of five working groups (WGs) described below. Given the obvious connection between insect physiology, microbiology and nutrient interactions these WGs are interconnected. Via an iterative process they will build a comprehensive nutritional understanding of the targeted edible insect species. These WGs have the following tasks:

- Provide comprehensive literature summaries of published scientific and grey literature, including unpublished and relevant anecdotal information to provide state-of-the-art knowledge of their

respective topic and build a roadmap to close knowledge gaps for maximising benefits of edible insect farming by nutritional optimization.

- Define cutting-edge methods and approaches and develop standard operating procedures to increase research reproducibility.
- Train doctoral and junior postdoctoral, as well as early career investigators (ECIs), in the selected areas around insect nutrition.
- Provide network and leadership opportunities for emerging talents to strengthen and build up excellent S&T communities.
- Drive scientific progress that will strengthen the European Research Area through the delivery of outputs needed to boost insect performance to compete with other alternative protein sources.

The figure below illustrates how the five WGs relate. Working groups 1-4 work on separate, but interconnected aspects of insect nutrition. They are supported by WG5, providing the energy required for the field to blossom. The foundation for this Action is laid by an inclusive, bottom-up community, willing to create and share knowledge.



WG1 - Comparative digestive physiology, microbiota, and feeding behaviour: Animal well-being and performance greatly relies on different nutrients. However, nutrient assimilation is firstly influenced by feeding behaviour and then by the digestive system, which is diverse among insects and plays a pivotal role in processing food, digesting macromolecules, and absorbing nutrients for metabolism and growth. Working group 1 will identify: aspects influencing feeding behaviour, digestive physiology, morphology and function, establish parameters for monitoring larval health and development during

mass rearing, employ nutritional immunology approaches to modulate systemic immunity through interventions of the diet, leverage genetic and genomic tools to select or produce strains with traits that enhance insect performance on specific substrates, and describe *in vitro* digestion models capable of predicting outcomes from *in vivo* feeding experiments. These aspects will be shared with WG1-4 to indicate possible interactions and inform WG5 for the development of materials and methods in insect research. A comparative nutrition workshop translating knowledge from well-studied livestock (e.g. poultry, pigs, and aquaculture) and well-studied insect-species, to the focus species within this Action will be organized in the second semester.

WG2 - Proteins and amino acids: The protein and amino acid profiles of feedstocks greatly affect animal development, growth and microbiota. Moreover, some amino acids are essential, while their provision is dependent on the feedstocks used for insect farming. Working group 2 will identify what is known on protein and amino acid requirements for the aforementioned edible insect species, and draft scientific protocols for determining unknown amino acid requirements thereby providing input for WG5. Moreover, WG2 will particularly collaborate with WG1 to better understand protein availability from different feedstocks as found in by-products used as feed.

WG3 - Lipids, carbohydrates (energy ratios), and fat-soluble compounds such as sterols: Carbohydrates and lipids are the two major energy sources. All animals use these macronutrients for growth and survival. Insects use these molecules for molt, flight, migration, diapause, starvation, nutrition of the embryo, synthesis of sex pheromones, cuticular wax, and production of various defensive secretions. Working group 3 will describe the major cell types involved in lipid and carbohydrate metabolism, evaluate the nutritional requirements in terms of quality and quantity for the selected insect species, and establish the key genes involved in lipogenesis and glycogenesis. WG3 will particularly collaborate with WG1 to evaluate the effect of nutrient digestibility and with WG5 to redact the experimental protocols.

WG4 - Vitamins and mineral requirements: Apart from macro- and other micronutrients, vitamins exert a key role in insect development, i.e., being co-factors in important biochemical pathways and contributing to defensive reactions (e.g., serving as antioxidants). Similarly, minerals are known to be involved in various metabolic processes and have multiple functions (e.g., role in muscle function and energy use, insect immunological responses, etc.). Working Group 4 will explore and describe the symptoms associated with vitamin and mineral deficiencies and develop recommendations about the minimum requirements of vitamins and minerals for edible insect species considered in this Action. As with WG2 and WG3, WG4 will contribute knowledge to WG1 and WG5 in terms of digestibility and experimental protocols.

WG5 – Materials and Methods in insect nutrition research: As research on the nutrition of feed- and food-producing insects is growing exponentially, it must be acknowledged that today's research is not limited in quantity. However, the knowledge generated in research labs is still highly variable and difficult to compare. To some extent, current research may fail in reproducibility because of unstandardized terminology, variable and poorly described experimental setups, improper statistical considerations and analytical methods, and the overall lack of harmonised methods. Working Group 5 will address current challenges and highlight opportunities in insect nutrition research through contributions to the improvement of experimental designs and setups, exploration of (new) tools for nutrient analysis, evaluation and studies on insect metabolism, in close collaboration with WGs 1-4.

In addition, the Action will set up the following Horizontal Groups:

HG1 –Communication, organisation and dissemination: This will include an Online Communicator, who facilitates an online resource in the form of a Knowledge Hub (Action website), which will provide timely updates on the project. This knowledge hub will function as a repository for Action Outputs, such as outputs from training schools & workshops for the open dissemination and sharing of knowledge within the Action and the wider network. Moreover, it will contain a stakeholder platform to allow open communication. The Online Communicator will select and manage relevant content and share it appropriately within the different stakeholder networks (e.g. academia, private sector, policy, wider public).

HG2 – Grant Awarding Committee: This group will be led by the Grant Awarding Coordinator and will coordinate Short-Term Scientific Missions and other grants to be awarded by the MC.

HG3 – Event Coordination: This group supports the organisation of training schools and special sessions at external events (e.g. EAAP, IFW, INSECTA, BSFCon, etc.).

4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

The GIN-TONIC deliverables include written or visual outputs in the form of:

- Peer-reviewed publications summarising the state-of-the-art, knowledge gaps and recommendations for future research (one each for WG1-WG4). These will be made publicly available in the last year of the COST action.
- A consensus paper on standardised terminology and research methods for edible insect nutrition (WG5). This will be made publicly available in the last year of the COST action.
- Documentation of the workshops, outreach events and training schools, made available via the Knowledge Hub. These will be made available within two months after the conclusion of the event (HG1).

4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

Risk analysis and Contingency plan	L	I	Ind
R: Difficulty of interaction between private and public sector	1	2	2
C: Firstly, all partners have agreed to share data. If information pertaining to diet composition is considered privileged, a focus on nutrients rather than ingredients can be a solution still allowing a fundamental approach. Moreover, meticulous agreements on confidentiality of underlying data provide further protection.			
R: Loss of key members (MC or/and WG leader)	1	4	4
C: Personal circumstances can prevent long term participation. When deciding on the MC or WG leader, this member will be required to identify a potential substitute.			
R: Imbalance in # of participants for target insect species	4	2	8
C: Some species are relatively understudied. Firstly, further participants specialised in these species will be added. Secondly, gaps will be filled via a comparative nutrition approach.			
R: Cultural differences and communication barriers	1	4	4
C: Differences in language, culture, and styles can create misunderstandings and misinterpretations, hindering effective collaboration and progress. This is mitigated by establishing communication protocols, outlining preferred communication channels and response times, and by maintaining diverse representation in decision-making processes and project teams.			
R: Unequal Contributions and Expectations	1	4	4
C: Disparities in resources, expertise, and contributions between partners can lead to conflicts. Hence, roles, responsibilities, and expectations from all involved parties will be clearly defined.			
R: Difficulty in sustaining focus	2	4	8
C: Maintaining a focused research agenda can be challenging in large collaborative groups. Divergent interests and goals among participants could lead to a lack of cohesion and direction in the pursuit of common objectives. Clear responsibilities, delegated ownership, frequent management and working group meetings as well as a continuous communication within the Action will ensure focus and delivery of the outlined objectives.			

Legend: R, risk; C, contingency plan; L, likelihood; I, impact; Ind, Index (LxI). L and I are considered on a scale from 1 to 4.

4.1.4. GANTT DIAGRAM

Description	Actor	Year 1	Year 2	Year 3	Year 4				
		Semester							
		1	2	3	4	5	6	7	8
Kick off – identification of MC and WGs leaders	ALL								
Establishment of Knowledge Hub	HG1								
Management Committee meeting	MC								
Working groups meeting	WGs								
Report of the meetings	ALL								
Workshop comparative nutrition	WG 1								
Update on nutritional requirements	WGs 1-5								
Call open for Short-term scientific missions	WGs 1-5								
Workshop by working groups	WGs 2-5								
Training schools	WGs 1-5								
Outputs training schools & workshops	HG1								
General meeting - interaction between WGs	ALL								
Outreach activity at an international event	HG1								
Publish reviews	WGs 1-5								

Legend: Green = meetings; Pink = deliverables; Orange = activities; Blue = milestones

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